Received: 28 March 2022 | Accepted: 6 September 2022

DOI: 10.1111/ppa.13646

Plant Pathology WILEY

ORIGINAL ARTICLE

Xylella fastidiosa subsp. *pauca* ST53 exploits pit membranes of susceptible olive cultivars to spread systemically in the xylem

Vito Montilon | Angelo De Stradis | Maria Saponari | Raied Abou Kubaa | Annalisa Giampetruzzi | Giusy D'Attoma | Pasquale Saldarelli

Consiglio Nazionale delle Ricerche, Institute for Sustainable Plant Protection, Bari, Italy

Correspondence
Pasquale Saldarelli, Consiglio Nazionale delle Ricerche, Institute for Sustainable Plant Protection, via Amendola 122/D, 70126 Bari, Italy.
Email: pasquale.saldarelli@ipsp.cnr.it

Funding information
EU H2020, Grant/Award Number: 727987

Abstract

Xylella fastidiosa subspecies *pauca* strain De Donno (XfDD) ST53 is the causal agent of olive quick decline syndrome, a severe disease first described in Apulia, Italy. Although the two local cultivars Cellina di Nardò and Ogliarola Salentina showed high susceptibility, traits of resistance to the bacterium were found in the cultivar Leccino. Previous studies in field-grown olives suggested that vascular occlusions and anatomophysiological properties of the different cultivars played a role in the olive response to XfDD. The present investigation reports observations at the early stage of the infection on artificially inoculated olives. Electron microscope studies showed that XfDD exploits the pit membranes (PMs) of the susceptible cultivar Cellina di Nardò to spread systemically. In this cultivar, PMs were degraded upon XfDD infection, suggesting activity of bacterial cell wall-degrading enzymes. Moreover, occluded vessels contained an amorphous electrondense matrix resembling gum. Conversely, in Leccino, occluded vessels were mainly filled by callose-like granules that tightly entrapped XfDD cells. In addition, PMs from Leccino had a compact undegraded structure that was not permeable to XfDD. Our study suggests that exploitation of PMs is a key event in the infection process of *X. fastidiosa* subsp. *pauca* ST53 in susceptible olive cultivars.

KEYWORDS
microscopy, olive, pit membranes, *Xylella*

1 | INTRODUCTION

Xylella fastidiosa is an aerobic, non-spore-forming, gram-negative phytopathogenic bacterium of the *Xanthomonadaceae* family (Wells et al., 1987), which multiplies and colonizes the xylem of host plants (Hopkins, 1989; Purcell & Hopkins, 1996). Under natural conditions, transmission of *X. fastidiosa* occurs via various genera and species of xylem-feeding insects of the Cicadellidae and Aphrophoridae families (belonging to Auchenorrhyncha) (Hill & Purcell, 1997). The long-distance spread of the bacterium is mediated by infected plants or infectious vectors. To date, six different subspecies of *X. fastidiosa* have been reported, name subspecies *fastidiosa*, *multiplex*, *pauca*, *sandyi*, *tashke* and *morus*, that infect a wide host range (European Food Safety Authority [EFSA], 2022) including important crops such as grapevine, peach, almond, citrus, plum and coffee (Chang et al., 1993; Davis et al., 1980; Li et al., 2007; Mircetich et al., 1976; Raju et al., 1982; Wells et al., 1983).

In 2013, a strain of *X. fastidiosa* subsp. *pauca* (strain De Donno; XfDD; ST53) was identified in Apulia, in the Salento area (Martelli, 2013), in tissues of olive, oleander and almond trees with symptoms of leaf scorching (Saponari et al., 2013, 2019). The extreme severity of the desiccation symptoms in olive led to the description of

olive quick decline syndrome (OQDS; Martelli et al., 2016; Saponari et al., 2019), a new disease that ultimately causes the death of susceptible cultivars Cellina di Nardò and Ogliarola Salentina. Its discovery was the first report of *Xylella* infection in the open field in Europe and in the Mediterranean basin, becoming a serious phytosanitary emergency that caused enormous damage to the Salento olive sector as well as to the landscape.

Xylella is characterized by a wide polyphagia, with a range of over 600 host plant species (EFSA, 2022). In several species, it multiplies and colonizes the vascular system without causing symptoms and therefore can provide inoculum for the transmission of the bacterium to susceptible plants. While in other hosts, it induces alterations including leaf scorching and desiccation, which, in some cases, can result in the death of the plant (Purcell, 2013; Purcell & Hopkins, 1996). To date, the host colonization process and the mechanisms that lead to the development of symptoms are not fully elucidated. It is generally agreed that water transport is impaired, thereby causing desiccation, by occlusions of xylem vessels by bacterial aggregates embedded in an exopolysaccharide matrix, and tyloses and gums produced by the plant in response to infection (De la Fuente et al., 2008; Rapicavoli et al., 2018; da Silva et al., 2001). Indeed, in grapevines with Pierce's disease (PD) caused by *X. fastidiosa*, a decrease in hydraulic conductivity is associated with vascular occlusions of the secondary xylem of stems. The extent of these occlusions, mainly consisting of tyloses, is dependent on the grapevine cultivar, as PD-resistant genotypes had less vascular obstruction than susceptible genotypes (Sun et al., 2013). Moreover, it was observed that tylose occlusions did not prevent the spread of *Xylella* as their production was probably late during the infection. Therefore, tylose production is not a primary event in the decline of hydraulic conductivity, but their presence affects the water transport that is initially disturbed by the pathogen diffusion in the xylem network. In this process, *Xylella* needs to cross through pit membranes (PMs) to move between adjacent vessels and spread in the xylem. PMs are porous structures of 5–20 nm in diameter made of hemicellulose, cellulose microfibrils and pectins, and connect adjacent vessels. In their intact state, they limit the passage of the bacterium as well as any gas bubbles, thus protecting plants from embolism. During infections, the porosity of PMs increases, due to the degradation of their polysaccharide components by a set of *Xylella* cell wall-degrading enzymes (CWDEs), necessary for the bacterium to move systemically. The effects of CWDE activity were recently investigated by Fanton and Brodersen (2021) in grapevine, who showed that the sole activity of polygalacturonase, the role of which in *Xylella* infections was previously ascertained by Roper et al. (2007), induces a significant decline in hydraulic conductivity that can be aggravated by the successive production of tyloses (Sun et al., 2013).

