9th IOBC-WPRS meeting on Integrated Protection of Olive Crops

BOOK OF ABSTRACTS

26th-29th October, 2021, Lisbon, Portugal

IOBC-WPRS

9th IOBC - WPRS Meeting on Integrated Protection of Olive Crops

Associação Portuguesa de Horticultura
Instituto Nacional de Investigação Agrária e Veterinária, I.P.
INSTITUTO POLITÉCNICO DE BRAGANÇA Escola Superior Agrária
INSTITUTO SUPERIOR DE AGRONOMIA Universidade de Lisboa
COTHN CENTRO OPERATIVO E TECNOLÓGICO HORTOFRUTÍCOLA NACIONAL CENTRO DE COMPETÊNCIAS

IOBC OILB
WPRS International Organisation for Biological and Integrated Control West Palaearctic Regional Section
SROP Organisation Internationale de Lutte Biologique et Integrée Section Régionale Ouest Palaéarctique

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This publication brings together the works presented at the 9th IOBC-WPRS meeting on Integrated Protection of Olive Crops in the form of abstracts.

Index

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Scientific programme

Tuesday, 26 October 2021

18.00-20.00h	**Welcoming cocktail at the Instituto Superior de Agronomia, room "salão nobre"** Placing of posters

Wednesday, 27 October 2021

08.00-20.00h	**Field trip (visit to olive groves and mills)** Fundação Eugénio de Almeida (Évora)

Thursday, 28 October 2021

8.30-9.30h	**Registration** Main Building of Instituto Superior de Agronomia, room "sala de atos"
9.30-10.00h	**Opening Ceremony** Organizing Committee – Helena Oliveira IOBC Representative – Andrea Lucchi Convenor of the WG – Paula Baptista President APH – José Alberto Pereira Welcome address by Prof. António Brito, President of the Instituto Superior de Agronomia, University of Lisbon
10.00-10.30h	**Open conference** The olive crop in Portugal. Past, present and future *José Alberto Pereira*
10.30-11.00h	**Coffee break**
11.00-12.30h	**Session 1 – Integrated management of olive pests** Chairpersons: Argyro Kalaitzaki and António Ortiz

11.00- 11.30h	***Plenary lesson*** Precision pest management in olive groves *N.T. Papadopoulos*
11.30- 11.45h	Three-year field trials for a sustainable approach in the control of olive fruit fly, *Bactrocera oleae* *Elisabetta Gargani, Silvia Guidi, Franca Tarchi, Donatella Goggioli, Riccardo Frosinini, Andrea Rocchini, Claudia Benvenuti, Matteo Guidotti, Alessandro Caselli, Stefano Econdi, Pio Federico Roversi, Ilaria Cutino*
11.45- 12.00h	Comparing olive fruit fly (*Bactrocera oleae*) density, fruit damage levels and parasitism rates in organic and conventional olive orchards in Crete, Greece *Giannoula Bogka, Nikolaos Volakakis, Dimitrios Kollaros, Bart Pannebakker, Nina Fatouros, Emmanouil Kabourakis*
12.00- 12.15h	Aldehyde-containing clays and zeolites: a sustainable approach in the control of olive fruit fly, *Bactrocera oleae* *Matteo Guidotti, Alessandro Caselli, Rinaldo Psaro, Stefano Econdi, Chiara Bisio, Stefano Marchesi, Salvatore Giacinto Germinara, Elisabetta Gargani*
12.15- 12.30h	Effect of olive fruit size on the parasitism rates of *Bactocera oleae* (Diptera: Tephritidae) by the figitid wasp *Aganaspis daci* (Hymenoptera: Figitidae), and first pilot field releases of adult parasitoids in an organic olive grove *C.A. Moraiti, C.S. Ioannou, G. Kyritsis, N.T. Papadopoulos*
12.30- 14.00h	***Lunch***
14.00- 15.30h	**Session 2 - Biological and biotechnological control of olive pests** Chairpersons: Patrizia Sacchetti and Dionyssios Perdikis
14.00- 14.30h	***Plenary lesson*** The use of semiochemicals in the olive grove: An essential tool *António Ortiz*
14.30- 14.45h	Towards an RNA-based biocontrol approach of olive fruit fly *Maria Feka, Antonia Spanomitrou, Konstantinos Mathiopoulos, Kalliope Papadopoulou, Athanasios Dalakouras*
14.45- 15.00h	Do endophytes play a role in the olive tree susceptibility to fruit fly infestation? *Teresa Lopes, Vitor Ramos, Cristina Cameirão, José Alberto Pereira, Paula Baptista*

15.00-15.15h	Effects of γ-irradiation on *Bactrocera oleae* adults: survival, behavioural parameters and presence of the endosymbiont *Candidatus* Erwinia dacicola *Gaia Bigiotti, Andrea Faggioli, Marzia Cristiana Rosi, Roberta Pastorelli, Silvia Arnone, Raffaele Sasso, Alessia Cemmi, Antonio Belcari, Patrizia Sacchetti*
15.15-15.30h	Field evaluation of the susceptibility of olive cultivars to *Bactrocera oleae* in Western Crete (Southern Greece) *Argyro Kalaitzaki, Ioannis Koufakis, Ekaterini Papadaratsaki, Ioanna Manolikaki, Georgios Koubouris*
15.30-16.00h	***Coffee break***
16.00-17.00h	Poster session
17.00-18.00	**Session 2 - Biological and biotechnological control of olive pests (continued)** Chairpersons: Isabel Calha and David Horta Lopes
17.00-17.15h	*Dittrichia viscosa* as a reservoir of *Bactrocera oleae* parasitoids *Dionyssios Perdikis, Nikolaos Ktenas, Argyro Kalaitzaki, Evangelos Koutsoukos, Vasilios Bournakas, Ioannis Koufakis*
17.15-17.30h	Mating Disruption of the olive moth (*Prays oleae*, Bernard) in olive groves using aerosol dispenser with synthetic sex pheromone. *Ortiz A., Porras A., Martí J., Tudela A., Rodríguez-González A., Sambado P.*
17.30-17.45h	A selection of wild flowers to enhance *Prays oleae* natural enemies *Fátima Gonçalves, María Villa, Anabela Nave, Laura Torres, José Alberto Pereira*
17.45-18.00h	The impact of super-high density olive orchard management system on the main insect pests in Tuscany: a three-year survey *Silvia Landi, Ilaria Cutino, Sauro Simoni, Stefania Simoncini, Claudia Benvenuti, Fabrizio Pennacchio, Francesco Binazzi, Silvia Guidi, Donatella Goggioli, Franca Tarchi, Pio Federico Roversi, Elisabetta Gargani*
20.00h	***Conference dinner***

Friday, 29 October 2021

9.00-10.30h	**Session 3 – Integrated management of olive diseases**
	Chairpersons: Jesús Mercado-Blanco and Helena Oliveira
09.00-09.30h	***Plenary lesson***
	Geographic and temporal population dynamics of the olive anthracnose pathogens
	Pedro Talhinhas, Andreia Loureiro, Helena Azinheira, Ana Cabral, Teresa Nascimento, Nuno Conceição, Helena Oliveira
09.30-09.45h	Fungal community diversity in leaves from two olive tree cultivars dissimilarly susceptible to anthracnose and to olive fruit-fly
	Vitor Ramos, Helgeneusa da Costa, José Pereira, Paula Baptista
09.45-10.00h	Massive screening of natural and biological compounds to select the best candidates for biocontrol of Verticillium Wilt of Olive in the Mediterranean basin
	Ana López-Moral, Carlos Agustí-Brisach, Antonio Mulero-Aparicio, Ángela Varo, Luis F. Roca, M. Carmen Raya, Joaquín Romero, Fco. Javier López-Escudero, Antonio Trapero
10.00-10.15h	Investigating decline symptoms of Super-High-Density Oil Olive in the Northern San Joaquin Valley of California
	Mohamed Nouri, Florent Trouillas
10.15-10.30h	The status of Neofabraea leaf and shoot lesions, Pleurostoma decline and Anthracnose in super-high-density oil olive orchards in California
	Florent P. Trouillas, Mohamed T. Nouri, Renaud Travadon, Daniel P. Lawrence, Juan Moral
10.30-11.00h	***Coffee break***
11.00-12.30	**Session 4 – Integrated management of *Xylella fastidiosa* in olive orchards**
	Chairpersons: Francisco Javier López Escudero and Célia Mateus
11.00-11.30h	***Plenary lesson***
	Xylella fastidiosa - State of Play in Portugal and Measures in Place
	Cláudia Sá
11.30-11.45h	Behaviour response of *Philaenus spumarius*, the main vector of *Xylella fastidiosa*, to traditional Portuguese olive cultivars

	Isabel Rodrigues, Marta Madureira, Jacinto Benhadi-Marín, Paula Baptista, José Alberto Pereira
11.45-12.00h	Effect of temperature and host plant species on the juvenile development of *Euscelis ohausi* (Hemiptera: Cicadellidae), a potential insect-vector of plant pathogens *Ioannis E. Koufakis, Maria L. Pappas, Argyro P. Kalaitzaki, Antonios E. Tsagkarakis, Despina K. Tzobanoglou, George D. Broufas*
12.00-12.15h	Bacteriome of *Philaenus spumarius* genitalia and its implication on insect's host reproduction *Cristina Cameirão, Daniela Costa, José Rufino, Teresa Lino-Neto, José Alberto Pereira, Paula Baptista*
12.15-14.00h	***Lunch***
14.00-15.30h	**Session 4 – Integrated management of *Xylella fastidiosa* in olive orchards (continued)** Chairpersons: Pedro Talhinhas and Kalliope Papadopoulou
14.00-14.15h	Development of a hybridization-based capture NGS assay to assess genome-wide diversity in *Xylella fastidiosa* infected samples *Velasco-Amo María Pilar, Arias-Giraldo Luis F., Imperial Juan, Landa Blanca B.*
14.15-14.30h	Plant endotherapy treatments enable the modification of xylem microbiome composition in olive trees *Manuel Anguita-Maeso, Guillermo León-Ropero, José L. Trapero-Casas, Juan A. Navas-Cortés, Blanca B. Landa*
14.30-14.45h	Involvement of specific traits of olive beneficial rhizobacteria to protect against biotic and abiotic stresses *Nuria Montes-Osuna, Carmen Gómez-Lama Cabanás, Antonio Valverde-Corredor, Pilar Prieto, Jesús Mercado-Blanco*
14.45-15.15h	Presentation of the 3 best posters
15.15-16.00h	***Coffee break***
16.00-17.30	**Workshop** Exogenous RNAi in plants: mechanisms and applications in pathogen and pest control, including olive fruit fly

	Athanasios Dalakouras
17.30-18.00h	**Concluding remarks**
	APH – Ana Cristina Ramos
	IOBC Representative – Andrea Lucchi
	Election of new WG convenor
	Meeting of the WG – Future developments – Voting on the next Venue

POSTERS

1. Population genetics and bacterial endosymbionts of Aphrophoridae putative vectors of *Xylella fastidiosa* and other Auchenorrhyncha species in Greece
   Michael Papamargaritis, Despoina E. Kapantaidaki, Ioannis Koufakis, Aris Ilias, Argyro Kalaitzaki, Stefanos Andreadis, Dimitrios Papachristos, Panagiotis Milonas, Anastasia Tsagkarakou

2. Diversity and population dynamics of *Xylella fastidiosa* potential vectors in olive groves with different management systems
   David Theodorou, Ioannis Koufakis, Zoi Thanou, Argyro Kalaitzaki, Ekaterini Chaldeou, Dimitrios Afentoulis, Antonios Tsagkarakis

3. Traditional and intensive olive growing have differences in the approaches to defense against the most common pests: the results of the DI. OL. Project
   Elisabetta Gargani, Ilaria Cutino, Sauro Simoni, Silvia Landi, Valeria Francardi, Fabrizio Pennacchio, Gian Paolo Barzanti, Veronica Vizzarri, Gaetana Mazzeo, Agatino Russo, Gaetano Siscaro, Lucia Zappalà, Salvatore Nucifora, Salvatore Giacinto Germinara, Antonella Marta Di Palma, Matteo Guidotti, Rinaldo Psaro, Pio Federico Roversi

4. The Impact of super-high density olive orchard management system on soil plant-parasitic nematodes in Central and South Italy
   Silvia Landi, Giada d'Errico, Rossella Papini, Ilaria Cutino, Stefania Simoncini, Andrea Rocchini, Giorgio Brandi, Roberto Rizzo, Giovanni Gugliuzza, Giacinto Germinara, Gaetana Mazzeo, Pio Federico Roversi.

