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Exploring a sustainable solution to control *Xylella fastidiosa* subsp. *pauca* on olive in the Salento Peninsula, Southern Italy

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ABSTRACT

The effectiveness of NuovOlivo®, a natural detergent made from plants oils and extracts of multi botanical species plus sodium and calcium hydroxide, and sulphur, activated with sodium bicarbonate (patent n. 102017000109094 Ministero dello Sviluppo Economico, Italy) was tested in the control of Olive Quick Decline Syndrome associated with *Xylella fastidiosa* subsp. *pauca*. Tests were conducted in two naturally infected olive groves located in the province of Lecce, Apulia, Italy). The first experiment included 70 to 75-year-old olive trees cv Cellina di Nardò, in Montesano Salentino. Here the percentage of McKinney's disease index was reduced to 2.5% and plants produced drupe fruits after seven treatments. Olive trees were sprayed twice per year (in March and October) and pruned in winter. The second experiment included 60 to 65-year-old olive trees cv Ogliarola salentina growing in Sternatia which initially showed a disease index of 90.88%. This figure was reduced to 4.0% after two years of three spray treatments. In both olive cultivars, qRT-PCR resulted in lower levels of *X. fastidiosa* DNA in the treated trees. The leaves of treated plants showed a low total phenolic content and no cell membrane damage associated to lipid peroxidation and electrolyte leakage. NuovOlivo® works as a curative product limiting and/or stopping the destructive epidemic caused by this bacterium.

1. Introduction

A spectral landscape characterizes the Salento Peninsula of Apulia in Southern Italy from Brindisi to Gallipoli and Santa Maria di Leuca. Diseased and withered olive (*Olea europaea* L.) trees will be uprooted, destroyed and practically erased from the Apulian culture. In the Classical Greek period, the philosopher Aristotle (384–322 BC) stated in the Athenian Constitution: "… *anyone who digs up or cuts down a sacred olive-tree, is to be put on trial by the council of Areopagus, and, if found guilty, he is to be put to death* …" (https://oll.libertyfund.org). Today, the xylem-limited bacterium *Xylella fastidiosa* Wells, Raju, Hung, Weisburg, Parl & Beemer is one of the most harmful plant pathogens worldwide (Hopkins, 1989). It is the silent executioner that is killing olive trees in the Salento Peninsula. This bacterium attacks several plant species that are highly profitable such as almond, cherry, myrtle-leaf milkwort and oleander (Saponari et al., 2013; Cariddi et al., 2014). The infected olive groves covered 8000 ha in 2013 and this increased to 750,000 in 2018 (https://www.camera.it/temiap/documentazione/temi/pdf/1161584.pdf?_1567979659615).

X. fastidiosa subsp. *pauca* is the etiological agent of "rapid olive decline complex" ("complesso del disseccamento rapido dell'olivo", Co. Di.R.O.) formerly known as "Olive Quick Decline Syndrome" (OQDS) in the Salento area on cv Ogliarola di Lecce (≡ 'Ogliarola salentina') and 'Cellina di Nardò' (Saponari et al., 2013; Cariddi et al., 2014; Martelli et al., 2015). Mismatches in the genomes of Salento-1, Salento-2 and De Donno strains, lead to the hypothesis that micro-evolutionary forces started to produce some variations in the Salento population of *X. fastidiosa* (Ramazzotti et al., 2018). Included on the list of A1 EPPO quarantine organisms, since September 2017, *X. fastidiosa* has been on the A2 list (n 166) as a locally present bacterium (http://www.eppo.int/QUARANTINE/listA1.htm).

Olive plants affected by OQDS show leaf scorch, the desiccation of

Abbreviations: ANOVA, Analysis of variance; BCYE, buffered charcoal yeast extract agar; C, conductivity; CTAB, Cetyl trimethylammonium bromide; DI, disease index of McKinney's; diam, diameter; EDTA, Ethylenediaminetetraacetic acid; GAE, gallic acid equivalents; GLM, General Linear Model; LSD, Least Significant Difference; MDA, Malondialdehyde; PCR, Polymerase Chain Reaction; OQDS, Olive Quick Decline Syndrome; qRT-PCR, quantitative real-time PCR; sd, standard deviation; TCA, trichloroacetic acid; TE buffer, Tris HCl and EDTA.

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twigs and branches which prevail in the upper canopy in the early stages of infection and on the whole plant in the later stage. Leaf tips and edges turn from dark yellow to brown. Drupes mummify. Leaves with leaf scorch and mummified drupes remain attached to shoots (EPPO, 2018; Catalano et al., 2019). Twigs, branches and trunk show an irregular discolouration of xylem vessels (Nigro et al., 2013). Apulian olive cultivars infected by *X. fastidiosa* subsp. *pauca* strain CoDiRO express genes related to drought resistance stress (Giampetruzzi et al., 2016). The ionome composition of the plants plays a role in protecting olive against OQDS (D'Attoma et al., 2019).

In Italy, *Philaenus spumarius* L. transmits the bacterium from tree to tree (Cornara et al., 2016). Molecular analyses have ascertained the presence of *X. fastidiosa* in *Neophilaenus campestris* (Fallén), *Euscelis lineolatus* Brullé and *Ph. italosignus* Melicha (Elbeaino et al., 2014).

No effective means for *X. fastidiosa* management is available worldwide. Generally speaking, to control bacterial diseases, an important point is prevention by avoiding the introduction of infected plant material. When the bacterium is already present, an integrated disease management approach (resistant or tolerant host plants, treatments against the vector, and cultivation practices) may limit the spread of the pathogen. Copper compounds and antibiotics are efficient against plant-pathogenic bacteria. The application of antibiotics in plant protection is restricted worldwide. According to FRAC, copper has a multi-site action, and thus it is low risk in terms of resistance. But, copper resistance is encoded by a copper-inducible operon (copABCD) in *Pseudomonas syringae* pv. *tomato*, *Xanthomonas citri* subsp. *citri* and *X. alfalfae* subsp. *citrumelonis* (Behlau et al., 2011; Mellano and Cooksey, 1988). Biological control agents, antibacterial peptides, bacteriophages and systemic acquired resistance inducers were shown to affect plant-pathogenic bacteria. T3SS apparatus, quorum sensing cell-to-cell communication and bacterial biofilms are the targets of innovative control methods, while nanoparticles are bactericides or carriers of antimicrobials (Sundin et al., 2016). Microbial endophytes, natural fungal products, bacteriophages and inoculation of weakly virulent/avirulent strains of *X. fastidiosa* could provide protection against Pierce's disease of grapevine (Rolshausen et al., 2018; Bragard et al., 2019).

In the Salento area of Southern Italy, control management of *X. fastidiosa* is nullified because of the high susceptibility of the olive cultivars, extensive olive monoculture, the xylem localization of the bacterium, and the high transmission efficiency of vector insects.

Two cultivars, Leccino and FS-17 (Favolosa), proved symptomless with a low presence of bacteria in the xylem (Catalano et al., 2019). These cultivars are also used as scions on old trees (https://www.eurone ws.com/2019/02/18/europe-steps-up-fight-to-stop-deadly-olive-tree-disease).

Infected olive trees treated with resistance inducers show an increase in vegetation, and decreased symptoms (Carlucci et al., 2016). Fosetyl aluminium, acibenzolar-S-methyl, chitosan-oligosaccharides mixed with oligogalacturonides, σ-β harpin protein, cerevisanae or N-acetylcysteine caused a partial decrease in disease incidence and severity (Dongiovanni et al., 2017). Six spray treatments per year with Dentamet® (a zinc, copper and citric acid bio-complex) reduced OQDS severity and *X. fastidiosa* in infected olive trees (Scortichini et al., 2018). The above mentioned control measures were able to reduce symptoms caused by *X. fastidiosa* (Bragard et al., 2019).

In this research, we will report on the effects of NuovOlivo® in two olive groves severely affected by OQDS. Quantitative real-time PCR was used to compare *X. fastidiosa* density between treated and control olive plants. The effects on some morfological and physiological features, such as leaf surface, total phenol content, and membrane integrity were also evaluated.

2. Materials and methods

2.1. Products, plant material and treatments

NuovOlivo® (Ministero dello Sviluppo Economico patent n 102017000109094, Italy) is a natural detergent made with plant oils and water-infusion from multi botanical species, plus sodium and calcium hydroxide, in addition to sulphur. NuovOlivo® includes *Thymus vulgaris* L., *Petroselinum crispum* (Mill.) Fuss, *Crataegus monogyna* Jacq., *Rosmarinus officinalis* L., *Salvia officinalis* L., *Origanum vulgare* L., *Matricaria chamomilla* L., *Malva sylvestris* L., *Salix babylonica* L., *Capsicum annuum* L., *Piper nigrum* L. and other Mediterranean plant species.

Trials were performed on naturally infected olive plants showing 85–95% of the canopy with OQDS symptoms at two sites near Lecce (Apulia, Southern Italy). At the first site in Montesano Salentino, 70-75 year-old plants of 'Cellina di Nardò' were used during the four-years of testing. The rows were labelled from A to F and the plants in each row were numbered from 1 to 7. Plants were heavily pruned at the end of January 2016 except for plants B7, C3, D4, E7 and F3 that were preserved with leaf scorch and withered branches. The plants in rows E and F were used as non-treated controls, while the others were treated with NuovOlivo® in early April and early October 2016 and in the following three years. Ten blocks, each with plants in randomized positions, were performed (Fig. 1). Every winter, the dried branches were removed except for plants B7, C3, D4, E7 and F3. At the second site in Sternatia, 60-65 year-old 'Ogliarola salentina' plants were tested. Among the diseased plants in this grove, 33 were chosen for a two-year trial and numbered. Twenty-one plants were heavily pruned in February 2018, whereas the other twelve were not. Six pruned trees received two treatments in early June 2018 and early April 2019; 15 (9 pruned and 6 unpruned) were treated three times: in early June and early September 2018, and in early April 2019; 6 were used as pruned controls; and 6 as unpruned controls. For each variable (pruned-control, pruned-treated-twice, pruned-treated-three-times, unpruned-control, and unpruned-treated-three-times), trees were randomly included in three blocks (Fig. 1). In both groves, plants for testing were sprayed (canopy and trunk) using a hydraulic sprayer at 22 ± 2 bar equipped with a high-pressure-operated lance and having a 1.5 mm diameter nozzle. Single trees received nearly 10 L of water containing 2% NuovOlivo® and 1% sodium bicarbonate as an activator added to the solution just before use. In both groves, the population of *P. spumarius* was controlled by mechanically removing wild plant hosts of the vector, according to EU regulation 2015/789.

