

PROGRAM AND ABSTRACT BOOK



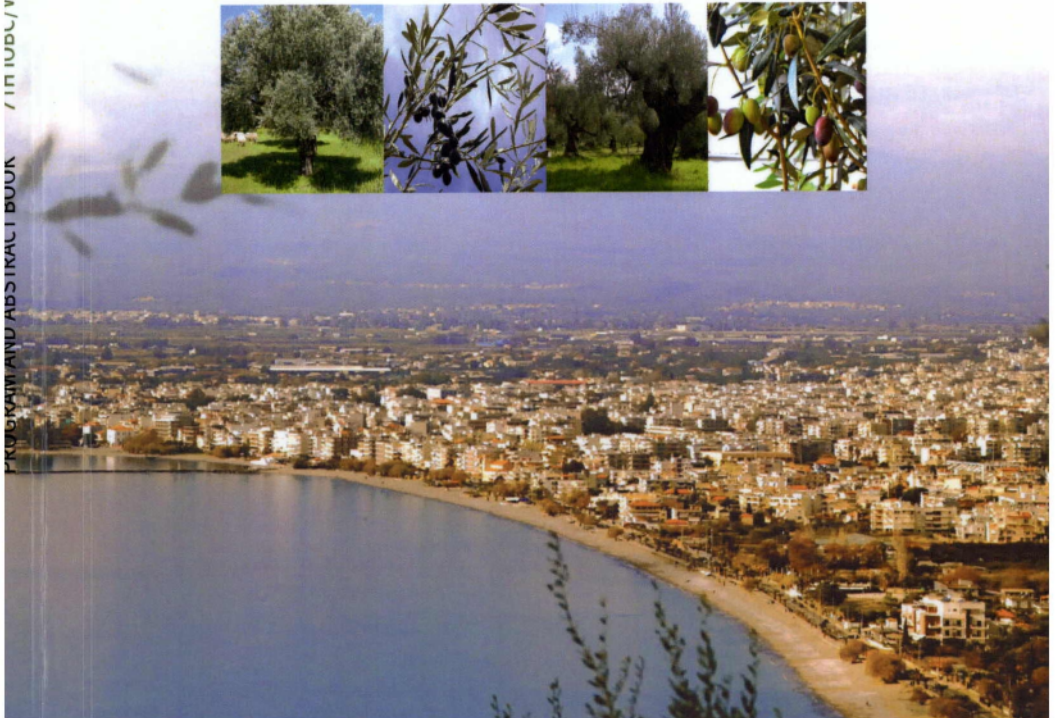
IOBC-WPRS

INTEGRATED PROTECTION OF OLIVE CROPS

11-14
May
2015



7th IOBC/WPRS • KALAMATA GREECE



7th Meeting of the IOBC/wprs
Working Group
**“Integrated Protection
of Olive Crops”**

Organized by:



**7th Meeting of the IOBC/wprs WG Integrated Protection of Olive Crops
Kalamata, Greece 11-14 May 2015**

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Dear Colleagues,

It is with great pleasure that we welcome you to the **7th Meeting of Integrated Protection of Olive Crops**, which will be held in Kalamata (Messinia), Greece, 11-14 May 2015. We would like to thank the International Organization for Biological Control, West Palaearctic Regional Section (IOBC/WPRS) for the honor to organize this meeting. We also would like to thank the co-organizers who are the Hellenic Entomological Society and the Technological Educational Institute of Peloponnese.

Messinia, the place that hosts the meeting, is one of most important olive oil production regions in Greece with 60,000 olive oil producers, 14,799,000 productive olive trees and an annual production of about 50,000 tons of olives. This is the birthplace of the olive cultivars "Kalamon" and "Koroneiki" as well as an area with archaeological sightseeing.

Sixty four scientists from 12 different olive growing countries (Spain, Italy, Portugal, Greece, USA, Israel, Iran, Brasil, Croatia, Egypt, Tunisia, Saudi Arabia) are meeting here in Kalamata, in order to present their research activities concerning the Integrated Protection of Olive Crops. In total, 69 scientific papers will be represented; 35 will be presented orally and 34 in poster format.

The meeting is focused on the exchange of knowledge and expertise on research and implementation of control strategies against olive pests and diseases, with the aim to minimize the impacts on the environment, increase sustainability and support the production of higher quality olive products. Another goal of the group is to promote cooperation among scientists, advisors, students and company representatives dealing with all aspects of olive tree protection.

We thank the members of the organizing committee, the members of scientific committee as well as the secretariat of the meeting, for all their efforts.

We also thank BASF, Captain Vassilis Foundation, Dow Agro-Sciences, Elanco Hellas and Alfa Agricultural Supplies who have provided financial support.

We wish you a good stay and profitable work in Kalamata.

Dr George Stathas
Chairperson

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**PROGRAM OF THE 7TH MEETING
OF THE IOBC/wprs
WORKING GROUP "INTEGRATED PROTECTION
OF OLIVE CROPS"**

Sunday 10 May 2015

**20:00 Welcoming cocktail in the congress hotel
(Elite City Resort)**

Monday 11 May 2015

8:30 -10:00 Registration

10:00-11:00 Welcome address
Organizing Committee – *George Stathas*
Convenor – *Dionyssios Perdikis*
IOBC Representative – *Nikos Papadopoulos*
Other Representatives

11:00-11:20 Opening lecture
**Overview of olive oil production in
Messinia: a socio-economic approach to
the application of integrated management
systems**
Vasilios Demopoulos, Anna Milionis

11:20-11:50 Coffee break

**Session I – Information and Communications Technology
(ICT) and Large Scale Monitoring in Olive Crop Protection**

Moderators: Emannouil Kabourakis, Jesus Mercado-Blanco

**11:50-12:20 Remote management of olive fruit fly
based on a Location Aware System (LAS)**
Theodore Tsiligiroidis, Invited Speaker

- 12:20-12:35** **Development of an innovative spatial decision support system for olive fruit fly monitoring and control**
Costas Pontikakos, Dionyssios Perdikis, Theodore Tsiligiridis
- 12:35-12:50** **The use of monitoring network for estimating early infestation of *Bactrocera oleae* at large scale**
Susanna Marchi, Diego Guidotti, Massimo Ricciolini, Ruggero Petacchi
- 12:50-14:30** Lunch

Session II – Research in Olive Diseases and Pests

Moderators: José Alberto Pereira, Antonio Ortiz

- 14:30-14:45** **Seasonal monitoring of olive fruit fly, *Bactrocera oleae* (Diptera: Tephritidae) in different agroecological zones in Greece**
Nikolaos Volakakis, Giannoula Bogka, Vassileios Gkisakis, Emannouil Kabourakis
- 14:45-15:00** **Damage potential of *Euphyllura phillyreae* on olives**
Dionyssios Perdikis, Nikolaos Gyftopoulos, Panagiotis Anastasopoulos, Theodoros Vaiopoulos, Konstantina Arvaniti
- 15:00-15:15** **Oviposition preference of *Bactrocera oleae* (Rossi) (Diptera: Tephritidae): influence of cultivar (cvs. Cobrançosa, Madural and Verdeal Transmontana) and maturation process**
Ricardo Malheiro, Susana Casal, Lara Pinheiro, Paula Baptista, José Alberto Pereira

- 15:15-15:30** Electrophysiological responses (EAG) of *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) and its natural enemies *Chrysoperla carnea* Steph. (Neuroptera: Chrysopidae) and *Psytallia concolor* (Szépligeti) (Hymenoptera: Braconidae) to ammonia
Antonio Ortiz, M^o Jose Hervás, Antonio Estevez
- 15:30-15:45** Effects of probiotic diets on the olive fly fitness
Patrizia Sacchetti, Luca Becherini, Antonio Belcari
- 15:45-16:00** Effect of natural vegetation management practices on *Prays oleae* (Bernard) and its parasitoids
Maria Villa, Rosalina Marrão, António Mexia, Albino Bento, José Alberto Pereira
- 16:00-16:15** Evaluation of various commercial trap types for *Bactrocera oleae* (Diptera: Tephritidae) in field and laboratory studies
Arayro Kalaitzaki, Eleni Malandraki
- 16:15-16:30** The electronic McPhail Trap and a potential revision of the decision protocol
Ilyas Potamitis, Panagiotis Eliopoulos, Dimitrios Kontodimas
- 16:30-17:00** Coffee break

17:00-17:35 POSTER SESSION

- 1. *Bactrocera oleae* (Rossi) phenology of wintering populations in Liguria (Italy)**
Ruggero Petacchi, Susanna Marchi, Simona Federici, Giorgio Ragagnini

2. **Natural enemies of *Bactrocera oleae* in different geographic regions of Greece**
Argyro Kalaitzaki, Dionyssios Perdikis, George Stathas, Panagiotis Skouras, Ioannis Zarboutis, Evanthia Pitika, Konstantina Spanou
3. **From genome to behavior: investigating the molecular basis of olfaction as target to interrupt the sexual communication for population control of the olive fruit fly, *Bactrocera oleae***
Konstantina Tsoumani, Alexandros Belavilas-Trovas, Kostas Mathiopoulos
4. **Studies on the damage potential of *Rhynchites cribripennis***
Dionyssios Perdikis, Spyridonas Reppas
5. **Preference of *Prays oleae* for oviposition among olive varieties and different quadrants of the olive tree canopy**
Dionyssios Perdikis, Argyro Kalaitzaki, Panagiotis Anastasopoulos, Theodoros Vaiopoulos
6. **Color of fruits and leaves of Portuguese olive cultivars (cvs. Cobrançosa, Madural and Verdeal Transmontana) and their relation to *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) oviposition preference**
Ricardo Malheiro, Susana Casal, Nuno Rodrigues, Paula Baptista, José Alberto Pereira
7. **High-light on certain olive pests and their control in Egypt**
Mohamed Abdel-Rahman Mohamed Amro

Tuesday 12 May 2015

9:00-18:00 Professional Tour

Wednesday 13 May 2015**Session III - Research In Olive Diseases and Pests and Their Control***Moderators: Leah Tsrer, Paula Baptista*

- 9:00-9:30** **The threat posed by *Xylella fastidiosa* to olive trees in the Mediterranean region: the first outbreak in Apulia, Italy**
Anna Maria D'Onghia, Invited Speaker
- 9:30-9:45** **Endophytic fungi of olive tree and its potential application in protection against *Colletotrichum acutatum***
Fátima Martins, José Alberto Pereira, Albino Bento, Paula Baptista
- 9:45-10:00** **Relationship between phyllosphere fungal communities and tolerance of olive tree cultivars to olive leaf spot disease**
Teresa Gomes, José Alberto Pereira, Teresa Lino-Neto, Paula Baptista
- 10:00-10:15** **Genomic analysis of two *Pseudomonas* spp. strains displaying endophytic lifestyle in olive roots and effective biological control of *Verticillium dahliae***
Pedro Martínez-García, David Ruano-Rosa, Elisabetta Schiliro, Carmen Gómez-Lama Cabanás, Cayo Ramos, Pablo Rodríguez-Palenzuela, Jesús Mercado-Blanco
- 10:15-10:30** **Influence of irrigation frequency on the onset and development of *Verticillium* wilt of olive**
Mario Pérez-Rodríguez, Nicolás Serrano, Ignacio Lorite, Octavio Arquero, Francisco Orgaz, Francisco Javier López-Escudero
- 10:30-11:00** Coffee break

Session IV - Research in Olive Diseases and Pests and Their Control (continued)

Moderators: John Vontas, Filitsa Karamaouna

- 11:00-11:15** **Ground cover as a practice to support pollinating insects and natural enemies in olive groves**
Filitsa Karamaouna, Vaya Kati, Nikolaos Volakakis, Kyriaki Varikou, Leonidas Economou, Nikolaos Garantonakis, Athanasia Birouraki, Fotis Andrinopoulos, Voula Kalliakaki, Emilia Markellou
- 11:15-11:30** **Identification and molecular characterization of pyrethroid resistance in the olive fruit fly *Bactrocera oleae***
Nena Pavlidi, Anastasia Kampouraki, Nicky Wybouw, Wannes Dermauw, Thomas Van Leeuwen, John Vontas
- 11:30-11:45** **A novel approach to evaluate efficacy of baited insecticide applications for the olive fruit fly control**
Emmanouil Roditakis, Maria Grispou, Marianna Stavrakaki, Nikos Roditakis, Giannis Livadaras, John Vontas, Ralf Nauen, Nigel Godley, Emmanouil Filippou, Emmanouil Manousakis, Angeliki Karataraki
- 11:45-12:00** **SAGE10-Establishment of an impact assessment procedure as a tool for maintaining the sustainability of Mediterranean olive agro-ecosystems**
Emilia Markellou, Irini Mantzouni, Antigoni Akrivou, Evangelos Karanasios, Filitsa Karamaouna, Katerina Kyriakopoulou, Eleftheria Kapaxidi, Eleni Karassali, Dimitrios Papachristos, George Michalopoulos, Evangelos Hatzigiannakis, Helena

Hadjicharalambous, Dionyssios Perdikis, Styliani Malliaraki, Aikaterini Angelaki, Chrysoula Manolaraki, Georgia Giakoumaki, Spyridonas Reppas

- 12:00-12:15 GELSURA and AgBalance: from sustainability assessment to continuous improvement of olive cultivation**

Notis Aristeidis, Cesar Blanco Ruiz, Sebastian Schultze, Markus Frank, Stergios Bitivanos

- 12:15-12:30 Spinetoram (Delegate™ 250WG), a new insecticide for the control of *Prays oleae* (Bernard) in olives**

Aris Chloridis, Maria Torne, Enzo Tescari, Vassilis Apostolidis

12:30-13:15 POSTER SESSION

- 8. Evaluation of "Insect Spy" approach for monitoring *Xylella fastidiosa* in symptomless olive orchards in the Salento Peninsula (Southern Italy)**

Issam Eddine Ben Moussa, Franco Valentini, Donato Lorusso, Valerio Mazzoni, Michele Digiario, Leonardo Varvaro, Anna Maria D'Onghia

- 9. Diversity of epi- and endophytic fungi on asymptomatic and symptomatic olive tree phyllosphere**

Teresa Gomes, José Alberto Pereira, Teresa Lino-Neto, Paula Baptista

- 10. The potential of indigenous bacteria from the olive rhizosphere as biological control agents against a broad range of phytopathogens**

David Ruano-Rosa, Antonio Valverde-Corredor, Carmen Gómez-Lama Cabanás, Rafael Sesmero, Jesús Mercado-Blanco

11. **Updated check list of scale insects (Hemiptera: Coccoidea) on olive trees in Croatia with special regard on Black araucaria scale - *Lindingaspis rossi* (Maskell 1891)**
Tatiana Masten Milek, Mladen Simala, Mario Bjeliš, Maja Pintar, Vjekoslav Markotic
12. **Preliminary studies on the management of the under canopy flora as a potential means to control the olive bug *Closterotomus (Calocoris) trivialis***
Dionvssios Perdikis, Spyridonas Reppas, Konstantina Arvaniti, Chrysaugi Reppa
13. **Biodiversity of soil beneficial arthropods in olive groves of different crop systems in three regions in Greece**
Eleftheria Kapaxidi, Maria Chatzaki, George Partsinevelos, Filitsa Karamaouna, Dimitrios Papachristos, Styliani Malliaraki, Aikaterini Angelaki, Chrysoula Manolaraki, Georgia Giakoumaki, Spyridonas Reppas
14. **"Teasing" the reproductive system of *Bactrocera oleae* as a potential tool for pest control**
Maria-Eleni Gregoriou, Spyros Tastsoglou, Martin Reczko, Kostas Mathiopoulos
15. **Remating of olive fruit fly (*Bactrocera oleae*) in the wild**
Christos Gerofotis, Antonis Augustinos, Maria-Eleni Gregoriou, Kostas Mathiopoulos, Nikos Papadopoulos
16. **Using Geometric morphometrics to investigate wing dimorphism in Iranian populations of *Bactrocera oleae* (Rossi) (Diptera: Tephritidae)**
Mitra Moezipour, Jamasb. Nozari, Parvaneh Azmayesh Fard, Kostas Mathiopoulos
17. **Geometric morphometric analysis of wing shape variation in world populations of *Bactrocera oleae***
Mitra Moezipour, Jamasb. Nozari, Parvaneh Azmayesh Fard, Kostas Mathiopoulos

13:15-14:45 Lunch

Session V – Control of Diseases and Pests

Moderators: Kostas Mathiopoulos, Anna Maria D'Onghia

- 14:45-15:00** Effects of semi-natural habitats on spiders in olive groves and their potential role against *Bactrocera oleae* (Rossi) (Diptera: Tephritidae).
Malayka Picchi, Martin Entling, Ruggero Petacchi
- 15:00-15:15** Functional responses of soil predators fed on pupae of *Bactrocera oleae* (Rossi)
Ana Dinis, Jacinto Benhadi-Marin, José Alberto Pereira, Sônia Santos
- 15:15-15:30** An autoparasitoid inferior at resource exploitation outcompetes primary parasitoids and controls their common host (*Saissetia oleae*) in the field
Rosalina Marrão, José Alberto Pereira, Alejandro Tena
- 15:30-15:45** Effects of natural occurring plants in olive agroecosystems and insect honeydews on the survival of *Chrysoperla carnea* (Neuroptera: Chrysopidae)
Maria Villa, Jacinto Benhadi-Marin, Antonio Mexia, Albino Bento, José Alberto Pereira
- 15:45-16:00** Production of alkaline protease by a Tunisian *Brevibacillus brevis* strain used as biopesticide against *Prays oleae*
Imen Blibech, Mohieddine Ksantini, Sami Aifa
- 16:00-16:15** The outbreak of the olive leaf gall midge populations and the importance of indigenous natural enemies in its control
Dionyssios Perdikis, Konstantina Arvaniti, Styliani Malliaraki, Aikaterini Angelaki

- 16:15-16:30** **A novel insecticidal formulation based on α -cypermethrin against *Bactrocera oleae* (Rossi) (Diptera: Tephritidae)**
Nickolas Kavallieratos, Stergios Bitivanos, Anastasios Klitsinaris, Efstratios Tzirtzilakis, Ioannis Rallis, Ioannis Zarboutis, Dimitra Markoyannaki, Dimitrios Kontodimas
- 16:30-16:45** **Population dynamics of the olive fly in Kyparisia, Greece under an area wide management program**
Athanassios Liakatas, Antonios Paraskevopoulos, Spyros Fountas, Nikoleta Dionysopoulou, Nikos Papadopoulos
- 16:45-17:15** Coffee break