It is therefore unsurprising that recent studies of healthy and naturally XfDD-infected field-grown olives reported a higher proportion of occluded vessels in the stems of the susceptible Ogliarola Salentina and Cellina di Nardò than in the resistant Leccino cultivars. De Benedictis et al. (2017) observed that occlusions were mainly composed of tyloses, gums and pectin gels rather than XfDD cell aggregates. Similarly, Cardinale et al. (2018), using a fluorogenic probe specific to *Xylella*, reported a low concentration of bacterial cells in vascular occlusions of the stems of Ogliarola Salentina, but a greater presence of bacterial aggregates in the leaf petioles of the same cultivar. In these field-grown olives, high percentages of occluded vessels were found of about 16%–53% (De Benedictis et al., 2017) and 50.2 ± 4.3% (Cardinale et al., 2018). Further evidence of resistance in cv. Leccino came from the studies of Sabella et al. (2019), which showed that, compared with the susceptible cultivar Cellina di Nardò, Leccino has a lower susceptibility to cavitation (formation of air bubbles in the xylem) due to a better ability to counteract the decline of hydraulic conductivity imposed by the bacterium. Indeed, this cultivar has a more efficient strategy of xylem refilling that is based on starch hydrolysis, as shown by the presence of starch grains in the vessel of infected plants.

While the above studies were carried out in naturally infected field-grown olives, whose timing of infection was not defined, we investigated the frequency and distribution of vascular occlusions in the secondary xylem in stems of healthy or artificially infected olives belonging to the susceptible and resistant Cellina di Nardò and Leccino olive cultivars (Boscia et al., 2017). Indeed, it has been shown that xylem occlusions can decrease the hydraulic conductivity of infected plants to a lesser extent in resistant cultivars (Sun et al., 2011, 2013), a fact that may have a direct impact on the screening of olive germplasm for resistance to *Xylella*. Our evaluations were carried out using optical and transmission electron microscopy (TEM) at a single time point on plants showing early symptoms of desiccation in order to understand better the initial events of the infection.

2 | MATERIALS AND METHODS

2.1 | Plants and *Xylella* infections

Vascular occlusions of the secondary xylem were studied in stems of potted plants of Cellina di Nardò and Leccino, mock inoculated and inoculated with XfDD (maintained in our collection). Three-year-old plants were purchased from a local nursery and their sanitary status was certified as pathogen-tested according to National Regulations for Certified Plant Material. Plants were grown in 10 L pots and maintained throughout the experiments in a greenhouse under controlled conditions at 25°C and 70% relative humidity, irrigated twice per week and fertilized with a slow-release fertilizer (NPK Original Gold; Compo). Inoculum was prepared from 8- to 10-day-old colonies of XfDD scraped from PD3 agar plates and dispersed in sterile potassium phosphate buffer (0.05 M, pH 7.2) to form a turbid cell suspension of approximately 10^9 cells/ml; this was used immediately for inoculations by the pin prick method (Saponari et al., 2017). Plants of the cultivar Leccino were inoculated in 2014, whereas plants of the cultivar Cellina di Nardò were inoculated in 2018. Three twigs per plant were punctured with a needle after placing a 10 µl drop of bacterial culture suspension on the surface (Saponari et al., 2016). Two plants per cultivar were inoculated with XfDD (Table 1; Leccino 1, Leccino 2, Cellina 1 and Cellina 2) while one plant was mock

inoculated with phosphate-buffered saline (Table 1; Leccino 3 and Cellina 3). At 12 months after the inoculations, each plant was individually tested by harvesting at least four leaves, whose petioles were excised and used for total DNA extraction and XfDD detection. XfDD was detected in plant tissues by quantitative PCR (qPCR) using total DNA purified with the Maxwell RSC PureFood GMO (Promega) protocol. qPCR was performed with TaqMan Fast Advanced Master Mix (Thermo Fisher Scientific) on a CXF 96 Real-Time System (Bio-Rad) following the EPPO diagnostic protocol (European and Mediterranean Plant Protection Organization [EPPO], 2019) and the primer/probe set from Harper et al. (2010).

2.2 | Optical and TEM

Studies were performed on 3-year-old Cellina di Nardò and 6-year-old Leccino olives, 1 and 4 years, respectively, after inoculation. Twigs from two XfDD-infected plants per cultivar were selected for qPCR testing to confirm bacterial colonization; for those of Cellina di Nardò, one had early symptoms of desiccation and the other was symptomless, whereas both those of Leccino were without symptoms. For controls, one mock-inoculated plant per cultivar was analysed. Only twigs that were qPCR-positive for XfDD were used for microscopy observations together with twigs of the same age from the mock-inoculated plant. Ten transverse sections, c.1–2 mm thick, of stem tissues were manually cut from each 1-year-old twig of c.0.5 cm in diameter from healthy or XfDD-inoculated olives of the cultivars Leccino and Cellina di Nardò. Sections were stained for 4 min in 0.05% toluidine in 80% ethanol and successively destained by four washes with 80% ethanol and distilled water before observing under a stereomicroscope Eclipse TI (Nikon). Regions containing occluded vessels were processed later for electron microscopy. Vessel occlusions were counted at 20× magnification on 50 randomly captured images of the cross-sections for a minimum of 500 vessels per cultivar. The micrographs were captured with a Bresser Fotocamera Mikrokular-Imaging by software CamLabLite (Bresser GmBH). For TEM, 1–2 mm^2 thin sections of stem tissues from the transverse sections were excised and processed according to standard embedding procedures (Martelli et al., 1984). Briefly, stem tissues were fixed in 4% glutaraldehyde in 0.05 M potassium phosphate buffer (pH 7.2) for 2 h and postfixed at 4°C in 1% osmium tetroxide in the same buffer for 2 h. Sections were bulk stained overnight in 0.5% aqueous UA-Zero EM stain (Agar Scientific Ltd), dehydrated in graded ethanol dilutions, and embedded in TAAB Spurr resin (TAAB Laboratories). Observations were performed with a Philips Morgagni 282D (Thermofisher) transmission electron microscope at 80 kV after staining with 4 mM lead citrate solution. Statistical analysis was performed with the RStudio software: Integrated Development for R (RStudio). The number of occlusions from the two groups, inoculated with XfDD or mock inoculated, were statistically compared using an unpaired Student's *t* test.

