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

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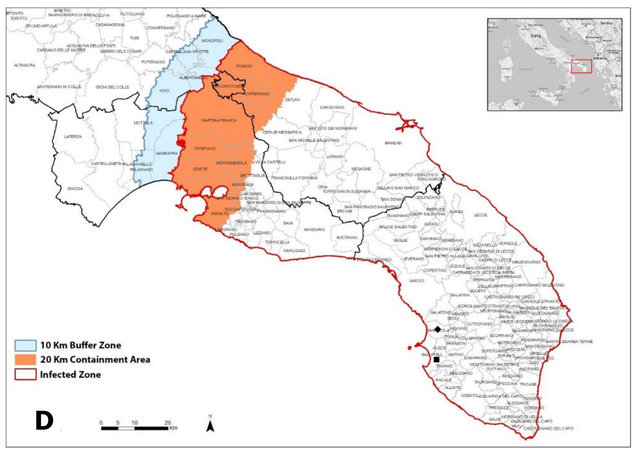
INTRODUCTION

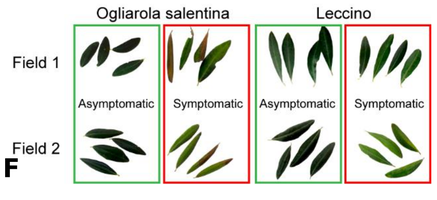
Xylella fastidiosa (Xf) is a vector-borne plant pathogen bacterium known to cause serious diseases on many crops of agronomic relevance, i.e. Pierce's disease on grapevine, citrus variegated chlorosis (CVC) and almond leaf scorch (ALS). Diseases caused by Xf had been confined to the Americas up to the very recent years, when Saponari et al. (2013) reported the first confirmed detection of the pathogen in the European territory. The pathogen was identified as the aetiological agent of a severe disease on olive trees, named "Olive quick decline syndrome" (OQDS), rapidly spreading throughout the southern part of the Apulia Region (Italy) (Saponari et al., 2017). The disease is characterized by initial leaf scorch and scattered desiccation of small branches that over time get worse and extend to the whole canopy (Martelli, 2016).
While seeking effective methods to contrast and prevent a further spread of the disease, several field observations in the OQDS-affected area, have demonstrated a different varietal response to bacterial infection in terms of incidence of infection and severity of symptoms. In particular olive cvs 'Cellina di Nardò' and 'Ogliarola salentina' are highly susceptible showing progressive desiccation of the canopy, that often culminating in the death of the plant, while the resistant cv 'Leccino' shows milder symptoms that often do not advance (Boscia et al., 2017).
The mechanism by which *Xylella fastidiosa* causes the disease is not yet completely understood. The most reliable hypothesis is that the bacterium is responsible for blocking xylem vessels and thus transporting water and nutrients from the roots to the canopy, resulting in desiccation symptoms. Several studies have been done to understand how the plant interacts with the pathogen and how tolerance and/or resistance phenomena can develop.
A number of experiments have demonstrated important roles of mineral elements for Xf in vitro and during infection in planta. The analysis of the ionome of field-grown grapevines, blueberry and pecan and greenhouse-grown *Nicotiana tabacum* plants showed significant changes in mineral element contents among infected and non-infected samples (De La Fuente et al., 2013). Similarly, virulence traits such as adhesion, biofilm formation and motility are modulated by mineral elements (Leite et al., 2002; Cruz et al., 2012).
Based on these recent findings, a field survey was carried out on naturally infected olive groves to determine the leaf ionome profile of two cultivars showing a different response to Xf infection. This study allows us to suggest hypotheses about the elements that may be involved in the success of the infection and/or in the development of symptoms.

***A**) Leaf scorching symptoms in olive; **B**) Initial desiccation in Xf-infected trees of the cv Ogliarola s.; **C**) Xf-infected trees of the cvs Leccino (left) and Ogliarola (right).*

MATERIALS AND METHODS



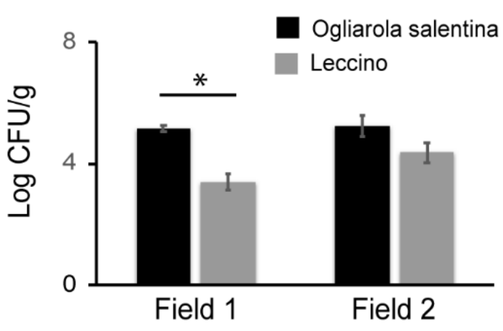
FIELD	VARIETY	N° TREES	N° REPLICATES	TOT SAMPLES
FIELD 1	LECCINO	10	5 ASYMP - 5 SYMP	100
	OGLIAROLA	10	5 ASYMP – 5 SYMP	100
FIELD 2	LECCINO	4	5 ASYMP - 5 SYMP	40
	OGLIAROLA	4	5 ASYMP - 5 SYMP	40
E				280