17:15-18:00 **POSTER SESSION**

- 18. Arthropoda associated to the olive crop in Southern Portugal (Algarve)**
Maria Goncalves
- 19. Biodiversity of arthropods in a landscape mosaic dominated by organic olive groves in Alentejo (south-east of Portugal)**
Cláudia Gonçalves, Maria Isabel Patanita, Paula Nozes, Ruben Mateus, José Alberto Pereira, Sônia Santos
- 20. Scale insect mortality in olive trees in Rio Grande do Sul (Brasil)**
Gabriela de Souza, Luiza Redaelli, Vera Wolff
- 21. Diversity and parasitism of scale insects (Hemiptera: Coccoidea) in ten varieties of olive trees (*Olea europaea* L.) in Southern Brazil**
Vera Wolff, Caio Efrom, Daniele Campos da Silva, Adilson Tonietto

22. **Genomic and functional approaches for understanding the adaptation of the olive fruit fly *Bactrocera oleae* to olives**
Nena Pavlidi, Nicky Wybouw, Wannes Dermauw, Yannis Livadaras, Thomas Van Leeuwen, John Vontas
23. **Ecosystem services for the control of *Bactrocera oleae* in different landscape spatial contexts**
Manuel González-Núñez, Ismael Sánchez-Ramos, Marta Ortega, Alejandro Rescia, Susana Pascual
24. **Estimation of the number of olive fruit fly *Bactrocera oleae* (Diptera: Tephritidae) attracted and killed by various bait spray solutions**
Kyriaki Varikou, Nikos Garantonakis, Athanasia Birouraki
25. **Composition and biodiversity of weed flora in established olive groves of southern Greece**
Garyfallia Economou, Dionysios Kalivas, Petros Vahamidis
26. **Insect pests associated with olive crop and virulence of native entomopathogenic nematodes to olive kernel borer, *Prays oleae* (Lepidoptera: Hyponomeutidae) in Saudi Arabia**
Magdy El-Kholly, Abdellah Abdel-Moniem, Sadek Salem

21:00

Gala dinner

Thursday 14 May 2015

Session VI – Control of Diseases and Pests (continued)

Moderator: Franco Nigro, Polymnia Antoniou

9:00-9:30

Verticillium wilt of olive - Current status and Management*Leah Tsrur (Lahkim). Invited Speaker*

- 9:30-9:45** **Serious anthracnose outbreak starting from inflorescence infection of olive trees in west Greece**
Eleftherios Tjamos, Maria Iliadi, Dimitrios Tsitsigiannis, Polymnia Antoniou
- 9:45-10:00** **Biological control of olive anthracnose**
Franco Nigro, Ilaria Antelmi, Rossella Labarile, Valentina Sion, Isabella Pentimone
- 10:00-10:15** **Current problems in managing Verticillium wilt of olives in Greece and the prospective of non chemical and biological control of the disease in olive orchards**
Polymnia Antoniou, Sotirios Tjamos, Dimitrios Tsitsigiannis, Eleftherios Tjamos

10:15-11:00 **POSTER SESSION**

- 27. Screening of organic amendments, plant extracts and microorganisms for the control of Verticillium wilt in olive trees**
Angela Varo, Carmen Raya, Luis Roca, Antonio Mulero, Mustafa Adem, Francisco Javier López-Escudero, Antonio Trapero
- 28. Effect of the irrigation dose on Verticillium wilt of Olive**
Mario Pérez-Rodríguez, Ignacio Lorite, Francisco Orgaz, Francisco Javier López-Escudero
- 29. Endophytic bacteria as biocontrol agents against Verticillium wilt on olive tree: an overview**
Diogo Mina, José Alberto Pereira, Albino Bento, Paula Baptista
- 30. A preliminary study of potential use of olive mill wastes as biocide against pupae of *Bactrocera oleae***
Arqyro Kalaitzaki, George Koubouris, George Psarras, Emmanouil Markakis, Eleni Malandraki

31. **SuccessTM 0.24 CB, the Naturalyte Insecticide for the control of *Bactrocera oleae* (Gmelin) by bait spray in Mediterranean countries**
Vassilis Apostolidis, Maria Torne, Enzo Tescari, Aris Chloridis
 32. **Pruning residue management associated pathogens in olive**
Emmanouil Markakis, George Koubouris, Nektarios Kavroulakis, George Psarras, Chryssa Sergeantani, Aravro Kalaitzaki
 33. **Quick decline syndrome and anthracnose: emerging and reemerging diseases of olive posing new challenges for an integrated control approach**
Franco Nigro, Ilaria Antelmi, Antonio Ippolito
 34. **Risk assessment of *Xylella fastidiosa* for olive growing area of Croatia**
Mario Bjeliš, Dario Ivič
- 11:00-11:30 Coffee break**
- 11:30-12:00 Concluding remarks**
Nikos Papadopoulos, Eleftherios Tjamos
- 12:00-12:30 Meeting of the WG – Future developments – Voting on the next Venue**
Convenor – *Dionyssios Perdikis*
IOBC Representative – *Nikos Papadopoulos*

OPENING SESSION



**Overview of olive oil production in Messinia:
a socio-economic approach to the application
of integrated management systems**

V. Demopoulos, A. Millionis

*Kalamata Olive Oil Taste Laboratory, Technological Educational
Institute of Peloponnese, 24 100 Antikalamos, Kalamata, Greece*

Olive trees are a historical, cultural, social and economic asset in Messinia. Nearly every home in Messinia uses its own produced olive oil in an extravagant way pointing to the fact that Greece is the highest per capita olive oil consuming country, in the world. Potato chips are fried in extra virgin olive oil, which is not used more than twice. The issue of which oil has the highest smoking point is not a concern. Olive oil is the cornerstone of the Messinian diet. However, the vast majority of households have family-run olive orchards that produce modest quantities of olive oil. The surplus olive oil is sold through a personal network of relatives and friends at a reasonable price or in bulk to Italy. The contribution of this income to the average family budget ranges from medium to small. Often the main income of the family comes from other sources. Olive groves are cultivated mainly by people who have other main occupations and take leave from their work to harvest their trees, or wait for the weekend to prune, fertilize or spray. As a result, the interest of these people to participate in cooperatives or other collective forms is from low to nil. The control of the olive fruit oil fly, the most important pest in the region, is centralized and carried out by ministerial authorities with questionable results. So while olive oil represents more than 50% of the agricultural production in volume and olive trees occupy more than 60% of the farming land in Messinia, statistics and narratives demonstrate that there is wide scope of social structure issues and economic factors that have to be considered when designing and applying integrated management systems.

Key words: olive oil production, socio-economic issues, integrated management systems, Messinia

SESSION I

INFORMATION AND COMMUNICATIONS TECHNOLOGY (ICT) AND LARGE SCALE MONITORING IN OLIVE CROP PROTECTION



Remote management of olive fruit fly based on a Location -Aware System (LAS)

Theodore Tsiligridis

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Informatics, Department of Agricultural Economy and Development
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11855 Athens, Greece*

Recent advances in Information and Communications Technologies (ICTs) allows the implementation of Location Aware Systems (LASs) almost in every activity that has spatial characteristics. Scientific issues and especially Pest Management (PM) problems with spatial features can be significant assisted by the design, development and implementation of a suitable LAS. *FruitFlyNet* project aims to develop an innovative LAS geared to prevent and reduce the environmental risk factors at the Mediterranean Sea basin level. It contributes to the development, implementation and demonstration of environmentally effective e-monitoring and ground spraying control solutions based on prototypes, technological innovations and knowledge transfer for specific key-pests in the Mediterranean, in order to increase the quality and quantity of available fruit to local consumers at lower prices. The system will be developed, deployed, and tested for *Bactrocera oleae* (in Spain, Jordan and Greece), *Ceratitidis capitata* (in Italy), *Rhagoletis cerasi* (in Greece), *Dacus ciliatus* and *Bactrocera zonata* (in Israel). In this talk we provide the general overview of the system under development concentrating more on the case of olive fruit fly. The LAS under deployment is based on an innovative trap capable of semi-automatically/automatically counting each insect trapped, identifying the species and send the data wirelessly. It will also be able to function autonomously during the whole fruiting season, it has an operative life-time of at least one year and it can be adapted to handle other fruit flies. Agriculture and related industries are fields that can greatly benefit by the adoption of new, harmonized practices. For example, the surveillance and monitoring of the pest population in order to timely apply bait-sprays is the most important activity for pest management. At farm level, operators perform periodical surveys of the traps distributed through the field. This is a labor-, time- and cost- consuming activity and requires a high level of expertise and accuracy. Therefore, it would be of great advantage to have an affordable location-aware

system like the one provided by the *FruitFlyNet*, doing this task semi-automatically/automatically in an accurate and more efficient way.

Key words: ICT, *Bactrocera oleae*, *Ceratitis capitata*, *Rhagoletis cerasi*, *Dacus ciliatus*, *Bactrocera zonata*, automatic insect counting, pest management

Development of an innovate spatial decision support system for olive fruit fly monitoring and control

**Costas Pontikakos¹, Dionyssios Perdikis²,
Theodore Tsiligiridis¹**

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In this work an innovate Spatial Decision Support System (S-DSS) developed for the management and optimization of the monitoring and control of the olive fruit fly (*Bactrocera oleae*) is presented. The developed S-DSS is based on a geo-spatial database architecture that combines different data sources such as Location Aware Systems (LAS), Wireless Sensor Networks (WSN), mobile GIS, e-trapping and geospatial risk assessment. The S-DSS aims to facilitate the decision making process in each stage of the monitoring and control of the olive fruit fly. Based on this approach the S-DSS consists from components that provide decisions related to the timing of sprays, the area to be sprayed and the proper spraying process according to the risk level maps, the effectiveness and accuracy of the spraying applications and the protection level of the environment. For each component the input data, the output data and the decision making framework are presented and described.

Key words: olive fruit fly, spatial decision support system, location aware system, monitoring, control

The use of monitoring network for estimating early infestation of *Bactrocera oleae* at large scale

**Susanna Marchi¹, Diego Guidotti², Massimo Ricciolini³,
Ruggero Petacchi¹**

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Bactrocera oleae (Rossi) population dynamics strongly depend, like other pest, on weather pattern, in particular air temperature. In Tuscany (central Italy), the regional extension service has set a monitoring network at farm level to follow *Bactrocera oleae* infestation on olive fruits from July to October, during the last fourteen years. Starting from the agrometeorological network database, we have calculated indices based on temperature and precipitation to describe the weather pattern during three periods of the year. These were selected according to *Bactrocera oleae* annual cycle. We implemented a prediction model relating the percentage of infestation observed at the field level to selected agrometeorological indices. To choose representative indices and avoid collinearity we applied principal component analysis (PCA). Only temperature-based indices were significant in predicting *B. oleae* infestation, in particular minimum and average temperature of winter preceding the summer infestation. Warmer winters sustained high infestation level in the following summer. Temperatures of the previous winter and spring explained 66% of variance of early-season infestation. Although a correct application of integrated pest management requires the monitoring of *B. oleae* infestation at the field level, the development of predictive models can provide early-warning signals of severe outbreaks of this pest and a well-timed set of control strategies.

Key words: *Bactrocera oleae*, integrated pest management, insect pest outbreaks, pest-monitoring network

SESSION II

RESEARCH IN OLIVE DISEASES AND PESTS



Seasonal monitoring of olive fruit fly, *Bactrocera oleae* (Diptera: Tephritidae) in different agroecological zones in Greece

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This study monitored the seasonal occurrence of adult olive fruit fly *Bactrocera oleae* (Rossi) activity and the levels of fruit infestation in 24 commercial olive orchards for two successive years. These orchards represented the main agro-ecological zones of olive oil production; including four sites located in the plain areas and another four sites located in the foothills of the West Messara valley, South Crete, Greece. Adult olive flies were weekly monitored using McPhail traps, while the levels of fruit infestation by the flies were determined based on fruit sampling once every two weeks from July until harvest. Weather conditions and management practices of the orchards were also recorded. High numbers of adult flies were captured in spring, but the numbers decreased from July to mid September, and then increased after September. Numbers of captured flies were higher in the hilly orchards than the plain orchards, but the levels of fruit infestation were similar in the hilly and plain orchards. High mortalities of olive fly adults, eggs, and larvae from July to September could result from sustained high summer temperatures. These results suggest that control strategies for olive fruit fly may need to take into account the impact of climate factors such as high summer temperatures.

Key words: *Bactrocera oleae*, agroecological zone, temperature, humidity, climate

Damage potential of *Euphyllura phillyreae* on olives

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The olive psyllids commonly occur on olive trees but their pest status has been little searched. In this paper efforts were developed to search the damage potential of the *Euphyllura phillyreae* (Hemiptera: Aphalaridae) in regard to its population densities on olive stems. For this reason stems at the period of early inflorescence development were covered with muslin cages and the number of inflorescences per stem was recorded. The intensity of the pest infestation on the enclosed stems was followed till the fruit development and classified in very intense, intense, moderate and control, according to the covering rate of the stem and the inflorescences with the cottony waxy secretions of the psyllid. In example, in the very intense treatment the entire stem was covered with the secretions during the experimental period. The experiments were initiated on 5th April 2013 and 14th March 2014, on trees of the olive variety "Koroneiki", in the campus of Agricultural University of Athens. According to the results the intense infestation reduced fruit load in 2013, but not significantly in comparison to the other treatments (0.23, 0.25, 0.17 and 0.02 fruits per inflorescence in stems without, moderate, intense and very intense infestation, respectively). The number of individuals per inflorescence was 0.65 on 5th April and the mean number of inflorescences developed per stem was 13.25 to 19.25. In 2014, a similar effect was found among the treatments (0.88, 0.70 and 0.53 fruits per inflorescence in stems without, moderate and intense infestation, respectively). These results indicate that this pest may cause a reduction in olive fruit setting which however was not significant.

Key words: olive psyllid, Aphalaridae, Koroneiki

Oviposition preference of *Bactrocera oleae* (Rossi) (Diptera: Tephritidae): influence of cultivar (cvs. Cobrançosa, Madural and Verdeal Transmontana) and maturation process

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Olive fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) is a key pest of olive crop, causing severe production losses, diminishes olive products quality, composition and properties, affecting all links of olive chain: producers, industrials and consumers. This pest display an oviposition preference all over the world, reporting some cultivars systematically low infestation levels, while others are heavily infested every crop campaign. In the present study the oviposition preference of olive fly towards three Portuguese olive cultivars (cvs. Cobrançosa, Madural and Verdeal Transmontana) was assessed in different oviposition bioassays. Olives at different maturation indices (MI) (MI 2, MI 3, and MI 4) were also assayed to evaluate the impact of maturation in olive fly preference. One-choice oviposition bioassays revealed high preference of olive fly by cv. Verdeal Transmontana, followed by cv. Madural, being cv. Cobrançosa the less preferred by olive fly. Nearly 78% of olives from cv. Verdeal Transmontana were infested, while 59% and 45% of olives from cvs. Madural and Cobrançosa were attacked respectively. A lower percentage of pupae/adults were recovered from cv. Cobrançosa olives (51%) while in cvs. Madural and Verdeal Transmontana the percentage recovered pass 60%. In the oviposition bioassays to determine the impact of olives maturation only cvs. Cobrançosa and Madural were tested at MI 4. It was clear that from MI 2 and MI 3 to MI 4 the number of ovipositions decrease considerably: a decrease of 63% and 36% in cvs. Cobrançosa and Madural respectively. Clearly olive fly prefer to oviposit in olives at green or reddish color. The present study concluded that olive fly has an oviposition preference to cvs. Verdeal Transmontana and Madural,

while cv. Cobrançosa is less preferred. Maturation stage influence olive fly oviposition with high maturation stages, at MI 4, deterring oviposition. Physical parameters, like olives color, as well as olives volume and elongation could be attributed to the oviposition preference of olive fly towards cv. Verdeal Transmontana. Olives from cv. Verdeal Transmontana also emit higher lightness (L^* values) than the remaining olive cultivars, fact probably related to chemical aspects, namely epicuticular waxes in the surface of olives.

Key words: *Bactrocera oleae*, olive cultivar, maturation, oviposition preference

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Electrophysiological responses (EAG) of *Bactrocera oleae* (Rossi) (Diptera:Tephritidae) and their natural enemies *Chrysoperla carnea* Steph. (Neuroptera: Chrysopidae) and *Psytallia concolor* (Szépligeti) (Hymenoptera: Braconidae) to ammonia

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Food baits are commonly used in traps for monitoring the olive fruit fly *Bactrocera oleae* (Rossi), but many of these baits are not specific to the fly. The lures attracting the olive fruit fly are volatiles from fermentation of protein and/or ammonia. Gaseous ammonia is one of the key compounds of the attractants, but little is known if it also attracts *B. oleae* natural enemies (i.e., non-target impact). In this study, we used electroantennogram (EAG) bioassay to quantify the responses of *B. oleae* and two of its natural enemies, *Chrysoperla carnea* and *Psytallia concolor* to ammonia. The EAG tests showed the strongest responses by the adults of each of three species to ammonia, when compared to their responses to other active components. The relationships between the strength of EAG response and ammonia dose were best fit by hyperbolic models. Over a range of doses tested (0.001 to 1 N) in all three species, female responded stronger significantly than male to ammonia. A 5% solution of diammonium phosphate (which releases ammonia) has been used for years as a food bait for monitoring and mass trapping of *B. oleae*. But the mass trapping effectiveness is highly variable and it probably does not provide adequate pest control when used alone. The release rate of ammonia from aqueous solutions is dependent on the pH values and temperature. In an initial field test, McPhail-type traps baited with ammonium biphosphate as a synthetic attractant were used for green lacewings monitoring. The results showed a positive correlation between the number of captured *Chrysoperla carnea* adults and the pH value of the solution.

Key words: electroantennogram, EAG, *Bactrocera oleae*, *Chrysoperla carnea*, *Psytallia concolor*

Effects of probiotic diets on the olive fly fitness

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Sterile Insect Technique (SIT) is a powerful tool in modern IPM strategies that has been applied to control several insect pests, especially fruit flies. Presently SIT cannot be employed to control the olive fly, the primary insect pest of olive fruits, mainly due to the high cost procedure and low quality of mass reared flies. Lack of symbiotic microorganisms in lab reared colonies might be the limiting factor in olive fly mass production. Since the use of preservatives and antimicrobials in artificial diets likely causes the olive fly to lose its endosymbiont, *Candidatus* *Erwinia dacicola*, the hypothesis that the role of the symbiont may be replaced in lab reared flies by supplying culturable bacteria was explored. The effects of bacteria on the olive fly physiology were evaluated feeding adults of a *Bactrocera oleae* lab strain with probiotic diets based on two bacteria that have been found associated with the fly: *Pseudomonas putida* and *Acetobacter tropicalis*. In order to highlight possible probiotic effects of the microorganisms on the olive fly fitness, live and heat-killed bacteria have been supplied at two different concentrations. Laboratory bioassays showed how live *P. putida* colonies positively affected longevity and fecundity, moreover the female survival was increased by the twofold concentration of the bacterium. On the contrary, probiotic diet containing heat-killed *P. putida* did not positively affect adult life span, indirectly proving that live bacteria are involved in the host metabolism and consequently they may play an important role within the host gut. In general, flies fed on live *A. tropicalis* showed a negative effect on olive fly survival compared to those fed on sugar, although the probiotic diet with the bacterium at the lower concentration enhanced egg production. Concerning fecundity, flies fed on full protein diet always laid the highest number of eggs, compared to sugar or to probiotic diets.