3 | RESULTS

3.1 | Evaluation of vascular occlusions of plants grown in a controlled environment

XfDD-infected Leccino (without symptoms) and Cellina di Nardò (showing symptoms of desiccation) plants were selected (Table 1), and two 1-year-old twigs (approx. 0.5 cm in diameter) per plant were sampled. For cultivar Cellina di Nardò, twigs with or without symptoms of desiccation were selected from the infected plants

TABLE 1 Numbers of occluded and open xylem vessels in twigs of Leccino and Cellina di Nardò olive trees inoculated with *Xylella fastidiosa* subsp. *pauca* strain De Donno or mock-inoculated

Condition	Twig	Symptoms	Occluded vessels	Open vessels	Total vessels	Occluded vessels (%)	C_q
Infected	Leccino 1-1	N	836	4975	5811	14.4	21.25
	Leccin 1-2	N	12	5922	5934	0.2	27.26
	Leccino 2–1	N	2	4510	4512	0.04	24.99
	Leccino 2–2	N	692	5683	6375	10.8	24.07
	Total		1542***	21,090	22,632	6.81 (avg)	24.39 (avg)
Infected	Cellina 1-1	Y	219	7555	7774	2.8	20.51
	Cellina 1-2	Y	506	4162	4668	10.8	19.66
	Cellina 2-1	N	51	6808	6859	0.74	21.72
	Cellina 2-2	N	1520	2967	4487	33.9	20.34
	Total		2296***	21,492	23,788	9.65 (avg)	20.55 (avg)
Mock	Leccino 3	N	1	5371	5372	0.02	N/D
Mock	Cellina 3	N	11	7258	7269	0.15	N/D

Note: Infections were confirmed by quantitative PCR, whose C_q values, performed on the same twigs, are reported. Two twigs, showing (Y) or not showing (N) initial symptoms of desiccation, were selected from the infected plants, while only one was sampled from the mock-inoculated plants of the same cultivar. (avg): average; N/D: not determined.

***$p < 0.001$, significant differences in total occluded vessels between infected and mock-inoculated plants of the respective cultivars.

(Table 1). qPCR assays on small portions of the selected twigs indicated a lower quantity of XfDD in the resistant Leccino compared with Cellina di Nardò, with average C_q values of 24.39 in Leccino and 20.55 in Cellina di Nardò (Table 1). No qPCR product was obtained from mock-inoculated plants. The percentages of completely occluded vessels were visually determined by optical microscopy on toluidine blue-stained sections (Figure 1). Occlusions were negligible in mock-inoculated plants while they were significantly higher in XfDD-infected plants, reaching the highest value (9.65%) in the susceptible cultivar Cellina di Nardò, with only 6.81% in Leccino. In addition, the distribution of occluded vessels was not uniform among individual twigs of the same cultivar, ranging from 1% to 34% in Cellina di Nardò and 0.044% to 14% in Leccino, and were not correlated with the presence of symptoms. However, the total numbers of occluded vessels from infected olive trees were statistically different from those of corresponding mock-inoculated plants (Table 1).

3.2 | Electron microscopy studies of *Xylella*-infected olives

Tissue portions in which occluded vessels were found were embedded in resin to characterize, by TEM, the nature of the vascular occlusions. Observations were performed on a total of 420 and 380 sections from Leccino and Cellina di Nardò, respectively. Electron microscope observations in twigs of Cellina di Nardò-infected plants showed that the lumen of xylem vessels was occluded by aggregates of XfDD cells embedded in an amorphous