5. Emergence of *L. rigidum* with cover crops – contribution for IWM
   Isabel M Calha, Milagros Saavedra, Juan Antonio Lezaun, Aritz Royo

6. Weed diversity in olive groves – shelther for insect vectors of *Xylella fastidiosa*

Aritz Royo, Isabel M Calha, Célia Mateus, Conceição Boavida, Paula Sá-Pereira

7. Remote Sensing: Assessing the differences between NDVI obtained in spring and autumn, in a mountain olive grove

   Natália Roque, Catarina Lourenço, Paulo Fernandez

8. Looking for insect vectors of *Xylella fastidiosa*: finding them and other insects. A survey in Portuguese olive groves

   Célia Mateus, Ana Carina Neto, Conceição Boavida, Liliana Sargento, Isabel Patanita, Paula Sá-Pereira

9. A tritrophic interaction model for an olive tree pest, the olive moth – *Prays oleae* (Bernard)

   Sonia Pappalardo, María Villa, Sonia A.P. Santos, Jacinto Benhadi-Marín, José Alberto Pereira, Ezio Venturino

10. New insights about *Ageniaspis fuscicolis* var. *praysincol*a, specific parasitoid of the olive moth, a key-pest of the olive tree

    María Villa, Sónia Santos, Jacinto Benhadi-Marín, José Alberto Pereira

11. Ground cover vegetation composition predicts the abundance of *Sphaerophoria scripta* (Linnaeus, 1758) (Diptera: Syrphidae) in olive groves from Trás-os-Montes region (Portugal)

    Marta Madureira, Isabel Rodrigues, Maria Villa, José Alberto Pereira

12. Biological treatments enhancing the plant immune system of olive (*Olea europaea*) against *Verticillium dahliae*

    Ana López-Moral, Eugenio Llorens, Loredana Scalschi, Pilar García-Agustín, Antonio Trapero, Carlos Agustí-Brisach

13. Selection of endophytes as antagonists of *Colletotrichum acutatum* and elucidation of their mode of action

    Liliana Pires, Teresa Lopes, Paula Baptista

14. Behavioral responses of female *Bactrocera oleae* flies to honeydew

    Argyro Kalaitzaki, Ioannis Koufakis, Ioannis Kasapakis, Emmanouela Kapogia, Dionyssios Perdikis

15. Development of a PCR-based diagnostic method to detect of DNA of *Philaenus* (Hemiptera: Aphrophoridae), vector of *Xylella fastidiosa*, in the gut of spiders

    Isabel Rodrigues, Vítor Ramos, Jacinto Benhadi-Marín, José Alberto Pereira, Paula Baptista

16. Phylogenetic analysis of Hypocreales strains isolated from olive trees, a fungal order known to harbour promising biocontrol agents

Vitor Ramos, Helgeneusa da Costa, José Alberto Pereira, Paula Baptista

17. Screening of potential biocontrol endophytes and epiphytes against olive knot disease

Ana E. Cunha, Diana Mora, Pedro A. Casquero, José A. Pereira, Paula Baptista

18. Assessment of physiological traits of olive trees infected by *Xylella fastidiosa* subspecies

Miguel Román-Écija, Concepción Olivares-García, Juan Carlos Rivas, Pilar Velasco-Amo P, Juan Antonio Navas-Cortés JA, Blanca B Landa

19. Predatory potential of Forficulidae (Dermaptera) on the olive fly *Bactrocera oleae*

Marco Neto, Jacinto Benhadi-Marín, Fátima Gonçalves, Maria Villa, José Alberto Pereira

20. Contribution to the study of the beneficial fauna of olive tree parcels in Porto Martim, Terceira Island, Azores

Cristina Moules, Elisa Tarantino, David Horta Lopes, Paulo A. Vieira Borges

21. A micronutrient fertilizer affects the survival of *Bactrocera oleae* adults and puparia emergence in laboratory

Patrizia Sacchetti, Marzia Cristiana Rosi, Daniela Noferini, Gaia Bigiotti, Roberta Pastorelli, Antonio Belcari

22. Identification of endophytes and of their volatile compounds with biocontrol potential towards olive anthracnose

Yosra Sdiri, Ana Cunha, Teresa Lopes, Kevin Silva, Nuno Rodrigues, José A. Pereira, Paula Baptista

23. Exploitation of endophytes of centenarian olive trees in the management of olive knot disease

Nawal Moudjeber, Gilcimar Candeias, Cristina Cameirão, Vitor Ramos, José A. Pereira, Paula Baptista

ABSTRACTS

Session 1 – Integrated management of olive pests

Session 1 – Plenary lesson

Precision pest management in olive groves

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Managing olive pests has become a challenging operation, because of unpredicted climatic conditions, changes in cultivation schemes, ban of commonly used insecticides, increased environmental concerns and socioeconomic aspects such as major fluctuations in produce prices. Technological advances, in recent years, offer new opportunities for an environmentally and economically sound management of olive pests. For example, Geographical Position Systems, advanced mapping tools, detailed landscape characterization, electronic insect traps, real time weather forecast are increasingly exploited to assist pest management efforts. Careful and judicious use of some or many of the above tools may generate reliable temporal and spatial predictions of harmful insect populations and be used to direct "surgical" interventions to suppress them. In addition, application of Integrated Pest Management (IPM) tools can be assisted by Unmanned Aerial Vehicles (UAV) to treat specific delimited hot spot areas, reducing cost of operation and the environmental footprint of pest management practices. So far, the above technology has been considered to develop IPM systems for couple of the major pests of olives. However, the list of olive pests is quite long and apart of the major ones like the olive fruit fly, *Bactrocera oleae* (Diptera: Tephritidae) and the olive moth, *Prays oleae* (Lepidoptera: Hyponomeutidae) includes additional important and diverse ones (e.g scale insects, mites) that can occasionally cause severe damages and yield losses in olives cultivation. Adoption of the new technology towards establishing precision pest management approaches in olive groves is data and knowledge demanding and still requires extensive field work. The current paper presents precision pest management approaches for the olive pests, demonstrates the use of DSS and discusses opportunities and challenges.

Keywords: Integrated pest management, pesticides, bait sprays, UAV, Decision Support Systems, IPM algorithm

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Session 1 – Oral communication

Three-year field trials for a sustainable approach in the control of olive fruit fly, *Bactrocera oleae*

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The implementation of novel environmentally friendly control methods of olive fruit fly is attracting an ever-growing attention, especially after the recent reduction in the use of the synthetic insecticides increasingly supported by European and national legislation. Treatments against olive fly populations can be preventive or curative: the former are the most used in organic production and their target are the adults. Some substances can physically mask the foliage of the plant, dusting it, making the fruits less attractive for the egg laying and inhibiting the bacterial populations on the leaves' surface. During the years 2018-2020, inorganic solids, with high specific surface area and ion-exchange capacity, were prepared starting from mineral bentonite, with high montmorillonite content, and zeolite clinoptilolite of natural origin, both subjected to a cation exchange treatment with copper (II) salts or, for the bentonite only, after deposition of a bioactive principle based on organic insect-repellent compounds: aliphatic aldehydes, namely, hexanal, heptanal, (*E)*-hex-2-enal and (*E)*-hept-2-enal. The solids were applied on olive trees by spraying, as an aqueous 3 wt.% suspension, on the whole crown of the plants, in three treatments in the period between July and September. The tests were carried out in olive crops in Central Italy (Tuscany), in two farms located in Siena district an in a third farm in Florence district. In each field trial, controls on olive trees were made before treatment and, every ten days, after each treatment, evaluating the percentage of active infestation and total infestation. A reduction in the *B. oleae* infestation of about 98% was observed in 2018 on the trees treated with bentonite with saturated and unsaturated aldehydes. After one week after the second treatment, in 2019, clinoptilolite exchanged with Cu (II) showed excellent efficacy against *B. oleae*, with a drastic reduction of the percentage of infestation on olive trees which reaches 1.1% for olive trees treated with new formulation, compared to over 20%, for the untreated plants control. This result is significantly better than that obtained with other commercial products currently in use. The montmorillonite materials, in the presence or absence of Cu(II) species, on the other hand, showed less performance with respect of clinoptilolite, promising, however, satisfactory results and in line with other reference formulations available on the market.

Keywords: inorganic solids, montmorillonite, zeolite, sustainable control strategies, olive fruit fly.

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Session 1 – Oral communication

Comparing olive fruit fly (*Bactrocera oleae*) density, fruit damage levels and parasitism rates in organic and conventional olive orchards in Crete, Greece

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The Olive Fruit Fly (OFF), *Bactrocera oleae* (Diptera: Tephritidae), is the main pest in olive agroecosystems causing quantitative and qualitative yield losses. Pest control is mainly done in conventional olive orchards by applying synthetic chemical pesticides. To reduce the use of pesticides and prevent pesticide resistance by the OFF, sustainable farming methods to control the OFF are needed. Here, we monitored OFF activity, fruit infestation levels, and parasitism rates by the larval parasitoid wasp *Psyttalia concolor* (Hymenoptera: Braconidae) for two consecutive years in twelve commercial olive orchards under two different farming systems, the conventional vs. organic/biodiversity-oriented. The orchards were located in plain and hilly agroecological zones of the Messara valley, South Crete. The aim of this study was to assess the effects of the pest management strategies of the two different farming systems. We found a higher OFF activity in organic orchards in both years. Fruit damage levels were higher in organic orchards only during the first year, while in the second year they were similar in both farming systems, despite that conventional orchards were sprayed with pesticides to suppress the OFF. Interestingly, we found higher *P. concolor* parasitism rates in organic orchards which could explain reduced fruit damage as conditions in organic olive agroecosystem are favorable for maintaining beneficial insects. Our findings suggest that even if higher OFF activity occurs in organic orchards, the more complex habitat may support the presence of the natural enemies of the OFF, leading to similar fruit infestation rates. This study may contribute to a holistic olive agroecosystem management and a more environmentally sound way to control the OFF.

Keywords: *Bactrocera oleae*, *Psyttalia concolor*, insect populations, olive agroecosystem, organic orchards, conventional orchards, farming systems, integrated pest control, sustainable agriculture.

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Session 1 – Oral communication

Aldehyde-containing clays and zeolites: a sustainable approach in the control of olive fruit fly, *Bactrocera oleae*

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The implementation of innovative and environmentally-friendly control methods against the olive fruit fly, *Bactrocera oleae*, has attracted an ever-growing attention, especially after 2014, 2016 and 2019 when the Italian olive oil production suffered from qualitative and quantitative losses. In this aim, two series of solids were prepared: 1) copper(II)-exchanged clinoptilolite zeolites and 2) organically modified montmorillonite clays, over which aliphatic aldehydes, *[E]*-hex-2-enal; *[E]*-hept-2-enal, *n*-hexanal; *n*-heptanal, have been deposited. The final goal is to set up a composite oxidic material capable to host a bioactive compound able to effectively disrupt the host-plant selection process of *B. oleae* adults featuring a targeted and constant release for a long-term treatment of the olive fruits. A maximum aldehyde loading of 4.7 wt.% and 13.5 wt.% was obtained for aldehyde/bentonite (C6-Ben, C7-Ben) and aldehyde/zeolite (C6-Zeo, C7-Zeo), respectively. The solids were fully characterized by physico-chemical analysis and a specific adsorption of the aldehyde into the interlayer space of the clay was evidenced (Fig. 1a). Tests for the evaluation of the release capability showed a smooth aldehyde desorption up to 40 days, under ambient conditions. Large kilogram-scale batches of the aldehyde/bentonite material were tested under real conditions, in an open-field trial on an olive tree orchard in Southern Tuscany (Fig. 1b). Extremely promising results were obtained with Cu-zeolite and with both aldehyde-containing bentonite-like montmorillonite clays, in terms of reduction of both fly infestation (up to 98% reduction) and damages on the olive fruit. These materials proved to be easily prepared, cost effective, environmentally-friendly, stable to rainwater leaching and led to a remarkable diminution in the use of bioactive species (and copper-containing ingredients) for on-field applications.

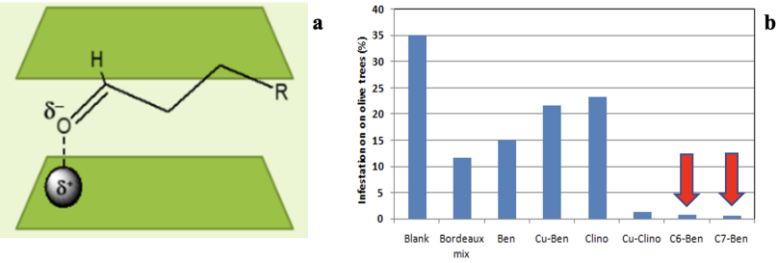

*Figure **a:** interaction between aldehyde and inner surface of montmorillonite; **b**: Percentage of infested olive trees under open-field conditions after 3 treatments. Blank: untreated trees, formulation: 3 wt.% suspension of the solid in drinkable water.*

Keywords: Organic/inorganic hybrid materials, clays, clinoptilolite zeolite, pest control, olive fruit fly.

Acknowledgements: financial support by the Italian Ministry MIPAAF through the Project "DIOL" (grant prot. no. 23774) is acknowledged.

Effect of olive fruit size on the parasitism rates of *Bactrocera oleae* (Diptera: Tephritidae) by the figitid wasp *Aganaspis daci* (Hymenoptera: Figitidae), and first pilot field releases of adult parasitoids in an organic olive grove

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The olive fruit fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) is the most serious pest of the olive production worldwide. *Aganaspis daci* (Hymenoptera: Figitidae), is a larval- prepupal endoparasitoid that successfully attacks several fruit fly (Tephritidae) species. To assess the potential of *A. daci* as a biological control agent against *B. oleae*, we studied, under laboratory conditions, the effect of olive fruit size on parasitism rates of *A. daci* on 2nd and 3rd instar larvae of *B. oleae*. In addition, we conducted pilot scale releases of *A. daci* females in a 0.5 ha organic olive grove. We released 1000 *A. daci* females/week from July to October. Extensive olive fruit sampling was performed to to estimate olive fruit infestation levels and the parasitism rates of *A. daci*. Laboratory trials revealed that both the size of the fruit and the larvae instar were significant predictors of parasitism success of *A. daci* when the cultivar Chalkidikis was considered. Parasitism rates were higher in small-size fruit compared to bigger and medium ones. The 3rd instar larvae of *B. oleae* were parasitized in higher levels compared to younger instars. Interestingly, no successful parasitism was observed in the wild olives irrespective of the developing larvae instar (2nd or 3rd). The pilot field trials revealed no recovery of *A. daci* adults from the sampled olive fruits that were infested by the olive fly. Apparently, higher number of parasitoids should be considered in future trials aiming to successful control of *B. oleae* in the wild. We discuss the importance of biological control for the management of the olive fruit fly.

Keywords: biological control, larval instar, parasitism, olive fruit fly, parasitoids, integrated pest management.

Acknowledgements: This research is co-financed by Greece and the European Union (European Social Fund- ESF) through the Operational Program «Human Resources Development, Education and Lifelong Learning 2014-2020» in the context of the project "Biological control of the olive fruit fly with the parasitoid *Aganaspis daci* (Hymenoptera: Figitidae)" (MIS 5004126).