Key words: olive fly, artificial diet, *Pseudomonas putida*, *Acetobacter tropicalis*

Effect of natural vegetation management practices on *Prays oleae* (Bernard) and its parasitoids

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The olive moth (*Prays oleae* Bernard) is generally considered as the most serious pest of olive trees in northern Portugal. In this region, there is a rich and diverse parasitoid complex of this pest. Spontaneous vegetation may provide food, alternative hosts and shelters for these parasitoids. This study investigated the effects of different spontaneous vegetation management practices on the moth's antrophagous generation and its parasitism. A total of 14 and 15 olive groves with different spontaneous vegetation management practices (herbicide application, tilling, and conservation of vegetation) were sampled in 2011 and 2013, respectively. In 2012, the pest population levels were extremely low probably due to the serious drought. In each grove, 10 olive trees were randomly selected and 20 olive moth larvae per tree were collected. Larvae were kept in laboratory at controlled conditions and emerging moths and parasitoids were recorded. Generalized Estimation Equations (GEE) with a Logit link function and exchangeable correlation structure were used to fit the response variables. HeatmapFit was used to assess the quality of the models. The estimated probability of presence of olive moth in 2011 (range: 0.15 to 0.20) was significantly lower than in 2013 (range: 0.55 to 0.70) but did not differ among different management practices. In contrast, the estimated probability of parasitism in the groves with spontaneous vegetation (0.50) was significantly higher than in the groves with herbicide application (0.36) in 2011. The parasitism experienced a dramatic decrease in 2013 and was significantly lower in the groves with spontaneous vegetation (0.045) than in the tilled groves (0.13). *Ageniaspis fuscicollis* (Dalman) accounted for the majority of the parasitism and followed by *Elasmus flabellatus* (Fonscolombe). These results suggest that, presence of spontaneous vegetation has probably increased the abundance of parasitoids and conservation of spontaneous vegetation, combined with other field management practices, may

be considered as an important strategy for the control the olive moth. Nevertheless, a significant decrease of the olive moth population suffered from the 2012 drought had also seriously reduced the abundance of parasitoids. This fact emphasizes the value of maintaining an equilibrium between the abundance of natural enemies and pests.

Key words: conservation biological control, ecological infrastructure, olive grove, parasitism

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**Evaluation of various commercial trap types for
Bactrocera oleae (Diptera: Tephritidae) in field
and laboratory studies**

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Five types of commercially available traps (Dakofaka, Glass McPhail, Plastic McPhail and Plastic Elkofon with food attractants and Ecotrap with food and sex attractants) were compared for their attractiveness to olive fruit fly *Bactrocera oleae* (Gmelin) (Diptera: Tephritidae) adults in the field and laboratory. Field studies were carried out during the seasons when adult flies were active in an olive grove in Chania, Crete (S. Greece) during 2012 and 2013. Laboratory experiments (choice and non choice) were conducted to determine the attractiveness of the traps to the fly under a controlled fly density (50 or 25 pairs). In the field the Ecotrap was most attractive to adult flies, followed by the Dakofaka and the Glass McPhail traps, while the Plastic McPhail trap and the Plastic Elkofon trap were least attractive. The Ecotrap captured 2 to 70 times more olive fruit flies than the rest traps tested during the fall in both years. Laboratory tests showed that, both Ecotrap and Dakofaka traps captured ≈30% of the fly population present in the tests, whereas the Glass McPhail trap, Plastic McPhail trap and Plastic Elkofon trap captured 12.3%, 7.9% and 4.2% of the population, respectively. This study also demonstrated that traps baited with attractants in solid form were more effective than other forms for mass trapping. Furthermore, the Ecotrap provides an effective tool for monitoring olive fruit fly than the currently used Glass McPhail trap, especially during the fall.

Key words: *Bactrocera oleae*, commercial traps, attractiveness

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The electronic McPhail Trap and a potential revision of the decision protocol

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For insect species that are of large economic impact such as the olive fly, *Bactrocera oleae* (Diptera: Tephritidae) there is a protocol to be followed on when to initiate a spraying procedure based on pest population monitoring using traps. The monitoring process for countries of large production of olive oil where olive oil production is a countable figure of the state's income, such as Spain, Italy and Greece is coordinated either by the state and sometimes assisted by large cultivators and olive-oil associations. In Europe, the olive fruit fly is controlled with ground pesticide sprays, but their efficiency depends on knowing the population level and location of the pest as early as possible in order to initiate the spraying procedure. This protocol is costly, complicated, it involves a large number of people that are not always qualified to carry the task but most importantly easily bypassed by practitioners. Therefore, large economic loss is often reported because of the past and this is usually attributed by expert entomologists not to the inefficiency of the protocol, but to its opportunistic application that often leads to an 'educated guess' of when and where to start the spraying procedure. The review of this protocol which is tested and shaped for decades is not the issue of this paper. The main point is that a large number of glass McPhail traps are placed in olive orchards and a large number of people should place, inspect the traps on a 5-day basis from the end of spring till the end of fall. The pest-managers must recognize adults of the olive fruit fly in the mass of trapped insects and even extract and deliver these counts to authorities as regards the Greek case. The weak point of this process is that it is difficult to find a large number of qualified and reliable personnel that can carry out efficiently the timing, counting and delivering of insects. The electronic McPhail trap does not revisit other aspects of the protocol, but only replaces two stages of it:

a) The classification of the insect is done automatically as it flies-in and collective results are transmitted to the central monitoring agency. There is no manual checking involved for at-least a month and we are currently testing traps that are power-sufficient for a larger time-scale.

b) The traps carry a GPS sensor and, therefore along with the insect counts transmit their coordinates. Therefore, one can be sure that the insects counted come from the placed located and not from some easier to reach the point.

In this work we describe a novel, fully operational stand-alone system for remote monitoring of insects of economic and social importance in the context of the decision protocol.

The electronic McPhail trap is a typical McPhail type plastic trap that is modified into an electronic one. Insect's fly-in the box-shaped traps that hang from trees in response to chemical signals they receive from inside the trap. As they fly-in, an opto-electronic sensor composed of an array of phototransistors that acts as a receiver and an infrared led on the opposite site of the circular entrance guard the entrance. As the insect flies on its wings interrupt the flow of infrared light from emitter to receiver. In this work we show:

a) The signal of the wing-flap received is of very high quality and resolves the fundamental frequency of the wing-flap as well as several overtones up to 2 kHz.

b) The analog signal of the wing-flap recording received from the opto-electronic sensors is directed to a microprocessor embedded in the trap that analyses the spectrum of the recording. The aim is to extract the fundamental frequency and the way the energy is distributed on the overtones of the recording. We show that this information extracted from a 50-100 msec duration flight is enough to reveal the species identity of the entering insect.

c) The detection results are transmitted through an embedded SIM card using the GSM mobile network. Therefore the recognition results and counts can be emitted as far as to another continent with minor cost.

d) We discuss how the decision protocol and the initiation of spraying can be benefited from such device.

We believe that once optimized the opto-acoustic sensor and the standalone recognizer has the potential to revolutionize the way insect monitoring is carried out for a series of insects with large economic impact (either positive or negative) such as fruit flies, bees and mosquitoes.

Key words: electronic McPhail trap, precision agriculture, remote monitoring of flying insects, computational ecology

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***Bactrocera oleae* (Rossi) phenology of wintering populations in Liguria (Italy)**

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In two representative areas of oil production in Liguria (northern Italy), we tested the consistency of a degree-day model for predicting the *Bactrocera oleae* (Rossi) day of spring emergence during two consecutive years. Starting from October oviposition, *B. oleae* needs to accumulate 379.01 ± 41.264 degree-day for completing its cycle from egg to adult, with a lower threshold of 8.99 °C. We measured the differences between the day of the year predicted by the model for the peak of adult spring flight and that observed in the field. In order to apply Area Wide Pest Management (AWPM) protocols at regional scale, the model was validated and used to simulate and spatialize the day of adult emergence in spring, with the support of software GIS and regional agrometeorological network of Liguria. Two different spatialization procedures were compared in order to map the model output: geostatistical and regressive. Geographic parameters considered as elements of variability were: altitude, aspect, and distance from the sea. The regressive model provided a more accurate indication of *B. oleae* behaviour and climate diversity at the local scale. The model results were utilized to plan pest monitoring network at the regional scale

Key words: *Bactrocera oleae*, pest phenology, degree-day model

Natural enemies of *Bactrocera oleae* in different geographic regions of Greece

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This study surveyed the natural enemy complex of olive fruit fly *Bactrocera oleae* (Gmelin) (Diptera: Tephritidae) as well as the percentages of parasitism in olive groves in 6 geographic regions of Greece during 2013 and 2014. Samples of 500 fruits, which showed evident symptoms of *B. oleae* infestation, were taken from olive groves at biweekly or monthly intervals during the olive season. Samplings started when the first infested olive fruits were recorded and continued until harvest. From each sample, 400 olives were placed into emergence cages at 25±5°C until the last *B. oleae* or natural enemy adult emerged and the rest 100 fruits were dissected in the laboratory under stereoscope for estimation of parasitism rate. Adult natural enemies were identified using taxonomic keys. The natural enemy complex of preimaginal stages of *B. oleae* included the ectoparasitoids *Eupelmus urozonus* Dalman, *Pnigalio mediterraneus* Ferrière & Delucchi, *Eurytoma martelli* Domenichini, *Cyrtoptyx latipes* Rondani, the endoparasitoid *Psytalia concolor* (Szépligeti), and the predator *Lasioptera berlesiana* Paoli. Among them, the dominant species was *E. urozonus*, that was found in all geographic regions, followed by *L. berlesiana* which was recorded in 4 geographic regions. *P. concolor* and *P. mediterraneus* were found in three geographical regions, while only a few individuals of *E. matrelii* and *C. latipes* emerged from the samples in Chania, Crete (S. Greece). The rate of parasitism was highly variable across the sampled regions. Higher percentage of parasitism (ectoparasitism, endoparasitism) of *B. oleae* was found in olive groves of Chania, Crete (reached up to 54.4%) compared to the other geographic regions of Greece.

Key words: *Bactrocera oleae*, parasitoids, predators

Acknowledgements

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From genome to behavior: investigating the molecular basis of olfaction as target to interrupt the sexual communication for population control of the olive fruit fly, *Bactrocera oleae*

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The olive fruit fly, *Bactrocera oleae*, is the major threat of the olives worldwide, causing significant yield and quality losses. Alternative control methods such as semiochemicals have been developed in an attempt to reduce or eliminate the principal chemical suppression of its populations. A promising improvement of such strategies targets mating disruption. This aspect of insect life is regulated mainly by olfaction, a sense highly engaged to the insect's survival and reproduction, since both life traits are relied on chemosensory cues. It is therefore necessary to understand olfaction and its associated behaviors in order to improve pest control. The goal of our research is to decipher the molecular dialogue governing the interaction between the flies of the opposite sex. We focus on the identification of the genes underlying variation in the olfactory responses with regard to the species sexual communication. These genes include odorant receptors, pheromone and odorant binding proteins that are thought to be directly associated with odor detection and signal transduction, as well as genes that are reported to be implicated in the olfactory process. Selected candidates were further functionally analyzed in the heads of males and females, where the main olfactory organs are located. The regulation of these genes was investigated by qRT-PCR analysis comparing the behavioral responses of both sexes at several time points and conditions of odorant exposure and mating status. The influence of environmental cues and developmental/physiological requirements on the expression pattern of the analyzed genes was evaluated, showing functional diversity. Although a complex set of reactions seems to occur, a preliminary estimate of their possible role was enabled. Our study provides the first holistic insight into the molecular key elements that are implicated in olfactory-driven sexual consequences in the olive fly. Further behavioral phenotypes via gene knockdown that lead to communication interference will possibly provide a basis to

design new tools taking the effective genetic manipulation of the species a step towards.

Key words: Diptera, Tephritidae, pheromones, olfactory system, reproduction

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Studies on the damage potential of *Rhynchites cribripennis*

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Rhynchites cribripennis (Coleoptera: Attelabidae) is an olive pest that is commonly recorded in olive groves in Southern Greece and in certain years may cause damage of economic importance if not properly controlled. In this work, the damage potential of this pest was examined on the olive variety "Mavrolia", which is a major variety for olive oil production in Messinia. In May 2011, twigs with olives were enclosed in net cages with 1 or 2 adults of the pest. The presence of the pest and the damage caused was also followed in marked twigs on the trees which had not been enclosed in cages. The percentage of the fruits damaged was higher in the cages with 2 adults. However, it was similar between the cages with 1 adult and the twigs on the tree. The average density of the pest recorded on the twigs was 0.89 individuals. Therefore, the damage caused on the "free" twigs was similar to that in the caged twigs where a similar number of pest adults occurred. Thus, at the conditions of this study, the damage potential is not altered by enclosing the adults in the cages. The effect of the damage (feeding holes in the flesh of the fruit) to the diameter of the fruit was examined and it was proved a negative correlation, which however, was not much intense. In 2012, the percentage of fruits dropped due to the activity of the pest was 43, 26 and 30%, in the cages with 2, 1 and 0 individuals. On each twig 304 fruits had been initially developed, in average. Therefore, *R. cribripennis* can cause a significant effect on the production of the olive variety "Mavrolia".

Key words: pest, olive variety, *Rhynchites cribripennis*, mavrolia, damage

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Preference of *Prays oleae* for oviposition among olive varieties and different quadrants of the olive tree canopy

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The olive moth, *Prays oleae* (Lepidoptera: Hyponomeutidae) is a pest of olives widespread in Greece that may cause serious damages in certain areas. In this study, the preference of this pest for oviposition was examined among 4 olive varieties (Kalamon, Konservolia, Koroneiki and Mastoidis) grown in an olive grove of the Institute for Olive Tree Subtropical Crops and Viticulture in Chania. Fruit samples were collected weekly from May 21 to June 20, 2014 and the percentage of fruits with eggs per variety was recorded. The population of the pest was monitored using delta pheromone traps. The results shown that in the first sampling there was a preference for "Konservolia", but in the next samplings there was not recorded a difference among the varieties. However, "Konservolia" was infested first, then "Kalamon" and "Koroneiki", whereas "Mastoidis" shown a similar infestation among the sampling dates. The male captures were similar in the traps placed in the plots with "Kalamon" and "Konservolia" trees. Those results indicate that *P. oleae* shows a preference for "Konservolia", in the early period of fruit development. The preference of the pest to lay its eggs among the different sites of the olive tree was searched in an olive grove of "Manaki" variety in the area of Athens. Each sampled tree was divided into sampling quadrants (north, south, east and west) and fruit samples were taken from a height of 1-2m, within each quadrant. Samples were collected on June 25 and July 4, 2014. In each sampling, 6 trees were sampled and 25 fruits were selected per quadrant and tree. The results shown that the eastern part of the tree was preferred for egg oviposition. The importance of these results in more effective management of this pest is discussed.

Key words: olive moth, within tree distribution, olive variety, Kalamon, Konservolia

Color of fruits and leaves of Portuguese olive cultivars (cvs. Cobrançosa, Madural and Verdeal Transmontana) and their relation to *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) oviposition preference

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Different pests and diseases affect production of olives each year. Among pests, olive fly is an increasing menace to olive groves affecting not only olives production but also olive products quality. Nevertheless, olive fly has preference for cultivars settled within the same agronomic and edaphoclimatic conditions. In this sense the present work intend to study the impact of physical factors, namely color of olives and leaves and their possible relation to olive fly oviposition preference. For that fruits and leaves from cvs. Cobrançosa, Madural, and Verdeal Transmontana were collected during olives maturation and color was measured with a colorimeter. Maturation index and infestation levels were also measured. Olives from cv. Verdeal Transmontana were the most susceptible (24.5% infestation) followed by cvs Madural (17.5%) and Cobrançosa (8.5%) (data from harvest moment, 1st week of November). Olives reported a faster maturation process with a maturation index (MI) of 3.0 at the end of the study while cvs. Madural and Verdeal Transmontana reported respectively 2.0 and 0.9. In leaves, color of down pages revealed no important differences among cultivars, but it was observed a significant higher luminosity (L^* values) of upper pages in cv. Verdeal Transmontana. This observation was also observed in fruits. Olives from the three cultivars at the same MI report significant difference of L^* values, mainly in cv. Verdeal Transmontana. With the advance of maturation L^* values decreased significantly in cv. Cobrançosa and Madural, while in cv. Verdeal Transmontana L^* values increased during maturation. By a principal component analysis is possible to verify that olives and leaves color can distinguish olive cultivars, a fact that could intervene in the oviposition preference of olive fly. The luminosity

verified in both leaves and olives from cv. Verdeal Transmontana is a crucial factor in the oviposition preference of olive fly. This aspect allied to the slower maturation stage of cv. Verdeal Transmontana turns this cultivar susceptible for longer periods comparatively to cvs. Madural and Verdeal Transmontana. In the cultivation of new olive orchards this information can be used in order to avoid the plantation of sensitive olive cultivars.

Key words: *Bactrocera oleae*, olive cultivar, maturation, oviposition preference

Acknowledgements

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High-light on certain olive pests and their control in Egypt

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This work aimed to determine the olive insect pests and their associated natural enemies recorded in Egypt by using the available literatures. Determination of the mites associated with olive groves is also took a beam of light. The collected articles exhibited the presence of 15 hexapod species belonging to 14 genera, 9 families and 4 orders in association with olive groves. Three predatory Acarid species were also encountered. From the obtained data it is clear that in Homoptera order included the 60% of the recorded arthropod pests. However, orders Lepidoptera, Diptera and Coleoptera harbored 20%, 13.33% and 6.67% of the total recovered species, respectively. Fifty five parasitoid species belonging to 9 Hymenoptera families were recorded in association with arthropods infesting olive trees. The obtained data showed that more abundant family is Aphelinidae since comprises the 30.90% of the total identified parasitoids, followed by family Encyrtidae (27.27%). The remaining families can be arranged in descending order as follows: Encyrtidae by 10.91% > Braconidae by 9.10% > both of Eurytomidae and Trichogrammatidae by 7.27% > Eupelmidae by 3.64% > both of Bethylidae and Chalcididae by 1.82%. The role of biological and chemical control procedures used to manage olive pests was determined. Parasitism of certain parasitoid species on the main olive arthropod pests was investigated. The impact of chemical and safe alternative compounds to reduce olive pests and their associated natural enemies has been discussed in more details.