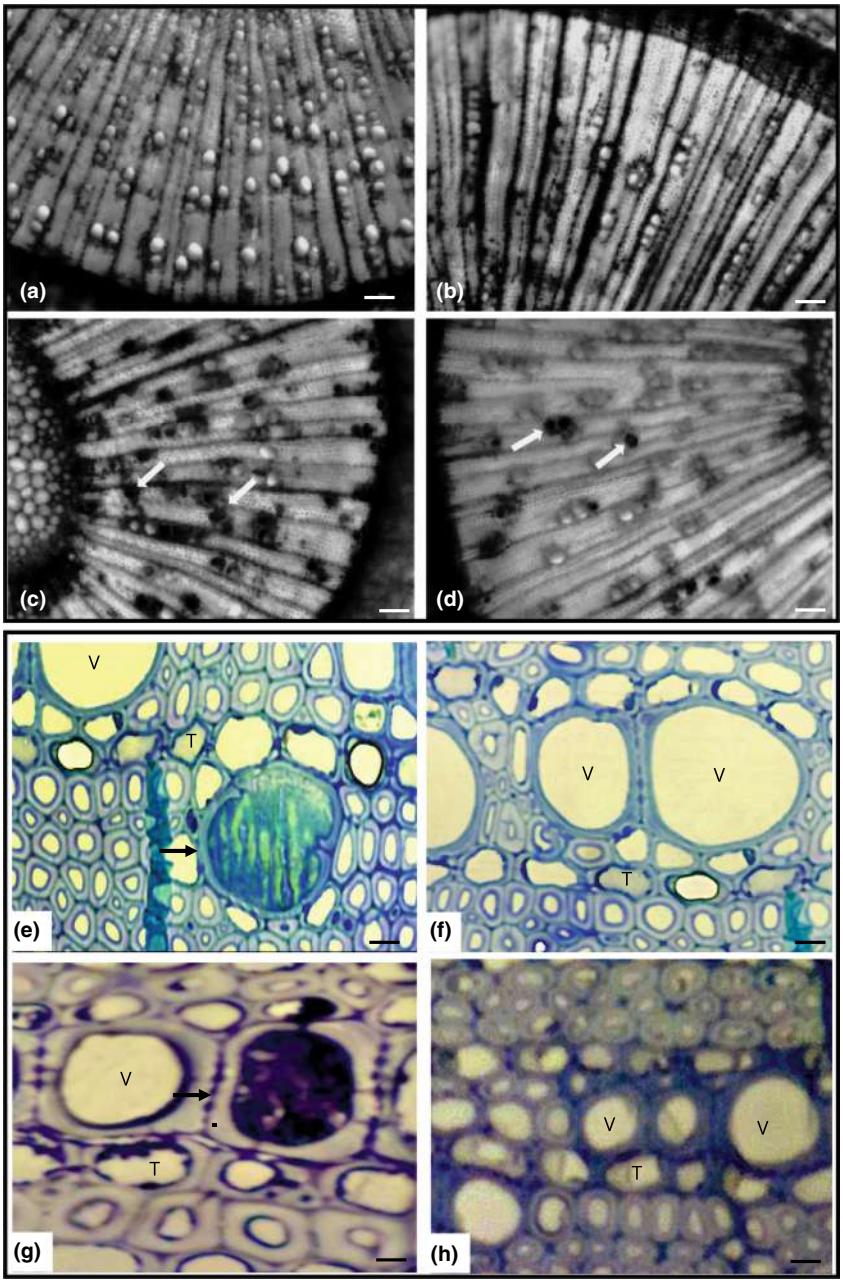

FIGURE 1 Stem cross-sections observed by fluorescence (a–d) and bright field (e–h) microscopy. (a, b) Mock-inoculated olive cultivars Cellina di Nardò (a) and Leccino (b); (c, d) olive cultivars Cellina di Nard' (c) and Leccino (d) infected with *Xylella fastidiosa* subsp. *pauca* strain De Donno (XfDD). Arrows indicate occluded xylem vessels. (e–h) Stem sections of cv. Cellina di Nardò (e, f) and cv. Leccino (g, h) after toluidine blue staining, showing occluded xylem vessels (arrowed) in XfDD-infected (e, g) plants and empty vessels (V) and fibre-tracheids (T) in mock-inoculated plants (f, h). (a–d) Bar = 50 μm, (e–h) bar = 20 μm. [Colour figure can be viewed at wileyonlinelibrary.com]