Session 2 – Biological and biotechnological control of olive pests

Session 2 – Plenary lesson

The use of semiochemicals in the olive grove: An essential tool

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Semiochemicals are natural products involved in chemical communication between living organisms. In an initial classification, semiochemicals are subdivided into pheromones and allelochemicals depending on whether the interactions are intraspecific or interspecific, respectively. Pheromones are released by one member of a species to cause a specific behavior to another member of the same species. This chemical language of insects has been the subject of deep research in the field of chemical ecology for the past fifty years. Pheromones are classified based on the interaction mediated, such as sexual attraction, alarm, recognition, aggregation, and many others. Insect sex pheromones have been most widely applied in integrated pest management (IPM) but other behavior-modifying semiochemicals have had success from a commercial point of view too. In many cases, pheromone-based methods have been successful; however, after years of applied research, it has become clear, that the development of semiochemical-based strategies for controlling insect pests was harder than we thought. There are many factors such as the ethology, the population of the target insect, the technical development of the active compound, or economic reasons, which determine the success/failure of its final application. Usually, the complete research work takes five to ten years.
In order to prevent the excessive use of synthetic insecticides in olive groves causes food residues, environment pollution, insect resistance problems or secondary pest resurgence, during the last three decades, pheromones and other semiochemicals are usually used in olive groves Integrated Pest Management programs. Following the identification of the sex pheromone of the olive pest (*Prays oleae*) in 1979 and, in 1980 the female sex pheromone of *B. oleae*, expectations for its potential use in IPM programs and sustainable agriculture were huge. The most widespread use of pheromones in olive groves has been for monitoring adult insect pest populations, so until now, the main uses of semiochemicals in olive groves IPM are monitoring and attractant-kill techniques. Even though mating disruption is probably, the semiochemical-based technique most successfully used in IPM, in our crop is still under development and not fully implemented.
Many factors such as the ethology or population of the target insect, the technical development of the active compound or economic motives determine the success or failure of its application. What are we missing to obtain better results with the use of semiochemicals in olive orchards? What new challenges do we face?
There are many questions to answer and to illustrate these issues; examples of successes and failures in the application of semiochemicals in olive groves will be briefly described.

Session 2 – Oral communication

Towards an RNA-based biocontrol approach of olive fruit fly

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Olive fruit fly (*Bactrocera oleae*) is the most serious insect pest of olive (*Olea europea*) fruits in the world with causing severe crop yield and monetary losses. The control of *B. oleae* remains almost exclusively based on insecticides, an approach that is dangerous for the environment. Clearly, alternative approaches for olive fruit fly control is an urgent need. Exogenous RNA interference (exo-RNAi) is a promising tool in modern pest management which offers extreme specificity of action and minimal environmental risk (Dalakouras et al., 2020). RNAi is a conserved eukaryotic mechanism that is triggered by double stranded RNAs (dsRNAs) that are processed into 20-25-nt small RNAs (sRNAs) which target complementary mRNAs for degradation and/or translational arrest. Exogenous application in plants of such RNA molecules designed to target insect essential genes can be taken up by them and, ideally, lead to their lethality. In order to prove the concept and investigate whether sRNAs can indeed be applied in olive trees and transported to the fruits so as to be taken up by the *B. oleae* larvae, we introduced by trunk injection 22-nt sRNAs (chemically synthesized, CY3-labelled). Confocal microscopy revealed that the oligonucleotides were transported through the xylem to the fruits already 48 hrs post application and, importantly, taken up by the *B. oleae* larvae. Future studies optimizing formulation and delivery methods and selecting the most suitable gene targets should pave the way towards the development of novel RNA-based biocontrol compounds for olive fruit fly management.

Keywords: olive fruit fly, RNA interference, small RNAs, trunk injection

Acknowledgements: This work is funded from PRIMA programme (INTOMED project), supported under Horizon 2020, the European Union's Framework Programme for Research and Innovation.

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Session 2 – Oral communication

Do endophytes play a role in the olive tree susceptibility to fruit fly infestation?

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Olive fruit fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) is the most important olive pest worldwide, by damaging olives due to oviposition and drastically decreasing the crop yield. In Portugal, it has been found that different olive cultivars exhibited different propensities to fruit fly infestation, being the cv. *Cobrançosa* less susceptible than the cv. *Madural*. This work aims to disclosed the role play by fruit-associated endophytes in conferring these different host plant susceptibilities to fruit fly infestation. Accordingly, the endophytic microbial composition of infested ad non-infested fruits from cultivars *Cobrançosa* and *Madural* were studied, by using a culture dependent PCR-based identification approach. Overall, 61 operational taxonomic units (OTUs) were obtained, being identified until the species/genera level a total of 21 bacteria and 23 fungi. The bacterial community was mostly composed by members of phyla Proteobacteria and genus *Pseudomonas*, while fungal community was dominated by Ascomycota and *Sarocladium* members. No significant differences were observed on the diversity of endophytes among the two cultivars or infestation level. Still, distinct endophyte community's composition was detected among cultivars, being this factor explaining 2.1% and 2.3% of bacterial and fungal composition variation, respectively. Endophytic communities from infested and non-infested fruits also differs significantly, but with greater differences in the most susceptible cv. *Madural* than in cv. *Cobrançosa*. Specific bacterial/fungal taxa were found to be positively associated to the most resistant cultivar and non-infested fruits. Their potential role in conferring host plant protection against olive fly attack will be discuss.

Keywords: *Olea europaea* L., *Bactrocera oleae*, infestation level, bacteria, fungi.

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Effects of γ-irradiation on *Bactrocera oleae* adults: survival, behavioural parameters and presence of the endosymbiont *Candidatus* Erwinia dacicola

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Bactrocera oleae, the olive fruit fly, is well known for the damage caused to olive crops, affecting both quantity and quality of drupe and olive oil productions. For decades conventional control methods have been relied on the use of organophosphates as larvicidal sprays. In view of defining more sustainable strategies, many low-impact methods have been tested and applied. Among them, the Sterile Insect Technique (SIT) attracted renewed interest since it is considered as the most effective control method against fruit flies, appropriate for an area-wide application and without environmental impact. Unfortunately, the mass production of olive fruit flies is still hindered by several technical and biological issues. As a matter of fact, adults reared on cellulose-based diet showed aberrant behavior and physiological deviations while released sterile males should be competitive with wild populations. Since in nature olive fruit fly fitness depends on the bacterium endosymbiont *Candidatus* Erwinia dacicola, a study aimed at evaluating the effects of irradiation on survival, behavior and retention of *Ca.* E. dacicola was carried out in symbiotic *B. oleae.*
Olive fruit fly puparia obtained from infested olives were irradiated with Co_{60} as source of γ−rays applying irradiation doses of 100 and 150 Gy. Adults emerged from irradiated puparia were used in lab experiments to evaluate their survival, content of the endosymbiont and behavioral parameters in comparison to non-irradiated flies. Flies irradiated at 150 Gy survived significantly less than the other two treatments. Also mating competitiveness bioassays showed a less performing behavior of the adults irradiated at 150 Gy respect to the other two treatments. Irradiation induced a different response also in olfactometer bioassays, when males were exposed to the pheromone. Adults irradiated at 150 Gy showed less walking activity and moved more slowly. The content of *Ca*. E. dacicola was assessed at four ages through Real Time PCR. One day old irradiated flies at 150 Gy showed a significant lower bacterial content in comparison to the other treatments. Overall, *Ca*. E. dacicola content increased as flies aged, with more evident trend in 100 Gy rather than in other treatments. The unexpected recovery in the endosymbiont content in flies irradiated at the lower irradiation dose, together with the

behavioral data and survival, highlight a possible development of SIT for the olive fly control.

Keywords: SIT, mating competitiveness, symbiosis.

Session 2 – Oral communication

Field evaluation of the susceptibility of olive cultivars to *Bactrocera oleae* in Western Crete (Southern Greece)

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The susceptibility of 27 olive cultivars to *Bactrocera oleae* (Rossi) from the major European Mediterranean olive-producing countries, was evaluated during two consecutive years, under field conditions of western Crete (Southern Greece), where environmental conditions are largely favorable for the reproduction and development of its population. Olives were collected from the National Olive Germplasm Bank of Greece of the Institute of Olive Tree, Subtropical Crops & Viticulture, Hellenic Agricultural Organization (ELGO-DIMITRA) in Chania. The samples (one hundred olives per cultivar per date) were collected every fortnight at eight different times during fruit ripening, from June to October, during 2019 and 2020. The observations were carried out by detecting the sterile oviposition stings, alive preimaginal stages (eggs, larvae and pupae), dead preimaginal stages (larvae and pupae) and emergence holes. The results of this study confirm that female olive fruit flies exhibited a strong ovipositional preference when presented with a choice of olive cultivars in the field. Among the olive cultivar tested, 'Manzanilla Isr'. 'Manzanilla' and 'Konservolia' were the most heavily infested during the two consecutive years. The next most heavily infested were 'Picual', 'San Francesco', 'Valanolia' 'Frantoio Rodou', Frantoio' and 'San Agostino' while 'Kalamon', 'Koroneiki', 'Tragolia', 'Pikrolia', 'Mavrelia', 'Rachati', 'Myrtolia', 'Koutsourelia' and 'Thiaki' show the lowest susceptibility to olive fruit fly attacks. Even though the olive fruit fly damaged all tested cultivars those showing indications of relative resistance could be tested for their suitability in new plantations in areas with high *B. oleae* populations as well as to be employed as prebreeding material.

Keywords: genotype, olive fruit fly, plant phenotyping, susceptibility.

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Session 2 – Oral communication

Dittrichia viscosa as a reservoir of *Bactrocera oleae* parasitoids

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The false yellowhead *Dittrichia viscosa* (L.) W. Greuter (Asteraceae) is a widespread plant species in the Mediterranean region. Several studies have reported its role as an alternative host plant of certain olive fruit fly parasitoids. These parasitoids parasitize the larvae or pupae of *Myopites stylatus* (Fabricius) (Diptera: Tephritidae) that causes galls on the flowers of this plant. However, relatively little effort has been devoted to investigating the number of larvae or pupae present per gall, the parasitism level of *M. stylatus* and the period that each parasitoid species emerges from the galls. In the current work, *D. viscosa* plants bearing galls of *M. stylatus* were located in the area of Chalkis, central Greece. On 14 February 2018 and on 23 November 2019, 20 galls were collected and were dissected under the stereomicroscope to record alive and parasitized larvae or pupae of *M. stylatus* per gall. In the first and second sampling, in average, each gall had 2.55 or 1.95 chambers, 62 or 71% of them were occupied by alive larva or nymph of *M. stylatus* and nymphs consisted 48 and 9% of the population, respectively. Larvae but mainly pupae of parasitoids were recorded in the galls. The parasitism level was 32% and 33%, respectively. On 14 February 2018 galls were enclosed in cages and transferred in the laboratory where were kept under room conditions to monitor the emergence of parasitoids. Parasitoids firstly emerged on 20 February and their emergence continued till September 2018. In the samples *Eupelmus urozonus*, *Eupelmus* sp., *Eurytoma martelli*, *Eurytoma* sp., *Ormyrus* sp., Torymidae, Pteromallidae and Eurytomidae were recorded. Among them, *E. urozonus* and *E. martelli* are important parasitoids of the olive fruit fly. *Eupelmus urozonus* emerged from 20 February to April 3, 2018. Therefore, this work shed light on aspects of the role of *D. viscosa* and *M. stylatus* in the conservation of olive fruit fly parasitoids and further research should focus on more detailed evaluation of their importance in olive fly parasitization.

Keywords: olive fruit fly, *Dittrichia viscosa*, *Myopites stylatus*, *Eupelmus urozonus*, parasitoid, banker plant, biological control

Session 2 – Oral communication

Mating Disruption of the olive moth (*Prays oleae*, Bernard) in olive groves using aerosol dispenser with synthetic sex pheromone.

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Mating disruption of the olive moth (OM), *Prays oleae* (Bern.) (Lepidoptera: Yponomeutidae) using its sex pheromone Z(7)-14:Al was tested for two successive years in Andalucía (South Spain) olive groves, to evaluate the efficacy of mating disruption (MD) to control the OM from first to third generation. Pheromone trap catches, percentage of leaves and olive fruits infestation were measured in both MD and untreated control olive groves. The reduction of attraction of male adults to pheromone traps was significantly lower in the MD treatment plots. Fruit damage in the MD plots was significantly lower compared with the untreated plots. Results of two-year field trials carried out in Andalucía demonstrated the potential of Mister P X841 aerosol devices as effective tool to control the olive moth *Prays oleae*.

Keywords: Mating disruption, sex pheromone, *Prays oleae*, olive moth, aerosols, IPM.

Session 2 – Oral communication

A selection of wild flowers to enhance *Prays oleae* natural enemies

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The diversity of vegetation in agroecosystems can provide arthropod natural enemies with food sources (pollen, nectar and alternative hosts and preys) and shelter (for matting and overwintering), potentially enhancing natural pest control and reducing dependence on chemical pesticides. Accordingly, the establishment of agroecosystems with flowering plants able to provide the resources needed by natural enemies to maintain and enhance their reproduction, survival and efficacy and without favour pests is an important challenge. The olive moth, *Prays oleae* (Bernard) is an important pest of olive groves, developing three generations per year each one damaging one organ of the tree (flowers, fruits, leaves). This pest is naturally controlled by several insects able to reduce its populations, including the predator *Chrysoperla carnea* s.l. (Stephens) and the parasitoids *Ageniaspis fuscicollis* (Dalman), *Chelonus elaeaphilus* Silvestri and *Elasmus flabellatus* (Fonscolombe). In the last years, the effect of flowering plants, frequently found in Portuguese olive groves, on the survival and reproduction of those natural enemies were analysed in several laboratory studies. Based on the compilation of the results of those studies, we present a set of plants to be installed in field studies in order to ascertain their effect in enhancing natural enemies and their efficacy as natural control agents of *P. oleae*. According to the plant adequacy to common management practices carried out in olive orchards, the selected set of plants can be classified in species suitable for olive interrows (e.g. *Andryala integrifolia* L., *Borago officinalis* L., *Echium plantagineum* L., *Lamium purpureum* L., *Malva sylvestris* L., *Silene gallica* L., *Tolpis barbata* (L.), *Veronica persica* Poir) and species suitable for olive edges or small flowering patches (e.g. *Conopodium majus* (Gouan) Loret, *Foeniculum vulgare* Mill., *Lonicera hispanica* Boiss et Reut., *Daucus carota* L., *Lavandula stoechas* L.).

