3rd European Conference on
Xylella fastidiosa and XF-ACTORS final meeting

In vitro and in vivo effects of ammonium chloride on *Xylella fastidiosa*, subsp. *pauca* infecting olives

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INTRODUCTION

The devastating impact of *Xylella fastidiosa* infections in olives in Apulia (southern Italy) raised major concerns and prompted an intense activity for testing formulations, synthetic molecules, mucolytics, bactericides, microbial antagonists, that could reduce *Xylella*-induced symptoms and/or inhibit the bacterial multiplication in the host plants.

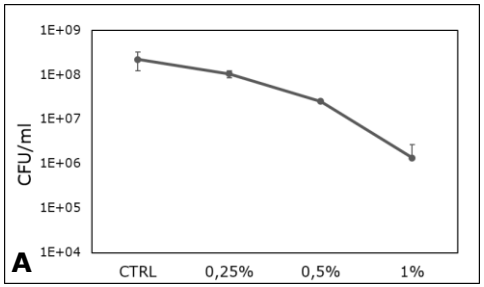
- **Ammonium salts** are water-soluble ionic compounds. Quaternary ammonium compounds are antimicrobials organic cations whose effectiveness has been previously proved against several bacteria, e.g. *Pseudomonas aeuruginosa*, *Staphylococcus aureus*, and *Escherichia coli*. The inorganic ammonium salts are mainly used in food industry and in agriculture as nitrogen fertilizer.

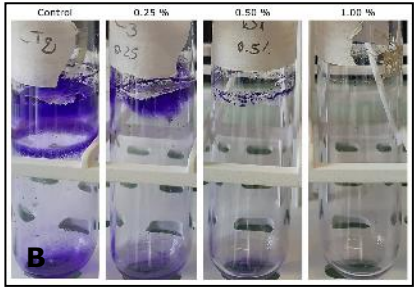
- In this work the effects of the use of ammonium chloride (NH_4Cl) against *Xylella fastidiosa subsp. pauca* ST53 were tested both ***in vitro*** **and under field conditions** in the infected demarcate area of Apulia.

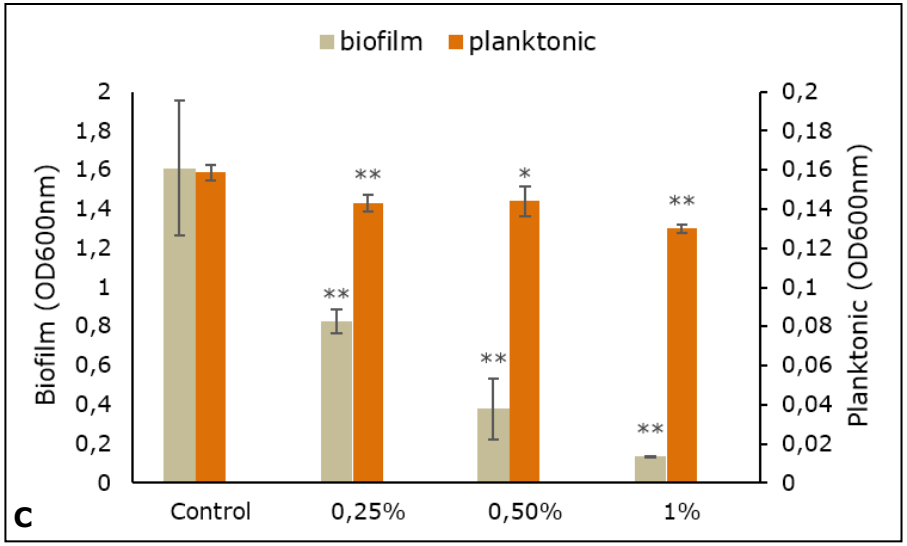
IN VITRO STUDIES

- **Methodology**: Three concentrations of NH_4Cl were tested to evaluate its impact on the growth of *Xylella fastidiosa* strain 'De Donno'. NH_4Cl was added to a liquid culture and after 3 days, planktonic cell growth was evaluated by plating on PD3 agar plates. The effect of NH_4Cl on cell adhesion and biofilm formation was determined after six days by using 0.1% crystal violet staining.