Key words: olive pests, biological and chemical control, Egypt

SESSION III

**RESEARCH IN OLIVE DISEASES AND PESTS
AND THEIR CONTROL**



**The threat posed by *Xylella fastidiosa* to olive trees
in the Mediterranean region: the first outbreak
in Apulia, Italy**

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Olive trees (*Olea europea*) have been landmarks of the Mediterranean region for thousands of years. Unfortunately, this precious resource is seriously threatened by *Xylella fastidiosa*, a harmful organism included in the list A1 of EPPO, which has long been known to affect a large number of plant species in the American continent, primarily grapevine, citrus and stone fruits. In October 2013, its presence was reported for the first time on olive trees in Apulia, the main olive-producing region of Italy, in association with the olive quick decline syndrome (OQDS). This devastating pathogen did not occur in Europe and in the Mediterranean region before. According to OQDS aetiology, *X. fastidiosa* seems to be the main responsible agent, as shown by the preliminary results of pathogenicity tests, even though the symptomatic olive trees are generally affected by a complex of organisms that include fungal species belonging to the genus *Phaeoacremonium* and *Phaeoniella*, and *Zeuzera pyrina* (leopard moth). The *X. fastidiosa* strain found in Italy (named "Codirot") belongs to the subspecies *pauciflora* and infects at least 15 host species, primarily olive, almond, cherry and oleander. Although the study on the host range is still in progress, apparently the Codirot strain does not infect grapevine and citrus spp.. The assessed vector of the Apulian Codirot strain is *Philaeus spumarius*, a polyphagous Auchenorrhyncha insect widely distributed in the Euro-Mediterranean region. There is no record of successful eradication of *X. fastidiosa* once established outdoors due to its broad range of plant hosts and vectors. Control strategies are mainly based on the prevention of introduction of the pathogen from areas where it is present and on the containment of the outbreak. In Italy a large-scale monitoring has been carried out in an attempt to contain the rapid spread of the pathogen and the correlated disease, which is destroying century-old olive trees. Efficient and innovative detection tools and monitoring methods have been developed (i.e. setting up of diagnostic tools for the *in situ* rapid detection of the bacterium, photointerpretation of aerial images for the identification of OQDS suspected trees, *X. fastidiosa*-detection on 'spy insects' for assessing the presence of

the pathogen before symptom development, etc.). Some of them are already included in the official monitoring scheme of the pathogen in Italy. Based on these tools, an urgent pathogen surveillance programme should be envisaged in the Mediterranean countries to immediately monitor the presence of OQDS and leaf scorch symptomatic trees as well the xylem-feeding insects as potential pathogen vectors.

Key words: olive quick decline syndrome, *Codiro* strain, tracheomicotic fungi, spy insect, vector, surveillance

Endophytic fungi of olive tree and its potential application in protection against *Colletotrichum acutatum*

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Endophytes are a group of microorganisms that reside asymptotically within most living plant tissues. Several reports have indicated that endophytes are able to protect their host against phytopathogens, suggesting their agricultural application as biocontrol agents. Thus, in this work we intend to assess the diversity of fungal endophytes in three olive tree cultivars with different susceptibilities to one of the most damaging diseases, the olive anthracnose, and select the strains with the greatest antagonistic effect against *Colletotrichum acutatum*, the main causal agent of this disease. The fungal endophytes were isolated from roots, leaves and twigs of healthy trees of cvs. Cobrançosa (moderately resistant), Picual (tolerant) and Galega (susceptible), and identified by rDNA sequencing. The *in vitro* effect of the isolates obtained against *C. acutatum* was analyzed by the dual-culture method. The three cultivars clearly differed according to the endophytic fungal communities. The frequency of endophytic colonization and fungal diversity were greater on cv. Galega, followed by cv. Picual and cv. Cobrançosa, in all of the plant tissues analyzed (roots, leaves and twigs). Among the species identified in the cv. Cobrançosa, *Paecilomyces lilacinus*, *Fusarium oxysporum* and *Trichoderma gamsii* were the most frequently isolated. *Fusarium oxysporum*, *F. rodolens* and *F. nematophilum* were the most dominant species in cv. Picual, whereas in the cv. Galega were *Phomopsis columnaris*, *F. oxysporum* and *Macrophomina phaseolina*. Among the fungi isolated from cv. Cobrançosa, *P. lilacinus*, *Hypocrea lixii* and *Penicillium roseopurpureum*, significantly inhibited the growth, sporulation and germination of *C. acutatum*. By contrast, only one species from cv. Galega (*T. gamsii*) and another from cv. Picual (*Ceratobasidium albasitensis*) were inhibitory to *C. acutatum*. These findings indicate that some fungal endophytes might be involved in the protection of olive tree against this pathogen. This knowledge is relevant for exploring endophytic fungi as biocontrol agents and for

elucidation of the mechanism of enhanced disease resistance in olive tree cultivars.

Key words: *Olea europaea*, olive anthracnose, biological control, plant resistance, fungal diversity

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Relationship between phyllosphere fungal communities and tolerance of olive tree cultivars to olive leaf spot disease

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Spilocaea oleaginea is the causal agent of olive leaf spot (OLS) disease on olive trees, which causes significant yield losses in many countries, including Portugal. The disease incidence is variable according to the olive cultivars, being cv. Cobrançosa more tolerant to OLS than Verdeal-Transmontana. The present work aimed to elucidate the role of endo- and epiphytic fungal communities inhabiting the olive tree phyllosphere of these two cultivars, in what concerns the mediation of plant host tolerance to OLS. For this purpose, leaves and twigs were collected from seven olive trees of each cultivar (Cobrançosa and Verdeal-Transmontana), present in a single olive grove located in Mirandela (Northeast of Portugal). The endo- and epiphytic fungi inhabiting the collected plant material were isolated in both PDA and PCA media. OLS incidence (% infected leaves) was assessed by evaluating the percentage of infected leaves per tree. The obtained isolates were further identified by sequencing the ITS region of rDNA. Both endo- and epiphytic fungal communities differ significantly between cultivars, especially within endophytes (85% dissimilarity). Cv. Cobrançosa was distinguished by the presence of *Chromelosporium carneum*, *Pseudocercospora cladosporioides*, *Hyalodendriella betulae* and *Plectania rhytidia*, within endophytic community, and by *Placopsis rhodophthalma*, *Plenodomus enteroleucus*, *Cladosporium* sp. and *Penicillium* sp., within the epiphytic community. The Verdeal-Transmontana was distinguished by exhibiting *Phaeosphaeria avenaria* and *Fusarium* sp. within the endophytic and epiphytic community, respectively. Cultivar Cobrançosa showed lower disease incidence (3%) than cv. Verdeal-Transmontana (15%). A correlation between data regarding fungal community and disease incidence displayed by both cultivars was performed by using multivari-

ate analyses. The results indicate that some fungal species, either epiphytes or endophytes, may have a direct effect on olive tree tolerance to OLS.

Key words: endophyte, epiphyte, tolerance/susceptibility, olive leaf spot, *Spilocaea oleaginea*

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Genomic analysis of two *Pseudomonas* spp. strains displaying endophytic lifestyle in olive roots and effective biological control of *Verticillium dahliae*

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Pseudomonas fluorescens PICF7 and *Pseudomonas putida* PICP2 are two effective biocontrol strains against *Verticillium* wilt (*Verticillium dahliae* Kleb.) of olive. In addition, both bacteria can colonize endophytically olive roots, even simultaneously. However, our knowledge on bacterial traits involved in biological control and endophytic lifestyle is scarce. In this study, we have sequenced and annotated the complete genomes of strains PICF7 (1 contig, 6.14 Mbp) and PICP2 (63 contigs, 5.60 Mbp). A comparative bioinformatics analysis was carried out aiming to identify genes potentially involved in (i) biological control, (ii) plant-bacteria interaction, and (iii) endophytism. The analysis has revealed that, among other determinants frequently associated to biocontrol and/or plant-bacteria interaction, the genome of strain PICF7 harbours the genetic information to encode two type VI (T6SS) and one Type III (T3SS) secretion systems, the T3SS effectors *avrE1* and *hopB1*, the siderophores pyoverdine, pyochelin and the hemophore HasAp, diverse adhesion factors, a battery of cell-degrading enzymes (protease, cellulase and lipase), as well as detoxifying systems such as CopA, KatB, Dps and Cbb. On the contrary, the genome of strain PICP2 showed, among other differences, no coding region for T3SS. Some of the phenotypes predicted by genome analysis have been demonstrated by biochemical and microbiological analyses. The availability of both genomes will help to understand both biocontrol activity and the ability to colonize and persist within olive root tissues. Supported by grants P07-CVI-02624, P10-AGR-5797, P12-AGR-667 (Junta de Andalucía, Spain), and AGL2011-30343-CO2-01 from the MINECO (Spain), all co-funded by ERDF from EU.

Key words: biocontrol, endophytic bacteria, *Verticillium* wilt, siderophores, detoxification systems

Influence of irrigation frequency on the onset and development of *Verticillium* wilt of olive

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The Spanish Research Group AGR-216 from the University of Córdoba have conducted experiments during the last four years for studying the effect of irrigation on the onset and development of *Verticillium* wilt of olive, caused by *Verticillium dahliae*. First works, carried out in naturally-infested microplots, demonstrated that disease was significantly more encouraged if trees were daily irrigated compared to weekly, biweekly and deficit watering schedules in the susceptible cultivar 'Picual'. In the present work, we have confirmed by field experiments conducted in Andalucía (southern Spain) from 2013 to 2015 in olive orchards planted with 'Picual' that short watering frequencies favor disease development. First experiment (Exp I) was planted in Andújar (Jaen province) on a naturally-infested field (5 microsclerotia/gram of soil, MS/g), in June 2013, with 6 blocks (tree rows), 3 irrigation treatment per block (daily, biweekly and deficit), and 9 trees per treatment. The second experiment (Exp II) was established in an experimental plot at the University of Córdoba in December 2013, planting the olive trees in excavated trenches (70 m long, 1 m wide, 70 cm deep) filled with a naturally infested soil (1.2 MS/g) collected from the Guadalquivir marshes (Seville province), with 4 blocks (tree rows), the same irrigation treatments than in Exp I per block, and 5 trees per treatment. First symptoms were consistently observed 48 and 42 weeks after planting at Exp I and II, respectively. During the evaluation period results from both fields clearly demonstrated that irrigation treatments encouraged VWO development when compared to the deficit irrigation. However, the importance and duration of differences between the three treatments varied over time and was influenced by the natural rainfall registered in both fields. Thus, in Exp I, up to 52 weeks after planting, values of the area under disease progress curve (AUDPC) were significantly higher in daily irrigated trees (15.8%)

than in olive plants of the other two treatments (5.0%). Thereafter, during following four months, disease developed till reaching similar values for this parameter in the daily and biweekly irrigated trees (average of AUDPC = 20.0%). On the contrary, by this time the AUDPC value of trees subjected to watering deficit remained significantly lower, accounting for 6.9%. In the Exp II, disease parameters have developed with a similar trend for the two first treatments, but there are not affected trees under the deficit treatment yet. It is probably that differences on disease between studied irrigation treatments will accentuate over time during next months of the current spring.

Keywords: irrigation frequency, olive, *Verticillium dahliae*, Verticillium wilt of olive

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SESSION IV

**RESEARCH IN OLIVE DISEASES AND PESTS
AND THEIR CONTROL (CONTINUED)**



Ground cover as a practice to support pollinating insects and natural enemies in olive groves

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Sustainable agriculture endorses protection and enhancement of biodiversity in agro-ecosystems. In this work, ground cover management with selected plants was studied as a practice to create or maintain habitats of desirable insect communities (pollinating insects and natural enemies of crop pests) in olive groves. For this, during three years (2011-2013), in an olive grove in the Messara plain (Crete, Greece), selected plant species were grown, namely *Sinapis alba* L. (Brassicaceae) *Chrysanthemum segetum* L. and *C. coronarium* L. (Asteraceae), *Vicia sativa* L. and *Pisum sativum* L. (Fabaceae), *Borago officinalis* L. (Boraginaceae) and *Coriandrum sativum* L. and *Pimpinella anisum* L. (Apiaceae). In autumn or early spring, patches of 3m² were sown between trees along tree lines, using patches of native vegetation as control. A native biotype of *S. alba* also emerged and was the main flowering species in the control. During the flowering period, the percentage of flowering, the attracted Hymenoptera pollinators (honey bee *Apis mellifera*, bumble bee *Bombus* spp., Megachilidae, mining bees) and the number of beneficial insects in the patches and in the canopy of the adjacent olive trees were evaluated. During all experimental years, the sown patches were able to attract pollinating Hymenoptera, especially mining bees and honey bees, but also megachelids and bumble bees. *Sinapis alba*, both in the sown mixtures and the control, attracted large numbers of pollinators, mainly mining bees and honey bees. The patches of *C. sativum* and *B. officinalis* attracted high numbers of mining and honey bees. The flowering mixture with *C. sativum* and *B. officinalis* was more attractive to bees than the one with *C. coronarium* as main flowering species but they were both equally

attractive to mining bees. Hymenoptera parasitoids, primarily Braconidae and Chalcidoidea were observed in low number in the olive grove. The species *Opius concolor* (Hymenoptera: Braconidae), was recorded only on the olive trees. Also large numbers of predators, namely *Orius* sp. and lacewings (Neuroptera: Chrysopidae), were recorded in the patches, principally in those with the mixture containing mostly *S. alba*, in 2011 (spring sowing). No interaction between the ground cover and the olive pests *Bactrocera oleae* (Diptera: Tephritidae) and *Closterotomus trivialis* (Hemiptera: Miridae) were observed. Our results suggest that ground cover with suitable flowering species could be part of a sustainable olive crop management providing food and refuge for pollinating insects and beneficial arthropods.

Key words: biodiversity, ground cover, sustainable management, pollination, beneficial arthropods

Acknowledgements

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**Identification and molecular characterization
of pyrethroid resistance in the olive fruit fly
*Bactrocera oleae***

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During a Hellenic Ministry of Rural Development and Food project, we monitor insecticide resistance levels in *Bactrocera oleae*. The data are deposited in an Ontology based Pesticide Resistance Database (Galanthus, <http://www.galanthos-prd.gr>), that has been developed to systematically store and retrieve pesticide resistance data, in order to support decisions for resistance management strategies. A significant increase of pyrethroid applications for the control of *Bactrocera oleae* is taking place in Greece, particularly in Crete, in the last couple of years. We conducted detailed transcriptomic analysis and subsequently constructed a full genome Agilent microarray tool, to analyse differential gene expression in olive flies, resistant and susceptible to pyrethroid insecticides. Two P450s were identified to be strongly associated with pyrethroid resistance phenotypes, and their functional characterization, including their capability to metabolise different pyrethroid molecules, is currently conducted. The presence of target site resistance mutations has been also investigated. The operation of P450-based detoxification resistance may require the introduction of additional/specific strategies for the management of resistance, which may differ from the empirical rotation of various insecticide classes applied to overcome target site resistance.

Key words: insecticide resistance, diagnostic, detoxification, cytochrome P450, integrated pest management

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A novel approach to evaluate efficacy of baited insecticide applications for the olive fruit fly control

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The olive fruit fly, *Bactrocera oleae* (Diptera: Tephritidae) is the most important pest of olive crops. Regional management of the pest is implemented by the Hellenic Ministry of Rural Development and Food and relies on control of adults via application of baited insecticides. Evaluation of the insecticidal efficacy of the chemicals used is an aspect of critical importance. We are presenting a novel bioassay method that was developed to simulate field exposure under controlled lab conditions. The method offers high accuracy, rapid result evaluation and most important a strong linkage with practice by simulating the actual exposure pathway in the field. The 'field simulation' method has been validated with a number of field populations collected mainly from Crete. The insecticides tested were alpha-cypermethrin, beta cyfluthrin, deltamethrin, dimethoate and spinosad. The lethal concentration LC₅₀ as well as actual % mortality levels at the recommended label rate (RLR) were estimated. Responses / mortalities of the field strains were compared to the susceptible reference strain and the resistance factors (RF) were calculated. The resistance factor ranged from 66- to 530- fold for alpha-cypermethrin (n=6), 21- to 157- fold for deltamethrin (n=11), 7- to 49- fold for beta cyfluthrin (n=5) and 17- to 28- fold for dimethoate

(n=6) with one notable exception (RF=291). The % mortality for alpha-cypermethrin at the RLR (300 ppm) was found lower than 36% in most cases with one exception. For deltamethrin the mortality at the RLR (85 ppm) was found between 36% and 70%, with only three cases under 36%. For beta cyfluthrin the mortality at the RLR (87.5 ppm) was found between 51% and 100%, for dimethoate (2500 ppm) between 57% and 100% and finally for spinosad (7.9 ppm) between 76% and 96% (n=7). The reference strain exhibited 100% mortality in all cases. The 'field simulation' method indicated that alpha-cypermethrin developed the highest resistance levels compared to the other chemicals, potentially due to its extensive use over the past 10 years, while the low % of adult mortality at the label rate is suggesting poor performance under field conditions. From a practical perspective, our findings are in accordance with observations from records of the Ministry of Rural Development and Food indicating insufficient control of the pest in the field. The 'field simulation' method provides an accurate indication of the efficacy of chemicals and could serve as an extremely useful tool for large scale efficacy monitoring programs. Further investigations are planned, particularly regarding the elucidation of the resistance mechanisms and their impact on resistance management tactics.