Keywords: conservation biological control, flowering plants, *Chrysoperla carnea* s.l., *Chelonus elaeaphilus*

Acknowledgements: This work was funded by the European Social Fund through the Regional Operational Program North 2020, within the scope of the Program "Contratação de Recursos Humanos Altamente Qualificados" (Oleachain project "Skills for sustainability and innovation in the value chain of traditional olive groves in the Northern Interior of Portugal" - Norte-06-3559-FSE-000188), and by Horizon 2020 (NovaTerra project "Reducing the negative impact of pesticides through innovation in Mediterranean olive groves and vineyards" - H2020-SFS-2018-2020). F.G, M.V. and J.A.P. are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support by national funds FCT/MCTES to CIMO (UIDB/00690/2020). F.G., A.N. and L.T. are grateful to the FCT for financial support, by national funds

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Session 2 – Oral communication

The impact of super-high density olive orchard management system on the main insect pests in Tuscany: a three-year survey

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Super-high density (SHD) olive orchards are recently planted in Italy with the aim of reducing production costs and increasing, at the same time, yields per hectare. To date, an area corresponding to less than 5% of total olive orchards has been converted to the SHD olive orchard system. However, specific research on the impact of SHD on well-known and widespread pests of olive orchards has not been carried out yet. The aim of the present study was to assess the impact of SHD olive orchards on *Bactrocera oleae* and other species of "minor" importance, such as *Prays oleae*, *Palpita vitrealis*, and *Otiorhynchus cribricollis* in three sites (in the districts of Florence and Siena, in Tuscany), characterized by different soils, climate and various cultivars. In each site, the SHD management system was compared to an adjacent olive orchard traditional system in which the same soil and phytosanitary managements were applied. The SHD management system differed from the traditional one as cultivars were characterized by small size (SHD – Leccio del Corno, Diana, Tosca, Pendolino, Correggiolo, Maurino selection Vittoria, Arbequina; Traditional – Frantoio, Leccino, Moraiolo, Pendolino) and a likely complementary irrigation requirement. Samplings of twigs and fruits were carried out during 2018-2020, every two weeks from spring to fall, in three different marked rows of both management systems of each study area. Moreover, on each tested olive orchard pheromone traps were used for monitoring *B. olea, P. oleae,* and *P. vitrealis.*
The results of the three-years survey seem to indicate some differences between the two olive orchard management systems in terms of infestations caused by the investigated pests. The catches of *B. olea* and its infestation percentage on olive fruits have always been higher in the traditional olive orchard system than in the SHD one. Conversely, *O. cribricollis* infestation was often higher in the SHD system than in the traditional one even though the damage was negligible. *Palpita vitrealis* showed discordant results and the infestation rate was very high in the SHD system in only one site (about 40% compared with 15% in the traditional one). Although *P. oleae* has shown higher catches in the traditional management system, plant infestation has always been low and with no difference between the two managements. Concerning the differences in *B. oleae* infestation in the two investigated managements, it should consider the intrinsic characteristics of the cultivars, as well as the possible difference in their productive load. Moreover, the greater incidence of vegetation on plants in SHD olive orchards may have favoured the infestation of *O. cribricollis* and, in some cases, of *P. vitrealis*.

Keywords: *Bactrocera oleae, Palpita vitrealis, Prays oleae, Otiorhynchus cribricollis.*

Acknowledgements: Work done under the Project DI.OL "Difesa dagli organismi nocivi nelle coltivazioni olivicole tradizionali e intensive" funded by the Ministero delle Politiche Agricole, Alimentari e Forestali.

Session 3 – Integrated management of olive diseases

Session 3 – Plenary lesson

Geographic and temporal population dynamics of the olive anthracnose pathogens

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Portugal is likely the country in the world where olive anthracnose is most relevant. The causal agent was first identified here in 1899 by J. Veríssimo de Almeida and the first record of symptoms compatible with olive anthracnose was produced by the Arab agronomist Bū'l-Jayr in the XIth century in South-western Iberia. Mild and wet autumn conditions (due to Atlantic exposure) and the widespread cultivation of the highly appreciated but highly susceptible 'Galega Vulgar' are referred as factors explaining high disease incidence and severity in the country. We now know that the causal agents are genetically diverse and cluster in different species of *Colletotrichum*, a knowledge that has in part contributed to the reformulation and update of the taxonomy of *Colletotrichum*, which in turn has contributed to a more sustained knowledge of olive anthracnose aetiology. The species *C. nymphaeae* is the most frequent in Portugal, but it is rarely associated to olive anthracnose elsewhere. In turn, *C. godetiae* is common throughout the Mediterranean basin but in Portugal it is only frequent in the North-east of the country (Trás-os-Montes). A third species, *C. acutatum*, common in the southernmost region (Algarve), is appearing with increased frequency in other parts of the country as well as in other Mediterranean countries, where it even seems to be replacing *C. godetiae* as the most common pathogen. Several other species are also identified, but are less frequent. Comparative studies have depicted *C. nymphaeae* and *C. acutatum* as the most virulent species towards olive fruits, along with *C. theobromicola*, a common olive pathogen in Australia but that has recently been associated to olive anthracnose also in South America. Any of these species occur on multiple hosts and affect several crops and thus there is potential for cross-infection to and from olive. In this presentation we will update the knowledge on the population dynamics of the olive anthracnose pathogens, bearing in mind the current taxonomic framework in *Colletotrichum* as well as the host range, life styles and geographic distribution of each species, in order to foresee future population dynamics and to anticipate better informed protection strategies.

Keywords: olive anthracnose, *Colletotrichum*, aetiology.

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Session 3 – Oral communication

Fungal community diversity in leaves from two olive tree cultivars dissimilarly susceptible to anthracnose and to olive fruit-fly

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Aiming to glimpse a biocontrol management approach anchored to the olive tree-associated resident fungal microbiota, in this work we studied the diversity, structure, and composition of epiphytic and endophytic fungi inhabiting in and on leaves from two important NE Portuguese cultivars (Cobrançosa and Madural). These cultivars exhibit dissimilar susceptibilities related to anthracnose disease and to a pest, the olive fruit-fly *Bactrocera oleae*, being cv. Madural more susceptible to anthracnose and more infestedby the fly than cv. Cobrançosa. Through a culture-dependent approach, and by sampling leaves (from the inner and outer canopy) from five trees of an organic orchard, we obtained 203 isolates corresponding to 161 taxa. A comparison of epiphytic and endophytic fungal communities, at the tree level, revealed significant differences in species richness and diversity. Epiphytes were most rich and diverse (6 subphyla, 11 classes, 21 orders and 36 families) when compared to endophytes (5 subphyla, 10 classes, 15 orders, 27 families). In terms of composition, the fungi community from leaves of cv. Cobrançosa was different than that from cv. Madural, markedly regarding the epiphytic community. On the other hand, the endophytic community was not different in terms of composition neither between cultivars, nor by canopy location. Implications of these findings for the use of endophytic and/or epiphytic fungi in these two cultivars are discussed, in the light of possible biocontrol practices to manage both anthracnose (*Colletotrichum* spp.) and the olive fruit-fly.

Keywords: *Olea europaea*, disease, pest, leaves, biological control agents.

Acknowledgements: This work is supported by FEDER funds through the COMPETE (Operational Programme for Competitiveness Factors) and by National funds through the FCT (Foundation for Science and Technology) within the POCI-01-0145-FEDER-031133 (MicOlives) project and the Mountain Research Center - CIMO (UIDB/00690/2020).

Session 3 – Oral communication

Massive screening of natural and biological compounds to select the best candidates for biocontrol of Verticillium Wilt of Olive in the Mediterranean basin

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Verticillium wilt of olive (*Olea europaea*; VWO), caused by *Verticillium dahliae*, is the most serious biotic challenge to this crop in the Mediterranean basin. Due to the lack of a truly efficient method to control VWO, an integrated management strategy is needed to reduce both pathogen dispersal and disease incidence in olive orchards. Thus, the use of natural or biological compounds arises as a potential complementary and eco-friendly control tool against VWO. In this context, the Agroforestry Research Group AGR-216 from the University of Cordoba (Spain) has been developing an extensive research line on biocontrol of VWO since 2011, which has contributed markedly in generating knowledge on such topic towards to obtain potential biological solutions against the disease in the near future. To reach this goal, more than 250 compounds including microorganisms (fungi, bacteria), organic amendments (waste from animals and food industry), natural substances (essential oils, plant extracts), biostimulants, nutrients and resistance inductors have been tested through a massive screening under controlled conditions by i) *in vitro* sensitivity tests or dual cultures to evaluate their effect on mycelial growth; ii) in naturally infested soil evaluating their effect on microsclerotia viability; and iii) *in planta*, by the effect on the infection of olive potted plants. The most promising compounds from this screening have been also tested under natural field conditions considering representative scenarios of the different olive-growing regions of Andalusia. Based on the results already obtained, the non-pathogenic strains of *F. oxysporum* FO12, and the grape marc compost CGR03 have resulted among the most promising candidates, reaching around 100% of the inhibition of the disease in both under controlled and in naturally infested soil conditions. More recently, new insights into the biological control of the disease have been obtained determining the action of plant biostimulants and microorganism as host resistance inductors. The strain *Aureobasidium pullulans* AP-08 produces a strong accumulation of Jasmonic acid and Jasmonic acid-isoleucine in treated olive plants, enhancing the plant immune system. Altogether, this work will be useful to select the best candidates towards the biocontrol of VWO, matching great within the frame of the European Green Deal.

Keywords: biocontrol, biostimulants, microorganisms, *Olea europaea*, organic amendments, *Verticillium dahlia.*

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Session 3 – Oral communication

Investigating decline symptoms of Super-High-Density Oil Olive in the Northern San Joaquin Valley of California

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Over 95% of the olives grown in the United States are grown in California. In 2019, California's total bearing acreage for olives was estimated at 37,500 acres producing 164,650 tons of olives with a total value reaching nearly $130 million. While oil olives are a growing industry in California, olive production continue to face major challenges such as unexpected freezes, droughts, pests and diseases. Over the last three years, we have received several calls from olive growers in the Northern San Joaquin valley of California reporting issues of olive trees with declining symptoms in – super-high-density (SHD) orchards – including branch wilt and dieback. In some cases, we suspected autumn freeze to be the major cause of decline. In other situations, we confirmed trees were affected by Verticillium wilt. Other dieback symptoms, associated with fungal pathogens, have occasionally been found. Overall, diagnosis has been challenging due to the strong overlap of Verticillium wilt symptoms with those of freeze injury. With SHD oil olive plantings have increased recently, most of the orchards were planted after solanaceous crops (i.e. tomato), and the occurrence of Verticillium wilt is a growing concern to the California oil olive industry. We conducted a survey of olive orchards with trees showing decline to get a closer look at the various symptoms. We diagnosed several Super SHD orchards affected by Verticillium wilt. The disease was observed in young (~ 6-year-old) trees of the three cultivars Arbequina, Arbosana and Koroneiki. On the other hand, during fall, winter, and spring, several of these new-planted orchards were at risk of injury caused by cold weather. Damage can occur at temperatures below 29ºF depending on the age of the tree, whether the tree has had a chance to harden, the specific temperature at ground level around the tree, and the duration of the cold snap. We usually try to differentiate between injury during the growing season, which is referred to as frost injury and the freeze damage that occurs in late fall or winter. The term frost injury is restricted to damage due to freezing temperatures during the growing season while the tree is not dormant, which is due to a late spring frost. This was not the case for the past three years – based on our farm calls and minimum air temperature data (°F) collected from weather stations located near damaged olive orchards – trees were damaged with a late fall or early winter freeze, and symptoms observed were sometimes confused with Vertcillium wilt disease. Symptoms were more pronounced in the Koroneiki cultivar, which may be due to its vigor. Lilac borer, commonly known as ash borer, was also recently found in young SHD olive orchards. The leaves of affected trees turn uniformly yellowish-green. The stem of those trees were girdled 1-2 feet from the ground resulting in wilting and dieback of twigs and branches, then trees eventually died.

Keywords: super-high-density, freeze damage, Verticillium, fungi, Lilac borer.

Session 3 – Oral communication

The status of Neofabraea leaf and shoot lesions, Pleurostoma decline and Anthracnose in super-high-density oil olive orchards in California

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California produces more than 95 percent of the olives grown in the United States. In 2019, the bearing acreage for olives was 14,700 hectares with a total of 164,650 tons of olives produced at a value of nearly $130 million. While these figures indicate a growing industry, olive oil production could be challenged by new and emerging diseases in olive orchards. During the winter of 2016, Neofabraea leaf and twig lesions was first detected in super-high-density (SHD) oil olive orchards in California. Affected trees revealed numerous leaf and shoot lesions, and cankers in branches, which developed at wounds caused by mechanical harvester. Two species, namely *Phlyctema vagabunda* and *Neofabraea kienholzii*, were found to be consistently associated with the disease and Koch's postulates were completed. The cultivar 'Arbosana' was highly susceptible to the disease, whereas cultivars 'Arbequina' and 'Koroneiki' appeared to be tolerant. Field trials indicated that several fungicides could reduce disease incidence and management strategy guidelines were implemented to limit further spread of the disease. Pleurostoma decline of olive trees caused by the fungal pathogen *Pleurostoma richardsiae* was also recently detected in SHD olive orchards. Symptoms of Pleurostoma decline in olive trees included leaf yellowing and browning, leaf drop as well as wilting and dieback of twigs and branches, as well as brown to dark discoloration of the wood, while severely affected trees died. *Pleurostoma richardsiae* was also isolated from collar rot symptoms in olive trees. Field observations suggested that infections by *P. richardsiae* initiate at wounds in trunks and branches caused by field equipment and large insect borers. Following a state-wide survey of olive orchards in California, olive anthracnose was not detected in SHD olive orchards. However, the disease was observed in an orchard of Gordal-Sevillana olives located at the Kearney Agricultural Research and Extension Center. Main symptoms in this orchard included fruit rot characterized by circular sunken lesions with acervuli forming in concentric rings. Fungal isolates obtained from the olive fruits in this orchard were identified as *Colletotrichum fioriniae* in the *C. acutatum* species complex based on DNA phylogenetic analyses. These analyses also indicated that *Colletotrichum* isolates from olive were genetically similar to those obtained from pistachio and almond, suggesting the olive anthracnose inoculum is present in California. However, the absence of olive anthracnose in commercial SHD oil olive orchards in California suggests that the disease does not thrive in olive under the environmental conditions of California's Central valley.