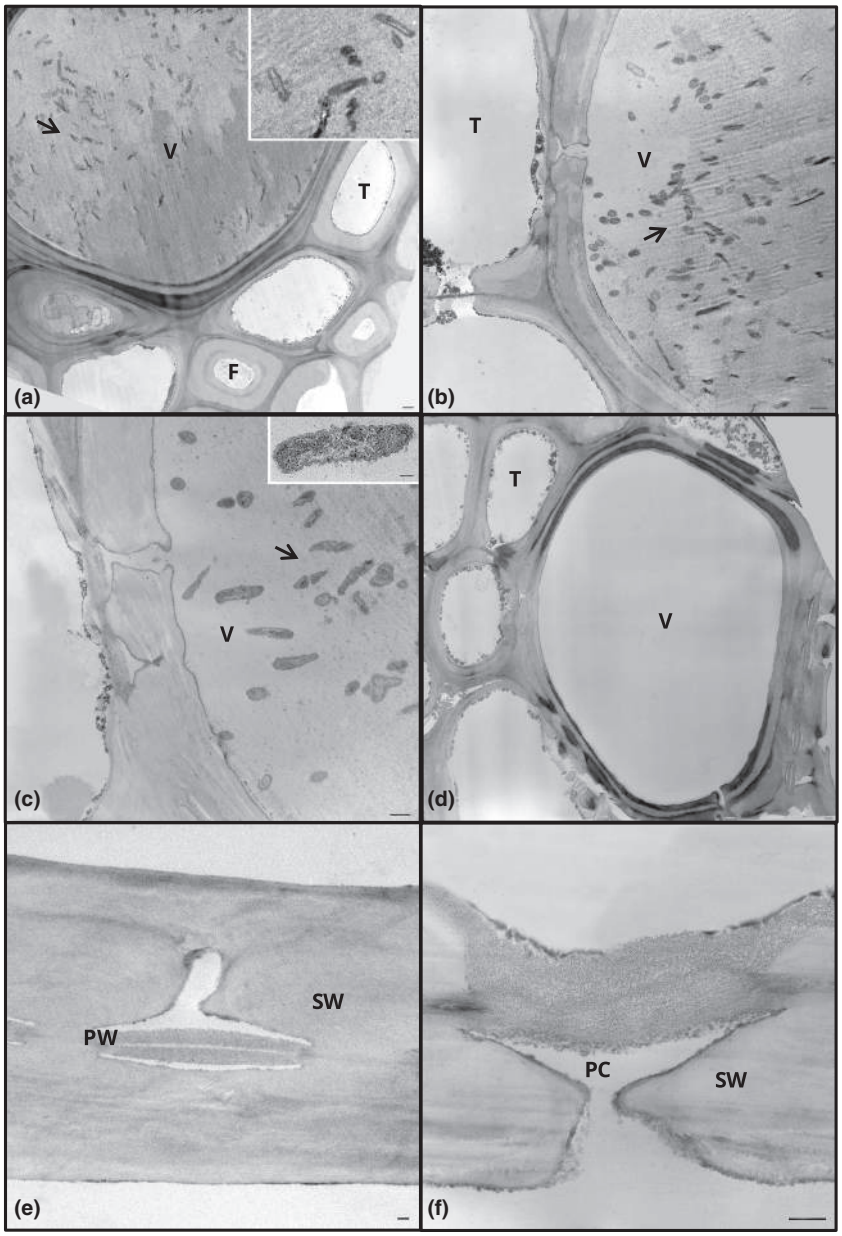

FIGURE 2 Transmission electron micrographs showing aggregates of bacterial cells (arrow) in the lumen of xylem vessels of olive cultivar Cellina di Nardò infected by *Xylella fastidiosa* subsp. *pauca* strain De Donno (a–c), compared with an empty vessel of a mock-inoculated plant (d). Insets highlight a group of bacterial cells (a) and a single bacterial cell with a characteristic wrinkled cell wall (c). Structure of a bordered pit connecting two adjacent vessels (e) and a half-bordered pit connecting a vessel with a fibre tracheid, in mock-inoculated plants. V, vessel, T, fibre-tracheid; F, fibre; PW, primary wall and middle lamella; SW, secondary wall; PC, pit chamber. Bars: 1 μm (a–d); 250 nm [inset (a, e, f)]; 100 nm [inset (c)].

electron dense matrix (Figure 2a–c), resembling gum, which was never observed in vessels from healthy olives (Figure 2d). Bacterial cells were not present in fibre tracheids (Figure 2a–c), which may have been due to the structure of half-bordered pits that interconnected the fibre tracheids with vessels (Figure 2f) rather than the bordered-pits connecting adjacent vessels (Figure 2e). Indeed, XfDD cells were also observed within these pits interconnecting adjacent vessels (Figure 3a–c). In these tissues, an uneven and degraded texture of the PMs (Figure 3b,c) was observed, which contrasted strikingly with the compact and intact structure of those from mock-inoculated olives (Figure 3d,e) of the cultivar Cellina di Nardò. Conversely, in XfDD-infected Leccino, deposition of callose-like granules represented the main component of vessel occlusions (Figures 4 and 5). This callose also infiltrated pits (Figure 5e), tightly entrapping XfDD cells (Figure 5a,b). Callose-like granules presented a more electron-dense central part (Figure 4d) and the matrix was organized into the form of "wads" that, in some cases, completely filled the vessel lumen. Moreover, only in Leccino, fibre tracheids contained crystal structures and depositions of electron-dense material (Figure 4e). As with Cellina di Nardò (Figure 2d), healthy plants of Leccino did not show such structures in the xylem vessels (Figure 4f). As a general observation, in cv. Cellina di Nardò vessel colonization completely filled the vessel lumens and proceeded along vessels and between contiguous vessels, with XfDD cells crossing the pits (Figure 3a,b). Indeed, in this cultivar, we observed strong structural modification of bordered pits interconnecting two adjacent vessels, showing fragmented middle lamellas and parts of the primary wall (Figure 3c). In cultivar Leccino, pits were more electron dense than in Cellina di Nardò, indicative of a higher density and compactness of the tissue, and they also appeared more intact (Figure 5c,d); no cells of XfDD were observed within these pits.