Key words: field simulation, adulticide, resistance, alpha-cypermethrin, beta cyfluthrin, deltamethrin, dimethoate, spinosad, olive fruit fly, bait, control

SAGE10-Establishment of an impact assessment procedure as a tool for maintaining the sustainability of Mediterranean olive agro-ecosystems

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The SAGE10 Project aimed at developing an innovative Impact Assessment Procedure (IAP) for the assessment of environmental footprint of olive cultivation with a view to provide a tool for targeted advisory services to the olive growers to maximise the yield on the ground of available resources and the benefit of the environment. The IAP was designed to prioritize the expected environmental impacts of olive cultivation based on certain selected components which were categorized and combined in triplets: the 'aspects' (elements of agricultural activities e.g. crop protection), the 'impacts' (e.g. pollution, toxicity, resources depletion) and the 'compartments' (soil, man, biodiversity). The method was implemented in 600 olive groves, which were located in three regions in Greece. The impacts were correlated with a range of parameters which are related to either activities and practices followed by the growers (e.g. application of the recommended dose of pesticides, management of agricultural waste, spray remnants management) or to the environment of an olive grove (e.g. distance of the parcel from a water body, soil type, slope). The parameters were selected on the basis of predefined criteria and were assigned with different weights in different triplets so as to attribute their con-

tribution to specific impacts according to the magnitude of their effect. The end users of IAP (advisors and/or growers) recorded the applied practices in every parcel. Data were collected through interviews, sampling, in-field measurements and recording. The basic result of IAP use was the prioritisation of the different triplets according to a score, as an indicator of significance. In this way the practitioner of IAP had the opportunity to see which of the parameters had been responsible for the high score; the environmental or the farmer's ones. An improvement plan was hence designed by the advisors for every single parcel. This plan was handed to the grower to decide which of the most '*responsible*' parameters, under his control, could be changed in order the negative environmental impacts to be minimized whilst the production capacity of his parcel(s) to be increased (where possible).

Key words: environmental impact, olive culture, grower's cultural practices, targeted instruction

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GELSURA: A new 'lure & kill' insecticide concept for the sustainable control of olive fruit fly

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The concern about the reduction of the quantity of insecticides applied in the environment drives research for the discovery of new technologies with potential use against pests under Integrated Production Management systems. Behavior-modifying chemicals (BMC) are being used in pest management either alone as in mass trapping or in combination with insecticides, termed "lure and kill". Lure and kill control method uses semiochemicals or feeding attractants in combination with insecticides. Although, the combination of an insecticide product and a bait attractant has many advantages, there are still shortcomings related mainly to the application and the residual activity of the baits. To overcome those shortcomings, BASF has developed what can be considered as the most advanced "lure and kill" product, GELSURA. It is a concentrated gel formulation based on the intelligent combination of two attractants, a sexual attractant and a food attractant, with the well-known active ingredient *a*-cypermethrin, and the innovative BASF technology of the superabsorber polymers. This new concept of product also leads to a new concept of application. Thanks to the hygroscopic characteristics of the polymer matrix and the more powerful attractants, GELSURA can be applied at low volume up to 3 l/ha of water-product solution in form of spot application in the tree canopy. This can be considered as precision agriculture, and therefore the correct application of the product, the determination of pest population densities and the accurate dose application are valuable points. For these reasons BASF has developed a new application equipment devoted exclusively to GELSURA, that will allow the best performance of the product. AgBalance™ is a methodology based on Life Cycle Assessment, which evaluates the sustainability of agricultural production systems and technologies over the entire life cycle. Thereby, the economic and ecological impacts of a technology such as Gelsura can be assessed. BASF used the AgBalance™ methodology to analyze the impact of Gelsura on the sustainability of olive production in comparison with three other application methods against olive fruit fly. The drivers underlying the sustainability performance of the Gelsura-based production system will be discussed. In addition, results from field trials comparing olive production with conventional application methods and other "lure and kill" control methods will be presented.

Key words: precision agriculture, GELSURA, AgBalance, sustainability, lure and kill, olive fruit fly

Spinetoram (Delegate™ 250WG), a new insecticide for the control of *Prays oleae* (Bernard) in olives

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Delegate™ 250WG is a wettable granule insecticide containing 250 g per litre spinetoram for use in pome, stone fruit and olives for the control of lepidopterous pests, thrips and psyllids. Spinetoram belongs to the chemical family of spinosyns which form the group 5 of the IRAC insecticide classification scheme. Delegate™ 250 WG is currently under zonal review by ANSES of France (serving as the Rapporteur) for authorization in France, Spain, Portugal, Italy and Greece. One of the lepidopterous pests controlled by Delegate 250 WG is the olive fruit moth *Prays oleae* (Bernard). This moth is common in Southern Europe where it attacks olive trees, as well as other plants of the *Olea-ceae* family. It completes 3 generations per year and each one is associated to a different stage of olive development. The larvae of the 1st generation attack the flower buds. The 2nd generation larvae grow inside the olive stone kernels, while the larvae of the 3rd generation are leaf miners. The type of damage depends on the attacked tissue. The most significant damage is observed by the fruit (or carpophagous) generation larvae which causes the premature drop of olives when they bore into the kernel of the olive fruit or later when they vacate the fruit to pupate. A series of 10 field trials were conducted between 2008 and 2011 in Greece (3), Italy (5) and Spain (2) to study the efficacy of Delegate™ 250WG against *P. oleae*. Applications targeted the carpophagous generation of *P. oleae* in the Greek and Italian trials and the anthophagous generation in the Spanish trials. The trial results indicated that Delegate™ 250WG, at rates between 18.75 and 25.0 g active substance/ha, is highly effective for the control of *P. oleae*, and compared favourably to all reference products. It is suggested that applications with Delegate™ 250 WG should be made when infestation levels exceed the economic threshold and when the fruitlets begin to grow while they should target the newly hatched larvae of the carpophagous generation.

Key words: spinetoram, Delegate 250WG, efficacy, *Bactrocera oleae*, *Olea europaea*

Evaluation of "Insect Spy" approach for monitoring *Xylella fastidiosa* in symptomless olive orchards in the Salento Peninsula (Southern Italy)

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The gram negative and Xylem inhabiting bacterium, *Xylella fastidiosa* (*Xf*) has been discovered on olive trees associated with "olive quick decline syndrome" in Apulia region in October 2013. *Xylella fastidiosa* at moment seems limited to the west coast of the Salento Peninsula and has not yet been recorded in the rest of Italy and Europe. The quick dissemination of *Xylella fastidiosa* requires an effective approach for monitoring this bacterium in the symptomless areas surrounding the infection sites (buffer zone). Since the bacterium is vector borne, an evaluation of the possibility to utilize the *Xf*-positive insects found in the buffer zones (so called "Insect Spy") as indicators of the presence of the bacterium in apparently uncontaminated areas, was carried out. From May to June 2014, insects were collected from a designed buffer zone in Trepuzzi area, which is a punctiform *Xf*-infected zone in the Lecce province. The study focused on species in the Auchenorrhyncha, a group that includes known vectors of *Xylella fastidiosa*. The bacterium was successfully detected by PCR in 3 out of 6 species of Auchenorrhyncha captured in the buffer zone i.e. *Phileanus spumarius*, *Neophileanus campestris* and *Euscelis lineolatus*. Infected adults of these species were detected throughout the buffer zone at a distance of 1 Km far from the infection site. Therefore, adults of these three species have an important role as "insects spy" to early reveal the presence of the pathogen in apparently free areas before symptoms become visible on the plants.

Key words: Auchenorrhyncha, PCR, indicators, buffer zone

Diversity of epi- and endophytic fungi on asymptomatic and symptomatic olive tree phyllosphere

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The phyllosphere supports a large and complex microbial community that can live both epiphytically and endophytically. There is increasing evidence that this microbial community can protect host plants against pathogens. The olive leaf spot (OLS), caused by the fungus *Spilocaea oleaginea*, is one of the most important disease on olive trees worldwide, including Portugal. The present work aimed to compare the endo- and epiphytic fungal community inhabiting asymptomatic and symptomatic leaves of olive trees (*Olea europaea* L.) that showed typical symptoms of OLS. The plant material was collected from seven olive trees, in a grove located in Mirandela (Northeast Portugal), being used to isolate endo- and epiphytic fungi. The fungal isolates were identified by sequencing the ITS region of rDNA. Results showed that the endo- and epiphytic fungal communities differ greatly among asymptomatic and symptomatic leaves. Diversity and richness of both endophytic and epiphytic communities were significantly lower in symptomatic than in asymptomatic plant material. In symptomatic/asymptomatic leaves the endophytic fungal communities were dominated by *Alternaria* sp. and *Pseudocercospora cladosporioides*, whereas *Fusarium* sp. and *Cladosporium* sp. were the most abundant species within epiphytic population of symptomatic plant material, *Penicillium* sp. and *Hyalodendriella betulae* being the dominant species within epiphytic asymptomatic community. This finding indicates that the presence of OLS on leaves clearly affects fungal community composition, which in turn may have implications on olive tree protection against this disease. It is expected to identify predictable microbial species that could explain the reduction of OLS disease infection in asymptomatic leaves.

Key words: phyllosphere, *Spilocaea oleaginea*, olive leaf spot, cultivar, fungal diversity

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The potential of indigenous bacteria from the olive rhizosphere as biological control agents against a broad range of phytopathogens

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Verticillium wilt (*Verticillium dahliae* Kleb.) is one of the main biotic constraints affecting olive trees in the Mediterranean Basin. The difficulty to control this disease has urged researchers to seek effective measures within an integrated management framework. Biological control, alone or combined with other approaches, emerges as one of the most promising alternatives to confront this pathogen. In this study, a collection of indigenous culturable bacteria (>300) from olive roots was generated. First selection was performed based on their antagonistic activity against *V. dahliae*. As a result, 189 isolates were selected which were: (i) tested against a battery of relevant olive pathogens (*V. dahliae*, *Pseudomonas savastanoi* pv. *savastanoi*, *Rosellinia necatrix*, *Colletotrichum godetiae* and *Colletotrichum nymphaeae*); (ii) identified molecularly; and (iii) screened for the presence of phenotypes usually associated to antagonism and/or plant growth promotion (PGP). Results showed predominance of phyla Proteobacteria and Firmicutes, *Pseudomonas* and *Bacillus* being the most abundant genera. Although none of the strains showed effectiveness against all tested pathogens, many of them presented a broad antagonistic activity, particularly strains of the genus *Paenibacillus*. Phenotypic characterization revealed enzymatic activities usually associated to biocontrol and PGP, such as production of catalase and siderophores, among others. This fact, together with the observed broad antagonist activity allow us to conclude that some of these bacteria are good candidates to be used as biological control agents in future bioformulations against diverse relevant olive pathogens. Supported by grants P12-AGR667 (Junta de Andalucía, Spain) and RECUPERA 2020 (MINECO-CSIC), all co-funded by ERDF from EU.

Key words: antagonism, biocontrol, plant growth promotion, rhizobacteria, *Verticillium dahliae*

Updated check list of scale insects (Hemiptera: Coccoidea) on olive trees in Croatia with special regard on Black araucaria scale - *Lindingaspis rossi* (Maskell 1891)

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During ten years of faunistic research on scale insects in Croatia (2005-2014), 11 scale insect (Hemiptera: Coccoidea) species belonging to three families were found on *Olea europaea* L. (Oleaceae): Asterolecanidae: *Pollinia pollini* (Costa, 1857); Coccidae: *Filippia follicularis* (Targioni Tozzetti, 1867), *Lichtensia viburni* Signoret, 1873, *Saissetia oleae* (Olivier, 1791) and Diaspididae: *Aonidiella aurantii* (Maskell, 1879), *Epidiaspis leperii* (Signoret, 1869), *Lepidosaphes flava* (Signoret, 1870), *Lepidosaphes ulmi* (Linnaeus, 1758), *Lindingaspis rossi* (Maskell 1891), *Parlatoria oleae* (Colvée, 1880) and *Pseudaulacaspis pentagona* (Targioni Tozzetti, 1886). The localities where scale insects were collected are listed and marked according to UTM system. This is the first record of *L. rossi* in Croatia. It is a polyphagous species, feeding on many ornamental trees and shrubs and some fruit species, citrus and olive in particular. So far this pest has been found in the following countries of the Mediterranean basin: Portugal, Spain, Egypt, Monaco, Italy and France.

Key words: scale insects, *Lindingaspis rossi*, olive, Croatia

Preliminary studies on the management of the under canopy flora as a potential means to control the olive bug *Closterotomus (Calocoris) trivialis*

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The olive bug *Closterotomus (Calocoris) trivialis* (Heteroptera: Miridae) is an olive pest that may reduce the olive fruit setting feeding on the inflorescence. Its damage potential has been associated with its movement from the under canopy flora to the olive trees at the pre-flowering period. The aim of this work was to record its population on major weeds but also to investigate the effect of weed management (cutting) before its population increase on suitable host-plants (weeds) naturally grown under the olive tree canopy, as a potential alternative means for its control. The experiments were conducted in the area of Chora Messinias, where this insect is a serious olive pest. In two olive groves the population densities of the pest were found high on the weeds *Sinapis arvensis* and on *Mercurialis annua* in 4 samplings in April 2013. In an olive grove in the area, the pest numbers were followed on olive trees and weed flora under the tree canopy, following three treatments: olive trees with *S. arvensis* plants (naturally grown) under their canopy, olive trees with *S. arvensis* removed (along with any other weed) and olive trees with non-host plants (grasses) under their canopy. It was proved that in the treatment where *S. arvensis* were kept under canopy a higher number of the pest was recorded on the trees reaching to 3 individuals per twig on April 27, whereas in the other treatments the number of the pest individuals on the trees was very low. These results indicate that the removal (cutting) of the weeds that host the pest early in the period of olive inflorescence development may offer an alternative control method. However, this has to be further investigated in regard to its efficacy under different conditions and years and to its integration in the appropriate weed management in olive groves.

Key words: weed management, *Sinapis*, *Mercurialis*, *Calocoris trivialis*, cultural control method

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Biodiversity of soil beneficial arthropods in olive groves of different crop systems in three regions in Greece

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The presence and diversity of soil beneficial arthropods was studied in olive groves under different crop systems (organic and Integrated Crop Management (ICM)) in three regions in Greece (Trifylia in Peloponnese; Mirabello and Peza in Crete) for three consecutive crop seasons (2012, 2013, 2014). Five study olive groves were located per region, which included three organic ones in Trifylia and one organic in Mirabello and Peza). Samplings of soil beneficial arthropods showed the presence of several Carabidae beetles, mostly in Trifylia (29 species) and secondly in Peza (19 species) and Mirabello (15 species), which can contribute to the control of the larvae of olive fruit fly and olive fruit curculionid. The soil spiders showed quite high biodiversity (index Shannon-Wiener) in all study areas (Mirabello>Trifylia>Peza) The main families recorded were Gnaphosidae, Lycosidae, Zodariidae, Agelenidae, Salticidae, Thomisidae and Orthognatha. The predominant families and their distribution in the spider communities differed among the study areas, whereas the abundance of spiders was affected by the weed management practice. Primarily different Carabidae species were found in the study areas, with *Carabus graecus* being the only common species among them. Trifylia and Peza exhibited similar values for the biodiversity index Shannon-Wiener in 2012, which was maintained in the majority of the olive groves of Trifylia in the next year (2013) (exception of decrease in an olive grove where chemical control of weeds was conducted) but decreased in Peza regardless of the applied weed management prac-

tice. In addition, the presence of mites that can be useful bio-indicators of changes in soil properties and impact of human activities (mainly the Order Cryptostigmata (Oribatida)) was recorded and differences in the number of mite species were found in relation to the different weed management practices in the olive groves. The number of mite species of the Order Cryptostigmata and the biodiversity indices were larger in the olive groves of Mirabello and Peza, where mechanical or chemical control of weeds was limited, compared to Trifylia where weed control was performed in most olive groves.

Key words: soil biodiversity, beneficial arthropods, sustainable agriculture, olive crop

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"Teasing" the reproductive system of *Bactrocera oleae* as a potential tool for pest control

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The olive fruit fly, *Bactrocera oleae*, is the major arthropod pest of commercial olive production, causing extensive damage to olive crops worldwide. After mating the female insect, *Bactrocera oleae*, deposits its eggs in the olive fruit where the developing larvae feed and grow. Insect reproduction is central to species survival but also a target for pest management methods. In male insects, sperm develop in the testes and, when mixed with secretions from accessory glands, travel through the female genital tract, to be stored for later egg fertilization. Manipulation of these processes would constitute an effective method for pest population control. However, the molecular basis of mating in olive fly is still unclear. In our current research we focused on the identification of genes that may play a role in reproduction of *Bactrocera oleae*. We performed transcriptomic analysis on female accessory glands and spermathecae and male accessory glands (MAGs) of olive fly before and twelve hours after mating. Additionally, we determined the expression profiles for some of the genes from day 0 to day 7 (just before mating). Candidate genes will be further analyzed for their functional role in reproduction through RNAi silencing technology.

Key words: reproduction, olive fly, accessory glands, transcriptomic

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Remating of olive fruit fly (*Bactrocera oleae*) in the wild

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The olive fruit fly (*Bactrocera oleae*) is a monophagous, multivoltine species that, following its recent invasion in North America and New Mexico, now threatens almost the entire olive producing industry of the world and causes enormous economic losses to olive growers. Control methods for the olive fruit fly rely mainly on cover or bait insecticide sprays and lure and kill techniques including mass trapping. Among the environmentally friendly pest control methods, the sterile insect technique (SIT) is receiving much attention. Sterile insect technique (SIT) relies on mating between mass-reared, sterilized males and wild females that ideally do not remate. Recent reassessment of the control of the olive fruit fly using the SIT has led to a resurgence of interest in the mating ecology of this species, with special emphasis on the remating frequencies of feral females. Microsatellites constitute very useful molecular/genetic markers that are particularly informative in the study of population processes and an important tool of choice for mating and remating studies. Here we explore the remating frequency of wild (field trapped) female olive fruit flies using microsatellite markers. Thirty females captured from locations in Larissa and Thessaloniki using McPhail type traps were transferred and maintained in individual cages in standard laboratory conditions, where they were provided with olive fruit for oviposition. Progeny were preserved in ethanol until DNA extraction. In order to study the remating frequencies, nine microsatellite markers of those available for the olive fly were used to genotype wild caught females from the specific locations and determine population allelic frequencies. The analysis performed verified that among the markers tested, the majority exhibits an adequate degree of polymorphism. These markers were selected for the analysis of progeny.

Key words: olive fruit fly, female remating, SIT, microsatellite markers

Using Geometric morphometric analysis to investigate wing dimorphism in Iranian populations of *Bactrocera oleae* (Rossi) (Diptera: Tephritidae)

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Bactrocera oleae is the main insect pest of olive fruits. As most tephritidae species, it is a pest of great economic importance. Being a highly monophagous species, *B. oleae*'s expansion is restricted to the cultivation areas of its host, *Olea europaea*. The southern areas of Caspian Sea have a long tradition of olive cultivation that was disregarded in the region's ancient history. During the last 15 years, in Iran the olive production area has increased more than tenfold, reaching to greater than 120,000 hectares today. Interestingly, the presence of the olive fly had not been reported until July 2004, as it was a quarantine pest. Presently, however, the olive fly has become a serious pest of olive cultivation in Iran. Moreover, no studies were conducted on the sexual shape and size in this species. In the current research, field-collected flies from different locations of the country were analyzed to investigate their morphological variations based on geometric morphometric. Geographic populations of olive flies were collected from eight locations of three provinces (Qazvin, Guilan and Zanjan), where this pest is established. Sexual dimorphism in the wing shape and size of olive fly in Iranian olive fly populations were detected by analysis of partial warp scores and centroid sizes. Wing shape and size were compared among the different populations using fourteen selected landmarks. Multivariate analysis of variance (MANOVA) was performed on partial warps that showed significant difference between sexes ($F=67.495$, $p=0.000$) and also among localities ($F=6.019$, $p=0.000$). Simple analysis of variance (ANOVA) indicated that the centroid size of females was significantly greater than that of males ($F=59.832$, $p=0.000$) in tested geographic populations. So, our results have shown that *Bactrocera oleae* presents wing sexual dimorphism with regard to centroid size. These results provide evidence of morphological differences among Iranian olive fly populations. Such studies can be combined with molecular data in order to give a comprehensive view of the structuring of olive fly populations in Iran and provide useful tools for pest management programmes and quarantine practices in the future.