Keywords: Diseases, Phlyctema, Neofabraea, Pleurostoma, Anthracnose, fungicides, super-high-density.

Acknowledgements: We are thankful to the Olive Oil Commission of California for funding this research.

Session 4 – Integrated management of *Xylella fastidiosa* in olive orchards

Xylella fastidiosa - State of Play in Portugal and Measures in Place

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Xylella fastidiosa is listed as a priority quarantine pest in the European Union as it is considered to have one of the most severe economic, social and environmental potential impacts in its territory.
As result of the official survey program in place covering the whole national territory, the bacteria was confirmed for the first time in Portugal in January 2019 in the metropolitan region of Porto, being currently present within an area of around 36 thousand hectares, where more than fifty different species were detected infected with *Xylella fastidiosa* subsp. *multiplex* ST7, including spontaneous, ornamental, forest and agricultural species. More recently, in July 2021, two additional outbreaks were detected, one in the metropolitan region of Lisbon and another in Algarve, where an intensive monitoring is taking place with no further detections up till now.
Since the first detection of the bacteria in Portugal, an action plan was set up aiming eradication in the areas where it was detected that includes an intensive survey, sampling and testing, destruction of the positive plants and the neighboring ones, control of the vectors and strict measures concerning the movement of host plants. For the rest of the country, a contingency plan is in place, being awareness campaigns and comprehensive monitoring a major part of it.
Further research on latency, diagnostic, epidemiology, resistant or tolerant varieties are crucial contributions for successful eradication measures.

Behaviour response of *Philaenus spumarius*, the main vector of *Xylella fastidiosa*, to traditional Portuguese olive cultivars

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In Portugal, *Xylella fastidosa*, a gram-negative phytopathogenic bacteria, has been detected in 2019 in the North of the country in urban areas, raising concerns with the possibility of this bacterium spread up to crops of economic importance such as the olive grove. This bacterium is transmitted by the xylem sap feeder *Philaenus spumarius* (Linnaeus, 1758). Understanding the olfactory response of *P. spumarius* to traditional olive cultivars of Portugal is essential to determining the most attractive olive cultivar to this vector. This could help to implement proper prevention and control measures against the spread of *X. fastidiosa* throughout Portuguese olive groves. The goal of this work was to evaluate the olfactory response of females and males of *P. spumarius* to five traditional Portuguese olive cultivars (Cvs. "Cobrançosa", "Negrinha de Freixo", "Santulhana", "Madural" and "Verdeal Transmontana"). The olfactory response to the cultivars was assessed by two trials on an eight-chamber olfactometer. In the first trial, the behaviour of 16 individuals towards the cultivars "Verdeal Transmontana", "Cobrançosa" and "Madural" was recorded. The second assay was used to assess the behavior of 16 individuals towards the most chosen cultivar in the first trial ("Cobrançosa") and the cultivars "Negrinha de Feixo" and "Santulhada". The insects were allowed to make their choice during 30 minutes for each trial. For each trial and sex, 20 repetitions were made. A stream of purified air was used as control. In the first trial, females and males were significantly more attracted by "Cobrançosa" ($P<0.001$). "Negrinha de Feixo" was the cultivar significantly most chosen in the second trial ($P<0.001$). No significant differences were observed between males and females ($P=0.38$). On average, two out of 16 individuals did not make any choice. Our results suggest that the "Negrinha de Feixo" could be the most attractive cultivar to the main vector of *X. fastidiosa*. However, assays of feeding preference and studies of presence and abundance of *P. spumarius* in olive groves must be conducted to confirm our results.

Keywords: olive groves, emerging plant diseases, "Negrinha de Feixo", "Cobrançosa", eight-chamber olfactometer.

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Effect of temperature and host plant species on the juvenile development of *Euscelis ohausi* (Hemiptera: Cicadellidae), a potential insect-vector of plant pathogens

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Euscelis (Hemiptera: Cicadellidae) is an economically important genus of the Deltocephalinae subfamily containing vectors of agricultural plant pathogens. In Greece, *Euscelis* species are widely distributed and abundant; particularly on the island of Crete, *Euscelis ohausi* has been recorded in high populations on the herbaceous cover in olive groves. To understand the effects of temperature and host plant species on biological parameters of *E. ohausi*, juvenile development and survival were studied at 15, 20, 25, 30 and 33 ºC on oat and at 25 ºC on vetch plants. Temperature affected both the developmental time and survival of *E. ohausi*. The shortest egg incubation period was recorded at 30 ºC and the longest at 15 ºC, whereas the highest percentage of nymphal hatchability was recorded at 25 ºC and the lowest at 30 ºC. The shortest developmental period was recorded at 30 ºC and the longest at 15 ºC. The lowest nymphal survival rate was 25 % at 33 ºC, while in the other temperatures it was above 60 %. Shorter nymphal developmental time was recorded on oat compared to vetch plants, however no differences were recorded in survival percentages at 25 º C. According to the linear model, the lowest temperature developmental threshold from egg to adult was 7 ºC. The assessment of the factors impacting juvenile development and survival is important for understanding the biology and phenology of *E. ohausi*.

Keywords: Auchenorrhyncha, Hemiptera, insect-vector, *Euscelis ohausi*, temperature, host-plant.

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Session 4 – Oral communication

Bacteriome of *Philaenus spumarius* genitalia and its implication on insect's host reproduction

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Philaenus spumarius has been increasingly studied in the last years as a result of being one of the principal vectors of *Xylella fastidiosa* in the European Union. *X. fastidiosa* is a phytopathogenic bacterium that infects several economically important crops, and for which there is no cure. One possible option to contain the spread of *X. fastidiosa* is through the management of its vector, *P. spumarius*. The natural microbiota population of an insect has important roles in the insect life, such as in immunity, feeding and reproduction. Thus, the characterization and exploitation of the insect-microbial associations can contribute to the control of disease vectors. Here, the bacterial community associated to the genitalia of *P. spumarius* was evaluated using both culture-dependent and –independent (metagenomic) approaches. The ability of the cultivable bacterial in altering the reproductive potential of their hosts, was additionally evaluated by using an insect model, the *Drosophila melanogaster*. Overall, 245 bacterial operational taxonomic units (OTUs) were identified, being 174 found in females and 244 in males. *Cutibacterium* was the most predominant genera found in the genitalia of both males and females. A high number of genera were found to be restricted to either male (28) or female (37), being only around 48% of genera shared among the two genders. A total of 5 cultivable bacterial were screened for their impact on *D. melanogaster* fitness, along three generations. Most of the tested bacterial reveal to significantly influenced the number of dead larvae, number of pupae, number of females, and size of the body and wings of both male and female, but with variable effects. Some bacteria were able to reduce the size of the body and wings of males; while others showed to promote the larvae dead, pupae and female number, and to increase the size of the body and wings of both males and females. Implications of these findings for *P. spumarius* management will be discussed.

Keywords: *Xylella fastidiosa*, insect fitness, bacterial community, biological control, *D. melanogaster*

Acknowledgements: This work was supported by the EU H2020 Research Project XF-ACTORS "*Xylella fastidiosa* Active Containment Through a multidisciplinary-Oriented Research Strategy" (Grant Agreement 727987) and by the Foundation for Science and Technology (FCT, Portugal) for funding CIMO (UID/AGR/00690/2020). Cristina Cameirão also acknowledges the PhD research grant (SFRH/BD/148586/2019) provided by FCT.

Session 4 – Oral communication

Development of a hybridization-based capture NGS assay to assess genome-wide diversity in *Xylella fastidiosa* infected samples

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Xylella fastidiosa (*Xf*) early detection and its assignment to subspecies and sequence type (ST) level is critical for its management since can help to determine the potential range of host plants of *Xf* populations present in epidemic foci and to take appropriate measures to its eradication, containment and monitoring. Currently, *Xf*-typing at the subspecies and ST level is based on the use of MultiLocus sequence typing analysis (MLST). However, the *Xf*-detection could fail or may be inconsistent due to interference of the DNA amplification with PCR inhibitors leading to not clear results or false negatives. In addition, genetic resolution at ST-level based on seven housekeeping genes does not provide sufficient phylogenetic resolution to determine dispersal paths or relationships among strains that are of biological and quarantine relevance. Consequently, the use of whole-genome sequence (WGS) data should probably be used specially when developing management strategies for *Xf* outbreaks. Unfortunately, when quickness is a need after detection of an outbreak it is not always possible to isolate the *Xf* strain to obtain its genome sequence, or the data obtained by direct NGS analysis of infected samples do not contain enough *Xf* reads to adequately identify the strain intercepted. In this study, we developed a *Xf*-Targeted Sequence Capture Enrichment (TSCE) in combination with High-Throughput Sequencing (HTS) procedure using an Illumina platform to provide efficient access to *Xf* reads to identify *Xf* at strain level. More than 7,000 baits targeting 140 *Xf* gene sequences present in the *Xf* chromosome or plasmids were selected to cover genomic markers of all subspecies and STs described to date. We showed that whereas < 0.25% of *Xf* reads were detected by direct WGS of host DNA this number increased up to 41-73% after using the TSCE-HTS approach in individual samples or in mixtures of up to four multiplexed plant samples. We were able to identify all seven loci commonly used for *Xf* MLST and correctly identify the subspecies and ST of all *Xf* strains present in mock-inoculate and naturally infected plant and insect samples. After assembly of captured reads we were able to identify up to 284 *Xf* coding sequences (CDS), indicating that more CDS than expected were captured. Furthermore, phylogenetic analysis of 90 captured and aligned genes correctly positioned the infected samples with the reference *Xf* strains known to be infecting the samples.

Keywords: olive vascular diseases, quarantine plant pathogens, *Xylella fastidiosa*.

Acknowledgements: This work was funded by Project E-RTA2017-00004-C06-02 (AEI-INIA Spain and FEDER), the Spanish Olive Oil Interprofessional and the Intramural Project 201840E111 from CSIC.

Plant endotherapy treatments enable the modification of xylem microbiome composition in olive trees

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Xylem-inhabiting plant pathogenic organisms, such as *Verticillium dahliae* and *Xylella fastidiosa*, compromise and seriously threat the health of olive crop worldwide by collapsing the xylem vessels and ultimately causing the death of the olive tree. Nowadays, the use of host resistance is the most practical and efficient control measure for vascular diseases in olive. However, research on endophytic microorganisms is gaining a special interest as a potential tool to confer protection against infection by xylem-inhabiting pathogens or suppress disease development. This work pursues i) the modification of xylem microbiome composition of cultivated olive plantlets ('Picual' and 'Arbequina') by transplanting an external xylem microbiome obtained from wild ('Acebuche') and cultivated ('Picual' and 'Arbequina') adult olive trees through endotherapy treatment, and ii) the evaluation of the changes in the xylem microbial composition of challenged plants over time. A total of 1,015 ASVs (grouped in 361 bacterial genera) were identified for all treatments. Globally, *Massilia* (12.91%) was the most abundant genus followed by *Hymenobacter* (9.62%), *Sphingomonas* (9.32%), *Curtobacterium* (9.05%) and *Methylobacterium* (6.09%). Furthermore, alpha diversity measure showed an increase of observed ASVs after endotherapy treatment from 'Picual' and 'Acebuche' xylem sap to 'Arbequina' olive plantlets whereas the endotherapy inoculation of 'Arbequina' and 'Acebuche' xylematic fluid to 'Picual' showed the opposite behaviour. Additionally, beta diversity using ASV frequencies showed some trends across the different sampling times in each olive genotype treated. These results indicated that consortia of xylem microorganisms can be artificially inoculated in xylem vessels of seedling plants modifying their native xylem microbiome with the aim to control diseases caused by xylem-inhabiting pathogens or modify olive plant physiology and growth.

Keywords: olive, xylem, microbiome, endotherapy.

Acknowledgements: study supported by Projects XF-ACTORS 727987 (EU-H2020) and AGL2016-75606-R (MICINN Spain and FEDER-EU).

Session 4 – Oral communication

Involvement of specific traits of olive beneficial rhizobacteria to protect against biotic and abiotic stresses

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Verticillium wilt of olive (VWO), caused by the fungus *Verticillium dahliae* Kleb., together with drought and salinity are considered serious (a)biotic constraints affecting olive cultivation worldwide. Different microorganisms have been identified as effective biocontrol agents (BCA) against *V. dahliae* and/or as useful tools to alleviate these stresses. A collection of olive beneficial rhizobacteria was screened, including the effective BCA against VWO *Pseudomonas simiae* PICF7 and *Pseudomonas* sp. PICF6, to assess them as protectants towards abiotic stresses. In this study, we pursued a double goal: i) on the one hand, to evaluate the potential involvement of selected phenotypes of strain PICF7 in root colonization and VWO biocontrol, and ii) on the other hand, to assess whether olive beneficial rhizobacteria can be used as protective agents to alleviate drought and salt stresses. For the latter objective we aimed to identify 1-aminocyclopropane-1-carboxylic acid deaminase (ACD) producers and tested the hypothesis that ACD producers lessen the effects of these abiotic stresses. A random transposon-insertion mutant bank of PICF7 was screened for the loss of phenotypes likely involved in rhizosphere/soil persistence (copper resistance), root colonization (biofilm formation) and plant growth promotion (phytase activity). Transposon insertions in genes putatively coding for the transcriptional regulator *CusR* or the chemotaxis protein *CheV* were found to affect copper resistance, whereas an insertion in the *fleQ* gene putatively encoding a flagellar regulatory protein hampered the ability to form a biofilm. However, these mutants displayed the same antagonistic effect against *V. dahliae* as the parental strain. Endophytic colonization of olive roots was only altered in mutants impaired in biofilm formation since they were never found inside olive roots. This rhizobacteria collection was *in vitro* screened for ACD activity. Strain PICF6 displayed this phenotype while PICF7 was defective, even though an ACD-coding gene was earlier predicted in its genome. An unidentified deaminase was confirmed instead. Greenhouse experiments with olive plants inoculated either with PICF6 or PICF7, or co-inoculated with both strains, and subjected to drought or salt stress were carried out. Several physiological and biochemical parameters increased in stressed plants (i.e., stomatal conductance and flavonoids content), irrespective they were previously bacterized. Finally, results demonstrated that the PICF7 phenotypes studied were irrelevant for VWO biocontrol and that neither PICF6 (ACD+) nor PICF7 (ACD-) lessened effects caused by these abiotic stresses, under our experimental conditions.