Disease severity was assessed by the data collector using a 0 to 5 rating scale in which 0 = no symptoms, 1 = 1–10% wilted canopy, 2 = 11–30% wilt, 3 = 31–60% wilt, 4 = 61–80% wilt, 5 = 81–100% wilted canopy. For each block, McKinney's disease index (DI) was calculated by applying the formula $DI = [\Sigma_{(d \times f)}/(N \times H)] \times 100$ where: d is the scale rate, f is the number of plants in a given scale rate, N is the total number of assessed trees, H is the highest scale rate. On 'Ogliarola salentina', OQDS symptoms were estimated in early June, August and September 2018, and early March, June, August and September 2019. On 'Cellina di Nardò', records were taken in early April 2016, and early September 2016, 2017, 2018 and 2019. DI percent decrease over the controls was calculated as 100 × [(DI in controls)-(DI in treated)]/(DI in controls).

2.2. X. fastidiosa assessments in plants

2.2.1. Indirect immunofluorescence

The indirect immunofluorescence test by Cariddi et al. (2014) was used to detect *X. fastidiosa* in leaf extracts.

2.2.2. PCR detection

The presence and quantification of the bacterium were determined by Polymerase Chain Reaction (PCR) and quantitative real-time PCR (qRT-PCR) respectively. Twigs from selected plants (Table 1) were

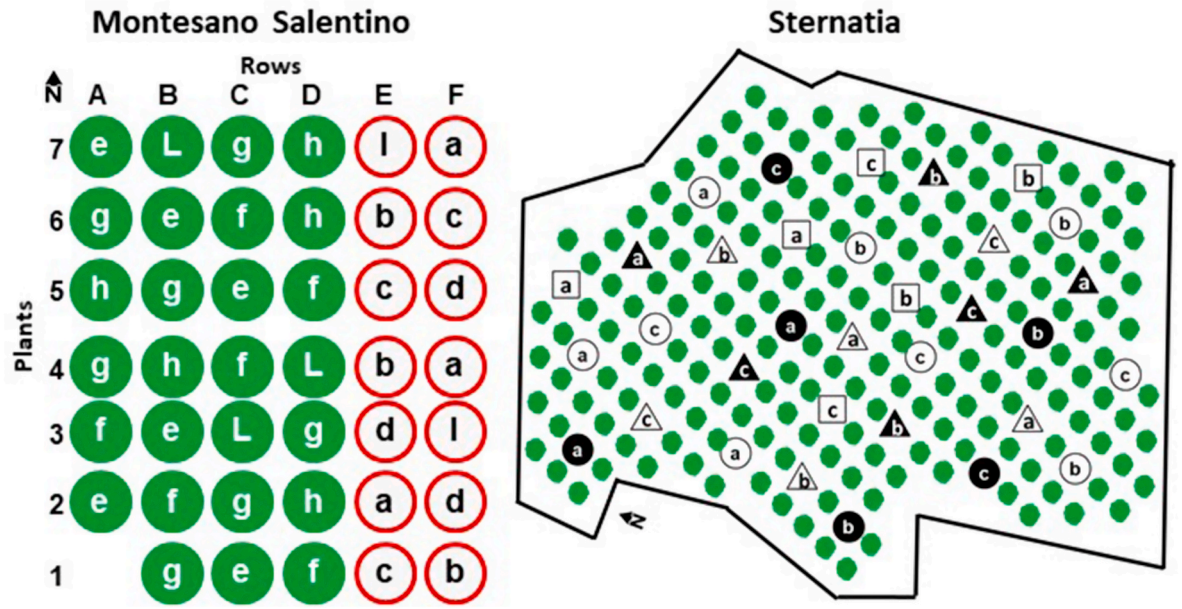

Fig. 1. Spatial distribution of olive plants. In the Montesano Salentino olive grove, 'Cellina di Nardò' trees were heavily pruned in January 2016 except those labelled as "I" and "L", those in rows E and F (○) were used as untreated controls, while plants in rows A-D (●) were treated in early April and October 2016, 2017, 2018, and 2019. 'Ogliarola salentina' plants in Sternatia were heavily pruned (empty symbols) in February 2018, whereas the others (full symbols) were not. Plants received two (◘), three (○) or no (□) treatments. Letters indicate the individual blocks. (For colour figure, the reader is referred to the Web version of this article)

Table 1
Selected plants used for Polymerase Chain Reaction (PCR) and quantitative real-time PCR assessment of *Xylella fastidiosa* DNA.

Thesis	Cellina di Nardò		Ogliarola salentina	
	Sternatia	Bari	Montesano	Bari
Pruned and untreated	S26		E3	
Pruned and treated 2 times	S10, S12			
Pruned and treated 3 times	S02, S05			
Pruned and treated 7 times			A3	
Unpruned and untreated	S28		F3	
Unpruned and treated 3 times	S31			
Unpruned and treated 7 times			C3	
Healthy control plants		CeN–V		Og-V

collected on 23 April and 16 September 2019 with the authorization of the Italian Ministry of Agricultural, Food and Forestry Policies. The protocol described by Valentini et al. (2017) was applied with minor adjustments. From each tree, twelve 15–20 cm long twigs were taken at three different heights of the canopy, i.e. bottom, middle and top. Canopy samples were collected every 30° as using a goniometer. The petioles, midribs and basal portion of 60 leaves per plant (5 per twig) were used for DNA extraction and crushed in a mortar with a pestle as an adjustment of EPPO (2018) procedure. Suspensions and solutions were made in sterile MilliQ water. CTAB-extraction-buffer (2% hexadecyltrimethylammonium bromide, 100 mM Tris-HCl, 1.4 M NaCl, 20 mM EDTA, and, added just before use, 1% polyvinylpyrrolidone and 0.2% β-mercaptoethanol) was used. About 700 mg of crushed material was suspended in 4 ml of CTAB-extraction-buffer, kept 15 min in boiling water, crushed with 50 mg of sterile quartz sand and maintained at 65 °C for 30 min. Material collected in September 2019 was extracted in the field, the extracts were sealed in plastic bags and stored in a cooler until it reached the lab. Plants debris and sand were removed by centrifugation (10,000 rpm, 4 °C, 5 min, SL8R centrifuge; Thermo Fisher Scientific, Waltham, MA, USA). The DNA was extracted with chloroform/isoamyl alcohol (24:1), precipitated with −20 °C isopropanol, rinsed twice with 1 ml of 70% ethanol (−20 °C), air-dried and dissolved in 50 μl of warm (40 °C) TE buffer (10 mM Tris pH 8.00, 1 mM EDTA). Control DNA was extracted from uninfected healthy 'Cellina di Nardò' and 'Ogliarola salentina' plants growing in Northern Apulia at the Experimental Station of the University of Bari in Valenzano (Bari province) 200 km north of the *Xylella*-infected area (Table 1). Positive DNA of *X. fastidiosa* was extracted from 1.7 10^8 cells of strain XFS25 recovered from plant S25 of 'Ogliarola salentina'. Twigs (4–5 cm long, 5 mm in diam) were surface-disinfected by soaking sequentially in a 2% NaClO (commercial bleach) in water for 2 min, then in 70% ethanol for 2 min followed by three rinses in sterile water. The cut ends were crushed with a mechanic's pliers and the sap blotted onto buffered charcoal yeast extract agar (BCYE) Petri dishes that were incubated at 28 °C in the dark (Cariddi et al., 2014; EPPO, 2018).

The quantities of DNA were determined by a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific). DNA with $OD_{(260/280)}$>1.9 and $OD_{(260/230)}$>1.9 were used for PCR and qRT-PCR analyses.

PCR amplification was performed as reported by EPPO (2018) in a programmable iCycler (Bio-Rad Laboratories, Hercules, CA, USA), using RST-31 (5′-GCG TTA ATT TTC GAA GTG ATT CGA TTG C-3′) and RST-33 (5′-CAC CAT TCG TAT CCC GGT G-3′) primers. The following PCR amplification conditions were run: 95 °C for 1 min, 40 cycles of (94 °C for 30 s, 55 °C for 30 s, 72 °C for 60 s), and a final step of 72 °C for 10 min. A total PCR volume of 25 μl was used, containing 12.5 μl Hot Start 2 × PCR mix (LeGene Biosciences, San Diego, CA, USA), 0.5 μl forward primer (10 μM), 0.5 μl reverse primer (10 μM), 4 μl of template DNA (≅ 50 ng) and 7.5 μl sterile water. TE buffer and water were performed as negative controls. To check PCR efficiency, DNA fragments were run on 1.0% agarose gel (Modesti et al., 2017; EPPO, 2018).