Key words: *Bactrocera oleae*, sexual dimorphism, geometric morphometric, geographic populations

Geometric morphometric analysis of wing shape variation in world populations of *Bactrocera oleae*

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The olive fruit fly, *Bactrocera oleae* (Rossi) (Dip.: Tephritidae), is a tephritid pest of olives in many parts of the world including areas in the Mediterranean basin, South Africa and North America (California). In Iran, olive fruit fly had been a quarantine pest. However, since its sudden emergence, in 2004, it has damaged extensively olive orchards in the country and is posing a serious threat to the olive industry. In the current research, field collected flies from different localities of the world were analysed to investigate their variations by using of landmark-based geometric morphometric. Wild olive flies were collected from eighteen localities around the world, including Iran, California, South Africa, Israel and the Mediterranean basin. Fourteen wing landmarks were selected for shape comparisons using Multivariate Analysis of Variance (MANOVA), Canonical Variate Analysis (CVA) and Discriminant Function Analysis (DFA) on the partial warps. MANOVA analysis showed significant variations among geographic populations ($F = 7.756$, $p = 0.000$). In DFA analysis, 859 individuals were investigated in left-hand wing. DFA showed that eighteen populations were differentiated in 48.2% of cases, i.e. among 859 individuals, 414 specimens were placed correctly. The geometric morphometric analysis showed significant variations among all geographic populations. Relationships between the geographic populations were analyzed through UPGMA algorithms. Results support the notion of a morphological differentiation of four groups: South Africa, the European part of the Mediterranean basin and Iran (except Manjil), Manjil (Iran) and North America (California). The results demonstrate that geometric morphometric techniques analysing wing shape, represent a promising complementary approach for discriminating between geographic populations of *B. oleae*. The results can be combined with other molecular data in order to obtain a more complete understanding of the fly's geographic differentiation.

Key words: olive fruit fly, geometric morphometric, geographic populations, multivariate analysis

SESSION V

CONTROL OF DISEASES AND PESTS



Effects of semi-natural habitats on spiders in olive groves and their potential role against *Bactrocera oleae* (Rossi) (Diptera: Tephritidae)

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Biological control in agriculture is an important ecosystem service mediated by natural enemies. To optimise pest control, it is essential to understand the relationship of predators and parasitoids to environmental factors such as management and landscape context of crop fields. The aim of this study is to clarify how semi-natural habitats, situated in a radius of 1 km around olive orchards, can support pest control. This study is part of the EU project QuESSA. In olive groves, spiders are the dominant predators in terms of species richness and abundance and they could potentially be effective against the olive fruit fly *Bactrocera oleae* (Rossi) (Diptera: Tephritidae), the key pest of olives in Italy and in the Mediterranean basin. During the summer of 2014 we sampled canopy spiders in 18 olive orchards of the Monte Pisano area (central Italy). In addition, their potential preys were sampled using sticky traps. A total of 72 species of spiders belonging to 15 families was sampled. The dominant species was the sheet web spider *Frontinellina frutetorum* (Koch) (Araneae: Linyphiidae). No clear landscape effect of semi-natural habitat proportion on olive spider community was observed, however, management practices such as chemical spraying reduced spider abundance and richness. Moreover, the activity of *B.oleae* was correlated negatively with the density of Linyphiidae family, suggesting that web-building spiders contribute to the suppression of this important pest.

Key words: semi-natural habitat, spider community, *Bactrocera oleae*, Linyphiidae, biological control

Functional responses of soil predators fed on pupae of *Bactrocera oleae* (Rossi)

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Edaphic arthropods can provide valuable services within agroecosystems, such as biological control of crop pests that spend part of their life cycle in the soil as *Bactrocera oleae* (Rossi). Functional response of a predator is an important factor regulating population dynamics of predator-prey systems. It represents the relationship between prey density and the number of prey consumed by an individual predator and an accurate description is important for practical and applied aspects of biological control. Functional responses of soil species, *Calathus granatensis* (Vuillefroy) and *Pterostichus globosus* (Fabricius) (Coleoptera: Carabidae) and *Ocypus olens* (O. F. Müller, 1764) (Coleoptera: Staphylinidae), were studied under laboratory conditions. Experiments were conducted on different densities of the prey (pupae of *B. oleae*), varying with soil species. After 24 hours, the number of pupae consumed was recorded and functional response curves were estimated. We compared the fit of two equations that describe functional responses: the Holling's disk equation that does not account for prey depletion and Rogers random-predator equation that accounts for prey depletion. All species tested exhibited a type II functional response determined by a logistic regression model. *Ocypus olens* showed shorter handling time on *B. oleae* pupae and a higher consumption of pupae before satiation than carabid species considering the Holling's disk equation. Rogers random-predator functions gave different parameters but functional curves obtained with both model were similar. Our results suggest that all the species can have important actions on *B. oleae* suppression in olive groves, being efficient predators of the olive fruit fly, especially at high pest population levels.

Key words: predation, functional responses, carabids, staphylinids

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An autoparasitoid inferior at resource exploitation outcompetes primary parasitoids and controls their common host (*Saissetia oleae*) in the field

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Autoparasitoids are intraguild consumers that attack and kill both heterospecific and conspecific parasitoids and immature stages of hemipteran hosts, such as soft scales. Field experiments assessing the importance of the interspecific competition between autoparasitoids and primary parasitoids, as well as its impact on host populations are scarce from the ecological literature. Using field data from 28 olive orchards with different parasitoid densities but similar host density at the beginning of the season, we examined the role of interspecific competition between primary parasitoids of genus *Metaphycus* and the autoparasitoid *Coccophagus lycimnia* on their population dynamics and that of their shared host *Saissetia oleae*. Our results reveal, for the first time, that an autoparasitoid, inferior at resource exploitation, can outcompete the primary parasitoid. *Metaphycus* parasitoids used smaller host than *C. lycimnia*, but they did not exploit this enemy-free space to outcompete *C. lycimnia*. On the other hand, *C. lycimnia* preferred to use *Metaphycus* females as secondary hosts for producing males rather than their own females at the beginning of the host life cycle, when parasitoids were scarce and the numbers of hosts were high. This mechanism might explain why the autoparasitoid was a superior competitor and it displaced the primary parasitoids as main parasitoid in all the olive orchards. Despite of the asymmetrical competition and the displacement of the primary parasitoids of genus *Metaphycus* by *C. lycimnia*, the latter suppressed host densities at the end of the life cycle of the scale. In May, *C. lycimnia* and host populations had almost reached the ratio 1:2, showing its high efficacy as biocontrol agent.

Key words: *Metaphycus*, *Coccophagus*, soft scales, interspecific competition, exploitative competition, intraguild predation, biological control

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Effects of natural occurring plants in olive agroecosystems and insect honeydews on the survival of *Chrysoperla carnea* (Neuroptera: Chrysopidae)

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Chrysoperla carnea s. lat. (Stephens 1836) larvae prey on insects including some olive pests, but adults are palyno-glycophagous feeding on pollen, nectar and insect honeydews. Knowledge about potential non prey foods for adult lacewings is crucial for establishing conservation biological control strategies in crops. In laboratory conditions, we tested the effects of: (i) eleven flowering plants that bloom sequentially during the year in olive orchards from the northeast of Portugal and (ii) honeydews from the secondary pests of olive trees, *Saissetia oleae* (Olivier 1791) and *Euphyllura olivina* (Costa 1839), on the survival of *C. carnea* s. lat. A pair of newly emerged *C. carnea* s. lat. were transferred into a glass cage (1.5 L) and provided with one of the food treatments and water. Each treatment had 27 to 35 replicates. Honey (10%) and sucrose (1M) solutions were used as a positive control while water was used as a negative control. Mortality was checked daily. Survival times among different food treatments were compared using survival analysis (log-rank test). If the overall log-rank test for the survival analysis was significant between group comparisons, the significance of each paired comparison was adjusted to a table-wide level of 5% using the sequential Bonferroni adjustment. *Chrysoperla carnea* s. lat. survived several weeks longer when *Veronica persica* Poir., *Lamium purpureum* L., *Malva sylvestris* L. flowers or both insect honeydews were provided than the negative control. Provision of *Ranunculus ollisiponensis* Pers., *Lonicera etrusca* Santi, *Foeniculum vulgare* L. or *Daucus carota* L. slightly increased the survival time of *C. carnea* s. lat.. The results suggest that establishment of these non prey foods, in olive groves could improve the survival of *C. carnea* s.

lat and consequently their performance as a conservation biological control strategy.

Key words: conservation biological control, survival analyses, natural enemies, non prey foods

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Production of alkaline protease by a Tunisian *Brevibacillus brevis* strain used as biopesticide against *Prays oleae* insect pest

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Brevibacillus brevis I13 (GQ397858) was isolated from olive cultivation in Tunisia and identified as strain with powerful larvicidal toxicity against *P. oleae* larvae and extra-cellular production of alkaline protease (AP) in the growth medium. AP crude preparations exhibited optimum activity at pH 10 and at temperature range between 65 and 70°C with notable decrease at high incubation temperatures. Different concentrations of lactose and bactopectone have been tested. The optimum medium composition was found to be: lactose 25 g/L; bactopectone Difco 10.0; NaCl 1.5g/L; MgSO₄ 7H₂O 0.15 g/mol; 0.01 M CaCl₂; 1.5 M K₂HPO₄; 1.5M Na₂SO₄; 0.01M MnCl₂ 4H₂O. The maximum alkaline protease production (5,000 uAPAM/ml) was attained after 72 hours of aerobic growth in shake flasks at 30°C. The crude AP enzyme activity exhibited quasi-linear response with enzyme concentrations up to 0.25 mg/mL. Interestingly, the maximum proteolytic activity was observed upon using a medium composed of 4% dried fodder yeast and casein as a substrate including gelatin, skim milk bovine and serum albumin. Toxicity against *P. oleae* larvae was determined with laboratory reared larvae (10 larvae per trial and three trial per bioassay) on fresh olive tree leaves. Ten-fold serial dilutions in distilled water were tested for toxicity screening. Constant level of larvicidal activity was obtained with higher concentrations of final alkaline protease culture (10⁻² and 10⁻³) which killed between 50 and 90% of the fourth and fifth larval instars of *P. oleae*. The LC₅₀ ranged from 5 x 10⁻⁶ and 8 x 10⁻⁶ with a highly potent toxicity against *P.oleae* larvae of first stages. The *B. brevis* strain can be used as a relatively inexpensive and important biopesticide with potent proteolytic production against *P. oleae* pest.

Key words: proteolytic enzymes, larvicidal activity

The outbreak of the olive leaf gall midge populations and the importance of indigenous natural enemies in its control

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The olive leaf gall midge *Dasineura oleae* (Diptera: Cecidomyiidae) is widespread in olive groves of Greece but its population levels are usually kept at very low densities. However, in 2011 and 2012 there was an outbreak of this pest in certain areas of Crete. In this study the population of *D. oleae* was recorded for 2 years, starting from May 8, 2012 to May 12, 2014, in two areas of Mirabello (Nisi Eloundas and Ampela), in North-East Crete. The number of galls per shoot was much higher in the olive grove of Elounda. In certain sampling occasions a really high number of galls per shoot was recorded showing a peak on the 2nd of July, 2012, when 32 galls were recorded per shoot, in average. As a consequence, the number of leaves developed on the infested shoots was much reduced. However, starting from the first sampling in 2013, parasitoids were recorded. At the samplings from Elounda the parasitization rate reached to 89%. Most of the parasitoids belonged to *Mesopolobus* sp. (Hymenoptera: Pteromalidae). Mostly due to the suppressing effect of the parasitoids, the incidence of the pest was much reduced i.e. on August 5, 2013, only 1 larva was recorded per 10 leaves in samples of the olive grove of Elounda, where the pest had developed high densities. These results indicate the significant role that native biological control agents play in the regulation of *D. oleae* outbreaks.

Keywords: *Dasineura oleae*, Cecidomyiidae, *Mesopolobus*, conservation biological control

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**A novel insecticidal formulation based
on α -cypermethrin against *Bactrocera oleae* (Rossi)
(Diptera: Tephritidae)**

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The novel insecticide Gelsura, which is a ready bait containing α -cypermethrin, was evaluated against olive fruit fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) in three regions of Central (Fthiotis) and Southern Greece (Lakonia, Chania). The formulation was tested in comparison with other three insecticides that are registered in Greece for use against *B. oleae*: α -cypermethrin and thiacloprid both with hydrolyzed protein as bait, as well as spinosad as ready bait. The study was conducted from 14 June until 31 October 2014. The occurrence of *B. oleae* adults was monitored by 12 McPhail traps in each region (3 traps by 4 treatments) which were checked every 7 days. The infestation level was estimated by collecting randomly 800 olive fruits from each region (200 fruits by 4 treatments) every 14 days. Three sprays were conducted in Fthiotis and Lakonia whereas in Chania region one more treatment was required. The results showed that all tested methods controlled successfully *B. oleae* in all regions. The sprays with Gelsura exhibited a notable comparative advantage; resources were saved, and the cost of application or carbon footprint was reduced because the total spray volume per hectare is 10 times less than the current practice with typical bait sprays.

Key words: ready bait, olive fruit fly

Population dynamics of the olive fly in Kyparisia, Greece under an area wide management program

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The olive fruit fly (OLF) *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) is the most damaging pest of the olive crop in the Mediterranean basin, where 96% and 90% of the world's olive oil and table olives is produced respectively. The OLF infestation may affect on average 40-50% of the total yield. The application of an Area Wide Control Program (AWCP) (operating and coordinated by the Greek Ministry of Rural Development and Food since 1953) usually succeeds to keep infestation levels between 5-15% in Greece. Despite a wealth of studies on population fluctuation of the OLF in the Mediterranean countries and elsewhere, there is limited published information regarding long-term effects of AWCP on population dynamics and infestation rates. Here we used adult trapping data obtained from approximately 1000 McPhail traps that ran for a period of 10 years in the frameworks of the AWCP against the OLF that has been established in the area of Kyparisia, Kalamata Greece to determine long term population fluctuation of the OLF under an intensive control program, considering climatic, landscape particularities of the area and fruit yield. The study area includes 2 million olive trees (98% of the cultivar Koroneiki), whereas the management strategy largely relies on bait sprays that are applied when the OLF population exceeds a threshold of one fly per trap per day (FTD>1). Our analyses reveal (a) a general tendency for a population increase during autumn months, where temperature becomes milder and humidity higher, (b) strong effects of landscape and distance from the coast on population fluctuation, (c) substantial effects of the last year olive fruit yield on population density over the subsequent year, and (d) a generally successful implementation of the AWCP towards keeping population density under the Economic Inquiry Level. The implication of our findings for understanding the ecology of the OLF in Mediterranean commercial olive orchards and for developing modern control strategies is discussed.

Key words: *Bactrocera oleae*, OLF, population dynamics, area wide program, bait sprays, McPhail trap

Arthropoda associated to the olive crop in Southern Portugal (Algarve)

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The objective of this work was to study the abundance and diversity of arthropods associated with the olive crop (*Olea europaea* L.), in southern Portugal. The trials were carried out in olive orchards under integrated mode of production, located in the Algarve region. The sampling techniques used in the trials consisted of pitfall traps, yellow sticky traps with and without sex pheromone, delta pheromone traps as well as collection of plant materials (branches with leaves, flowers, and fruits). The results obtained in the trials indicate that the arthropods associated with the olive crop belong to the following classes: Arachnida (order: Araneae), Chilopoda, Entognatha (order: Collembola) and Insecta. The class Insecta was the most representative, followed by Arachnida, Entognatha and Chilopoda. Regarding to the Insecta class the orders and families that inhabit the olive ecosystem are: Diptera (Syrphidae and Tephritidae), Coleoptera (Carabidae, Chrysopidae, Curculionidae and Staphylinidae), Hemiptera (Anthocoridae and Miridae), Homoptera (Coccidae and Psyllidae), Hymenoptera (Braconidae, Encyrtidae, Eulophidae, Trichogrammatidae and Formicidae), Lepidoptera (Hyponomeutidae), Neuroptera (Chrysopidae) and Thysanoptera (Phlaeothripidae).

Key words: diversity, arthropods, olive crop

Biodiversity of arthropods in a landscape mosaic dominated by organic olive groves in Alentejo (south-east of Portugal)

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Farming and the need to increased yield has given rise to more simple and homogeneous landscapes. An example is the increase of olive plantations with intensive and hedgerow systems, across the Mediterranean basin. In olive groves, the group of arthropods represents an important part of the biodiversity, where they can provide multiple ecosystem services, being one of the most important the biological control of crop pests. This service helps maintaining agricultural productivity and reduces the need of pesticide inputs. The objective of this work was to study the abundance and diversity of arthropods in a landscape mosaic dominated by organic olive groves in Alentejo, Portugal. Two landscapes consisting of four olive groves plots in organic production and a contiguous plot dominated by holm oak (*Quercus rotundifolia* Lam.) were selected. Olive grove plots were covered by spontaneous and/or sown vegetation and the holm oak plot was dominated by spontaneous vegetation. Three passive traps for interception of flying insects were installed in each plot, except for the third plot, where one trap was placed. These traps consisted of two Plexiglas plates (52 cm x 32 cm) arranged crosswise and placed vertically on a bright yellow plastic funnel (ø 34 cm) placed about 70 cm above the ground. The funnel was filled with 2.5 L of water and detergent. The collection of arthropods was held on the 3rd, 11th and 18th of June, and on the 9th, 16th and 23rd of October of 2014. In total 7809 specimens of arthropods were collected, belonging to 13 orders. Diptera was the most abundant ones representing 33.55%, followed by Thysanoptera with 23.83 % and Coleoptera with 20.69%. The abundance of Coccinellidae and Staphylinidae families was higher in the olive grove

compared with the holm oak plot that could be an indicator of their functions in olive grove agroecosystems.