Keywords: 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase (ACD); biocontrol; biofilm; chlorophyll; copper tolerance; endophyte; flavonoid; integrated disease management; *Olea europaea* L.; phytase activity; proline; *Pseudomonas* sp. PICF6; *Pseudomonas simiae* PICF7; stem water potential; stomatal conductance; Verticillium wilt of olive.

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Posters

Poster nº 1

Population genetics and bacterial endosymbionts of Aphrophoridae putative vectors of *Xylella fastidiosa* and other Auchenorrhyncha species in Greece

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Xylella fastidiosa is a Gram-negative bacterium which is responsible for many diseases in economically important crops such as olives. Its vectors are xylem fluid-feeding insects of the suborder Auchenorrhyncha (Order: Hemiptera). *Philaenus spumarius* is the main vector of the bacterium in Europe, responsible for the spread of *X. fastidiosa* in Apulia region of South-East Italy causing the olive quick decline syndrome in olive orchards. The knowledge of the distribution and the genetic structure of the potential insect vectors of *X. fastidiosa* or other pathogens is important in preventing such epidemics. Here, we investigated the genetic diversity and the infection status by five secondary symbionts of three Aphrophoridae (*P. spumarius*, *Neophilaenus campestris*, *P. signatus*) putative vectors of *Xylella* collected from olive orchards in 19 regions of Greece. Additionally, the polymorphism of two Deltocephalinae species (*Euscelidius variegatus* and *Euscelis lineolatus*) collected from ground vegetation of the olive orchards was also investigated. The genetic polymorphism of the sampled populations was determined based on the mitochondrial (mt) c*ytochrome c oxidase subunit I* (*COI*) and *cytochrome b* (*cytb*) genes. In total, 238 *COI* and *cytb* sequences obtained in the present study were analyzed together with 91 additional from GenBank. Analysis of the mtDNA sequences revealed high polymorphism Greek *P. spumarius*, (26 haplotypes for *COI* and 52 for *cytb)* and *N. campestris* (13 and 7 haplotypes for *COI* and *cytb,* respectively). In addition, the presence of secondary symbionts known for their interference in shaping the genetic structure of insect species was determined by PCR using species-specific primers for *Hamiltonella*, *Rickettsia*, *Arsenophonus*, *Cardinium* and *Wolbachia.* The infection statuses ranged from 0 to 100% depending on the sampled species and the secondary symbiont detected. Every species tested, harbored at least one secondary endosymbiont with *Arsenophonus* being found as the most prevalent one (overall 9.2% infection frequency). In *P. spumarius*, only *Hamiltonella*, *Rickettsia* and *Wolbachia* were present in equally low frequencies (4%). Regarding *N. campestris, Arsenophonus* had the highest frequency (35%) whereas *Cardinium* was not found in any individual of *N. campestris*. Moreover, to determine the overall bacterial composition of *P. spumarius*, we obtained Next Generation Sequencing

data of 16S metagenome by examining different parts of the insect body. The comparative analysis of populations of *P. spumarius* from several regions in Greece revealed constant differences in bacterial composition between the head and the rest of the insect body.

Key words: genetic diversity, endosymbionts, *Aphrophoridae, Deltocephalinae,* metagenome

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Poster nº 2

Diversity and population dynamics of *Xylella fastidiosa* potential vectors in olive groves with different management systems

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Diversity and population dynamics of *Xylella fastidiosa* Wells et al. potential vectors species and other Auchenorrhyncha were studied in olive orchards under three management systems: organic, conventional and abandoned. Auchenorrhyncha were monitored monthly in 6 olive groves at Istiea (Central Greece) and Chania (South Greece) for 13 months using Malaise traps and sweeping-net. Overall, *Philaenus spumarius* was the most abundant *X. fastidiosa* potential vector followed by *Neophilaenus campestris*, *Cercopis sanguinolenta*, *Lepyronia coleoptrata* and *Neophilaenus lineatus*. The abundance and diversity in *X. fastidiosa* potential vectors as well as in total Auchenorrhyncha were lower in olive orchards in Chania. The abandoned olive orchard hosted the highest population of potential vectors in Chania, but the lowest in Istiea, compared with the respective cultivated one. In both regions, the organic orchards displayed higher diversity in Auchenorrhyncha compared to the other management systems. Cultural techniques such as weed control provided the most probable explanation for the significant Auchenorrhyncha population fluctuation among olive orchards. However, insecticide applications against olive fruit fly did not affect Auchenorrhyncha abundance. The conventional orchard in Istiea displayed the highest abundance and species richness of the potential vectors of *X. fastidiosa*, mainly during autumn, when dried weeds remained in the orchard after herbicide application.

Keywords: Auchenorrhyncha, potential vectors, spittlebugs, Aphrophoridae, conventional, organic, abandoned.

Poster nº 3

Traditional and intensive olive growing have differences in the approaches to defense against the most common pests: the results of the DI. OL. Project.

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The project "DI.OL." - Defense from harmful organisms in conventional and intensive olive crops*"* had as primary objective, the study of a protection system of olive crops addressed to the olive growers to obtain a healthy and high-quality product both in traditional and in intensive olive growing. During the three-year period, the group involved in the work package aimed to define control strategies and containment of *Bactrocera oleae* in traditional olive groves and in high-density systems, carried out research on immobilization of bioactive substances with repellent effect against olive fly individuals: the active ingredients were inserted within nanostructured oxidic solids from mineral origin with controlled-release properties. A set of tailored hybrid materials was developed. They contain inorganic and/or organic species: copper (II) in limited quantities (up to 80% lower than the main commercial products) and aliphatic linear aldehydes, such as the ones contained in drupes or leaf surface or derived from the transformation processes of the product. Innovative diffusers and VOCs with attractive or repellent action towards the adults of the olive fly were tested: these substances can improve the attraction efficiency of current tools or, conversely, can interfere with the mechanisms regulating various behavioural aspects of the insect, such as feeding, mating and oviposition. Regarding conventional control strategy, the results obtained from the potential of the synergistic treatment, clay powders with repellent action and of the natural attractant (spinosad), gave promising results. Survey on other arthropods and nematodes were performed to verify the impact of high-density olive orchards on them. The results showed a greater density of *Saissetia oleae* in traditionally cultivated monitored olive trees, lower in the intensive and with minimal values in the super-intensive. Conversely, *Otiorhynchus cribricollis* infestation was often higher in the high-density system than in the traditional one. *Palpita vitrealis* showed discordant results and the infestation rate was very high in the high-density system in only one of the monitored sites. Although *Prays oleae* has shown higher catches in the traditional management system, plant infestation has always been low in the monitoring period and with no difference between the two managements were registered. As regard nematodes, the ratio of obligate plant-parasites

to bacterivores and fungivores were significantly higher in high-density than traditional olive orchard system in two sites located in Southern Italy and usually tillage. Finally, a survey aimed to verify the presence of xylophagous and phytophagic potential disease vectors, gave negative results in both situations.

Keywords: traditional olive grove, high-density olive grove, management.

Poster nº 4

The Impact of super-high density olive orchard management system on soil plant-parasitic nematodes in Central and South Italy

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Super-high density (SHD) olive orchards are spreading in Italy due to reduced production costs and increase yields per hectare. Although plant-parasitic nematodes in the olive orchards are usually very low, more specific studies on the impact of SHD on them are required. The aim of the present study was to assess the effect of SHD olive orchards on soil plant-parasitic nematode community in five sites located in the main areas suitable for the cultivation of the olive orchards (Apulia, Tuscany, and Sicily) with different soils, climate, and consequently various cultivars. In each site, the SHD management system was compared to the adjacent olive orchard traditional system, in which the same soil management was applied. Samplings were carried out in 2018 and 2019, in spring and fall, in three different marked rows of both management systems at 0-30 cm depth. Nematodes were extracted by cotton-wood filter method and identified to genus or family level. Soil nematode community was evaluated using the nematode taxa abundances and the ratio of obligate plant-parasites to bacterivores and fungivores [Pp/(B+F)].
Although the free-living nematodes (bacterial and fungal feeders and their predators) were prominent in each site, the high ratio plants/hectare, the different cultivars than traditional, and a higher irrigation requirement favoured the families of Telotylenchidae, Paratylenchidae, Meloidogynidae, and Criconematidae than Longidoridae, Heteroderidae, and Pratylenchidae. The ratio Pp/(B+F) was significantly higher in SHD than in the traditional olive orchard system in two sites located in Southern Italy and usually subjected to conventional tillage. This showed that the negative effects were mainly evident in environments already stressed by summer droughts and by conventional soil management. Although further studies are necessary, this investigation showed that the SHD olive orchard management system could change the soil plant-parasitic nematode community associated to olive orchards. However, using a conservative and sustainable soil management might maintain or improve the soil nematode community functionality and prevent the plant-parasitic nematode increase.

Keywords: free-living nematodes, plant-parasitic nematodes, soil management.

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Poster nº 5

Emergence of *L. rigidum* with cover crops – contribution for IWM

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Lolium rigidum Gaudin (anual ryegrass) is a major weed of olive groves in mediterranean countries but also commonly occurs as a weed of cereal crops. Its seeds have little dormancy and short term viability. Knowledge of the germination and emergence patterns of this species can play a major role in reducing soil seed bank. This study presents the results of a common experiment of emergence of several *L. rigidum* populations from the point of view of weed management. 200 seeds m^{-2} of each *L. rigidum* population were seeded in 0,5x0,5m plots in October 2020 with four repetitions. Periodic weed samplig was carried out from November until April. In three sites, from Spain (Andaluzia and Navarra) and Portugal (Ribatejo) the emergence of a common population was followed either alone or with two cover crops, *Hordeum vulgare* (300 seeds m^{-2}) and *Sinapis alba* (160 seeds m^{-2}). The emergence of *L. rigidum* was similar among the three regions but the effect of cover cropping was diferente among regions. Integrating a number of practices directed to various stages of the lifecycle is key to dealing with weeds such as *L. rigidum*. Cover cropping could be a cultural tool for Integrated Weed Management of *L. rigidum* particularly in olivegroves with herbicide-resistant populations.

Keywords: anual ryegrass, olive grove, barley, white mustard.

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Poster nº 6

Weed diversity in olivegroves – shelther for insect vectors of Xylella fastidiosa

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Xylella fastidiosa subsp. multiplex ST7 was firstly detected in Portugal in 2019, and until now about 50 plant species were identified as being infected. It is a very serious threat to agriculture and forestry, with great production losses in olive, vine, almond, *Quercus suber* and other crops. In all Europe, more than 600 plant hosts are potential reservoirs supporting the spread of this bacterium through insect vector feeding process. Some insects (Auchenorrhyncha: Cicadomorpha) that feed on the plants' xylem fluid are known vectors of this pathogen. They are polyphagous, frequently found on weeds and bushes undercanopy cover. It is of great importance to know which are the weed hosts of these insects. Some of them, the Cercopoidea spittlebugs, while in the nymph stage, produce a characteristic foam as the result of the feeding activity. In the demarcated area, in northern Portugal, *Philaenus spumarius* individuals were already detected as being infected with the same strain that infected the plant species, confirming the natural dissemination of these bacteria. In the course of the project *FCT856 – XF-Freeolive*, that was implemented from 2015 to 2019, weeds with foams were monitored in olive groves and other ecosystems frequently found next to them. In the laboratory, the spittlebug nymphs present inside the foam were separated from the foam. Weeds were identified through morphological characteristics and the nymphs by biomolecular methods. At the same time, adult insect vectors were also captured in three olive groves, in the north (Mirandela region), center (Abrantes region) and south of Portugal (Serpa region), by using yellow sticky traps and sweep net applied to the trees and to the weeds. They were identified by their morphological characteristics. *Philaenus spumarius* and *Neophilaenus campestris* were detected. All the samples collected were screened for *Xylella fastidiosa,* by qPCR (Harper protocol). The botanic families where the presence of spittlebug foam was most frequent were: Asteraceae (3 species); Boraginaceae (3 species); Fabaceae (2 species); Poaceae (2 species); and other families (3 species). All the samples (plants, nymphs and adults) tested negative for Xf in the regions in study. The biomolecular identification of nymphs using several molecular markers showed that they were *Philaenus* sp..

Keywords: *Olea europaea*, natural vegetation, host, xylem, bacterial disease, spittlebugs, nymphs.

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Poster nº 7

Remote Sensing: Assessing the differences between NDVI obtained in spring and autumn, in a mountain olive grove

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By providing a set of tools that allow the acquisition and processing of spatial data, Geographic Information Technologies (GIT) facilitate monitoring and management of olive parcels and enable precision olive growing, even in traditional mountain olive groves. The use of unmanned aerial vehicles (UAV) with multispectral cameras, for acquisition the geospatial data of terrain surface and the feature on its surface, allows monitoring the crop phenology evolution, as well as the differences between various phenological phases. In 2018 campaign, we used UAV, multispectral cameras, and photogrammetry software for drone mapping -Pix4D in a traditional mountain olive grove, whit 22.5 ha in Figueira de Castelo Rodrigo, during the project "Promoção e Valorização de Azeites de Montanha" (CENTRO-01-0246-FEDER-000004 / 6756). The data were processed with a set of tools for processing spatial data in ArcGIS software, which allowed analyze the differences between the Normalized Difference Vegetation Index (NDVI) obtained in two distinct phenological phases: at flowering (spring) and at harvest (autumn). The results showed us significant interzonal differences and between seasons, (spring: -0.49 ¬ 0,74 and autumn: -0.08 – 0.96). That allowed us to identify less vigorous and problematic areas, which need greater attention and investment by the farmer and the practice of other techniques, such as, for example, soil analysis and the corresponding fertilization advice. By focusing the investment only on areas identified as being weakened or less productive, this will allow the farmer using treatments that are more effective and reduce production costs.