2.2.3. qPCR analyses

X. fastidiosa quantification was assessed by qRT-PCR using the Bio-Rad CFX96 Real-Time System. Each sample amplification was performed in triplicate in a final volume of 20.0 μl including 2 μl of template DNA, 10 μl of 2 × qPCR master mix (BioRad), 0.6 μl of 10 μM Xf-F (5′-CAC GGC TGG TAA CGG AAG A-3′) forward primer, 0.6 μl of 10 μM Xf-R (5′-GGG TTG CGT GGT GAA ATC AAG-3′) reverse primer, 0.2 μl of 10 μM XF-P (5′-6FAM-TCG CAT CCC GTG GCT CAG TCC-BHQ-1-3′) probe, and 0.6 μl sterile MilliQ water. Amplification conditions included 50 °C

for 2 min, 95 °C for 10 min and 39 cycles of 94 °C for 10 s, 62 °C for 40 s (Scortichini et al., 2018; EPPO, 2018). Samples were considered positive when they produced a Ct (Cycle threshold) value < 35. The specificity of PCR products was checked using either DNA from healthy 'Cellina di Nardò' and 'Ogliarola salentina' plants or water. Standard curves were elaborated with *X. fastidiosa* DNA at 0.01, 0.05, 0.1, 0.5, 1.0, 2.0, 5.0, 10, 50, 100 ng. BioRad CFX Manager Version 3.1 software was used to analyse qRT-PCR data. The bacterial concentration detected in plants was expressed as ng mg^{-1} per gram of fresh weight (f wt).

2.3. In planta morphological and physiological effects

The effects of NuovOlivo® on the morphological and physiological aspects of olive plants such as leaf size, total phenolic content and cell membrane integrity were evaluated on leaves collected on 23 April 2019.

Mature leaves (5 per twig, 60 per plant) were photographed and leaf size was measured with an open-source image-processing program ImageJ (National Institute of Health, MD, USA).

Chemicals were purchased from Sigma-Aldrich (part of Merck Corporation, Burlington, MA, USA). Analyses were performed in triplicate.

Fresh leaf tissues (200 mg) were treated with 4 ml of 90% ethanol and 50 mg of sterile quartz sand on ice with a mortar and pestle. Ethanol extracts were centrifuged at 4000×g, 20 min, 4 °C. The total phenolic content was determined according to Buono et al. (2009) with minor adjustments. To 100 μl diluted leaf ethanol extract, 50 μl of 2 N Folin–Ciocalteu reagents and 450 μl of water were added and allowed to stand at room temperature for 3 min. Then 400 μl of 1M Na_2CO_3 were added and incubated in a water bath at 45 °C for 15 min. Absorbance was measured at 765 nm using a Beckman DU 640 (Beckman Coulter Inc., Brea, CA, USA) spectrophotometer and quantified using a gallic acid calibration curve. TPC was expressed as mg of gallic acid equivalents (GAE) g^{-1} leaf f wt.

Cell membrane damage was assessed by measuring lipid peroxidation and electrolyte leakage. Malondialdehyde (MDA), a lipid peroxidation product, was considered as an indicator of oxidative damage (Heath and Packer, 1968). Leaf samples (200 mg) were homogenized in 1 ml of 0.1% trichloroacetic acid (TCA) and 50 mg of sterile quartz sand on ice using a pre-killed mortar and pestle. After centrifugation (15, 000×g, 15 min, 4 °C), 200 μl of supernatant were added to 800 μl of 0.5% (w/v) thiobarbituric acid solution containing 20% (w/v) TCA. The mixture was heated to 90 °C for 30 min and the reaction was stopped by transferring the tubes into an ice bath. After centrifugation (12,000×g, 4 °C, 10 min), absorbance at 532 nm and 600 nm was measured. The non-specific absorbance (600 nm) was subtracted, and MDA concentration was determined using a 155 mM^{-1} cm^{-1} molar extinction coefficient. Results were expressed as nmol MDA per g of leaf f wt. The method devised by Bajji et al. (2002) was applied for electrolyte leakage. Leaf disks (15 per plant; 0.5 cm in diam) were rinsed 3 times (2–3 min) with water (conductivity 0.056 μS cm^{-1}) and floated on 15 ml of water. Each test tube was stirred and kept 16 h at 22±1 °C. Subsequently, the conductivity of the solution (C_i) was measured using a Eutech XS COND 110 (Eutech Instruments Europe B.V., Nijkerk, NL) conductimeter equipped with a 1 cm probe. Then the tubes were autoclaved (121 °C, 20 min) and the final conductivity (C_F) was measured after equilibration at 22 ± 1 °C, and was assumed as 100% electrolyte leakage. The percentage of electrolyte leakage was defined as $(C_i/C_F) \times 100$.

2.4. Statistical analysis

The experimental design of the field trials was adapted to the requests of the property owners, and for this reason, each plant was considered as a single experimental unit. Plants were also randomly combined to form blocks used to calculate disease index. The effects associated to the vector were not considered, because all the plants were naturally infected by *X. fastidiosa* and the vector population was ineffective in transmitting the bacterium. The normality of data and homogeneity of variances were evaluated using Shapiro-Wilk and Bartlett's tests respectively. Data were subjected to variance analysis (ANOVA), using the SAS/STAT version 9.0 (SAS Institute Inc., Cary, NC, USA) General Linear Model (GLM) procedure. The 'cultivar' factor, neither replicated nor randomized, was not included in the statistical analysis. Means were separated by Fisher's least significant difference (LSD) test at $P \leq 0.05$, when the *F*-test was significant. Percent values of McKinney's disease index and electrolyte leakage were transformed to arcsine before analysis. Results were presented with untransformed values as mean ± standard deviation (sd).

3. Results

3.1. OQDS symptom development

In September 2019, untreated check plants of 'Cellina di Nardò' cultivar developed leaf scorch and desiccation extending to the branches and the whole canopy which showed few green leaves (Fig. 2). On the other hand, treated plants produced new vegetation, flowers and drupes, about 20 kg per plant, in the 2019 growing season (Fig. 2). Untreated olive plants used as controls showed neither flowers nor drupes. In September 2019, DI reached 88.33% for pruned control trees, 94.01%

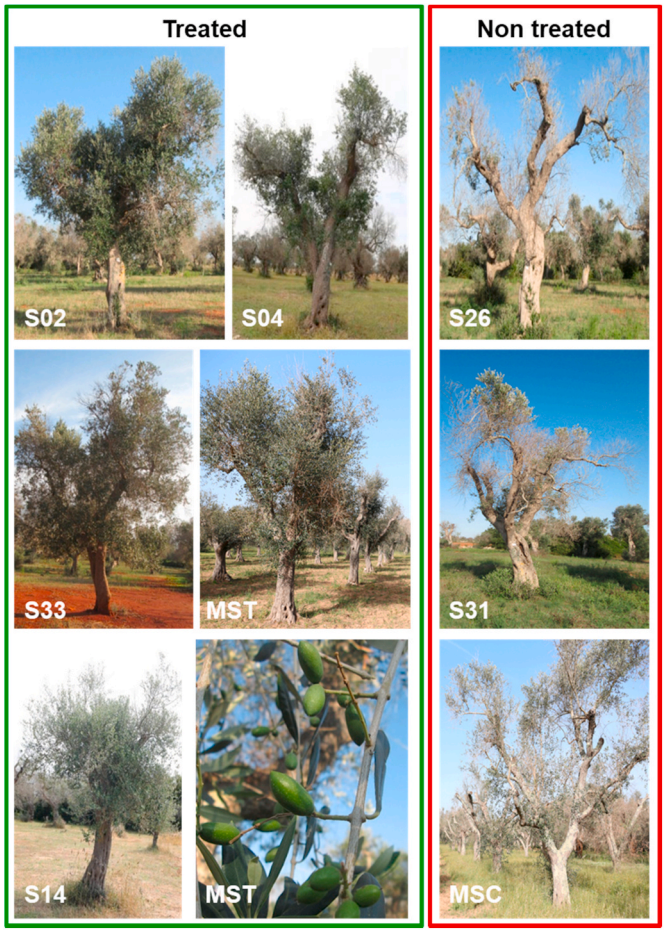

Fig. 2. Olive 'Ogliarola salentina' (S) and 'Cellina di Nardò' (MS) plants. Trees were pruned in February 2018 (S02, S04, S14 and-S26) or un-pruned (S31 and S33). Trees S02 and S04 were treated in early June and September 2018, and April 2019. Plant S14 received two treatments (June 2018 and April 2019). Plants S26 and S31 untreated controls. Plants of 'Cellina di Nardò' treated (MST) with NuovOlivo® in early April and October 2016, 2017, 2018, and 2019 and untreated controls (MSC). (For colour figure, the reader is referred to the Web version of this article).

for unpruned control plants, 2.51% for pruned treated plants, and 6.67% for unpruned treated plants (Fig. 3). The disease index decrease over the controls was reduced to 97.17% on pruned plants and 92.91% on unpruned trees (Fig. 3). Statistic analysis showed that treatment had a highly significant effect, whereas pruning ($P = 0.526$) or treatment and pruning interaction ($P = 0.879$) had no significant effects (Table 2).

Leaf scorch, desiccation and dry canopies were recorded on untreated control trees of 'Ogliarola salentina' cultivar (Fig. 2). In September 2019, DI reached 88.01% on pruned control trees and 96.67% on unpruned control plants. New vegetation, low OQDS symptoms and low DI also occurred on NuovOlivo® treated plants (Fig. 3). In the last survey, the disease index decrease over the controls was up to 58.62% for unpruned treated plants, and up to 92.42% and 95.45% for pruned plants treated two or three times respectively (Fig. 3). No significant effects of pruning or treatment and pruning interaction were shown by statistical analysis (Table 2).

3.2. *X. fastidiosa* assessments in plants

X. fastidiosa was detected by indirect immunofluorescence tests in 'Ogliarola salentina' and 'Cellina di Nardò' at the beginning of the experiments (Fig. 4A and B).