Two fields were selected in the *Xf*-infected area for the presence of both cultivars grown under the same agronomic regime. For each tree, five symptomatic and five asymptomatic branches were selected and mature leaves were used for the study. qPCR was performed to estimate the bacterial population within the tissues, by using a standard curve established with 10-fold serial dilutions of Xf DNA.
Symptomatic leaves and asymptomatic leaves were subjected to the analysis of the ions content by Inductively coupled plasma - optical emission spectrometry (ICP-OES), prior digestion in mineral-free concentrated nitric acid.

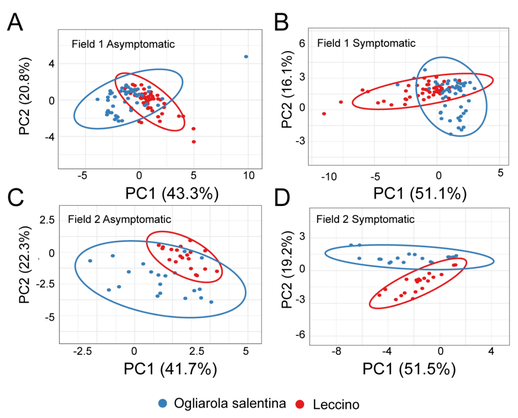
***D**) Location of plots sampled in the infected area; **E**) Summary table of samples analyzed with ICP-OES and qPCR; **F**) Rapresentative symptomatic and asymptomatic leaves used for the study.*

RESULTS



Population size of *Xylella fastidiosa* within the two cultivars

Xf detection showed that all plants selected for the analysis were infected by Xf. The estimation of the bacterial concentration showed that Xf was about one order of magnitude higher in the 'Ogliarola salentina' than in 'Leccino', although only in Field 1 the difference in Xf population was statistically significant ($p<0.05$). The quantification of the bacterial population in the two cultivars grown in the field under high infection pressure confirms the resistance of 'Leccino' to *X. fastidiosa* infection, since the growth of the bacterium is restricted and this leads to milder symptoms when compared to 'Ogliarola salentina'.



Principal component analysis

The analysis showed that 'Ogliarola salentina' and 'Leccino' have a different mineral profile independent of the disease status, since the two cultivars separated along both principal component 1 (PC1) and principal component 2 (PC2) in both symptomatic and asymptomatic samples.

		Ca	K	Mg	S	P	Fe	Mn	Na	Zn
Location#		**ASYMPTOMATIC**								
Field 1	% change	4*	**23**	14	-11	**-15**	**-57**	**43**	**30**	**39**
	p-value	0.53	**6.60E-04**	0.08	0.14	**6.66E-04**	**1.13E-07**	**7.64E-05**	**2.94E-02**	**6.52E-03**
Field 2	% change	**-40**	11	**-34**	-5	-10	**51**	**50**	-34	**-34**
	p-value	**6.6E-03**	0.10	**2.69E-03**	0.59	0.11	**6.42E-03**	**2.43E-03**	0.14	**4.12E-03**
		SYMPTOMATIC								
Field 1	% change	**21**	**35**	-1	4	**-23**	**-52**	**41**	-3	8
	p-value	**2.5E-02**	**9.79E-07**	0.93	0.59	**8.39E-07**	**3.39E-08**	**2.04E-03**	0.85	0.56
Field 2	% change	-22	13	-25	-7	-10	**45**	**100**	**-61**	**-45**
	p-value	0.24	0.25	0.17	0.68	0.24	**0.043**	**2.80E-04**	**1.17E-02**	**2.85E-03**

Changes in element content in olive 'Leccino' versus olive 'Ogliarola salentina'

Numbers indicate the percentage of change in element concentration in 'Leccino' compared to 'Ogliarola Salentina'. Numbers in bold indicate significant differences according to Student's t-test ($p<0.05$).
Assessing levels of nutrients to identify elements that could be relevant in the resistance of 'Leccino', manganese (Mn) is the only nutrient that consistently changed in both fields with an increase in 'Leccino' relative to 'Ogliarola salentina'. Therefore a potential protective role may be suggested for Mn. Also Zn and Fe levels are different among the cultivars but these elements are increased in one field and decreased in the other.