Keywords: NDVI, olive grove, remote sensing.

Acknowledgements: Data obtained through the Promotion and Valorization of Mountain Olive Oils project (CENTRO-01-0246-FEDER-000004 / 6756).

Poster nº 8

Looking for insect vectors of *Xylella fastidiosa*: finding them and other insects. A survey in Portuguese olive groves

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Xylella fastidiosa is a phytopathogenic bacterium transmitted by insects that inhabits the plants' xylem. Insects that feed on the xylem fluid are candidates to transmit it to other plants during their feeding activity. Such insects are found in some families within Auchenorrhyncha (Hemiptera) and are often polyphagous. Insects polyphagy may increase the risk of *X. fastidiosa* spreading. This pathogen causes diseases in several plants, some of which are strategic crops to the Portuguese economy, as the olive trees. A survey of the Auchenorrhyncha community was conducted in three Portuguese olive groves (in Mirandela region, Abrantes region and Serpa region), from 2017 to 2019. The objective was to contribute to fill the knowledge gap on the *X. fastidiosa* vectors in Portugal. We found two species of known competent vectors: *Philaenus spumarius* and *Neophilaenus campestris*. A list of the identified species is presented and considerations about their bioecology and the used monitoring methods are made.

Keywords: *Olea europaea*, *Philaenus*, *Neophilaenus*, Cicadomorpha, Fulgoromorpha.

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Poster nº 9

A tritrophic interaction model for an olive tree pest, the olive moth – *Prays oleae* (Bernard)

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The olive tree (*Olea europaea* L.) is among the oldest and most widespread crops in the Mediterranean basin. Portugal is the third olive producer in the European Union, and Trás-os-Montes region, located in the northeastern Portugal, is the second Portuguese producing olive region.

The olive moth, *Prays oleae* (Bernard) (Lepidoptera: Praydidae) is a key olive pest in Trás-os-Montes. This pest, is a natural host/prey of several organisms which include larvae of generalist and specialist parasitoids as well as generalist predators and entomopathogens. Its most abundant parasitoid is the specialist *Ageniaspis fuscicollis* (Dalman) (Hymenoptera: Encyrtidae) and this, in Trás-os-Montes region, is commonly followed by the facultative hyperparasitoid *Elasmus flabellatus* (Fonscolombe) (Hymenoptera: Eulophidae). Spiders represent a relevant group of generalist predators in olive agroecosystems and encompass an important predatory action in agroecosystems as well as an ability to reduce the populations of various insect pests. In this context, a mathematical model, considering the population of the olive moth, the two parasitoids populations and the spider population as the variables in our system, was constructed. The ecosystem steady states for feasibility and stability were assessed. The possible pesticide effects, that represent essentially extra mortality rates for each one of the insect populations, and potential abundance variations on their populations under a climate change scenario were included. Results indicate that the most important natural control agent is *A. fuscicollis*, but in certain conditions *E. flabellatus* or spiders may be relevant contributors for the pest reduction. This approach may provide a useful tool to assist the field researchers on this pest system and its management.

Keywords: Biological control, pest control, parasitoids, predators, modelling.

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Poster nº 10

New insights about *Ageniaspis fuscicolis* var. *praysincola*, specific parasitoid of the olive moth, a key-pest of the olive tree

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The olive moth, *Prays oleae* (Bern.) (Lepidoptera: Praydidae), one important pest of the olive tree, is naturally attacked by a large complex of parasitoids and generally the specific parasitoid *Ageniaspis fuscicolis* var. *praysincola* is the most abundant. Despite its importance, relevant aspects such as its feeding requirements (which are responsible for survival and reproduction traits), movement parameters (related with host encountering or dispersion), or cultivar preferences (related in some cases with processes of host searching) are understudied. Thus, in this work the goal was to provide with a baseline knowledge about *A. fuscicollis* var. *praysincola* traits. For that three approaches were followed: (i) adult parasitoids were fed with insect honeydews and artificial nectar composed by different concentrations of sugary solutions in order to analyze its feeding requirements; (ii) several parameters of movement -distance, velocity or angular velocity- were recorded using Noldus Ethovision XT 11.5 software; (iii), the varietal preference over healthy olive trees (Cobraçosa, Verdeal, Madural) was evaluated using a four arm olfactometer. As a result, beneficial sugars and honeydews for the parasitoid survival were identified, several movement parameters were calculated and varietal preference was determined, stablishing the basic knowledge for further research about *A. fuscicollis*.

Keywords olive crop, pest control, natural enemy, survival, movement, varietal preference

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Poster nº 11

Ground cover vegetation composition predicts the abundance of *Sphaerophoria scripta* (Linnaeus, 1758) (Diptera: Syrphidae) in olive groves from Trás-os-Montes region (Portugal)

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The spontaneous ground cover vegetation provides shelter and vital floral resources for the Hoverflies (Diptera: Syrphidae). Such resources can contribute to the growth, development, reproduction, and survival of syrphids, allowing them to maximize their function as natural pest enemies, pollinators, and decomposers of organic matter. So, identifying the plant families driving the abundance of *Sphaerophoria scripta* (Linnaeus, 1758), one of the main syrphids species present in the olive groves in the Mediterranean region, is essential to promote its presence and abundance. Here, we investigate how the percentage of plants in the flowering stage presents in the ground cover vegetation within two olive groves, under integrated production management located in Mirandela (Portugal), shapes the abundance of *S. scripta*. For that in 2018, weekly, from April to August, in both olive groves, ground cover vegetation was identified. Thirty sample units randomly distributed over a transect covering 1 ha were selected per sampling date and sampling site. In each sample unit was recorded the vegetative stage of the plants and the percentage of ground cover by each plant family. On the same dates, syrphids were also collected using an entomological net, nevertheless, only *S. scripta* was considered. The effect of the percentage of the plant families in the flowering stage in the abundance of *S. scripta* was studied through a Generalized Linear Model. The results showed that the abundance of the *S. scripta* is positively associated with the proportion of plants in the flowering stage of the families Asteraceae and Orobanchaceae and negatively with the plants of the families Boraginaceae and Polygonaceae. According to the developed model, the families Poaceae, Euphorbiaceae and Plantaginaceae did not influence the abundance of *S. scripta* in the olive grove. Our results suggest that the increase of the percentage of certain plant families and the decrease of others can promote the abundance of *S. scripta* in the olive groves in Trás-os-Montes.

Keywords: syrphids; pollinators; Asteraceae; Orobanchaceae.

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Poster nº 12

Biological treatments enhancing the plant immune system of olive (*Olea europaea*) against *Verticillium dahliae*

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The use of biostimulants and biological control agents enhancing the plant immune system results in a potential alternative strategy against Verticillium Wilt of Olive (*Olea europaea*; VWO). To this end, we evaluated a copper phosphite (CoPh), and two microorganisms (*Bacillus amyloliquefaciens* PAB-024 and *Aureobasidium pullulans* AP-08) for their effectiveness as resistance host inducers to 6-month-old olive plants ('Picual') against VWO. Foliar or irrigation applications were conducted by spraying 15 ml per plant or by irrigation with 350 ml per plant of the dilutions of each product (CoPh: 3 or 10 ml/l^{-1}, respectively; PAB-024: 10^8 UFC ml^{-1}; AP08: 10^6 conidia ml^{-1}). Treatments were conducted weekly from two weeks before inoculation to 10 days after inoculation moment. Plants were inoculated by means a cornmeal-water-sand mixture (1:2:9; w:w:v) colonized by *Verticillium dahliae*. Additionally, treated and non-inoculated, non-treated and inoculated, and non-treated and non-inoculated plants were included for comparative purposes. Disease severity progress and shoot fresh weight were assessed. Parameters involved in plant resistance were monitored through determination and quantification of reactive oxygen species (ROS) response (H_2O_2), and evaluation of hormones by gene expression analysis. *A. pullulans* and CoPh were the most effective in disease reduction *in planta* by foliar or irrigation application, respectively. Plants treated with CoPh by irrigation showed significantly highest shoot fresh weight in comparison with that from other treatments. Non-effect on ROS response was observed for any treatments. Concerning hormones evaluation, our results showed that there is a presence of Salicylic acid on leaves; however, none of the observed enhancements was significantly different than the observed in the positive control. Regarding to the gene expression related to salicylic acid, only the WRKY gene has shown a strong enhancement in the treatment with *B. amyloliquefaciens*. On the other hand, strong accumulation of Jasmonic acid and Jasmonic Acid-isoleucine in plants treated with *A. pullulans* was observed in all the tissues analyzed as well as in roots of plants treated with *B. amyloliquefaciens* and CoPh.

Keywords: biological control agents, biostimulants, Induced Resistance, Priming, Verticillium Wilt.

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Poster nº 13

Selection of endophytes as antagonists of *Colletotrichum acutatum* and elucidation of their mode of action

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Olive anthracnose, caused by several fungi of the genus *Colletotrichum*, is one of the main diseases affecting the olive tree worldwide. There is currently no effective treatment against it. A strategy for control this disease could rely on the use of naturally occurring endophytic antagonists, such as bacteria and fungi, as biocontrol agents. Thus, in this work, six endophytic bacteria and fungi previously isolated from olives of cultivar Cobrançosa (considered to be tolerant to anthracnose), were screened for their antagonistic activity against the causal agent *C. acutatum*, by using both dual-culture and bioassays with detached olive leaves. The involvement of lytic enzymes on the antagonistic activity displayed by the screened endophytes was also investigated. Among the six endophytes tested in dual-culture, three were able to reduce significantly the growth of *C. acutatum* on more than 10% when compared to single culture of *C. acutatum*, being the greatest inhibition displayed by *Microbacterium* sp. and *Bacillus* sp.. The qualitative detection of cellulase, protease, lacase and lipase produced by endophytes during the interaction with *C. acutatum*, suggested their involvement in the mechanism of antagonism. In detached leaves, the reduction of both incidence and severity of anthracnose ranged between 25% and 38% for the three most efficient endophytes *Bacillus cf. pumillus*, *Microbacterium* sp. and *Pseudomonas cf. cedrina*. These three endophytes are potential biological control agents of *C. acutatum*, whose effect in preventing pathogen infection in olive tree should be tested.

Keywords: Biocontrol, olive anthracnose, bacteria, fungi, enzymes.

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Poster nº 14

Behavioral responses of female *Bactrocera oleae* flies to honeydew

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Sugar and protein-rich natural products have long been used as baits for tephritid fruit flies. Honeydew, a sugar-rich secretion produced by Sternorrhyncha may serve as an alternative source of food-based lure for adult tephritids. In the present study, bioassays were conducted to determine the behavioral responses of female olive fruit fly to honeydew excreted by *Saissetia oleae* (Olivier) (Hemiptera: Coccidae), *Euphyllura* sp. (Hemiptera: Psyllidae), *Aleurothrixus floccosus* (Mask.) (Hemiptera: Aleyrodidae) and *Icerya purchasi* Maskell (Hemiptera: Monophlebidae) as well as to two commercially available attractants of hydrolyzed protein Dacus bait 100 SL and Entomela 50 SL. The behavioral responses of a female were continually observed for 24h after its introduction in a cage, in no-choice or two-choice laboratory tests. A digital camera system was used for long-term uninterrupted time-lapse photography and filming began immediately after the female was inserted into the cage. The behavioral responses recorded were: i) the initial choice/selection of the female (honeydew or the hydrolyzed protein); ii) the time required for first selection iii) the duration of feeding on each food resource and iv) the number of feeding events per food resource. The results indicated that female flies were more attracted and fed more frequently and for longer period on the honeydew excreted by *A. floccosus*, *I. purchasi*, *S. oleae,* or *Euphyllura* sp. than to hydrolyzed proteins Dacus bait and Entomela. Therefore, the tested honeydews showed promising results in the attraction of olive fly females and thus, their potential to be used as sources of new attractants should be further investigated.

Keywords: olive fruit fly, honeydew, behavioral response, time-lapse video.

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Poster nº 15

Development of a PCR-based diagnostic method to detect of DNA of *Philaenus* (Hemiptera: Aphrophoridae), vector of *Xylella fastidiosa*, in the gut of spiders

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In the last years, the phytopathogen *Xylella fastidiosa* (*Xf*) has been devastated olive groves throughout Southern Italy in the form of the disease known as "Olive Quick Decline Syndrome". Since there's no cure for *Xf*, vector control is recognized as the main protection measure to reduce the spread of this pathogen. However, the implementation of sustainable and ecological measures to control the population of *Philaenus spumarius*, the main vector of *Xf*, is required. Generalist predators, such as spiders, are naturally presented in the ecosystems and, they can play an important role in vector control. But the identification of predation in the field may not always be possible. So, the development of a PCR-based diagnostic method may be a reliable tool to accurately detect predation events and potential predators. Therefore, the main goal of this work was to design and evaluate taxon-specific primers within the mitochondrial cytochrome oxidase I (COI) and cytochrome b (cytB) genes, to be used for a PCR-based diagnostic method aimed to detect *Philaenus* sp. in the gut of the spider *Xysticus acerbus* Thorell, 1872 (Thomisidae). For that, a set of 20 primers were designed and tested for specificity, sensitivity, and efficiency to detect the presence of *Philaenus* sp. DNA in the gut of *X. acerbus*. Feeding trials with the *X. acerbus* were conducted to estimate the time at which prey DNA is detectable within the predator after ingestion. Finally, spiders were also collected in the field to confirming the predation of *Philaenus* sp. Among the pair primers tested, one showed great sensitivity and specificity for *Philaenus* sp. and when applied in the feeding assay the primer pair successfully amplified the degraded DNA of *Philaenus* sp. in the gut of the spider. According to the Probit regression, there is a 50% probability of detecting *Philaenus* sp. DNA 82 hours after ingestion. 20% of spiders collected in the field tested positive for *P. spumarius*. This study demonstrates that this molecular tool can provide an estimation of predator efficiency of spiders over *Philaenus* sp. in the field.