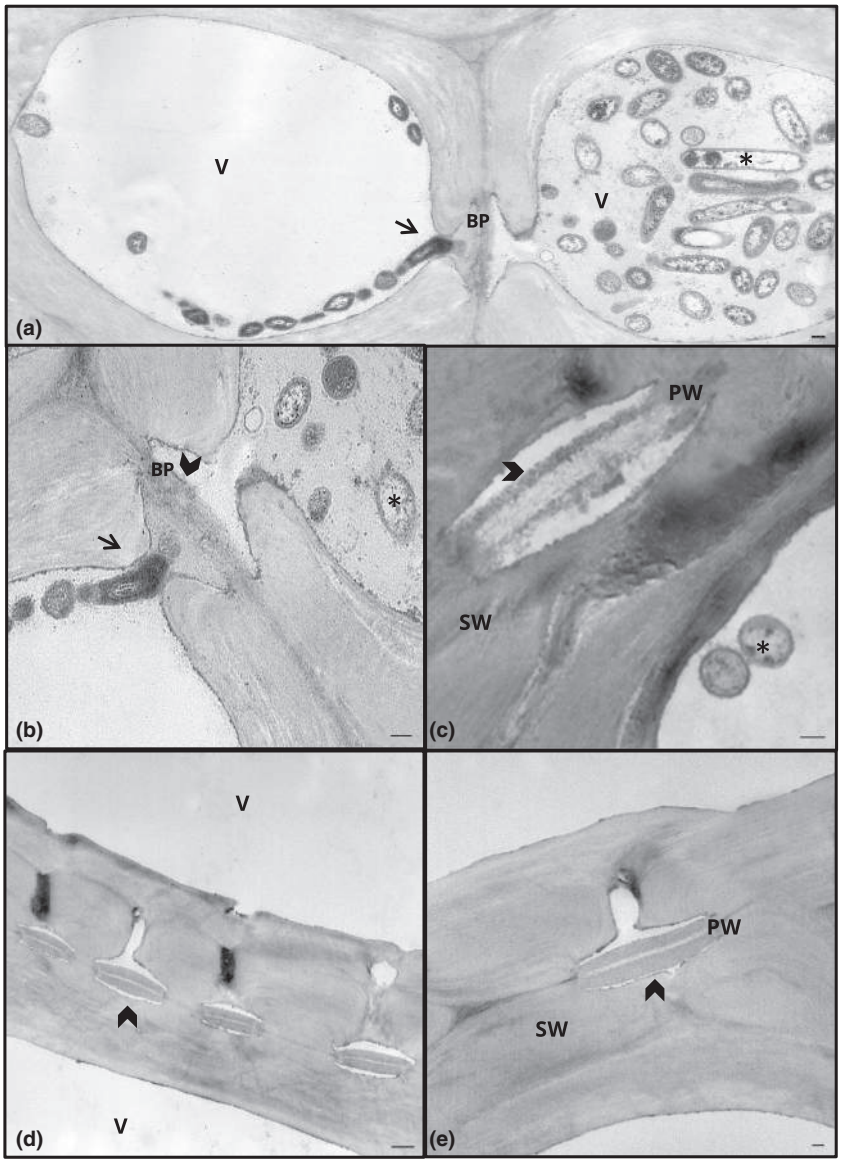

FIGURE 3 Transmission electron micrographs showing cells of *Xylella fastidiosa* subsp. *pauca* strain De Donno (*) in the lumen of xylem vessels of infected olive cultivar Cellina di Nardò (a–c). Arrows point to bacterial cells crossing the pits interconnecting two adjacent vessels (a, b) or free in the lumen (c). In infected tissues, an uneven pit texture was observed [arrowhead in (b, c)]; in healthy mock inoculated plants of the same cultivar (d, e), the pit structure was compact and intact [arrowhead in (d, e)]. V, vessel; BP, bordered pit; PW, primary cell wall and middle lamella; SW, secondary wall. Bars: 500 nm (b, d); 250 nm (a, c, e).

4 | DISCUSSION

Pathogenesis of *X. fastidiosa* is caused by a complex of mechanisms, including the plant anatomophysiology and immune response as well as virulence factors of the bacterium. A consensus of research argues in favour of occlusions playing a major role in the disease symptoms, based on the presence of a higher number of occluded vessels in the xylem of grapevines with PD compared with healthy vines. For example, Sun et al. (2013) found a higher rate of occluded vessels in susceptible (up to 60%) than in resistant (up to 20%) grapevine cultivars. With olive, De Benedictis et al. (2017) found a higher number of occlusions in field-grown olives of the susceptible Cellina di Nardò and Ogliarola Salentina compared with the resistant cultivar Leccino. However, the vessel blockage was not found to be correlated with the induction of symptoms. These authors found, for example a lower number of occlusions in the infected branches of Leccino in comparison with those from healthy trees of the same cultivar. Thus, based on their findings, they concluded that occlusions are not responsible for OQDS symptomatology. On the contrary, Cardinale et al. (2018) reached the opposite conclusion, suggesting a major role of the occlusions (accounting for more than 50% of the stem vessels) in the disease progression on susceptible trees of Ogliarola Salentina. They found that occlusions were made by bacterial aggregates in vessels in petioles and by tyloses/gums in vessels in branches.

Our investigations showed that occlusions were rare in twigs from greenhouse-grown mock-inoculated olives, while they were clearly induced in response to the bacterial infection in both the susceptible and resistant cultivar. The observed average proportion of occluded vessels in infected olives was 6.8% and 9.6% in Leccino and Cellina di Nardò, respectively. Although this is in line with the difference in susceptibility to XfDD of the two cultivars, no correlation can be drawn between the extent of occlusions and the occurrence of symptoms. In addition, there were striking differences in distribution of occlusions among different twigs, reflecting the erratic mode of colonization of the bacterium, which depends on its ability to move within the xylem vessel network. The percentages of occluded vessels found in our study are different from those reported in both previous studies, most probably because these earlier works