Keywords: Holm oak woodland, biodiversity, ecological infrastructures, Diptera

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Scale insects mortality in olive trees in Rio Grande do Sul (Brazil)

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The olive crop as a business is a recent activity in Rio Grande do Sul (Brazil), and there are few studies related to insects associated with this crop. In this study we intended to identify the species of scale insects present in olive tree (*Olea europaea* L.) and evaluate and quantify their natural mortality. The work was conducted in an olive orchard, variety Arbequina, six years old, located in Caçapava do Sul (30°33'29.79"S and 53°24'09.48"W), RS, Brazil. The grove with 475 plants per hectare was conducted in traditional management. From April 2012 to March 2013 monthly, we sampled 20 trees randomly selected and, from each quadrant of their canopies (northern, southern, eastern and western) two branches were randomly taken, one from inside and the other from outside the canopy, with a length of 20 to 30 cm and containing at least 20 leaves. Scale insects were identified and counted as living, dead and with parasitism evidence. It was found a scale insects complex comprising a Coccidae, *Saissetia oleae*, and five Diaspididae, *Aonidiella aurantii*, *Hemiberlesia cyanophylli*, *Acutaspis paulista*, *Aspidiotus nerii* and *Melanaspis obscura*, being *S. oleae* the most abundant scale species. For all species, the parasitism was not the main mortality factor. For *S. oleae* the highest mortality may be associated with intraspecific competition. The total parasitism rate was 3.86%. *Saissetia oleae* had the lowest rate, while *A. nerii* and *M. obscura* had the highest parasitism registered among all the species.

Key words: scale insects, mortality, parasitism

Diversity and parasitism of scale insects (Hemiptera, Coccoidea) in ten varieties of olive trees (*Olea europaea* L.) in Southern Brazil

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Commercial plantations of olive trees (*Olea europaea* L., Oleaceae) are being developed in the State of Rio Grande do Sul, southern Brazil. One recent problem with olive production in this region is the presence of scale insects (Hemiptera, Coccoidea). Scale insects suck the sap of host plants and can negatively affect olive trees and reduce the yields of olive fruit and oil. This study surveyed the scale insect species and their associated parasitoids in ten olive varieties, including Alfafara, Arbequina, Arbosana, Cipressino, Coratina, Frantoio, Koroneike, Leccino, Manzanilla and Picual in an olive grove located in the Research Center of State Foundation for Agricultural Research (Fundação Estadual de Pesquisa Agropecuária-Fepagro) (30°33'15" S, 52°23'45" W; 420 m a.s.l.), Encruzilhada do Sul, southern Brazil. The surveys were carried out twice in each season (spring, summer, fall and winter), from April 2013 to February 2014. For each sample, two branches (upper and lower canopy), each was 20-30 cm long and had at least 20 leaves infested with scale insects, from each of three randomly selected trees, were examined for the presence and number of scale insects. The material was examined under a stereomicroscope or in an optical microscope to identify the scale insect. Scale insects were separated from the substrate and quantified by living individuals, with parasite or with perforations in the body after the emergence of the parasitoid. The percentage of parasitism was evaluated without identifying species of parasitoids and without distinguishing between immature and adult stages. The following species of scale insects were determined: *Acutaspis paulista*, *Aonidiella aurantii*, *Aspidiotus nerii*, *Hemiberlesia cyanophylli*, *Hemiberlesia lataniae* and *Pinnaspis strachani* (Diaspididae); *Saissetia coffeae*, *Saissetia oleae* (Coccidae). The most common scale insect was *S. oleae* (88.15%) found in all varieties of olive trees, followed by *A. nerii* (5.85%), which also occurred in all the varieties. *Saissetia coffeae* (4.51%) occurred only in five varieties,

while the other scale insects occurred in a small percentage (< 1%) in the olive grove. More scale species were found in Arbequina, Frantoio and Cipressino and only one species was found in Frantoio. Parasitoids were found in five scale insect species, but the percentage of parasitism was higher than 1% only on *A. nerii* (31.86%), *A. paulista* (26.95%) and *P. strachani* (5%). The identification of the parasitoids will be carried out in a subsequent study.

Key words: Diaspididae, Coccidae, parasitoid, olive variety

Genomic and functional approaches for understanding the adaptation of the olive fruit fly *Bactrocera oleae* to olives

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The olive fruit fly, *Bactrocera oleae*, is the most important pest of olive orchards worldwide. The fly lays its eggs in the olive fruit and the larvae feed and grow in the mesocarp, a plant tissue with a particular distinct chemical composition, including very high content of fatty acid and several classes of phenolic compounds, which may vary across maturation stage and olive fruit varieties. We conducted a whole transcriptome analysis of *B. oleae* using *de novo* assembly of reads generated by using 454 sequencing technology. The assembled sequences have been used to discover genes belonging to several protein families, such as those related to xenobiotic detoxification and fatty acid metabolism. Based on the complete transcriptome, an Agilent microarray (8x60K) was constructed and used for the analysis of the gene expression profile in first and second instar olive fruit fly larvae during its development in olives (ripe, mature, with/without symbiotic bacteria). In addition, Illumina sequencing was performed in first instar larvae in the presence and absence of symbiotic bacteria. The differential expression of several genes has been recorded and the data are currently being validated, including functional analysis with the use of recombinant protein and RNAi-based approaches.

Key words: ranscriptomics, microarray, insect-plant interaction, RNAi, olive

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Ecosystem services for the control of *Bactrocera oleae* in different landscape spatial contexts

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Landscape structure can affect pest populations directly or indirectly through its effect on natural enemies of the pest and the ecosystem services provided, i.e. parasitism and predation. Rates of parasitism and predation on the olive fruit fly, *Bactrocera oleae* were measured in different landscape spatial contexts trying to elucidate the mechanisms responsible for previously reported lower densities of the insect associated with complex landscapes. The study was carried out in Madrid in autumn 2014, using 15 olive groves in a gradient of landscape complexity. Parasitism rates found were very low for *Psytalia concolor* (0-3.13%) and negligible for *Pnigalio mediterraneus* (0-0.86%). Values of a biocontrol service index (BSI) indicating intensity of predation ranged from 0 to 0.63. Landscape structure was characterized at four spatial scales: circular areas of 500, 750, 1000 and 1500 m radii around the olive groves. Neither parasitism nor predation showed a significant relationship with any of the calculated landscape indices (number of patches, mean patch size, total length of patch edges and Simpson diversity index). This suggests a direct effect of landscape structure on *B. oleae*, but other hypotheses are discussed in the context of conservation biological control.

Key words: conservation biological control, landscape complexity, multi-scale approach, parasitism, predation

Estimation of the number of olive fruit fly *Bactrocera oleae* (Diptera: Tephritidae) attracted and killed by various bait spray solutions

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Field trials concerning estimation of the number of attracted and killed *Bactrocera oleae* adults by various bait spray solutions were conducted in Chania during the last two years. Specifically solutions of trophic attractants of *Bactrocera oleae* (two formulations of hydrolysed proteins at 55% and 75% w/w) separately or mixed with various insecticides (alpha-cypermethrin and dimethoate) and a spinosad solution sprayed in olive foliage, were evaluated for their effectiveness during two periods in successive summers, under semi-field conditions. The results indicated that among all bait spraying solutions tested, the mean number of adults attracted per day for a 10-day period attracted and probably killed, was approximately 0.3 to 1.5. The highest number of flies was recorded on both proteins combined with or without alpha-cypermethrin solution, as indicated by the catches on sticky transparent panels. However into more realistic conditions, under large field trials of bait sprays, the mean number of adults attracted per week by a bait spray spot was approximately 3.8.

Key words: olive fruit fly, bait sprays, bait spray spot, number

Composition and biodiversity of weed flora in established olive groves of southern Greece

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A weed survey of 60 olive groves located in three study areas of Greece (X. Trifilias, Peza and Meramvello) was conducted over a 2-year period in order to estimate the floristic biodiversity. Weed flora diversity was mainly assessed using the four common diversity indices: Shannon's index, Simpson's index, evenness index and species richness. Significant differences regarding the composition and the diversity of weed communities were observed among the three study areas and also between the 2 years of observations according to a discriminant function analysis. Species richness was increased in 2014 in comparison to 2013. 5.2 weed species were recorded on average per olive grove in Peza and Meramvello and 7.33 in X. Trifilias in 2013 while the respected values in 2014 surveys were 12.5, 8.85 and 12.56. Although a greater number of species and a more equitable proportion of various species in the population increases the diversity measured by Shannon's and Simpson's indices, the first gives greater weight to rare species while the second index gives greater weight to common species. Both these indices exhibited a different trend between the 2 years of observations. Except from X. Trifilias, the average values of Shannon's diversity index were greater in 2014 compared to 2013 surveys. The observed averaged values of Shannon's index during 2013 were 1.14 for Peza, 1.07 for Meramvello and 1.48 for X. Trifilias. The respected values during 2014 were 1.53, 1.19 and 1.30. On the other hand the values of Simpson's index were decreased between the cultivation seasons and ranged from 0.65 to 0.84 in 2013 and from 0.53 to 0.79 in 2014. Evenness index standardizes the relative abundance and it can vary from near 0 to about 1 whether, respectively, most of individual plants belong to a few species or when species are nearly equally abundant. In all study areas evenness index was decreased in 2014 compared to 2013 surveys and ranged from 0.65 to 0.75 in 2013 and from 0.52 to 0.63 in 2014. The most frequently occurring weeds, regarding their relative abundance, in each area and cultivation period were *Oxalis pes-caprae* L. in Peza and Meramvello

and *Calendula arvensis* L. in X. Trifilias during the surveys in 2013, while *Anthemis arvensis* L. was recorded as the most frequent weed in X. Trifilias during 2014. Astraceae and Poaceae were the most important botanical families in terms of the number of plant species structuring the plant community of the studied olive groves.

Key words: weeds, biodiversity, Shannon's index, species richness, olive groves

Insect pests associated with olive crop and virulence of native entomopathogenic nematodes to olive kernel borer, *Prays oleae* (Lepidoptera: Hyponomeutidae) in Saudi Arabia

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Field survey of olive crop at Besaita, Dawmat Al-Jandal and Sakaka in Aljouf region, Saudi Arabia during 2013, revealed the occurrence of seven insect species belonging to orders Hemiptera, Coleoptera, Lepidoptera and Thysanoptera. Records of insect species associated with olive crop showed prevalence of *Parlatoria oleae*, *Zeuzera pyrina* and *Prays oleae*. Knowledge of seasonal abundance of *Parlatoria oleae*, *Zeuzera pyrina* and *Prays oleae* immature stages are of great value to assist planning a control program. Therefore, a monthly abundance study was performed. Pathogenicity of three native entomopathogenic nematode isolates against olive kernel borer was compared. These entomopathogenic nematode isolates extracted by greater wax moth, *Galleria melonella* larvae baiting technique from olive orchards were in one sample (*Steinernema* spp. SAK1) (Rhabditida: Steinernematidae) and in two samples (*Heterorhabditis* spp. SAK2 and SAK3) (Rhabditida: Heterorhabditidae) were identified due to morphological features. Two experiments were designed and conducted to determine the susceptibility of *Prays oleae* to each entomopathogenic nematode isolate. For both experiments, harvested infective juveniles were maintained in tap water at 25°C and used within two weeks of emergence. The nematode application rate was 50 IJs/cm², total moisture content of the sand soil was 10%, and five replicates were used per EPN isolate. In the first experiment, five larvae were added to each cup and allowed to burrow into the soil. The cups were covered and incubated at 25 ± 2°C for 48 hrs, after which the insect mortality was observed and the dead larvae were dissected to insure the presence of the nematodes. Infective juveniles of SAK1 isolate caused 100% mortality of exposed larvae; while SAK2 and SAK3 isolates caused lowest levels of mortality (68%). The second experi-

ment to confirm the results from first experiment using infested olives, plastic cups were filled with sand and potting soil to depth of 5 cm, followed by placing 30 olives on the surface of the soil. The olives were collected beneath olive trees during September and October, 2013. Nematodes were sprayed over the olives. Water was sprayed as a control. After 72 hrs, the olives were dissected and the soil was sifted to collect larvae and pupae, which were dissected to determine infection status, all three isolates of entomopathogenic nematodes caused higher mortality than the control treatment, in which no infection; SAK1 was the most virulence isolate, including 70% infection. Infected larvae were found 4 mm and larger (third and fourth larval instar), infected pupae were found only in the soil, the total number of dissected larvae and pupae differed among treatments.

Key words: *Steinemema*, *Heterorhabditis*, biological control, *Prays oleae*

SESSION VI

**CONTROL OF DISEASES AND PESTS
(CONTINUED)**



Verticillium wilt of olive - Current status and Management

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Verticillium wilt caused by the soilborne fungus *Verticillium dahliae* is one of the major olive diseases, widely distributed in all olive-growing regions of the world. Verticillium wilt on olive is an increasing problem because of changing conditions for olive growing, especially irrigation and high-density planting. Disease symptoms are associated with the *V. dahliae* pathotypes, the defoliating pathotype, distributed across large areas where cotton has been previously grown, and the non-defoliating pathotype, distributed across fields where mainly potato has been cultivated prior to olive planting. The disease can be spread by using *V. dahliae*-latently infected seedlings, by contaminated water, and by bad sanitation. Currently, no control means applied singly are effective enough for disease management. An integrated disease management (IDM) is required to reduce the disease, combining available means and developing new ones. It includes the use of preventive (*V. dahliae*-free soil and planting material), pre-planting (resistant cultivars, soil disinfestation, sanitation) and post-planting (cultural practices, soil solarization, organic amendments, green manure, biological control agents) measures. Control of Verticillium wilt on olive is an ongoing challenge which requires further research for understanding the pathogen-tree-environment system. an integrated approach combining all.

Key words: *Verticillium dahliae*, VCG, detection, biological control, chemical control

Serious anthracnose outbreak starting from inflorescence infection of olive trees in west Greece

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Olive fruit infection by fungi of the genus *Colletotrichum* sp. is very common and particularly destructive in olive groves of west Greece, affecting Lianolia of Corfu, Koroneiki and Kalamata cultivars from Corfu Island down to the end of south Peloponnese. Prolonged wet periods due to heavy rains in Aetolia region last year (2014) resulted in the destruction of almost the 25% of the expected production of Kalamata cultivar with a loss of 6 million Euros income for the growers. Local survey during January 2015 in the affected region showed the presence of infected fruits with rot symptoms and conidiosomata of the pathogen on the fruits which had started to become mummified. Similar symptoms and signs were easily observed in olive fruits already fallen on the soil. A second survey in the Zakynthos island on March 26, 2015 revealed that certain olive groves of the variety Koroneiki at the onset of blooming developed a dark red to brown discoloration due to the rot of all flower parts. Laboratory examinations demonstrated formation of acervuli (conidiosomata) mainly on anthers and stigmata of the diseased flowers 2-3 days after their placement under wet chamber conditions. Linear streaking of fungal spores from infected stigmata in PDA resulted in the formation of pink colour colonies. Further transfer of the fungus in Rose Bengal medium and acidified PDA initially concluded from the colour of the colonies and the dimension and form of the conidia that we deal with the species *Colletotrichum accutatum*. Similar symptoms were also observed in a third survey carried out in April 18 2015 in Kalamata county in Koroneiki variety. Molecular identification of the pathogen is under examination. As it has been reported by several researchers in Australia and Mediterranean countries blossom infection affects not only the percentage of flowers but also the extension of the infection period, thus changing dramatically the methods and seasons of disease management. Our research group is currently organizing field and lab experiments to further study the problem and find out the best possible measures and methods of disease management.

Key words: olive trees, disease survey, *Colletotrichum accutatum*, disease management

Biological control of olive anthracnose

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Olive anthracnose, caused by species of *Colletotrichum* genus, is responsible for severe yield losses and poor oil quality. Recent investigations have shown that isolates of *C. acutatum* species complex prevails in areas where the disease occurs epidemically, such as in Spain, Portugal and Southern Italy. The taxonomy of the *C. acutatum* complex has been revised and new species have been erected. Typical symptoms of anthracnose appear in autumn or early winter, when the drupes begin to ripe. Under favourable conditions, symptoms on branches and leaves can also occur, leading to chlorosis, severe defoliation, and death of woody organs. Symptomless infection of flowers and blights have been recently reported. These infections could play an important role as inoculum source for the autumn-winter epidemics. Several authors reported that fruitlet infections remain latent from June to the end of October, when they begin to enlarge, causing the typical rots. Orange conidial masses on mature fruits characterize the disease and lead to premature fruit drop or mummification. The epidemic outbreaks of anthracnose occurring in Apulia (Southern Italy) during the last years have been investigated focusing on some epidemiological and control aspects. In particular, data on the epidemiology of *Colletotrichum* spp. on drupes showed that the incidence of latent fruit infections can reach 60-70% in September. The newly gathered information on the etiology, disease cycle, and latency of anthracnose infections, raise substantial questions about the traditional chemical control practices, with reference to both, the active ingredients and the timing of fungicide applications. Protectant fungicides can be ineffective against pathogens that are able to survive within fruits or as mycelia under the epidermis. Application of systemic fungicides has proved effective in field trials, and spring sprays contribute to reducing the inoculum density for autumn infection. However, the use of chemical control measures alone may not be enough to guarantee protection against olive anthracnose. Moreover, public concerns about potential

risks on environment and human health and the occurrence of resistant strains, promoted the search for alternative and sustainable means to manage the disease. Biological control by using microbial organisms represents an effective and complementary approach to chemical fungicides. Therefore, the biocontrol activity of the commercial formulate Serenade®, based on *Bacillus subtilis*, and several endophytic isolates of the yeast-like fungus *Aureobasidium pullulans* were evaluated in field trials. *Bacillus subtilis* applied at the pre-blooming stage was effective as the chemical fungicides. Moreover, some endophytic *A. pullulans* strains provided high protection levels against *Colletotrichum* spp. when applied at the veraison stage. Among the most effective *A. pullulans* strains, L47 showed also high population level and survival rate on drupe surface.

