



IOBC-WPRS

7th WORKING GROUP MEETING
“Integrated Protection of Olive Crops”

CONCLUDING REMARKS: *PHYTOPATHOLOGICAL ISSUES*
prepared by Professor ERIS TJAMOS
Agricultural University of Athens

A. *Xylella fastidiosa*

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

Dr. Anna Maria D’Onghia from Istituto Agronomico Mediterraneo di Bari, Valenzano (BA), Italy (CIHEAM)

Analysed thoroughly the current situation of *Xylella fastidiosa* in Italy, (a known pathogen in Americas but not previously known in Europe) and stressed that the Mediterranean Olive industry is seriously threatened by *X. fastidiosa* subspecies *pauca* strain “*Codiro*”. Nearly two years ago the pathogen was reported for the first time on olive trees in Apulia, in association with the olive quick decline syndrome (OQDS).

Strain “*Codiro*” seems to be the main responsible agent, even though *Phaeoacremonium* and *Phaemoniella*, and *Zeuzera pyrina* could be also isolated or found.

She underlined the possible association of organic olive culture of almost abandoned unfertilised, neither irrigated nor sprayed trees on the outbreak of the disease. As for the host range the study is still in progress, but apparently the *Codiro* strain does not infect grapevine and citrus spp. **The assessed vector of the *Codiro* strain is *Philaenus spumarius*,** a polyphagous Auchenorrhyncha insect widely distributed in the Euro-Mediterranean region

- **There is no record** of successful eradication of *X. fastidiosa* once established outdoors due to its broad range of plant hosts and vectors.
- Control strategies are mainly based on the prevention of introduction of the pathogen from areas where it is present and on the containment of the outbreak. She mentioned that no nursery material is allowed to be moved out of Puglia
- **She noticed that** areas where the cultivation does not show yet the symptoms of the disease have been properly cultivated irrigated fertilised and sprayed with chemicals.
- **Large-scale monitoring** has been carried out in an attempt to contain the rapid spread of the pathogen and the correlated disease, which is destroying century-old olive trees.
- **Efficient and innovative detection** tools including monitoring methods have been developed (i.e. *X. fastidiosa*-detection on 'spy insects' for assessing the presence of the pathogen before symptom development, etc.). Based on these tools, an urgent pathogen surveillance programme should be envisaged in the Mediterranean countries to immediately monitor the presence of OQDS.
- **Neighbor countries should be in alert**
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2. Evaluation of "Insect Spy" approach for monitoring *Xylella fastidiosa* in symptomless olive orchards in the Salento Peninsula (Southern Italy)

**Issam Eddine Ben Moussa^{1,2}, Franco Valentini², Donato Lorusso², Valerio Mazzoni³, Michele Digiario², Leonardo Varvaro¹, Anna Maria D'Onghia² by Viterbo, and Valenzano
CIHEAM**

- They developed an effective approach for monitoring this bacterium in the symptomless areas surrounding the infection sites (buffer zone).
- Since the bacterium is vector borne, an evaluation of the possibility to utilize the *Xf*-positive insects found in the buffer zones (so called "Insect Spy") as indicators of the presence of the bacterium in apparently uncontaminated areas, was carried out. The bacterium was successfully detected by PCR in 3 out of 6 species of Auchenorrhyncha captured in the buffer zone i.e. ***Phileanus spumarius*, *Neophileanus campestris* and *Euscelis lineolatus***.
- **Infected adults of these species were detected throughout the buffer zone at a distance of 1 Km far from the infection site.**
- Therefore, adults of these three species have an important role as "insects spy" to early reveal the presence of the pathogen in apparently free areas before symptoms become visible on the plants

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

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- Recent outbreak of the harmful bacterium *Xylella fastidiosa* strain CoDiRO represents a potential risk for Croatian olive growing sector.
- The diversity of susceptible hosts in the Croatian sea coast and the fact that population of the coastal region is traditionally engaged in agriculture and tourism, facilitates the uncontrolled transmission. Furthermore five potential vector species [*Philaenus spumarius* L., *Phylaenus signatus* L., *Lepyronia coleoptrata* L. and *Aphrophora alni* F. (Hemiptera: Aphrophoridae) and *Cicadella viridis* L. have been described in olive growing areas of the under study area. Results of the 2014 surveys in Croatia, after extensive visual inspections and laboratory tests, indicated that *Xylella fastidiosa* strain CoDiRO is not present in Croatia.
- **Presence of potential and confirmed vectors of the pathogen, abundance and high biodiversity of host plants, favorable climate and high movement of plant material are important elements that should be taken into account for the risk assessment of the spread of pathogen in case of his undesirable introduction.**

4. Quick decline syndrome *Xylella fastidiosa* and anthracnose: emerging and reemerging diseases of olive posing new challenges for an integrated control approach

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- *Xylella fastidiosa* vectored by *Cicadellidae* insects, affects young and aged trees (100 years or older) growing in the Salento peninsula.
- Fungal species of the genus *Phaeoacremonium* and several other isolates similar to genus *Phaeomoniella* were obtained from the discolored wood.
- The role of these fungi, alone or in combination, in determining the symptoms observed on olive trees is currently under investigation.