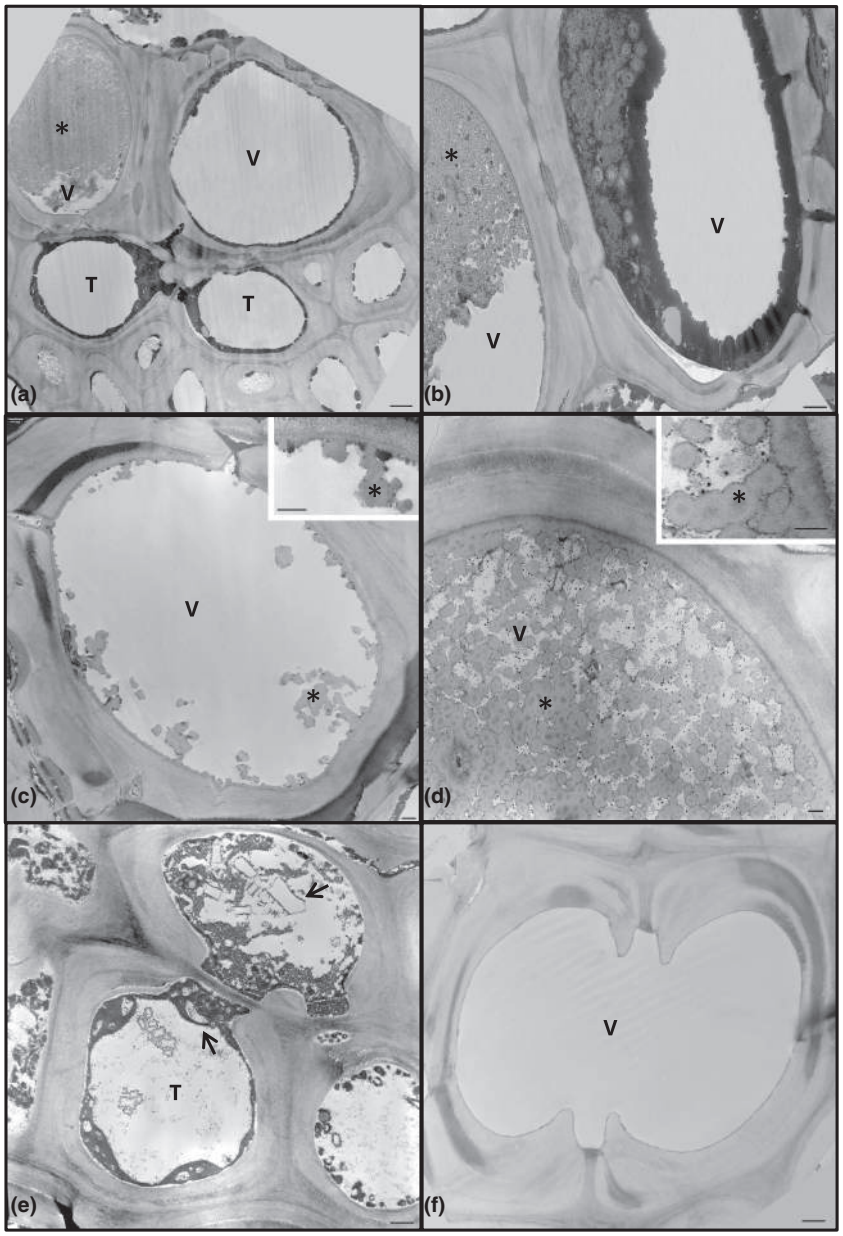

FIGURE 4 Transmission electron micrographs of vascular parenchyma of twigs from olive cultivar Leccino infected with *Xylella fastidiosa* subsp. *pauca* strain De Donno. Callose-like granules (*) occlude the xylem vessels (a–d) and some of these are full of dark matrix (b). Insets of the callose-like granules are shown in (c) and (d). Crystals and electron-dense material (arrow) are present in fibre-tracheids (e). (f) Xylem vessel from a mock-inoculated twig (f). V, vessel; T, fibre-tracheid. Bars: 1 μm (a, b) 500 nm (c–f) 250 nm [inset (c), inset (d)].

referred to twigs from field-grown olives, which were older (i.e., 25–30 years old) and under natural environmental conditions, compared to the potted plants used in our study.

Both De Benedictis et al. (2017) and Cardinale et al. (2018) reported that in twigs of field-grown infected olives, occlusions mainly consisted of tyloses/gums and pectin gels. In contrast, we noted that occlusions were composed of gums or callose-like structures in Cellina di Nardò or Leccino, respectively, and we never observed the presence of tylose-like structures in vessels of either cultivar. Again, we cannot exclude that the different tissue, plant and infection stage could be responsible of the lack of these plant outgrowth structures, although the existence of tyloses in *Olea europaea* has been questioned by Baas et al. (1988) because this plant has small vessel-parenchyma pits. Indeed, our study was performed in greenhouse controlled conditions, on pathogen-tested olives not exposed to biotic stresses and whose time of infection was known. Our observations of the presence of callose-like granules in the resistant Leccino fit better with the description of starch granules in the vessels of this cultivar by Sabella et al. (2019), which act as a refilling mechanism to avoid the loss of plant hydraulic conductivity and/or as a mechanism to entrap the bacteria. Significantly, the results indicate that XfDD exploits the PMs to diffuse systemically within the susceptible Cellina di Nardò. In this cultivar, we observed clearly degraded middle lamellas, which allowed the bacteria to pass to adjacent vessels through degraded PMs. Conversely, this phenomenon was not observed in Leccino, which had intact and more compact PMs, perfused with an electron-dense material, which probably maintained the impermeability of these structures to the bacteria. Such findings, which indicate a diverse susceptibility to XfDD of PMs of the two cultivars and an active role of Leccino in counteracting the infection, suggest a variation in the pectic and cellulose