Key words: *Olea europea L.*, anthracnose, latent infection, biocontrol agents, *Bacillus subtilis*, *Aureobasidium pullulans*

Current problems in managing *Verticillium* wilt of olives in Greece and the prospective of non chemical and biological control of the disease in olive orchards

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Verticillium wilt of olive trees is considered as the most serious and destructive disease of olive trees in Greece, particularly in regions where the susceptible variety Amphissis is cultivated. During the last two decades, the extensive replacement of 'Amphissis' with the tolerant variety Kalamata did not solve the problem of the disease. Indeed, the cultivation of 'Kalamata' in fields that had been previously cultivated with cotton, or at the first stages of olive plantation were co-cultivated with cotton or other *Verticillium* susceptible vegetables, increased the number of microsclerotia in the soil and consequently symptoms severity in olive trees. Varieties Kalamata in Aitolokarnania and Fthiotida regions, and Amphissis and Chalkithikis in Magnesia, suffered dramatically from the disease. *Verticillium* wilt is currently spreading in traditional olive-growing regions of Chalkidiki, Magnesia and Kalampaka. More recent data in the regions of Western Macedonia and Thrace also report heavy symptoms in olives orchards of the variety Chalkidiki. In these areas, the source of the inoculum appears to originate from cotton fields, a crop extensively cultivated in these regions. The recent establishment of olive tree orchards in dense or hyper-dense planting both in Aitolokarnania and Ilia regions, respectively, could potentially cause similar problems. Planting of the susceptible variety Manzanillo in Ilia in former potato fields can probably generate similar problems. Biological control of *Verticillium* wilt was attempted in heavily infested olive orchards of Kalamata variety by drenching the soil around the trees with bacterial suspensions of *Paenibacillus alvei* strain K-165, effective against *Verticillium* wilt of potatoes. It was shown that three soil drenchings of the biocontrol agent applied at 4 month intervals mitigated significantly symptoms of the disease and promoted recovery of affected trees or prevented infection of healthy ones. However, new infestation of the experimental orchards by the dispersal and incorporation of leaves from diseased trees diminished the efficacy of the biocontrol agent during the next years after the treatments, underlying the importance of the inoculum

level in overcoming the effectiveness of biological control agents. The application of soil solarization (alone or/and in combination with bio-control agents) not only as a pre-planting measure but also in already established olive orchards is a promising and effective method to decrease the soil inoculum level of the pathogen.

Key words: *Verticillium dahliae*, soil solarization, biological control

Screening of organic amendments, plant extracts and microorganisms for the control of *Verticillium* wilt in olive trees

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One of the most important challenges for olive growing is the sustainable control of *Verticillium* wilt caused by *Verticillium dahliae*. The lack of an effective control method motivates the search of alternative and environmentally friendly biological control treatments. In this study, we evaluated different organic amendments (waste of plants, animals and food industry), plant extracts and microorganisms (various fungi, bacteria and their extracts) for the control of *V. dahliae*. This massive selection of candidates was conducted in four stages: i) *in vitro*, by the effect on the mycelial growth and spore germination of the pathogen; ii) in naturally-infested soil, by the effect on the reduction of microsclerotia of the pathogen; iii) *in planta*, by the effect on the infection of olive plants under controlled conditions; and iv), in field, by the effect on *Verticillium* wilt of olive trees grown in a highly-infested soil. The assessment of 220 treatments under controlled conditions resulted in a selection of 20 candidates to evaluate in field conditions. Among the candidates evaluated in the field, stand out several non-pathogenic strains of *Fusarium oxysporum*, pomaces of grape and olives, and mixtures of bacteria and yeasts. The mechanisms of action and the optimization of application methods are being investigated for the best candidates in field trials.

Key words: biological control, olive, *Verticillium dahliae*, *Verticillium* wilt

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Effect of the irrigation dose on Verticillium wilt of Olive

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Verticillium wilt of olive (VWO) (caused by *Verticillium dahliae*) causes losses particularly severe under irrigation regimes. However, there is an important lack of information regarding how irrigation doses used by farmers influence on disease development. Therefore, during autumn 2012 and spring 2013 a survey comprising 70 olive orchards affected by VWO (53 irrigated and 17 dry-land plantations) was carried out in the Genil-Cabra Irrigation Community of Córdoba province (central Guadalquivir Valley, Andalucía, southern Spain). A 2-3 hours visit for each olive orchard, accompanied by the farmer or a technician of the Irrigation Community, was used for completing a survey that consisted of three parts: 1) general information about the crop, the environment and the agronomic practices in the orchards; 2) information about the disease; and 3) questions about the use of irrigation in the plantations (irrigation system, irrigation frequencies, watering periods, etc.). Thereafter, the disease incidence (DI) in a square of a hundred olive trees chosen at random in the olive orchard was assessed, collecting plant tissue samples from affected trees to confirm *V. dahliae* infection. Finally, the Irrigation Community central office provided the real value of water consumption for irrigation during the years 2012 and 2013 for each visited plot. 93.1% of the visited olive orchards were planted with susceptible olive cultivars to *V. dahliae* (mainly 'Picual' and 'Hojiblanca'). Mean DI of the orchards accounted for 14.9%. In almost all investigated plantations disease onset occurred just before farmers had introduced irrigation. In the case that it was present, the introduction of irrigation always encouraged VWO development. Indeed, disease incidence was positively associated with the number of years of irrigation, showing a linear trend ($P=0.0062$). VWO was significantly more severe in olive plantation irrigated with high irrigation doses. 47.1% of olive plantations were irrigated with average values of 1793.4 (in 2012) and 2059.9 (in 2013) m³/ha, and

showed the highest DI (21.42%). Plantations watered with lesser doses (28.6%) with average values of 712.55 (in 2012) and 741.53 (in 2013) m³/ha showed a DI significantly lower, which accounted for 13.3%. Finally, dry-land olive orchards (24.3%) showed the lowest DI (4.0%). Moreover, regardless of water doses, older trees (> 30 years old) were less affected by the disease.

Key words: irrigation dose, *Verticillium dahliae*, Verticillium wilt

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Diversity of bacteria endophytes in olive tree

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Over the last decades the number of publications concerning microorganisms as a tool to control plant diseases has increased dramatically. One group of microorganisms, that started to be better studied regarding its role in host protection against pathogens, is endophytes. Endophytic microorganisms, which include mainly fungi and bacteria, are those that inhabit the interior of plants, showing no apparent harm to the host. Olive tree (*Olea europaea* L.) has a great importance in the Mediterranean region. This plant is attacked by several diseases, being Verticillium wilt (caused by the fungus *Verticillium dahliae* Kleb), one of the most important worldwide due to their high incidence and related losses. Therefore, in this work we intend to assess the diversity of culturable bacterial endophytes in *O. europaea* and create a reservoir of strains with potential beneficial applications on olive tree protection against Verticillium wilt. Bacterial endophytes were isolated from roots, leaves and twigs of 35 olive trees cv. Picual, located in Granada (Spain). Pure bacteria cultures were identified morphological- and molecularly through sequencing of V1 to V4 regions from 16S rDNA, and further maintained in culture (PCA) and cryopreserved (-80°C in aqueous glycerol solution). A total of 35 taxa belonging to 14 genera were identified. The bacterial endophytes most frequently observed were *Alcaligenes faecalis* and *Pseudomonas aeruginosa*, found in 9% of roots and 80% twigs, and in 8% of roots, respectively. Root explants were the most colonized (96%), followed by twigs (3%) and leaves (1%). Correspondence analysis of the endophyte assemblages showed that the endophytes exhibited organ specificity. Thirty three species were consistently associated with root tissues, while one species was found predominantly in leaves. Many bacterial species are found to be restricted to root tissues, and knowledge of their interactions with *V. dahliae* might be useful in the control of this disease.

Key words: plant protection, *Olea europaea*, endophytic bacteria, biological control

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A preliminary study of potential use of olive mill wastes as biocide against pupae of *Bactrocera oleae*

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The common olive oil extraction process is a three-phase procedure, resulting in the production of olive oil, solid pomace (SP) and olive mill wastewater (OMW). During the last decade, two-phase decanters were introduced into the olive oil industry to reduce the volume of OMW, generating instead, a viscous sludge-like byproduct which contains wastewater and olive pomace (TPOMW), known as "alpeorujo". Although the phytotoxic and antimicrobial properties of olive mill wastes have been extensively investigated, their potential utilization as biocides has been barely investigated. However, the biocide capacity of olive mill wastes, which could be utilized to suppress plant pathogens and pests, would open new opportunities for the recycling of these bioactive byproducts. The aim of this work was to study the effect of olive mill wastes on pupal mortality of olive fruit fly, *Bactrocera oleae* (Gmelin) (Diptera, Tephritidae), the most serious pest of olives in Mediterranean basin. Samples of olive mill wastes were collected from three olive mills located in Crete and Peloponnesus in Greece. More specifically, two samples of OMW and three samples of SP as well as three samples of TPOMW were collected from three-phase and two-phase decanters respectively. Ten pupae of *B. oleae*, 2-5 days old, and OMW or SP or TPOMW were placed in a plastic petri dish (9 cm in diameter and 1 cm high) separately or in mixture with soil. Sample containing only 10 pupae of *B. oleae* separately or in mixture with soil was used as a control. Each treatment was replicated six times. The petri dishes were kept in laboratory conditions at 20°C. Mortality was determined by counting the number of dead pupae and emerged adults of *B. oleae*, 23 days after pupae were placed in the petri dishes. Among treatments significant higher pupal mortality was recorded at OMW and TPOMW samples compared to the other treatments. With regard to olive mill wastes in mixture with soil significant mortality was recorded at OMW treatments. Dry solid pomace did not cause significant mortality. Results from the study demonstrated the

potential use of OMW and TPOMW as biocides against pupae of *B. oleae* in the soil. Further investigation for use in integrated production systems is in progress through the application of OMW in commercial olive orchards in the framework of the project LIFE OLIVECLIMA.

Key words: olive mill wastes, biocide, *Bactrocera oleae*

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**Success™ 0.24 CB, the Naturalyte Insecticide,
for the control of *Bactrocera oleae* (Gmelin) by bait
spray in Mediterranean countries**

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Success™ 0.24 CB is a soluble bait concentrate, ready to use insecticide, which contains the active ingredient spinosad, at 0.24 g per litre and an attracting system at 99.76 g per litre. The attracting system consists of stabilizers to improve shelf life, plant protein to attract the flies, sugars as feeding stimulant for flies and humectant and adjuvant to improve longevity. Success™ is registered in many countries in Europe, USA, Asia and Africa for both ground and aerial applications against many Tephritid fruit fly species on various crops such as olives, citrus, stone fruit, mango, avocado, and others. Spinosad belongs to the chemical family of spinosyns which form the group 5 of the IRAC insecticide classification scheme. One of the fruit fly species controlled by Success™ is the olive fruit fly *Bactrocera oleae*. It is a monophagous species whose larva feed exclusively on olive fruit. It is found throughout the Mediterranean basin and is considered the most serious pest of olives, affecting the quality and quantity of olive oil and table olives. It completes 3-5 generations per year and its population varies throughout the growing season. Chemical control of olive fruit fly implemented on either killing the hatching eggs and larvae in the olive fruit (therapeutic method) or the adults before females laying eggs (preventive method). A large series of trials were carried out in South Europe to study the efficacy of Success™ 0.24 CB against *B. oleae*. The trials indicated that spinosad is highly effective to control *B. oleae* adults through foliar bait spray applications, at the very low rate of 1 lt pr/ha (0.24 g a.s./ha). In Greece and other countries of the Mediterranean basin the product is used with high effectiveness, since 2009. Success™ 0.24 CB approved also for use in organic agriculture by numerous national and international certifications. The specifications of the bait application are discussed in detail.

Key words: Dow AgroSciences, fruit flies, *Bactrocera oleae*, spinosad, olives

Pruning residue management associated pathogens in olive

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Within the framework of the LIFE+ project OLIVECLIMA, several alternative management practices are applied in commercial olive orchards at three Prefectures of Greece (Heraklion, Lasithi and Messinia), as a way to cope with the ongoing climate changes and improve the carbon balance within the orchard ecosystem. Applied practices focus on recycling organic materials removed during olive production process in either raw or composted form. In particular, use of trimmed pruning residues as soil mulch aims at increasing organic matter content and water retention capacity with overall positive results in soil fertility. However, in cases of diseased trees, recycling of pruning by-products might be associated with the dissemination of fungal diseases causing wood discoloration and decay. The main objective of the present study was to investigate whether recycling of raw or composted pruning residues in soil could potentially contribute to fungal pathogens dispersion, such as *Verticillium dahliae* and *Fomitiporia mediterranea*, within and between olive orchards. Two complementary methods (isolation on acidified potato dextrose agar medium-APDA and DNA extraction and polymerase chain reaction-PCR assay) were employed to detect the abovementioned pathogens in healthy and diseased plant tissues as well as in raw or composted plant materials. Both fungal species were detected in diseased tissues but not in composted materials. The outcome of these analyses indicates the low risk of pathogen dispersion in the fields following good agricultural practices and regular orchard monitoring.

Key words: pruning residues, *Verticillium dahliae*, *Fomitiporia mediterranea*, pathogen dispersion

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Quick decline syndrome and anthracnose: emerging and reemerging diseases of olive posing new challenges for an integrated control approach

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A new and devastating olive decline syndrome, involving the noxious quarantine pathogen *Xylella fastidiosa* vectored by *Cicadellidae* insects, is currently under investigation in Apulia (southern Italy). The decline affects young and aged trees (100 years or older) growing in the Salento peninsula. The disease is characterized by a rapid dieback of shoots, twigs and branches followed by death of the entire tree. Leaf tips and margins turn dark yellow to brown, a condition that spreads inward, eventually leading to desiccation. The margins of the necrotic area may be reddish brown with a chlorotic halo merging the green tissue. A pattern of concentric zones or waves of necrosis may also appear. Desiccated leaves and mummified drupes remain attached to the shoots. Trunks, branches and twigs viewed in cross section show more or less extensive discolorations of the vascular elements, particularly sapwood and vascular cambium. Fungal species of the genus *Phaeoacremonium* and several other isolates similar to genus *Phaeomoniella* were obtained from the discolored wood. The role of these fungi, alone or in combination, in determining the symptoms observed on olive trees is currently under investigation. Anthracnose has long been recognized as one of the most important and severe olive disease in the Mediterranean basin, mainly in Portugal, Italy, Spain, and Greece. The causal agents are *Colletotrichum gloeosporioides* (Penz.) Penz. et Sacc. and *C. acutatum* species complex, although previous studies had identified *C. gloeosporioides* (originally known as *Gloeosporium olivarum*) as the primary incitant. More recent investigations have shown that groups in the *C. acutatum* complex can be erected as distinct species, and prevail in areas where the disease occurs epidemically. In fact *C. acutatum sensu stricto* resulted the most frequent species in southern Italy, causing latent infection in the drupes during the flowering stage. The emerging quick decline syndrome and the reemerging anthracnose of olive, pose new challenges to the integrated protection approach. Quarantine pathogen *X. fastidiosa* requires stringent phytosanitary regulation at the EU level,

taking also into account the large host range of the pathogen and the specificity of the olive trees: centenarian plants cannot be considered as a common annual crop. Moreover, effective and sustainable methods to control the vector need to be investigated. As for anthracnose, the newly gathered information on the etiology, disease cycle, and latency of infections raise substantial questions about the traditional chemical control practices, with reference to both, the active ingredients and the timing of fungicide applications. Protectant fungicides can be ineffective against pathogens that are able to survive within fruits or as mycelia under the epidermis. Application of systemic fungicides has proved effective in field trials, and spring sprays contribute to reducing the inoculum density for autumn infection. However, potential risks on environment and human health and the occurrence of resistant fungal strains raise substantial public concerns and prompt the search for alternative, safe and sustainable means to manage the disease.

Key words: *Olea europea*, quarantine pathogens, integrated control, phytosanitary measures

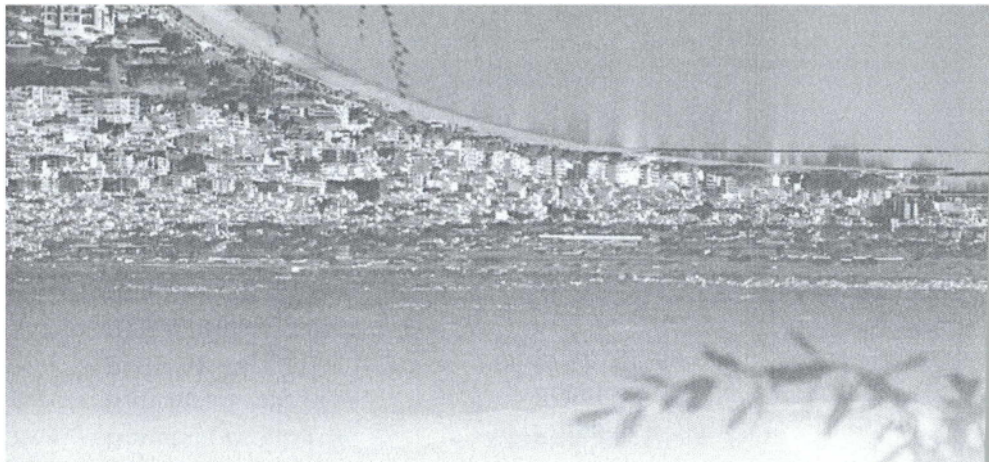
Risk assessment of *Xylella fastidiosa* for olive growing area of Croatia

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Traditional olive growing along the six thousands kilometers coastal and insular area of Croatia is an economically important source of income for the local population. Recent outbreak of the harmful bacterium *Xylella fastidiosa* strain CoDiRO over Salento peninsula in Italy represents a potential risk for Croatian olive growing sector. The main agro-ecological and social features of olive growing in Croatia in relation to its vulnerability to the CoDiRO strain of *X. fastidiosa* are discussed. Olive tree, *Olea europaea*, is considered as the most endangered crop of CoDiRO strain, and differences in susceptibility among olive cultivars are widely known. Thus, Croatian olive growing that includes over one hundred different varieties are potentially vulnerable to infestations to the CoDiRO strain.. Other subtropical plants that are part of the Mediterranean natural vegetation including cultivated, wild and ubiquitous species that have been proven to be hosts of CoDiRO strain and are growing next to olive orchards represent additional risk factors to infestation of the pathogen due to their high degree of biodiversity and abundance. Moreover, the fact that population of the coastal region is traditionally engaged in agriculture and tourism, facilitates the uncontrolled transmission and planting of plant material of host species in gardens, tourist centers and public spaces. Furthermore five potential vector species [*Philaenus spumarius* L., *Phylloxera signatus* L., *Lepyronia coleoptrata* L. and *Aphrophora alni* F. (Hemiptera: Aphrophoridae) and *Cicadella viridis* L. (Hemiptera: Cicadellidae)] have been described in olive growing areas of the under study area. Results of the 2014 survey in Croatia, after extensive visual inspections and laboratory tests, indicated that *Xylella fastidiosa* strain CoDiRO is not present in Croatia. Presence of potential and confirmed vectors of the pathogen, abundance and high biodiversity of host plants, favorable climate and high movement of plant material are important elements that should be taken into account for the risk assessment of the spread of pathogen in case of his undesirable introduction.

Key words: Croatian olive growing, vectors, host plants, risk assessment



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