DNA extraction yielded 116–401 mg of DNA per mg of plant material and 96.3 mg from strain XFS25 cells. PCR primers RST-31/33 amplified a fragment of about 733 bp when *X. fastidiosa* DNA was present (Fig. 4C). No DNA bands were detected on TE buffer, water and uninfected 'Cellina di Nardò' and 'Ogliarola' plants from near Bari, a latitude not reached by *Xylella*. The calibration curve $y = 0.4321 \times + 4.3329$ ($R^2 = 0.987$; $E = 93.33\%$) was produced with *X. fastidiosa* DNA by qRT-PCR in the range 0.1–50 ng. *X. fastidiosa* DNA values ranged from 0.006 to 1.1% in plant extract from treated plants and controls respectively. In the Salento area, regardless of cultivar and location, treated plants showed a 99.04% reduction in *X. fastidiosa* DNA content compared with the untreated controls. In leaves collected in September 2019 from NuovOlivo® treated plants showed a further 10–13% decrease in *X. fastidiosa* DNA, whereas untreated plants had a further 25–30% increase (Table 3). Statistic analysis showed that treatment resulted in a significant effect, whereas pruning or treatment and pruning were insignificant (Table 2).

3.3. *In planta* morphological and physiological effects

The leaves of untreated-control plants of 'Cellina di Nardò' and 'Ogliarola salentina' were significantly smaller than the leaves of NuovOlivo® treated plants (Fig. 5A).

Plants of both olive cultivars, when treated with NuovOlivo®, showed a total phenolic content 10 times lower than untreated ones (Fig. 5B).

NuovOlivo® treated 'Ogliarola salentina' showed an MDA concentration lower than the control plants. On 'Cellina di Nardò', MDA of control plants was 7.4-fold higher than on treated ones (Fig. 5C). Untreated plants had a higher MDA level in both cultivars.

The complete (100%) electrolyte leakage resulted in a conductivity of 485 ± 11.2 and 672 ± 8.5 μS cm^{-1} for cvs Ogliarola salentina and Cellina di Nardò, respectively. The untreated plants of both cultivars had the highest percentage of electrolyte leakage. Regardless of the number of spray treatments, treated plants had electrolyte leakage values 6–8 times lower than untreated control plants (Fig. 5D).

In both cultivars, for each feature considered, the statistical significance of the effect of treatment and the lack of significance of pruning or treatment and pruning were calculated by SAS/STAT procedure (Table 2).

4. Discussion

NuovOlivo® is a bioactive detergent. It contains plant oil and water extracts from various botanical species, sulphur, sodium and calcium hydroxide. In our research, it showed specific characteristics. Our investigations are based on a small limited number of samples. In addition, for systemic diseases in old trees, two or four years of data are not enough to draw definitive conclusions. Further experiments are thus

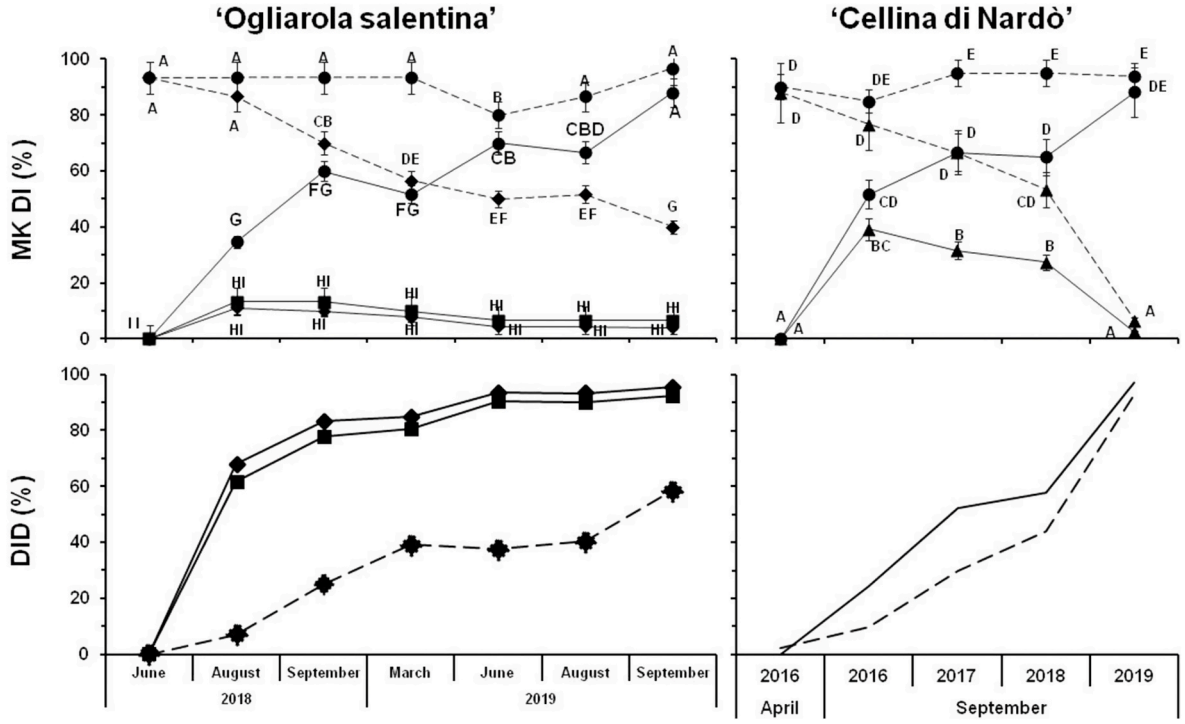

Fig. 3. Effects of zero (◇), two (●), three (■) or seven (♦) NuovOlivo® spray treatments on McKinney's disease index (MK DI) and disease index decrease over the controls (DID) of pruned (—) and unpruned (— —) 'Cellina di Nardò' plants in Montesano Salentino or 'Ogliarola salentina' plants near Sternatia. Data are mean ± sd. For each cultivar, values with the same letters are not significantly different according to Fisher's LSD post-hoc test $P < 0.05$.

Table 2
Statistical analysis considering treatment (Tr), pruning (Pr) and their interactions with McKinney's disease index, *Xylella fastidiosa* DNA, leaf area, total phenolic content, lipid peroxidation, and electrolyte leakage.

Source	df	Sum of square	Mean square	F	*P*-value	df	Sum of square	Mean square	F	*P*-value
	OGLIAROLA SALENTINA						CELLINA DI NARDÒ			
MCKINNEY'S DISEASE INDEX										
Model	4	20.029577124	5.00739281	81.04	<0.0001	3	8.91229382	2.97076461	27.96	<0.0001
Tr	2	5.91117937	2.95558969	47.86	<0.0001	1	3.49312371	3.49312371	32.88	<0.0001
Pr	1	8.92287352	8.92287352	144.48	0.2001	1	5.41292995	5.41292995	50.95	<0.5261
Tr × Pr	1	0.30094893	0.30094893	4.87	0.3296	1	0.00624016	0.00624016	0.06	0.8092
Error	100	6.17582220	0.06175822			76	8.07375371	0.10623360		
Total	104	26.20539343				79	16.98604753			
***XYLELLA FASTIDIOSA* DNA**										
Model	4	1820353.440	455088.360	1882.4	<0.0001	3	2991724.980	997241.660	8399.5	<0.0001
Tr	2	1557810.047	778905.023	3221.8	<0.0001	1	57364.075	57364.075	483.17	<0.0001
Pr	1	18881.356	18881.356	78.10	0.3001	1	2877633.904	2877633.904	24238	0.2212
Tr × Pr	1	14666.230	14666.230	60.67	0.5004	1	56727.000	56727.000	477.8	0.6541
Error	16	3868.106	241.757			8	949.801	118.725		
Total	20	1824221.546				11	2992674.780			
LEAF AREA										
Model	4	4036230.489	1009057.622	44.79	<0.0001	3	3251747.646	1083915.882	36.85	<0.0001
Tr	2	3930532.412	1965266.206	87.23	<0.0001	1	3023191.400	3023191.400	102.79	<0.0001
Pr	1	9500.761	9500.761	0.42	0.5174	1	89938.311	89938.311	3.06	0.8491
Tr × Pr	1	42818.558	42818.558	1.90	0.1707	1	138617.935	138617.935	4.71	0.3342
Error	115	2591055.082	22530.914			68	1999939.833	29410.880		
Total	119	6627285.571				71	5251687.479			
TOTAL PHENOLIC CONTENT										
Model	4	100511971936	25127992984	1002.6	0.0001	3	41362702705	13787567568	3361.9	<0.0001
Tr	2	98731040358	49365520179	1969.6	<0.0001	1	38522814556	38522814556	9393.4	<0.0001
Pr	1	83124822	83124822	3.32	0.7312	1	182532593	182532593	44.51	0.2547
Tr × Pr	1	145215646	145215646	5.79	0.5177	1	2912941	2912941	0.71	0.4023
Error	115	2882235172	25062914.53			68	278870723	4101040		
Total	119	103394207108				71	41641573428			
LIPID PEROXIDATION										
Model	4	327029.9113	81757.4778	866.34	<0.0001	3	246921.1238	82307.0413	1331.7	<.0001
Tr	2	317229.0905	158614.5453	1680.7	<0.0001	1	229855.0734	229855.0734	3719.1	<0.0001
Pr	1	86.5057	86.5057	0.92	0.3404	1	287.0487	287.0487	4.64	0.3247
Tr × Pr	1	623.6846	623.6846	6.61	0.1014	1	326.4603	326.4603	5.28	0.5246
Error	115	10852.7204	94.3715			68	4202.6024	61.8030		
Total	119	337882.6317				71	251123.7262			
ELECTROLYTE LEAKAGE										
Model	4	13.13776892	3.28444223	20648.	<0.0001	3	6.57410460	2.19136820	20951	<0.0001
Tr	2	12.77605161	6.38802581	40160	<0.0001	1	5.8475620	5.84756203	55907	<0.0001
Pr	1	0.00012351	0.00012351	0.78	0.3801	1	0.07382260	0.07382260	705.81	0.1354
Tr × Pr	1	0.01422969	0.01422969	89.46	0.6001	1	0.10724877	0.10724877	1025.3	0.5144
Error	115	0.01829230	0.00015906			68	0.00711235	0.00010459		
Total	119	13.15606122				71	6.58121695			

needed to verify the effectiveness of this control measure; at present, it represents a basis for further data collection.