B. Colletotrichum acutatum

1. Serious anthracnose outbreak starting from inflorescence infection of olive trees in west Greece *Colletotrichum accutatum*

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- *Anthracnose* is very common and particularly destructive in olive groves of west Greece, along the west coastal area and the Ionian Islands due to prolonged wet periods because of heavy rains. In Aetolia region last year almost the 25% of the expected production of Kalamata was destroyed. Orchard surveys last January showed mummified fruits on the trees. Similar symptoms and signs were easily observed in olive fruits already fallen on the soil.
- Surveys in Zakynthos island on March 26, 2015 revealed that certain olive groves of the variety Koroneiki at the onset of blooming developed a dark red to brown discoloration demonstrated formation of acervuli (conidiosomata). of the colonies and the dimension and form of the conidia that we deal with the species *Colletotrichum accutatum*.
- Similar symptoms were also observed in in April 18 2015 in Kalamata county in Koroneiki variety. Molecular identification of the pathogen is under examination.
- Our research group is currently organizing field and lab experiments to further study the problem and find out the best possible measures and methods of disease management.

2. Biological control of olive anthracnose

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- The biocontrol activity of the commercial formulate Serenade[®], based on *Bacillus subtilis*, and several endophytic isolates of the yeast-like fungus *Aureobasidium pullulans* were evaluated in field trials.
- *Bacillus subtilis* applied at the pre-blooming stage was effective as the chemical fungicides.
- Moreover, some endophytic *A. pullulans* strains provided high protection levels against *Colletotrichum* spp. when applied at the veraison stage.

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

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The diversity of fungal endophytes from roots, leaves and twigs of healthy trees from three olive tree cultivars with different susceptibilities to olive anthracnose were assessed to select strains with the greatest antagonistic effect against *Colletotrichum acutatum*. (identify them by rDNA sequencing)

They suggest exploring them as biocontrol agents and use them for elucidating the mechanism of enhanced disease resistance in olive tree cultivars.

C. Spilocaea oleagina

1. Relationship between phyllosphere fungal communities and tolerance of olive tree cultivars to olive leaf spot disease *Spilocaea oleaginea*

Teresa Gomes^{1,2}, José Alberto Pereira¹, Teresa Lino-Neto², Paula Baptista¹

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Spilocaea oleagina is the causal agent of olive leaf spot (OLS) disease on olive trees, A correlation between data regarding fungal community and disease incidence displayed by both cultivars was performed by using multivariate analyses.

Their results indicate that some fungal species, either epiphytes or endophytes, may have a direct effect on olive tree tolerance to ***Spilocaea oleaginea*** .

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

Teresa Gomes^{1,2}, José Alberto Pereira¹, Teresa Lino-Neto², Paula Baptista¹ *Spilocaea oleagina*

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- Their work aimed at comparing the endo- and epiphytic fungal community inhabiting asymptomatic and symptomatic leaves of olive trees (*Olea europaea* L.) that showed typical symptoms of *Spilocaea oleagina*
- The plant material was collected from seven olive trees, in a grove located in Mirandela (Northeast Portugal), being used to isolate endo- and epiphytic fungi.
- The presence of *Spilocaea* on leaves clearly affects fungal community composition, which in turn may have implications on olive tree protection against this disease. It is expected to identify predictable microbial species that could explain the reduction of OLS disease infection in asymptomatic leaves.

D. Verticillium dahliae

1. *Verticillium wilt of olive - Current status and Management*

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- She stressed that we need an integrated disease management (IDM) to reduce disease impact, by using **preventive** (*V. dahliae* -free soil and planting material), **pre-planting** (resistant cultivars, soil disinfestation, sanitation) and **post-planting** (cultural practices, soil solarization, organic amendments, green manure, biological control agents) measures.
- Control of *Verticillium* wilt on olive is an ongoing challenge which requires further research for understanding the pathogen-tree-environment

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

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- *Pseudomonas fluorescens* PICF7 and *Pseudomonas putida* PICP2 are endophytes and effective biocontrol strains against *Verticillium dahliae* of olive.
- They sequenced and annotated the complete genomes of strains PICF7. A comparative bioinformatics analysis was carried out aiming to identify genes potentially involved in
 - (i) biological control,
 - (ii) plant-bacteria interaction,
 - and (iii) endophytism.
- The analysis has revealed that, the genome of strain PICF7 harbours **the genetic information to encode two type VI (T6SS) and one Type III (T3SS) secretion systems, the T3SS effectors *avrE1* and *hopB1*, the siderophores pyoverdine, pyochelin and the hemophore HasAp**, diverse adhesion factors, a battery of cell-degrading enzymes (protease, cellulase and lipase), as well as detoxifying systems such as CopA, KatB, Dps and Cb), and AGL2011-30343-CO2-01 from the MINECO (Spain), all co-funded by ERDF from EU.

