

Search for crop species immune to *Xylella fastidiosa* subsp. *pauca*, ST53

**Montilon V.¹, Cavalieri V.², Altamura G.², Zicca S.², Savino V.^{1,3},
Potere O.¹, Susca L.¹**

¹Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi di Bari Aldo Moro, Bari, Italy

²CNR, Istituto per la Protezione Sostenibile delle Piante, Bari, Italy

³Centro di Ricerca, Sperimentazione e Formazione in Agricoltura "Basile Caramia", Locorotondo (Bari), Italy

INTRODUCTION

- Olive quick decline syndrome (OQDS) is a devastating disease emerged in 2013 in the Apulia region (Saponari *et al.*, 2013), in southern Italy, caused by *Xylella fastidiosa* subsp. *pauca* strain ST53 (*Xfp*) (Loconsole *et al.*, 2016). It is particularly aggressive on olive and on other species (oleander, *Acacia* spp, *Polygala myrtifolia*), causing severe desiccation that leads to the death of the plants (**Fig.1**). Instead, asymptomatic infections occur in some hosts (*Rhamnus alaternus*, *Myoporum insulare*, *Westringia glabra*) (Saponari *et al.*, 2019) that may have however an epidemiological



Fig.1: Symptoms of leaf scorch (left) and desiccation (right) on olive trees affected by OQDS.

role as a source of primary inoculum. Up to date 35 different host species have been reported susceptible to the Apulian strain of *Xfp*. On the other hand, our previous investigations demonstrated that this genotype was not able to infect *Vitis* spp., several species of *Citrus* and the *Prunus* hybrid GF677 (Saponari *et al.*, 2016).

In the last 3 years, we extended the investigations by performing a series of artificial inoculations on different species, with the aim of identifying additional immune species. These included *Pyrus communis*, *Juglans regia*, *Ceratonia siliqua*, *Mespilus germanica*, *Diospyros kaki*, *Citrus limon*, *Punica granatum*, *Persea Americana*. Moreover, vector-mediated transmission in open field conditions was also used for *P. americana*.

Inoculated plants were periodically sampled at different distance from the inoculation point and analyzed through qPCR to assess bacterial multiplication and host colonization. In addition, visual surveys were performed to detect any eventual appearance of symptoms of leaf scorching or stunting attributable to disease.

MATERIALS AND METHODS

■ Needle-inoculations under controlled conditions

10 plants for each species were used, in July 2017 for *P. granatum*; October 2017 for *C. siliqua*, *M. germanica*, *P. vera*, *P. americana*; April 2018 for *D. kaki*, *P. communis*, *J. Regia*; July 2018 for *C. limon*. The experiment was repeated for *P. americana* and *M. germanica* between July and August 2018. A suspension in phosphate buffered saline of *Xfp* ST53 colonies from 4-5 subculture grown on BCYE (Wells *et al.*, 1981) or PD3 (Davis *et al.*, 1980) medium, 1×10^9 CFU mL⁻¹ concentrated, was used. 10 µl of suspension was placed on the stem at the node level



Fig.2: *Xfp* ST53 colonies on PD3 medium (left), needle inoculation under controlled conditions (right)

and 4-5 needle punctures of the stem were performed to reach the xylem (**Fig.2**). Perwinkle plants were used as a susceptible control; 3 plants of each species were mock-inoculated and used as negative controls.

■ Vector-transmission under field conditions

In August 2018, 12 plants of *P. americana* were caged with an anti-insect net by introducing inside 5 adults of *Philaenus spumarius*, captured in olive groves with high incidence of OQDS. Recipient olive plants were used as susceptible controls.

■ qPCR assays and visual surveys

The occurrence of *Xfp* was assessed in leaf midrib, petioles while in wood stem lastly through qPCR. Visual inspections were performed to check for the appearance of symptoms. Inoculated plants were analyzed starting 5 months after the infection at the inoculation point (i.p.) and subsequently at 10cm and over 10cm from the i.p. after 6 and 8 months respectively. All plants exposed to natural infection were periodically inspected and tested after 5 and 15 months from the start of the transmission test by qPCR, by randomly sampling each plant. Also insects used in the transmission trials were tested by qPCR

RESULTS

Needle-inoculation under controlled conditions

Xfp was not detected from all samples distal (>10cm) from the i.p. and no symptoms of leaf scorching were observed in any of the plants of the evaluated species in both tests performed (**Tab.1**). Positive or dubious qPCR reactions were obtained in correspondence of the i.p.

Tab.1: qPCR results of artificial inoculation tests under controlled conditions in the first test (**a**) and in the second replicate of the experiment (**b**).

a.