Plant extracts with antibacterial activity are often considered a safe alternative for the control of bacterial plant disease (Dorman and Deans, 2000; Ichim et al., 2017; El-Hefny et al., 2017; Salem et al., 2018; Han et al., 2018). Based on this assumption, we tested the effectiveness of NuovOlivo® detergent in controlling *X. fastidiosa* in infected olive groves. Water extracts from *Brassica napus* L. seeds and *Solanum lycopersicon* L. fruits reduce diseases caused by *Pectobacterium carotovorum* subsp. *carotovorum*, *Burkholderia cepacia* and *B. gladioli* pv. *alliicola* on onions (Kowalska and Smolinska, 2008). In addition, aqueous extracts of *Hibiscus sabdariffa* L., *Punica granatum* L. and *Eucalyptus globulus* Labill. protect potato plants against *Ralstonia solanacearum* activating plant systemic resistance (Hassan et al., 2009). Extraction of water-soluble plant-active ingredients is usually obtained at room temperature. Roots, stems, leaves, fruits, seeds and whole plants used in NuovOlivo® preparation were treated at 50 °C. Extraction at higher temperatures denatures heat-labile compounds but improves the solubility of other bioactive plant components. Among the latter, anthocyanins, starches, tannins, saponins, terpenoids, polypeptides and lectins are effective in plant disease management (Gurjar et al., 2012). Soaps, oils, compost teas, acetic acid, micronutrients (Si or Zn), bicarbonates, copper and potassium-based products are also applied to control plant pathogens and pests, especially in organic agriculture (van Bruggen et al., 2016).

In our tests on two olive cvs Ogliarola salentina and Cellina di Nardò showing OQDS, NuovOlivo® spray treatments consistently reduced OQDS disease severity already after the second application. On untreated control plants, OQDS symptoms increased and near-complete defoliation occurred. Our control strategies allowed treated plants to produce flowers and fruits. The yield of drupes are not yet typical of the cultivar (about 45–60 kg per plant), but this simply regards the first new crop. Our experimental data also showed that pruning alone or in combination with treatment had no effect on the McKinney disease index, on *X. fastidiosa* DNA *in planta*, nor on the morphological and physiological features of olive plants such as leaf size, total phenolic content, lipid peroxidation and electrolyte leakage.

Generally, pruning could interfere with symptom expression and pathogen development in the trees. In fact, drastic pruning of *X. fastidiosa*-infected olive trees resulted in the emission of new sprouts, but so far, this has not been shown to cure the plants and prevent them from dying. Moreover, Carlucci et al. (2016) applied several bioactive compounds on olive plants vigorously pruned to remove dead branches. Treated trees produced new sprouts with leaves free from disease symptoms. On the contrary, severe pruning does not significantly contribute to the recovery of vines affected by Pierce's disease (Daugherty et al., 2018; Bragard et al., 2019).

Phenolics, flavonoids, and closely related compounds are the most common secondary metabolites in the plant kingdom and amount to 2%

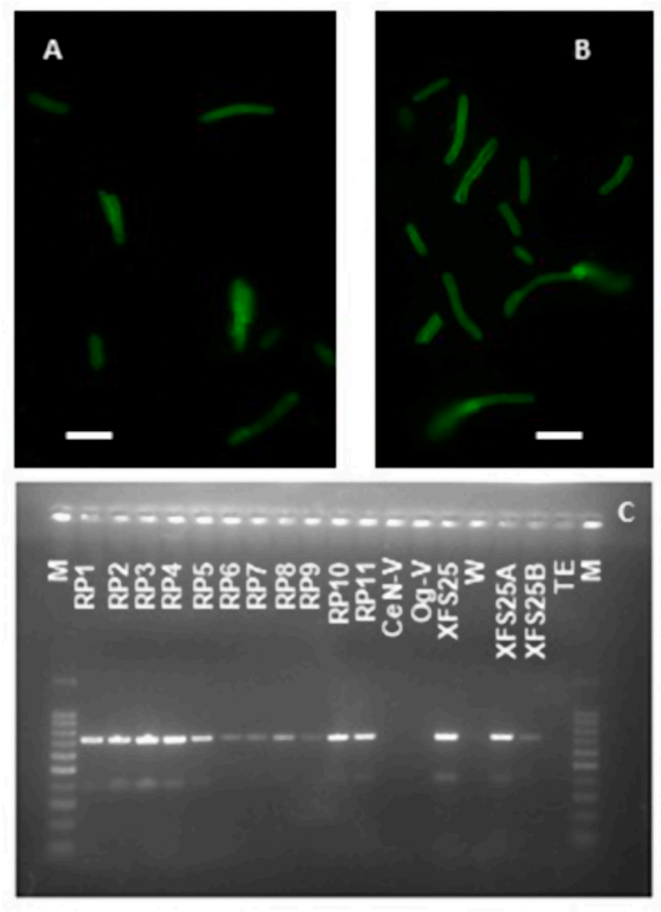

Fig. 4. Immunofluorescence-positive green-fluorescing cells of *Xylella fastidiosa* detected at the beginning of experiments. 'Ogliarola salentina' (A) and 'Cellina di Nardò' (B) plants. Bars = 10 μ. C) Agarose (1%) gel electrophoresis of 8 μl PCR products obtained from *Xylella fastidiosa* subsp. *pauca* isolate XFS25 (XFS25 = 5 μl, XFS25A = 4 μl, XFS25B = 2 μl), olive 'Cellina di Nardò' (RP1-RP4, CeN–V), 'Ogliarola salentina' (Rp5-RP11, Og-V) plants growing in Montesano Salentino (RP1-RP4), Sternatia (Rp5-RP11) or Valenzano (CeN–V, Og-V). W = Water negative control. TE = TE Buffer negative control. M = 100–1500 bp DNA ladder. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article).

of photosynthesized carbons (Robards and Antolovich, 1997). Plants under biotic and abiotic stress produce and accumulate phenols as antioxidants and chemical defence (Nicholson and Hammerschmidt, 1992; Sundin et al., 2016; Dresselhaus et al., 2018). Total phenols are significantly altered in *Xylella*-infected grapevine (Wallis and Chen, 2012). In olive, exogenous factors, i.e. sunlight, pathogens and insects, and endogenous factors of the plant itself, i.e. cultivar and age, stimulate the synthesis of bioactive compounds, especially polyphenols. Moreover, in olive, phenol concentration increases when infected with *Pseudomonas savastanoi* pv. *savastanoi* (Janse) Gardan et al., *Verticillium dahliae* Kleb. and other pathogens (Cayuela et al., 2006; Báidez et al., 2007; Markakis et al., 2010). OQDS symptomatic and symptomless trees of 'Cellina di Nardò' and 'Ogliarola di Lecce' did not change Secoiroid and Oleuropein concentrations but increased the concentration of some phenolics, including hydroxytyrosol glucoside and quinic acid (Luvisi et al., 2017; Sabella et al., 2018). The latter compound is also associated with symptoms of Pierce's disease in grapevine (Wallis et al., 2013). In our studies, a 10-fold decrease of total phenolics occurred in NuovOlivo® treated plants of the two cultivars.

The integrity of cell membranes is closely linked to MDA concentration, one of the final products of stress-induced lipid peroxidation of

Table 3
Quantitative real-time PCR assessment of *Xylella fastidiosa* DNA (ng mg^{-1} f wt) in olive leaves collected in Montesano Salentino, Sternatia and Valenzano.

Thesis	Plant	Collection time (2019)[a,b]	
		April	September
from diseased areas			
	'Cellina di Nardò' - Montesano Salentino		
Pruned and Treated 7 times	A3	8.82 ± 0.21^{A}	8.84 ± 0.31^{A}
Unpruned and Untreated	C3	9.21 ± 0.80^{A}	8.06 ± 0.42^{A}
Pruned and Untreated	E3	1126.89 ± 7.45^{B}	1444.61 ± 3.32^{B}
Unpruned and Treated 7 times	F3	1251.1 ± 2.45^{B}	1830.54 ± 2.51^{D}
	'Ogliarola Salentina' - Sternatia		
Pruned and Treated 3 times	S02, S05	12.32 ± 0.21^{A}	11.01 ± 0.45^{A}
Pruned and Treated 2 times	S10, S12	13.34 ± 1.16^{A}	11.67 ± 1.64^{A}
Pruned and Untreated	S26	1591.13 ± 1.27^{C}	1772.77 ± 2.33^{C}
Unpruned and Untreated	S28	21.13 ± 1.12^{A}	20.53 ± 0.82^{A}
Unpruned and Treated 3 times	S31	1730.74 ± 3.43^{D}	1926.73 ± 4.86^{E}
from disease free territory			
	'Cellina di Nardò' - Valenzano		
Uninfected control		not present	not present
	'Ogliarola' - Valenzano		
Uninfected control		not present	not present

[a] Data are the mean of three replicates ± sd.
[b] Within the column, values with the same apex letters are not significantly different according to Fisher's LSD test at $P < 0.05$.

polyunsaturated fatty acids (Ayala et al., 2014). Change in cell permeability is considered the basis of necrosis often associated with toxic metabolites produced by pathogens (Pennisi e Graniti, 1987). High lipid peroxidation could activate genes involved in water stress as proved for grapevine, mandarin orange and olive infected by *X. fastidiosa* (Choi et al., 2013; Rodrigues et al., 2013; Giampetruzzi et al., 2016; Rapicavoli et al., 2018). In our tests, however, NuovOlivo® treatments reduced electrolyte leakage in both olive cultivars, which suggests that this product performs a protective action against membrane damage by *X. fastidiosa* virulence factors. This action could also suggest that untreated plants, colonized by the pathogen, activate a biological response, e.g. necrosis and programmed cell death aimed to bar off the bacterium.