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

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- The Spanish Research Group AGR-216 from the University of Cordoba has conducted 4-year experiments for studying the effect of irrigation on development of Verticillium wilt of olive.
- During the evaluation period results clearly demonstrated that **irrigation** treatments encouraged Verticillium wilt development when compared to the **deficit irrigation**.
- They concluded that, up to 52 weeks after planting, values of the area under disease progress curve (AUDPC) were significantly higher in daily irrigated trees (15.8%) than in olive plants of the other two treatments (5.0%).

4. VERTICILLIUM DAHLIAE

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

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- Biological control of Verticillium wilt was attempted in heavily infested olive orchards of Kalamata variety by drenching the soil around the trees with bacterial suspensions of *Paenibacillus alvei* strain K-165, effective against Verticillium wilt of potatoes in the field. It was shown that soil application of the biocontrol agent applied at 4 month intervals mitigated significantly symptoms of the disease. New infestation of a year after was attributed to the incorporation of leaves from diseased trees during the next years after the treatments, underlying the importance of the inoculum level in overcoming the effectiveness of biological control agents.
- The application of soil solarization in already established olive orchards proved that heavy inoculum coming mainly from infested leaves are drastically reduced so solarization is a promising and effective method to decrease the soil inoculum level of the pathogen in young olive orchards.

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

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- Different organic amendments plant extracts and microorganisms (various fungi, bacteria and their extracts) for the control of *V. dahliae* were selected.
- The selection was conducted in four stages:
- i) *in vitro*, by the effect on the mycelial growth and spore germination of the pathogen;
- ii) in naturally-infested soil, by the effect on the reduction of microsclerotia of the pathogen;
- iii) *in planta*, by the effect on the infection of olive plants under controlled conditions; and iv), in field, by the effect on Verticillium wilt of olive trees grown in a highly-infested soil.
- The assessment of 220 treatments under controlled conditions resulted in a selection of 20 candidates to evaluate in field conditions. Among the candidates evaluated in the field, are non-pathogenic strains of *Fusarium oxysporum*, pomaces of grape and olives, and mixtures of bacteria and yeasts.

6. Diversity of bacteria endophytes in olive tree

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- Their work intends to assess the diversity of culturable bacterial endophytes in *O. europaea* and create a reservoir of strains with potential beneficial applications on olive tree protection against *Verticillium* wilt.
- Bacterial endophytes were isolated from roots, leaves and twigs of 35 olive trees cv. Picual, located in Granada (Spain). Pure bacteria cultures were identified morphological- and molecularly through sequencing of V1 to V4 regions from 16S rDNA, and further maintained in culture (PCA) and cryopreserved (-80°C in aqueous glycerol solution). A total of 35 taxa belonging to 14 genera were identified. The bacterial endophytes most frequently observed were *Alcaligenes faecalis* and *Pseudomonas aeruginosa*, found in 9% of roots and 80% twigs, and in 8% of roots, respectively. Root explants were the most colonized (96%), followed by twigs (3%) and leaves (1%).
- Correspondence analysis of the endophyte assemblages showed that the endophytes exhibited organ specificity.
- Thirty three species were consistently associated with root tissues, while one species was found predominantly in leaves.
- Many bacterial species are found to be restricted to root tissues, and knowledge of their interactions with *V. dahliae* might be useful in the control of this disease.

7. *VERTICILLIUM DAHLIAE*

Pruning residue management associated pathogens in olive

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- They investigated whether recycling of raw or composted pruning residues in soil could potentially contribute to fungal pathogens dispersal, such as *Verticillium dahliae* and *Fomitiporia mediterranea*, within and between olive orchards. (DNA extraction and polymerase chain reaction-PCR assay) were employed to detect the abovementioned pathogens in healthy and diseased plant tissues as well as in raw or composted plant materials. Both fungal species were detected in diseased tissues but not in composted materials.
- The outcome of these analyses indicates the low risk of pathogen dispersion in the fields following good agricultural practices and regular orchard monitoring.