subsp. *fastidiosa* isolates to tetracycline, streptomycin, gentamicin, chloramphenicol, ciprofloxacin and penicillin. Minimal Inhibition Concentration (MIC) (µg/ml) were generated using Etest® assay, a quantitative technique that has been used previously in slow-growing bacteria including *X. fastidiosa*, with reproducible results; a difficult task to achieve using other susceptibility determination techniques. Results show an overall susceptibility of the isolates to most of the antibiotic tested; however, differences in MICs within isolates for each antibiotic should not be overlooked. To evaluate whether the endemic *X. fastidiosa* subsp. *fastidiosa* population is capable of infecting and causing severe disease in *Nerium oleander* as seen for *X. fastidiosa* subsp. *pauca* (ST53), also present in Costa Rica, several isolates of *X. fastidiosa* subsp. *fastidiosa* representing different ST types, were assayed in a virulence test using Nerium *oleander*. Eight months after inoculation none of the tested isolates have induced evident symptoms of disease and bacteria were barely detected near the inoculation site six months post-inoculation. Monitoring is still ongoing.

Leaf ionome profile of susceptible and resistant olive cultivars infected by *Xylella fastidiosa*

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Abstract: *Xylella fastidiosa* (Xf) is continuing to emerge as a devastating bacterial pathogen for many economically relevant species. Xf subsp. *pauca* strain De Donno is associated with Olive Quick Decline Syndrome (OQDS), a destructive disease occurring in the southern area of Apulia (Italy). In susceptible olive cultivars, symptoms are characterised by initial leaf scorch and scattered desiccation of small branches that over time worsen and extend to the whole canopy. Greenhouse and field observations revealed that olive trees of the cultivar Leccino show milder symptoms, when compared to those observed in Ogliarola salentina. Ogliarola salentina have progressive, severe scorching and complete dieback. Moreover, the lower bacterial population size in Leccino confirmed the resistance of this cultivar to Xf infection, suggesting that it is able to limit pathogen multiplication.

To understand the role that mineral nutrition may play in host resistance to OQDS, a field survey of the leaf ionome was carried out in trees of two orchards located in the Xf-infected demarcated area, that showed clear differences in response to Xf infection. Infected leaf samples, classified as symptomatic and asymptomatic, were subject to the determination of the ion content by inductively coupled plasma – optical emission spectrometry (ICP-OES). Data were analysed in relation to the different cultivars and the presence or absence of symptoms.

The comparison between symptomatic and asymptomatic samples showed an increase of sodium levels in both cultivars and significantly higher calcium levels in the symptomatic tissues of Leccino, a response that had been found in other Xf-host pathosystems. Otherwise, Leccino trees had a significantly higher content of manganese, in both symptomatic and asymptomatic leaf tissues.

These field observations inform currently ongoing experiments under controlled conditions to investigate the relevance of these mineral ion changes in the development and progression of symptoms, and the potential involvement of manganese in resistance of the Leccino cultivar to Xf infection.

Acknowledgement

Monitoring of biofilm production in *Xylella fastidiosa* strain De Donno via biochemical signalling modulation

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Abstract: Diffusible lipid species are exploited by bacteria for regulating cell motility, cell-to-cell communication, activation of metabolism and proliferation. The most widely investigated lipid family is represented by diffusible signal factors (DSFs) responsible for quorum sensing. *Xylella fastidiosa* (Xf) uses the DSFs to coordinate genes involved in the expression of virulence and biofilm formation. These moieties are mainly cis-2-unsaturated fatty acids which directly enhance xylem infection and biofilm production, which are the two processes involved in the genesis of severe plant diseases such as the noticeable Olive Quick Decline Syndrome, associated with Xf subsp. *pauca* strain De Donno and affecting olives in the Apulia Region. Here we report the results of studies aiming to identify DSFs molecules of Xf De Donno and exploit strategies for modulating its biofilm formation.

We started inducing DSF expression in *Escherichia coli* using a plasmid vector recombinant for the *rpfF* gene of Xf De Donno and verifying the production of exogenous proteins and fatty acids. We set up extraction, mechanical treatments, and methyl ester derivatisation of the extracted crude oils from Xf and *E. coli* cultures. We compared the GC-MS profiles of fatty acids belonging to the metabolic activity of bacteria harbouring the bare and *rpfF*-recombinant plasmids. Completing previous studies, we speculated on the production of unsaturated fatty acids with a chain length of 12-18 carbon atoms, with α-unsaturated functions. Isolated and treated crude extracted oils obtained from the same bacterial sources, were tested *in vitro* to investigate their phenotypic effect on biofilm growth and the expression of key genes related to surface adhesion, biofilm formation and cell movement.

Furthermore, we set the synthesis of new, no commercially available, cis-2-unsaturated fatty acids with a chemical structure related to the DSFs family, in order to test the *in vitro* alteration of biofilm production in Xf De Donno. The exploited reaction was the stereoselective Still-Gennari olefination which leads to the synthesis of unsaturated fatty acids in cis (Z) conformation starting from commercial aldehydes.

Results of these activities will be presented.

Experimental confirmation that *Xylella fastidiosa* subsp. *pauca*, ST53, does not colonise grapes

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Abstract: *Xylella fastidiosa* is able to colonise a very large number of plant species, but considering each subspecies/phylogenetic clade the number of associated susceptible hosts is significantly reduced. Although strains genetically related most likely share similar host range, using phylogenetic relationships to infer information regarding the potential host