- **Results**: Plate count after three days of growth in PD3 supplemented with NH_4Cl, showed a gradual reduction in growth, positively correlated with the concentration of NH_4Cl. A lower fraction of planktonic cells with all three concentrations tested was observed after six-day incubation. Crystal violet assay exhibited a remarkable drop in biofilm formation, with OD 1.6 in the control versus OD 0.8, 0.4 and 0.15 in PD3-broth containing 0.25%, 0.5% and 1% of NH_4Cl, respectively.







Figures: A) Bacterial population (CFU/ml) after three days of incubation with NH_4Cl. **B**) Glass tubes stained with 0.1% Crystal violet. **C**) Biofilm quantification and planktonic growth of *X. fastidiosa* after six days incubation with NH_4Cl.

FIELD TRIALS

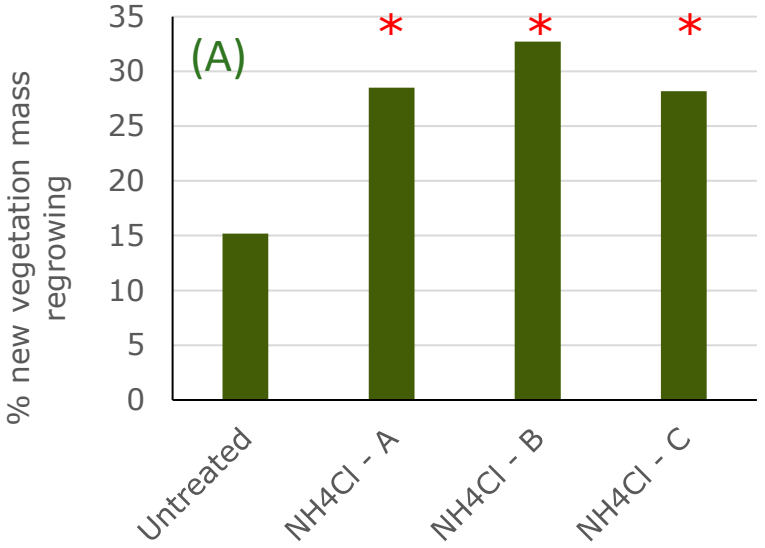
Field experiments started either in 2019 or 2020, using different formulations of NH_4Cl (A,B,C), applied alone or with bio-stimulants.

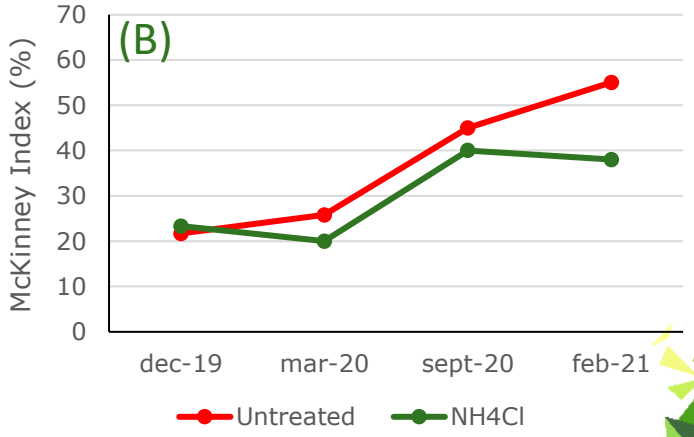
Trials included:

- 5 olive groves with ≠ levels of infection/symptoms
- 4 applications/year (March to October)

Preliminary results: (i) Regardless the NH_4Cl formulation, a significant increase of the new vegetation was recorded (Graph A); **(ii)** a reduction of the severity of symptoms was observed on treated plants, even if no statistical differences were recorded among treatments (Graph B); **(iii)** no difference was detected in the bacterial population size between treated and non-treated trees (data not shown)

Untreated plants - Treated plants





CONCLUSIONS

- The use of NH_4Cl in the culture media significantly affected the *in vitro* growth of *X. fastidiosa*. Its effect was particularly evident on biofilm-forming cells, given the remarkable aggregation and adhesiveness of *X. fastidiosa* strain De Donno.
- No effects of NH_4Cl were recorded on the bacterial population size of the treated trees.
- A reduction of the wilting and desiccation phenomena (particularly in the lower portion of the crowns) and an increase in the new vegetation of the trees were observed.

However, these trials require prolonged observations to achieve solid data at field scale.

Untreated plants - Treated plants

Funding: This work was done with the financial and scientific support of the EU Horizon 2020 research and innovation programme under Grant Agreement N. 727987 "Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy XF-ACTORS". The EU Funding Agency is not responsible for any use that may be made of the information it contains.