In our research in both olive groves, indirect immunofluorescence tests and PCR revealed the presence of *X. fastidiosa* DNA in diseased 'Cellina di Nardò' and 'Ogliarola salentina' plants. In the leaves of treated plants, *X. fastidiosa* DNA reached a 99% reduction in concentration when compared with untreated control plants.

NuovOlivo® appears to work in two directions. On the bacteria as partly shown by qRT-PCR, and on plant metabolism, as may be inferred to occur after the responses obtained with spray treatments in the field. NuovOlivo® limits *X. fastidiosa* DNA, and seems to act as a growth promoter, plant defence inductor and protectant for cell membrane integrity. NuovOlivo® applications significantly reduced the incidence of OQDS symptoms in both olive groves and in both olive cultivars.

The action of NuovOlivo® is likely to be related to its diverse phytochemical components, including primary and secondary plant metabolites and phytohormones. Alkaloids, cyanogenic glucosides, glucosinolates, non-protein amino acids, phenolics, phytoalexins, polyphenols, sterols, terpenes, terpenoids are examples of plant defence compounds, or elicitors active against pathogens *in planta* (Bennett and Wallsgrove, 1994; Mazid et al., 2011; Mhlongo et al., 2018; Isah, 2019). Simple phenols and phenolic acids act as enzyme inhibitors of membrane disruption and substrate deprivation (Bednarek and Osbourn, 2009; Gurjar et al., 2012). Various plant hormones exert antimicrobial activity (Abass, 2017).

A pivotal role in the interaction between plant and vascular pathogens is played by the integrity and efficiency of the plant's vascular system. Xylem tissue transports water and mineral nutrients and forms a complex reticulate network of many interconnected vessels. *X. fastidiosa* invades the xylem, restricts water movement, and triggers the drying out of the related parts of tree crown (Sun et al., 2013). In the case of OQDS, susceptible cvs Ogliarola salentina and Cellina di Nardò showed a higher

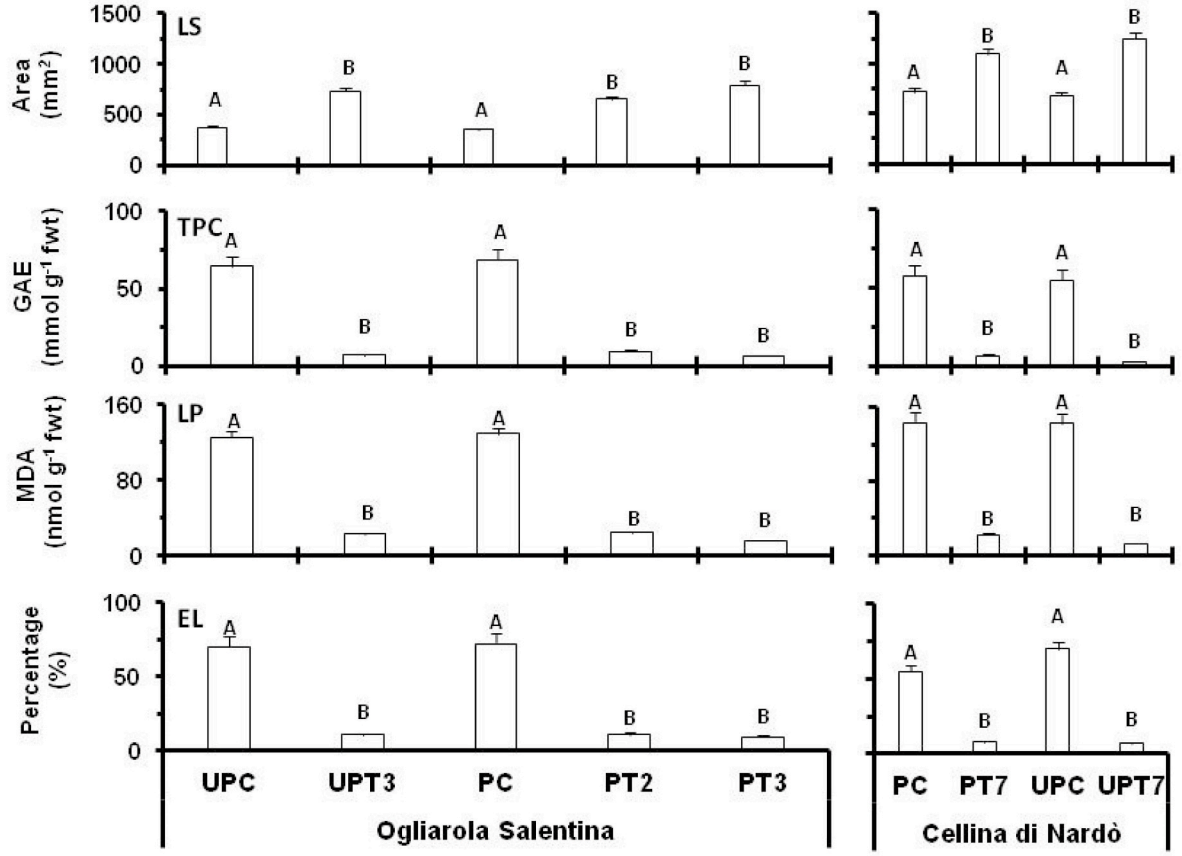

Fig. 5. Leaf surface (LS), total phenolics content (TPC), lipid peroxidation (LP) and percentage of electrolyte leakage (EL) of leaves from NuovOlivo® untreated (C) or treated two (T2: early June 2018 and early April 2019), three (T3: early June and September 2018, and early April 2019) or seven (T7: early April and October 2016, 2017, 2018, 2019) times from pruned (P) and unpruned (UP) 'Ogliarola salentina' and 'Cellina di Nardò'. Data are the mean of three replicates ± sd. For each parameter and cultivar, values with the same letters are not significantly different according to Fisher's LSD test $P < 0.05$.

number of occlusions than the more tolerant 'Leccino', and the severity of symptoms did not depend directly on the percentage of vessels occluded by tyloses and gums/pectin gels, and not by bacterial cell aggregates (De Benedictis et al., 2017). Bacterial cells are present in the tracheary element of leaf petiole (Cariddi et al., 2014).

The multicomponent mixture (e.g. oils, bio-active plant extract, vinegar) make NuovOlivo® a product with diverse physiological properties and explains the effects induced in the olive/*X. fastidiosa* system, such as antibacterial activity, plant growth promoter, and plant defence inductor. All these actions require the penetration of active components into the plant and diffusion into tissues including xylem vessels of leaves, twigs, branches and trunk. Any active ingredients of any pesticide, fungicide, nematocide, fertilizers and the other agrochemicals or plant extracts applied on the plant canopy must penetrate the epicuticular wax, the cuticle and the plasma membrane of epidermal leaf cells. Water and solutes penetrate through stomatal pores by diffusion in trans-stomata water clusters (Eichert et al., 2008) and peristomal cuticle (Schlegel et al., 2005). The activity of systemic foliar treatments with antimicrobial products is improved by adjuvant and surfactant agents (Ramsey et al., 2005; Wang and Liu, 2007). These agents contain non-polar and polar (or ionic) groups that lower tension between solutions and solid leaf surface. Several secondary plant metabolites have these chemical properties and may act as detergents, wetting, emulsifying, foaming dispersant agents, and promote foliar uptake of bioactive substances. Molecules like capsaicin, piperine and other capsaicinoids with antimicrobial properties, act as a typical surfactant structure and may improve permeability (Peter, 2012) to antimicrobials present in NuovOlivo®. Also, the use of fatty acids C7–C18 and C18 unsaturated potassium salt are authorized by EU regulation 540/2011 for plant disease control.

5. Conclusion

NuovOlivo® combines different chemicals. It is an effective bacterial defence inducer in olive plants and it could also work as a bacteriostatic. In four years at Montesano and two years at Sternatia, NuovOlivo® spray treatments have induced recovery from *X. fastidiosa* in olive plants.

Our present data clearly show that NuovOlivo® improved OQDS control in both 'Cellina di Nardò' and 'Ogliarola salentina' olive groves. Its effectiveness resulted in lower levels of *X. fastidiosa* DNA, larger leaf size, low total phenolic content and no cell membrane damage associated to lipid peroxidation and electrolyte leakage in treated trees.

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CRediT authorship contribution statement

Giovanni Luigi Bruno: Formal analysis, Writing - original draft, Data curation. **Corrado Cariddi:** Writing - original draft. **Luigi Botrugno:** Writing - original draft.

Declaration of competing interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Abass, M.H., 2017. *In vitro* antifungal activity of different plant hormones on the growth and toxicity of *Nigrospora* spp. on date palm (*Phoenix dactylifera* L.). Open Plant Sci. J. 10, 10–20. https://benthamopen.com/contents/pdf/TOPSJ/TOPSJ-10-10.pdf.

Ayala, A., Muñoz, M.F., Argüelles, S., 2014. Lipid Peroxidation: Production, Metabolism, and Signaling Mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal. Hindawi Publishing Corporation Oxidative Medicine and Cellular Longevity. https://doi.org/10.1155/2014/360438. Article ID 360438.