Keywords: Biological control, *Xysticus acerbus*, specific primers; generalist predators, cytochrome b.

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Poster nº 16

Phylogenetic analysis of Hypocreales strains isolated from olive trees, a fungal order known to harbour promising biocontrol agents

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CIMO-CC is a recently established microbial culture collection hosted at CIMO-IPB (Bragança, Portugal). This culture collection is a repository of relevant microorganisms for agriculture, namely olive crops, taking advantage of the numerous sampling and isolation campaigns being undertaken through time by team members of the AgroBioTec lab. Among fungi, the order Hypocreales is known to encompass species that are used as biological control agents (notably, *Beauveria bassiana* and *Trichoderma* spp.), but also some putative phytopathogens (e.g. *Fusarium* spp.). In this work, we expose the phylogenetic diversity of endophytic and epiphytic Hypocreales strains which were previously obtained from olive tree tissues, and are to be included in CIMO-CC. The 93 Hypocreales strains are distributed by seven families and include *Beauveria*, *Trichoderma*, and *Sarocladium*, as well as *Fusarium* species. The relevance of these innate, olive-associate fungi to be used as biocontrol agents in olive tree protection is discussed.

Keywords: *Olea europaea*, biological control agents, culture collection, bioprospection.

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Poster nº 17

Screening of potential biocontrol endophytes and epiphytes against olive knot disease

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Pseudomonas savastanoi pv. *savastanoi* (Psv) is an important pathogen of olive tree, causing the olive knot disease. Its control is very difficult, requiring preventive bactericide applications. Here, five endophytes and epiphytes previously isolated from olive tree were screened for the suppression of Psv growth by using a bioassay with detached olive branches. The effect of protein extracts from olive trees elicited by some of these microorganisms in inhibiting Psv was also evaluated. The isolates were identified by sequencing a portion of the16S (for bacteria) or ITS (for fungi) of rDNA region, as *Beauveria bassiana*, *Aureobasidium pullulans* and *Vishniacozyma victoriae*, *Alcaligenes faecalis* and *Bacillus amiloquiefaciens*. In detached olive twigs, the fungi *B. bassiana*, *A. pullulans* and *V. victoriae*, inhibited completely the development of tumors after 15 days of inoculation with the pathogen. At the same time, 50% of the branches inoculated with Psv had tumors. However, only the protein extracts from the olive tree inoculated with *A. pullulans*, and at the concentration of 0.625 and 0.3 mg protein/mL, significantly inhibited the growth of Psv (2.6 times), when compared with protein extracts from non-inoculated plants. Overall, *A. pullulans* seems to be a promising biocontrol candidates for controlling olive knot disease. Further experiments are needed to determine its effectiveness under field conditions and to identify protein compounds responsible for its antimicrobial activity.

Keywords: *Pseudomonas savastanoi* pv. *savastanoi*, antimicrobial activity, *Aureobasidium pullulans*, bioassays, proteins.

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Poster nº 18

Assessment of physiological traits of olive trees infected by *Xylella fastidiosa* subspecies

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The detection of the diseases caused by the plant pathogenic bacterium *Xylella fastidiosa* (*Xf*) is difficulted by the occurrence of asymptomatic infections, its long incubation period and non-specific and diagnostic symptoms. Early detection of infected plants based on the assessment of changes in physiological plant traits due to bacterial infection might be an important tool for pathogen detection, disease management and breeding for resistance to *Xf* in woody crops. Therefore, the objectives of this study were to evaluate the susceptibility to four Spanish *Xf* strains and the response to infection of three olive cultivars widely grown in Spain by measuring plant physiological traits with different leaf-level sensors and *Xf*-specific molecular diagnostic assays. Leaf measurements and petiole samplings were taken at the inoculation point, and at 5, 10, 15 and 20 cm above the inoculation points at 1, 3, 6, 12 and 18 months post-inoculation. Additionally, sampling of wood tissue was performed at 8 month post inoculation at the same plant sections of leaf sampling. Molecular diagnosis based on two qPCR tests was used to determine the presence and colonization level of the bacteria into the olive xylem tissues. The Dualex Scientific leaf clip sensor and two spectrometers (PolyPen RP400 and DLP NIRscan nano) covering the range of the electromagnetic spectrum from 340 and 1700 nm were used for leaf measurements to detect physiological changes. The Spanish strain XYL1961 from *Xf* subsp. *pauca* ST80 showed the highest colonization in the different olive cultivars as compared to strains from *Xf* subsp. *multiplex* from ST81 and ST6 from Spain and the Italian strain 'DeDonno' from *Xf* subsp. *pauca* ST53. On the other hand, from a total of 70 vegetation indices calculated related with chlorophyll, carotenoid and flavonoids content and structural related indexes a set was selected to discriminate between olive plants infected by *Xf* from those non-inoculated even in the absence of visible disease symptoms.

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Poster nº 19

Predatory potential of Forficulidae (Dermaptera) on the olive fly *Bactrocera oleae*

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Earwigs (Dermaptera: Forficulidae) are omnivorous insects, feeding on a variety of plants and animal material and are described as natural control agents of pests in several agroecosystems. Earwigs are abundant in olive orchards and their adult stage overlaps in time and space with the pupae stage of the main olive pest, the olive fly *Bactrocera oleae* (Rossi). However, the role of earwigs as natural control agents of *B. oleae* is almost unknown. The present work aimed to study the functional response of female and male earwigs on olive fly pupae in order to analyse their potential ability to limit this pest. Increasing densities of pupae were offered to starved earwigs in a controlled laboratory environment. After 24 h, the number of attacked pupae was recorded. The type of functional response, handling time, and attack rate of the earwigs was assessed. Our results disentangle the potentiality of earwigs as predators of *B. oleae* during the pupae stage and provide relevant information for further studies and applications.

Keywords: earwigs, natural enemies, functional response, biological control.

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Poster nº 20

Contribution to the study of the beneficial fauna of olive tree parcels in Porto Martim, Terceira Island, Azores

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The olive tree culture in Terceira Island occupies a strip in Porto Martim area whose fruit is very sought, mainly by tourists and restaurants. The olive tree is very affected by pests, such as the olive fly, which causes high losses and a drastic loss of production. Therefore, large quantities of pesticides are applied whose applications have a great impact, in particular, on beneficial organisms. In this work, the beneficial fauna present in olive groves was identified, through the technique of beatings and its subsequent identification and it was also possible to evaluate the impact of the application of pesticides on these beneficial insects present in olive trees with different levels of treatments. For this, orchards with intensive applications, with less intensive applications and one organic orchard were selected to studied and monitoring. It was verified that in the olive groves with intensive and semi-intensive pesticide applications the abundance of beneficial insects was unbalanced, with a low abundance of predatory insects and still high population densities of the olive cottonweed pest. The results obtained point to the fact that chemical treatments do not destroy the target pests and worst contribute to a reduction in auxiliary organisms. In the organic orchard was where we observed the highest abundance of predatory insects and the lowest abundance of herbivorous insects. These results showed that this type of orchard is in a natural balance and that this is the way to follow in preserving the presence of beneficial insects in the olive parcels because these are very important to keep low pest populations and permit its better control. The conclusions of this study point to the fact that producers can further increase their yield by taking the biological option in their daily practices that will have a lower impact on the beneficial fauna present in the olive parcels. At the same time will also permit to preserve the environment and the health of the applicators, producers and of the consumer by obtaining a product without any pesticide residues.

Keywords: beneficial fauna; predators; pests; olive tree; Azores.

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Poster nº 21

A micronutrient fertilizer affects the survival of *Bactrocera oleae* adults and puparia emergence in laboratory

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The olive fruit fly is the main insect pest of olive crops in all the countries where the species is spread. Conventional control strategy has been based on the cover sprays with synthetic chemical insecticides, mostly organophosphates. However, the ban of dimethoate, together with the increased awareness towards environmental safety, have been driving research and olive growers to evaluate more sustainable approaches. Several low-impact tools, such as copper products, kaolin and entomopathogenic fungi have been used in different countries. Among them, copper-based pesticides have the advantage to be inexpensive and easy to handle and operate. Studies conducted in recent years have shown that copper affects the survival of the olive fruit fly adults and larvae, due to the negative effects on its endosymbiont, the bacterium *Candidatus* E. dacicola. The symbiont is essential for adult survival and larval growth in unripe olives. On the other hand, the use of copper-based pesticides should be reduced because of their well-known drawback for the high content of metallic copper equivalent. In order to disrupt the bacterial symbiosis exploiting the bactericidal effect of copper, and to reduce the environmental impact of heavy metals as well, the fertilizer Dentamet®, containing very low percentages of zinc and copper, was tested in laboratory experiments. The effect of Dentamet® on wild adults of *B. oleae* obtained from infested olives was evaluated determining the survival of flies exposed to different dilutions of the product and to water as a control. Two sets of experiments have been carried out, the first one with the concentrations usually applied in the field (0.3% and 0.6 %) and the second with reduced concentrations (0.05% and 0.005%). Specimens of the three groups of flies were dissected and the heads were analysed through PCR to evaluate the presence of the symbiont *Ca.* E. dacicola. Moreover, infested olives were sprayed with Dentamet® at the concentrations used in the field and the numbers of puparia emerged from treated drupes were compared to those emerged from control olives. Adults provided with Dentamet® solutions survived less than the control ones, with significant differences among treatments. Also the number of puparia obtained from infested olives treated with the highest concentration of the fertilizer was significantly lower than the other treatments. The fertilizer does not appear to affect the presence of the symbiont in the treated adults. Further investigations are needed to ascertain the mode of action of this fertilizer, and possible synergistic effects among the components as well, however its influence on olive fruit fly survival seems to be promising and this product could be taken into consideration for a preventative management of the olive fruit fly.

Keywords: olive fruit fly, copper, zinc, adult mortality.

Poster nº 22

Identification of endophytes and of their volatile compounds with biocontrol potential towards olive anthracnose

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Anthracnose, mainly caused by *Colletotrichum acutatum*, is the main disease of olive fruit worldwide. Strategies for controlling this disease are mostly based on the use of copper-based pesticides. Besides its limited efficacy, this approach is not compatible with sustainable production systems. Therefore, there is a need to developed more environment-friendly alternatives to these pesticides. Accordingly, this work aims to study the effect of two endophytes previously isolated from olive tree, namely *Aureobasidium pullulans* and *Sarocladium summerbellii*, on anthracnose infection by using in *vitro* and *in vivo* assays. Endophyte-mediated changes in plant volatiles (VOCs) and their consequences for anthracnose disease development were also evaluated. Among the two endophytes tested, only *A. pullulans* showed capacity to inhibit significantly the growth (up to 1.3-fold), sporulation (up to 5.9-fold) and spore germination (up to 3.5-fold) of *C. acutatum* in relation to the control (self-paired *C. acutatum*) in *in vitro* dual-culture assays. Similarly, in inoculated olive assays, only *A. pullulans* was able to reduced significantly progress curve for incidence (up to 10-fold) and severity (up to 35-fold) of anthracnose, as well as both sporulation (up to 90%) and germination (up to 70%) of *C. acutatum*, in comparison with olives inoculated solely with *C. acutatum*. Gas chromatography–mass spectrometry analysis of olives inoculated with *A. pullulans*+*C. acutatum* and controls (olives inoculated with *C. acutatum*, *A. pullulans* or tween) led to identification of 29 VOCs, belonging to 10 different chemical classes, being alcohols the most diversified and abundant ones. The volatile profile of *A. pullulans* + *C. acutatum* treatment revealed quali- and quantitatively differences from the controls, due to the production of recognized antimicrobial compounds, namely 1-hexanol, 1-Heptanol and 2-methyl-1-propanol. These findings provide new viable possibilities of controlling olive anthtacnose using the endophyte *A. pullulans* and/or their VOCs. These volatile inhibitory metabolites should be further tested against anthracnose.

Keywords: Biological control, *Colletotrichum acutatum*, *Aureobasidium pullulans*, Gas chromatography–mass spectrometry

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Poster nº 23

Exploitation of endophytes of centenarian olive trees in the management of olive knot disease

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Endophytes are microorganisms that spend most of their life cycle within plant tissues and they are recognized to play important roles in plant protection. These features, have been attracted the interest of many researchers in the exploitation of endophytes to manage plant diseases and pests. Some recent studies have been showed that endophytes in wild and ancient plants include beneficial endophytes that are absent or underrepresented in domesticated/recent crops. We hypothesized that the loss of these beneficial endophytes may be remediated by transferring endophytes from wild/ancient relatives of crops to crop species. Thus, in this work the effect of the application of a consortium of endophytes, retrieved from centennial olive trees, on the development of olive knot (OK) disease, was evaluated. The mechanisms responsible for the control of OK by endophytes was also evaluated by studying the changes in the indigenous stem endophytic bacterial community, through 16S rRNA gene amplicon-based metagenomic analysis. Accordingly, endophytes were extracted from stems of centennial olive trees using a Histodenz gradient, and used to inoculated one-year-old olive plantlets. One week later, these plants were inoculated with the causal agent of OK, the bacterium *Pseudomonas savastanoi* pv. *savastanoi* (Pss). Results showed that the inoculation with endophytes reduced significantly both the incidence (up to 1.5-fold) and severity (up to 5.8-fold) of OK, when compared to plants inoculated exclusively with the pathogen Pss. The composition of stem associated-bacteria was significantly changed by the single inoculation with the pathogen Pss or endophytes, being the effect of the former greater. However, if plants have been previously inoculated with endophytes, the subsequent inoculation of Pss one week later did not affect the composition of indigenous stem endophytic bacterial community. Overall, the results suggest that the inoculation of olive plants with endophytes from ancient trees could be an innovative strategy to manage olive knot disease. Despite these promising results, further studies are needed to better understand their mechanisms of action.

Keywords: *Pseudomonas savastanoi pv. savastanoi*, biological control, bacteria, incidence, severity.

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