		Ca	K	Mg	S	P	Fe	Mn	Na	Zn
		Field 1								
'Leccino'	% change	**19***	9	-12	-9	3	10	2	**79**	2
	p-value	**1.61E-02**	5.96E-02	1.43E-01	7.10E-01	5.19E-02	1.65E-01	8.19E-01	**1.19E-03**	7.86E-01
'Ogliarola salentina'	% change	-3	-1	2	0	-12	-1	4	**139**	31
	p-value	6.74E-01	9.20E-01	8.51E-01	6.67E-02	9.69E-01	9.17E-01	7.57E-01	**1.08E-05**	1.20E-01
		Field 2								
'Leccino'	% change	**76**	-8	**56**	**8**	33	**38**	**81**	**220**	**45**
	p-value	**6.16E-04**	1.53E-01	**1.87E-02**	**6.12E-03**	1.97E-01	**7.80E-03**	**1.23E-04**	**7.23E-05**	**5.89E-03**
'Ogliarola salentina'	% change	37	-10	36	8	36	44	36	**449**	**72**
	p-value	1.53E-01	6.40E-01	9.22E-02	1.14E-01	7.30E-01	1.09E-01	7.86E-02	**5.07E-04**	**2.92E-03**

Changes in element content in symptomatic leaves versus asymptomatic leaves

Numbers indicate the percentage of change in element concentration in symptomatic leaves compared to asymptomatic leaves. Numbers in bold indicate significant differences according to Student's t-test (p-values < 0.05).
Data revealed a significant increase in Na levels in symptomatic leaves of both cvs in the two fields, suggesting a physiological role of this element. Moreover, the transition from asymptomatic to symptomatic phenotypes in leaves showed a remodeling of the ionome, particularly a higher Ca content in symptomatic leaves of 'Leccino'.

CONCLUSIONS

Our data suggest hypotheses about elements that may be involved in the success of the infection and/or in the development of symptoms. Ionomics revealed potential phenotypic differences between the susceptible and the resistant cultivar, extending the knowledge about the differential response to *X. fastidiosa* infection. This is relevant, as the discovery of traits of varietal resistance represents the most promising strategy to coexist with the pathogen in the area affected by the disease and prevent its spread in the European territory.
Experiments under controlled conditions are ongoing to test the hypotheses and further define the role of mineral elements in host-pathogen interaction.

ACKNOWLEDGEMENTS

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REFERENCES

De La Fuente, L., Parker, J. K., Oliver, J. E., Granger, S., Brannen, P. M., van Santen, E., & Cobine, P. A. (2013). The bacterial pathogen Xylella fastidiosa affects the leaf ionome of plant hosts during infection. PloS One, 8(5), e62945.

Saponari, M., Boscia, D., Nigro, F., & Martelli, G. P. (2013). Identification of DNA sequences related to Xylella fastidiosa in oleander, almond and olive trees exhibiting leaf scorch symptoms in Apulia (southern Italy). Journal of Plant Pathology, 95(3).

Saponari, M., Boscia, D., Altamura, G., Loconsole, G., Zicca, S., D'attoma, G., ... & Savino, V. N. (2017). Isolation and pathogenicity of Xylella fastidiosa associated to the olive quick decline syndrome in southern Italy. Scientific reports, 7(1), 17723.

Boscia D, Altamura G, Dongiovanni C, Giampetruzzi A, La Notte P, Loconsole G, Martelli GP, Morelli M, Palmisano F, Potere O, Saldarelli P, Savino VN, Susca L, Tavano D, Zicca S, Saponari M. 2017. Screening of olive germplasm for resistance to Xylella fastidiosa ST53: the state of the art. In: Proceedings of the European conference on Xylella fastidiosa: finding answers to a global problem, Palma de Mallorca, Spain, 13-15 November 2017, 12-13. https://doi.org/10.5281/zenodo.1051209.

Martelli GP. The current status of the quick decline syndrome of olive in southern Italy. Phytoparasitica. 2016; 44(1):1-10.

Cruz LF, Cobine PA & De La Fuente L. Calcium increases surface attachment, biofilm formation, and twitching motility in Xylella fastidiosa. Appl Environ Microbiol. 2012; 78(5): 1321-1331.

Cobine PA, Cruz LF, Navarrete F, Duncan D, Tygart M & De La Fuente L. Xylella fastidiosa differentially accumulates mineral elements in biofilm and planktonic cells. PLoS One. 2013; 8(1): e54936.

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