Báidez, A.G., Gomez, P., Del Río, J.A., Ortuño, A., 2007. Dysfunctionality of the xylem in *Olea europaea* L. plants associated with the infection process by *Verticillium dahliae* Kleb. Role of phenolic compounds in plant defense mechanism. J. Agric. Food Chem. 55, 3373–3377. https://doi.org/10.1021/jf063166d.

Bajji, M., Kinet, J.M., Lutts, S., 2002. The use of the electrolyte leakage method for assessing cell membrane stability as a water stress tolerance test in durum wheat. Plant Growth Regul. 36 (1), 61–70. https://doi.org/10.1023/A:1014732714549.

Bednarek, P., Osbourn, A., 2009. Plant-microbe interactions: chemical diversity in plant defense. Science 324, 746–748. https://doi.org/10.1126/science.1171661.

Behlau, F., Canteros, B.I., Minsavage, G.V., Jones, J.B., Graham, J.H., 2011. Molecular characterization of copper resistance genes from *Xanthomonas citri* subsp. *citri* and *Xanthomonas alfalfae* subsp. *citrumelonis*. Appl. Environ. Microbiol. 77, 4089–4096. https://doi.org/10.1128/AEM.03043-10.

Bennett, R.N., Wallsgrove, R.M., 1994. Secondary metabolites in plant defense mechanisms. New Phytol. 127 (4), 617–633. https://doi.org/10.1111/j.1469-8137.1994.tb02968.x.

Bragard, C., Dehnen-Schmutz, K., Di Serio, F., Gonthier, P., Jacques, M.-A., Jaques Miret, J.A., Fejer Justesen, A., MacLeod, A., Magnusson, C.S., Milonas, P., Navas-Cortés, J.A., Potting, R., Reignault, P.L., Thulke, H.H., Van der Werf, W., Civera, A. V., Yuen, J., Zappalà, L., Makowski, D., Delbianco, A., Maiorano, A., Muñoz Guajardo, I., Stancanelli, G., Guzzo, M., Parnell, S., 2019. Effectiveness of in planta control measures for *Xylella fastidiosa*. EFSA J. 17 (5), 1–17. https://doi.org/10.2903/j.efsa.2019.5666.

Buono, V., Paradiso, A., Serio, F., Gonnella, M., De Gara, L., Santamaria, P., 2009. Tuber quality and nutritional components of early potato subjected to chemical haulm desiccation. J. Food Compos. Anal. 22, 556–562. https://doi.org/10.1016/j.jfca.2009.01.001.

Cariddi, C., Saponari, M., Boscia, D., De Stradis, A., Loconsole, G., Nigro, F., Porcelli, F., Potere, O., Martelli, G.P., 2014. Isolation of a *Xylella fastidiosa* strain infecting olive and oleander in Apulia. Italy. J. Plant Pathol. 96, 425–429. https://doi.org/10.4454/JPP.V96I2.024.

Carlucci, A., Ingrosso, F., Faggiano, S., Raimondo, M.L., Lops, F., 2016. Strategie per contenere il disseccamento degli olivi. Inf. Agrar. 8, 58–63. http://www.ediagroup.it/bdo/BDO_popupAbstract.asp?D=124452.

Catalano, L., Shoki, A.-D., Boscia, D., Martelli, G.P., 2019. Guidelines for the prevention, eradication and containment of Xylella fastidiosa in olive-growing areas. Series Cairo FAO. http://www.fao.org/3/i5994en/I5994EN.pdf.

Cayuela, J.A., Rada, M., Rios, J.J., Albi, T., Guinda, A., 2006. Changes in phenolic composition induced by *Pseudomonas savastanoi* pv. *savastanoi* infection in olive tree: presence of large amounts of verbascoside in nodules of tuberculosis disease. J. Agric. Food Chem. 54, 5363–5368. https://doi.org/10.1021/jf060807w.

Choi, H.K., Iandolino, A., da Silva, F.G., Cook, D.R., 2013. Water deficit modulates the response of *Vitis vinifera* to the Pierce's disease pathogen *Xylella fastidiosa*. Mol. Plant Microbe Interact. 26, 643–657. https://doi.org/10.1094/MPMI-09-12-0217-R.

Cornara, D., Saponari, M., Zeilinger, A., De Stradis, A., Boscia, D., Loconsole, G., Bosco, D., Martelli, G.L., Almeida, R.P.P., Porcelli, F., 2016. Spittlebugs as vectors of *Xylella fastidiosa* in olive orchards in Italy. J. Pest. Sci. 90, 521–530. https://doi.org/10.1007/s10340-016-0793-0.

D'Attoma, G., Morelli, M., Saldarelli, P., Saponari, M., Giampetruzzi, A., Boscia, D., Savino, V.N., De La Fuente, L., Cobine, P.A., 2019. Ionomic differences between susceptible and resistant Olive cultivars infected by *Xylella fastidiosa* in the outbreak area of Salento. Italy. Pathogens 8, 272. https://doi.org/10.3390/pathogens8040272.

Daugherty, M.P., Almeida, R., Smith, R.J., Weber, E., Purcell, A.H., 2018. Severe pruning of infected grapevines has limited efficacy for managing Pierce's disease. Am. J. Enol. Vitic. 69, 289–294. https://doi.org/10.5344/ajev.2018.18003.

De Benedictis, M., De Caroli, M., Baccelli, I., Marchi, G., Bleve, G., Gallo, A., Ranaldi, F., Falco, V., Pasquali, V., Piro, G., Mita, M., Di Sansebastiano, G.P., 2017. Vessel occlusion in three cultivars of *Olea europaea* naturally exposed to *Xylella fastidiosa* in open field. J. Phytopathol. 165, 589–594. https://doi.org/10.1111/jph.12596.

Dongiovanni, C., Di Carolo, M., Fumarola, G., Ciniero, A., Tauro, D., Palmisano, F., Silletti, M.R., Pollastro, P., Altamura, G., Cavalieri, V., Morelli, M., Saldarelli, P., Boscia, D., Saponari, M., Faretra, F., 2017. Recenti sperimentazioni per il controllo di *Xylella*. Olivo e Olio 20 (4), 25–29. https://olivoeolio.edagricole.it/ricerca-scientifica/recenti-sperimentazioni-controllo-xylella/.

Dorman, H.J.D., Deans, S.G., 2000. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J. Appl. Microbiol. 88, 308–316. https://doi.org/10.1046/j.1365-2672.2000.00969.x.

Dresselhaus, T., Hückelhoven, R., 2018. Biotic and abiotic stress responses in crop plants. Agronomy 8, 267. https://doi.org/10.3390/agronomy8110267.

Eichert, T., Goldbach, H.E., 2008. Equivalent pore radii of hydrophilic foliar uptake routes in stomatous and astomatous leaf surfaces further evidence for a stomatal pathway. Physiol. Plantarum 132, 491–502. https://doi.org/10.1111/j.1399-3054.2007.01023.x.

Elbeaino, T., Yaseen, T., Valentini, F., Ben Moussa, I.E., Mazzoni, V., D'Onghia, A.M., 2014. Identification of three potential insect vectors of *Xylella fastidiosa* in Southern Italy. Phytopathol. Mediterr. 53 (1), 328–332. https://doi.org/10.14601/Phytopathol_Mediterr-14113.

El-Hefny, M., Ashmawy, N.A., Salem, M.Z.M., Salem, A.Z.M., 2017. Antibacterial activities of the phytochemicals-characterized extracts of *Callistemon viminalis, Eucalyptus camaldulensis* and *Conyza dioscoridis* against the growth of some phytopathogenic bacteria. Microb. Pathog. 113, 348–356. https://doi.org/10.1016/j.micpath.2017.11.004.

EPPO, 2018. PM 7/24 (3) *Xylella fastidiosa*. Bulletin OEPP/EPPO Bulletin 48 (2), 175–218. https://doi.org/10.1111/epp.12469.

Giampetruzzi, A., Morelli, M., Saponari, M., Loconsole, G., Chiumenti, M., Boscia, D., Savino, V.N., Martelli, G.P., Saldarelli, P., 2016. Transcriptome profiling of two olive cultivars in response to infection by the CoDiRO strain of *Xylella fastidiosa* subsp. *pauca*. BMC Genom. 17, 475. https://doi.org/10.1186/s12864-016-2833-9.

Gurjar, M.S., Ali, S., Akhtar, M., Singh, K.S., 2012. Efficacy of plant extracts in plant disease management. Agric. Sci. 3 (3), 425–433. https://doi.org/10.4236/as.2012.33050.

Han, J.W., Choi, G.J., Kim, B.S., 2018. Antimicrobial aromatic polyketides: a review of their antimicrobial properties and potential use in plant disease control. World J. Microbiol. Biotechnol. 34 (11), 163. https://doi.org/10.1007/s11274-018-2546-0.

Hassan, M.A.E., Bereika, M.F.F., Abo-Elnaga, H.I.G., Sallam, M.A.A., 2009. Direct antimicrobial activity and induction of systemic resistance in potato plants against bacterial wilt disease by plant extracts. Plant Pathol. J. 25 (4), 352–360. https://doi.org/10.5423/PPJ.2009.25.4.352.

Heath, R.L., Packer, L., 1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys. 125, 189–198. https://doi.org/10.1016/0003-9861(68)90654-1.

Hopkins, D.L., 1989. *Xylella fastidiosa*-xylem-limited bacterial pathogen of plants. Annu. Rev. Phytopathol. 27, 271–290. https://doi.org/10.1146/annurev.py.27.090189.001415.

Ichim, E., Marutescu, L., Popa, M., Cristea, S., 2017. Antimicrobial efficacy of some plant extracts on bacterial ring rot pathogen, *Clavibacter michiganensis* ssp. *sepedonicus*. EuroBiotech J. 1 (1), 85–88. https://doi.org/10.24190/ISSN2564-615X/2017/01.14.