Species	Inoculation	I assay qPCR - inoculation point			II assay qPCR - 10 cm			III assay qPCR - above 10 cm		
		Date	% positive	Ct mean	Date	% positive	Ct mean	Date	% positive	Ct mean
<i>Punica granatum</i>	July 2017	June 2018	88,9	26,1	May 2018	0	NA	June 2018	0	NA
<i>Ceratonia siliqua</i>	October 2017	March 2018	0	NA	March 2018	0	NA	June 2018	0	NA
<i>Mespilus germanica</i>	October 2017	June 2018	100	27,5	March 2018	0	NA	May 2018	0	NA
<i>Persea Americana</i>	October 2017	March 2018	20	31,8	March 2018	40	31,05	June 2018	0	NA
<i>Diospyros kaki</i>	April 2018	November 2019	42,8	29,4	November 2019	14,3	30,67	January 2021	0	NA
<i>Pyrus communis</i>	April 2018		-	-	March 2019	0	NA		-	-
<i>Juglans regia</i>	April 2018		-	-	November 2019	0	NA		-	-
<i>Citrus limon</i>	July 2018	March 2019	100	29,7	March 2019	0	NA	November 2019	0	NA

b.

Species	Inoculation	I assay qPCR - inoculation point			II assay qPCR - 10 cm			III-IV assay qPCR - above 10-15 cm		
		Date	% positive	Ct mean	Date	% positive	Ct mean	Date	% positive	Ct mean
<i>Mespilus germanica</i>	August 2018	February 2019	22,2	30,8	February 2019	0	NA	November 2019-July 2020	0	NA
<i>Persea Americana</i>	July 2018	January 2019	6,2	31,67	January 2019	5,5	29,5	September 2019-July 2020	0	NA

even several months after inoculation. In *D. kaki* and *P. americana*, positive amplification reactions were recorded for some samples at 10cm from i.p. after 19 and 5 months respectively. Nevertheless, isolation attempts to verify the viability of the *Xfp* cells from stem samples of 3 plants of *P. americana*, after 26 months since inoculation (September 2020), gave negative results. Periwinkle plants tested positive at different levels far from the i.p. without showing any symptoms throughout the duration of test.

Vector-transmission under field conditions

The results are in agreement with those obtained from artificial inoculations. No transmission of *Xfp* infection to *P. americana* plants exposed to vector infection was registered (**Tab.2**). Olive used as susceptible control gave positive reaction. The mean percentage of positive insect used in the transmission trial was of about 50%.

Tab.2: qPCR results related to vector-mediated infection tests under open field conditions.

Species	number of plants	start transmission test	end transmission test	I assay qPCR			II assay qPCR		
				date	% positive	Ct mean	date	% positive	Ct mean
<i>P. americana</i>	12	August 2018	September 2018	January 2019	0	NA	November 2019	0	NA

DISCUSSION

■ The diagnostic tests performed repeatedly during 3 years post-inoculation did not reveal the presence of *Xfp* in tissues above 10cm from the inoculation points in any of the inoculated plants. Detection occurred only when leaf petioles from the inoculation points were tested and in some samples of *D. kaki* and *P. americana* at 10 cm from the point of inoculation, due to bacterial cells inoculated or nucleic acids resulting from their degradation. Similarly, no positive detections occurred in the plants of *P. americana* exposed to infected insects. In addition, for *C. limon*, *P. granatum*, *P. communis* and *C. siliqua*, surveys

conducted in these last years in the heavily infected area did not reveal the occurrence of natural infected plants (Regione Puglia, 2015; Saponari *et al.*, 2016). Overall, the data suggest that these species do not support the multiplication and the spread of the bacterium. However, even if for *P. Americana* needle-inoculations were complemented with vector transmissions, for the remaining species mechanical inoculations should be integrated by vector-mediated transmission tests for conclusive assessment of their immunity.

BIBLIOGRAPHICAL REFERENCES

- Davis, M. J., Purcell, A. H., & Thomson, S. V. (1980). Isolation media for the Pierce's disease bacterium. *Phytopathology*, 70(5), 425-429.
- Loconsole, G., Saponari, M., Boscia, D., D'Attoma, G., Morelli, M., Martelli, G. P., & Almeida, R. P. P. (2016). Intercepted isolates of *Xylella fastidiosa* in Europe reveal novel genetic diversity. *European Journal of Plant Pathology*, 146(1), 85-94.
- Regione Puglia – Relazione su *Xylella fastidiosa*. Situazione a Marzo 2015. REGIONE PUGLIA. Area Politiche per lo Sviluppo Rurale.
- 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., D'Attoma, G., Cavalieri, V., Zicca, S., ... & Di Carolo, M. (2016). Pilot project on *Xylella fastidiosa* to reduce risk assessment uncertainties. *EFSA Supporting Publications*, 13(3).
- Saponari, M., Giampetruzzi, A., Loconsole, G., Boscia, D., & Saldarelli, P. (2019). *Xylella fastidiosa* in olive in Apulia: Where we stand. *Phytopathology*, 109(2), 175-186.
- Wells, J. M., Raju, B. C., Nyland, G., & Lowe, S. K. (1981). Medium for isolation and growth of bacteria associated with plum leaf scald and phony peach diseases. *Applied and Environmental Microbiology*, 42(2), 357-363.