Isah, T., 2019. Stress and defense responses in plant secondary metabolites production. Biol. Res. 52, 39. https://doi.org/10.1186/s40659-019-0246-3.

Kowalska, B., Smolinska, U., 2008. The effect of selected plant materials and extract on the development of bacterial disease on onion. Veg. Crops Res. Bull. 68, 33–45. https://doi.org/10.2478/v10032-008-0003-6.

Luvisi, A., Aprile, A., Sabella, E., Vergine, M., Nicolì, F., Nutricati, E., Miceli, A., Negro, C., De Bellis, L., 2017. *Xylella fastidiosa* subsp. *pauca* (CoDiRO strain) infection in four olive (*Olea europaea* L.) cultivars: profile of phenolic compounds in leaves and progression of leaf scorch symptoms. Phytopathol. Mediterr. 56 (2), 259–273. https://doi.org/10.14601/Phytopathol_Mediterr-20578.

Markakis, E.A., Tjamos, S.E., Antoniou, P.P., Paplomatas, E.J., Tjamos, E.C., 2010. Phenolic responses of resistant and susceptible olive cultivars induced by defoliating and nondefoliating *Verticillium dahliae* pathotypes. Plant Dis. 94 (9), 1156–1162. https://doi.org/10.1094/PDIS-94-9-1156.

Martelli, G.P., Boscia, D., Porcelli, F., Saponari, M., 2015. The olive quick decline syndrome in south-east Italy: a threatening phytosanitary emergency. Eur. J. Plant Pathol. 144, 235–243. https://doi.org/10.1007/s10658-015-0784-7.

Mazid, M., Khan, T.A., Mohammad, F., 2011. Role of secondary metabolites in defense mechanisms of plants. Biol. Med. 3 (2), 232–249. https://www.longdom.org/open-access/role-of-secondary-metabolites-in-defense-mechanisms-of-plants-0974-8369-3-128.pdf.

Mellano, M.A., Cooksey, D.A., 1988. Nucleotide sequence and organization of copper resistance genes from *Pseudomonas syringae* pv. *tomato*. J. Bacteriol. 170 (6), 2879–2883. https://doi.org/10.1128/jb.170.6.2879-2883.1988.

Mhlongo, M.I., Piater, L.A., Madala, N.E., Labuschagne, N., Dubery, I.A., 2018. The chemistry of plant-microbe interactions in the rhizosphere and the potential for metabolomics to reveal signaling related to defense priming and induced systemic resistance. Front. Plant Sci. 9, 112. https://doi.org/10.3389/fpls.2018.00112.

Modesti, V., Pucci, N., Lucchesi, S., Campus, L., Loreti, S., 2017. Experience of the Latium region (central Italy) as a pest-free area for monitoring of *Xylella fastidiosa*: distinctive features of molecular diagnostic methods. Eur. J. Plant Pathol. 148, 557–566. https://doi.org/10.1007/s10658-016-1111-7.

Nicholson, R.L., Hammerschmidt, R., 1992. Phenolic compounds and their role in disease resistance. Annu. Rev. Phytopathol. 30, 369–389. https://doi.org/10.1146/annurev.py.30.090192.002101.

Nigro, F., Boscia, D., Antelmi, I., Ippolito, A., 2013. Fungal species associated with a severe decline of olive in Southern Italy. J. Plant Pathol. 95 (3), 668. http://www.sipav.org/main/jpp/index.php/jpp/article/view/2985/1659.

Pennisi, A.M., Graniti, A., 1987. Cell permeability changes in tissues of Citrus trees infected by *Phoma tracheiphila* (Petri) Kanc. Et Ghik. Phytophatol. Mediterr. 26 (2), 142–145. https://www.jstor.org/stable/42685640?seq=1.

Peter, K.V., 2012. Handbook of Herbs and Spices, second ed. Woodhead Publishing.

Ramazzotti, M., Cimaglia, F., Gallo, A., Ranaldi, F., Surico, G., Mita, G., Bleve, G., Marchi, G., 2018. Insights on a founder effect: the case of *Xylella fastidiosa* in the Salento area of Apulia. Italy. Phytophatol. Mediterr. 57 (1), 8–25. https://doi.org/10.14601/Phytopathol_Mediterr-22263.

Ramsey, R.J.L., Stephenson, G.R., Hall, J.C., 2005. A review of the effects of humidity, humectants, and surfactant composition on the absorption and efficacy of highly water-soluble herbicides. Pestic. Biochem. Physiol. 82, 162–175. https://doi.org/10.1016/j.pestbp.2005.02.005.

Rapicavoli, J.N., Blanco-Ulate, B., Muszynski, A., Figueroa-Balderas, R., Morales-Cruz, A., Azadi, P., Dobruchowska, J.M., Castro, C., Cantu, D., Roper, M.C., 2018. Lipopolysaccharide O-antigen delays plant innate immune recognition of *Xylella fastidiosa*. Nat. Commun. 9, 390. https://doi.org/10.1038/s41467-018-02861-5.

Robards, R., Antolovıch, M., 1997. Analytical chemistry of fruit bioflavonoids. A review. Analyst 122, 11–34. https://pubs.rsc.org/en/content/articlepdf/1997/an/a606499j.

Rodrigues, C.M., De Souza, A.A., Takita, M.A., Kishi, L.T., Machado, M.A., 2013. RNA-Seq analysis of *Citrus reticulata* in the early stages of *Xylella fastidiosa* infection reveals auxin-related genes as a defense response. BMC Genom. 14, 676. https://doi.org/10.1186/1471-2164-14-676.

Rolshausen, P., Roper, C., Maloney, K., 2018. Greenhouse evaluation of grapevine microbial endophytes and fungal natural products for control of Pierce's disease. Final report of CDFA Agreement Number 16-0512-SA. https://static.cdfa.ca.gov/PiercesDisease/reports%2F2018%2FRolshausen%2016-0512-SA_Final%20Report.pdf.

Sabella, E., Luvisi, A., Aprile, A., Negro, C., Vergine, M., Nicolì, F., Miceli, A., De Bellis, L., 2018. *Xylella fastidiosa* induces differential expression of lignification related-genes and lignin accumulation in tolerant olive trees cv. Leccino. J. Plant Pathol. 220, 60–68. https://doi.org/10.1016/j.jplph.2017.10.007.

Salem, M.Z.M., EL-Hefny, M., Ali, H.M., Elansary, H.O., Nasser, R.A., El-Settawy, A.A.A., El Shanhorey, N., Ashmawy, N.A., Salem, A.Z.M., 2018. Antibacterial activity of extracted bioactive molecules of *Schinus terebinthifolius* ripened fruits against some pathogenic bacteria. Microb. Pathog. 120, 119–127. https://doi.org/10.1016/j.micpath.2018.04.040.

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). J. Plant Pathol. 95, 668. https://doi.org/10.4454/JPP.V95I3.035.

Schlegel, T.K., Schönherr, J., Schreiber, L., 2005. Size selectivity of aqueous pores in stomatous cuticles of *Vicia faba* leaves. Planta 221, 648–655. https://doi.org/10.1007/s00425-005-1480-1.

Scortichini, M., Chen, J., De Caroli, M., Dalessandro, G., Pucci, N., Modesti, V., L'aurora, A., Petriccione, M., Zampella, L., Mastrobuoni, F., Migoni, D., Del Coco, L., Girelli, C.R., Piacente, F., Cristella, N., Marangi, P., Laddomada, F., Di Cesare, M., Cesari, G., Fanizzi, F.P., Loreti, S., 2018. A zinc, copper and citric acid biocomplex shows promise for control of *Xylella fastidiosa* subsp. *pauca* in olive trees in Apulia region (southern Italy). Phytophatol. Mediterr. 57 (1), 48–72. https://doi.org/10.14601/Phytopathol_Mediterr-21985.

Sun, Q., Sun, Y., Walker, M.A., Labavitch, J.M., 2013. Vascular occlusions in grapevines with Pierce's disease make disease symptom development worse. Plant Physiol. 161, 1529–1541. https://doi.org/10.1104/pp.112.208157.

Sundin, G.W., Castiblanco, L.F., Yuan, X., Zeng, Q., Yang, C.H., 2016. Bacterial disease management: challenges, experience, innovation and future prospects. Mol. Plant Pathol. 17 (9), 1506–1518. https://doi.org/10.1111/mpp.12436.

Valentini, F., Cavallo, G., D'Onghia, A.A., 2017. Sampling procedures of plant material for the survey of *Xylella fastidiosa* in Puglia Region. Options Méditerranéennes: Série A. Séminaires Méditerranéens 121, 49–52. http://om.ciheam.org/om/pdf/a121/00007210.pdf.

van Bruggen, A.H.C., Gamliel, A., Finckh, M.R., 2015. Plant disease management in organic farming systems. Pest Manag. Sci. 72, 30–44. https://doi.org/10.1002/ps.4145.

Wallis, C.M., Chen, J., 2012. Grapevine phenolic compounds in xylem sap and tissues are significantly altered during infection by *Xylella fastidiosa*. Phytopathology 102 (9), 816–826. https://doi.org/10.1094/PHYTO-04-12-0074-R.

Wallis, C.M., Wallingford, A.K., Chen, J., 2013. Grapevine rootstock effects on scion sap phenolic levels, resistance to *Xylella fastidiosa* infection, and progression of Pierce's disease. Front. Plant Sci. 4, 502. https://doi.org/10.3389/fpls.2013.00502.

Wang, C.J., Liu, Z.Q., 2007. Foliar uptake of pesticides - present status and future challenge. Pestic. Biochem. Physiol. 87, 1–8. https://doi.org/10.1016/j.pestbp.2006.04.004.