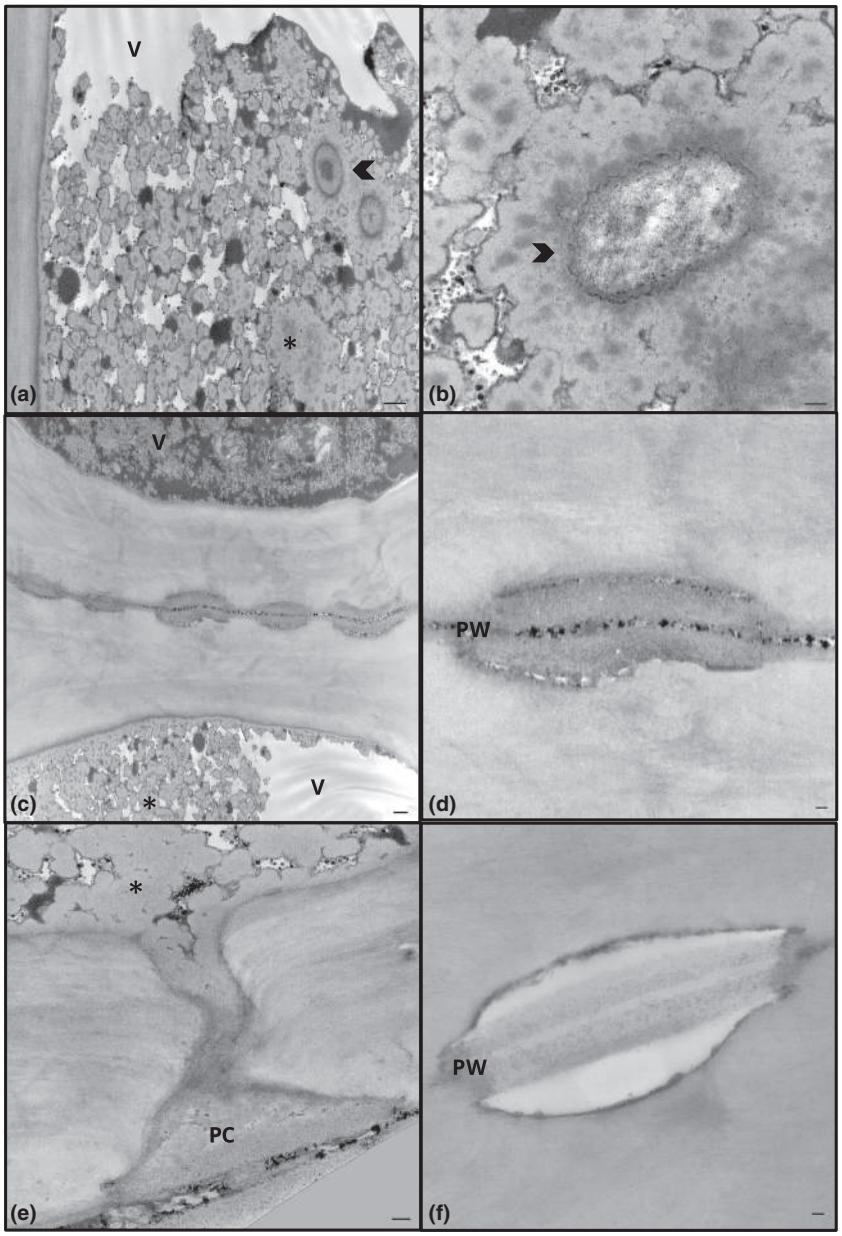

FIGURE 5 Transmission electron micrographs of the xylem vessel content and pit detail of twigs from olive cultivar Leccino infected with *Xylella fastidiosa* subsp. *pauca* strain De Donno (a–d) and from a healthy mock-inoculated twig of the same cultivar (f). A callose-like matrix (*) entraps bacterial cells [arrowhead in (a, b)] and fills the pit chambers (e). The bordered pits and surrounding primary wall between adjacent vessels have a more electron-dense middle lamella and appear intact (c, d) compared with that of the mock-inoculated plant (f). V, vessel; PW, primary wall and middle lamella; PC, pit chamber. Bars: 250 nm (a, c); 100 nm (e, f); 50 nm (b, d).

composition of PMs between Leccino and Cellina di Nardò; such a difference has also been observed between grapevine cultivars (Ingel et al., 2019; Sun et al., 2011) and deserves further study. Thus, our findings provide support to the hypothesis, mainly defined in the grapevine/PD pathosystem, that impairment of xylem conductivity is an initial event in the pathogenesis mechanism occurring in susceptible hosts (Fanton & Brodersen, 2021; Ingel et al., 2021; Pérez-Donoso et al., 2010) and is associated with the ability of the bacterium to spread systemically by crossing the PM barrier. Symptoms are therefore the undesired effect of the plant systemic colonization by the bacteria through the xylem network, whose vessels, interconnected by pores (pits) that are in their intact state not permeable to *Xylella* cells, are occluded by bacterial aggregates during infection (Pérez-Donoso et al., 2010). Previous reports have indicated that the bacteria can cause an increase in pit permeability (increased size and diameter) through the action of a battery of CWDEs that degrade the polysaccharide components of the middle lamella, thus enlarging the pore diameter and allowing systemic colonization (Fanton & Brodersen, 2021; Ingel et al., 2019). A side effect of this degradation is an increased risk of embolism, which leads to the disruption of water transport; such an effect is exacerbated in the susceptible olive cultivar Cellina di Nardò, which has a higher air-embolism vulnerability than the resistant Leccino because of the larger lumen of its xylem vessels (Petit et al., 2021). While we did not prove the direct involvement of CWDEs in olive infections, our observations clearly indicate that XfDD enlarges PMs of susceptible cultivars to spread systemically. Further investigations of the role in the infection process of bacterial CWDEs and the polysaccharide composition of PMs from different olive cultivars may further elucidate the mechanisms of resistance in olives, which can be exploited in future olive breeding programmes.

ACKNOWLEDGEMENTS

This work received funding from the EU H2020 Research Project XF-ACTORS '*Xylella fastidiosa* active containment through a multidisciplinary-oriented research strategy' grant agreement 727987. Open Access Funding provided by Consiglio Nazionale delle Ricerche within the CRUI-CARE Agreement.

[Correction added on 28 November 2022, after first online publication: CRUI-CARE funding statement has been added.]

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Pasquale Saldarelli https://orcid.org/0000-0002-8633-7813

REFERENCES

Baas, P., Esser, P.M., van der Westen, M.E.T. & Zandee, M. (1988) Wood anatomy of the Oleaceae. *IAWA Journal*, 9, 103–182.

Boscia, D., Altamura, G., Notte, P., Morelli, M., Saldarelli, P., Saponari, M. et al. (2017) Resistenza a *Xylella fastidiosa* in diverse cultivar di olivo. *L'Informatore Agrario*, 11, 59–63.

Cardinale, M., Luvisi, A., Meyer, J.B., Sabella, E., De Bellis, L., Cruz, A.C. et al. (2018) Specific fluorescence in situ hybridization (FISH) test to highlight colonization of xylem vessels by *Xylella fastidiosa* in naturally infected olive trees (*Olea europaea* L.). *Frontiers in Plant Science*, 9, 431.

Chang, C.J., Garnier, M., Zreik, L., Rossetti, V. & Bové, J.M. (1993) Culture and serological detection of the xylem-limited bacterium causing citrus variegated chlorosis and its identification as a strain of *Xylella fastidiosa*. *Current Microbiology*, 27, 137–142.

Davis, M.J., Purcell, A.H. & Thomson, S.V. (1980) Isolation media for the Pierce's disease bacterium. *Phytopathology*, 70, 425–429.

De Benedictis, M., De Caroli, M., Baccelli, I., Marchi, G., Bleve, G., Gallo, A. et al. (2017) Vessel occlusion in three cultivars of *Olea europaea* naturally exposed to *Xylella fastidiosa* in open field. *Journal of Phytopathology*, 165, 589–594.

De La Fuente, L., Burr, T.J. & Hoch, H.C. (2008) Autoaggregation of *Xylella fastidiosa* cells is influenced by type I and type IV pili. *Applied and Environmental Microbiology*, 74, 5579–5582.

European and Mediterranean Plant Protection Organization (EPPO). (2019) PM 7/24 (4). *Xylella fastidiosa*. *EPPO Bulletin*, 49, 175–227.

European Food Safety Authority (EFSA). (2022) Update of the *Xylella* spp. host plant database–systematic literature search up to 31 December 2021. *EFSA Journal*, 20, 7356.

Fanton, A.C. & Brodersen, C. (2021) Hydraulic consequences of enzymatic breakdown of grapevine pit membranes. *Plant Physiology*, 186, 1919–1931.

Harper, S.J., Ward, L.I. & Clover, G.R.G. (2010) Development of LAMP and real-time PCR methods for the rapid detection of *Xylella fastidiosa* for quarantine and field applications. *Phytopathology*, 100, 1282–1288.

Hill, B.L. & Purcell, A.H. (1997) Populations of *Xylella fastidiosa* in plants required for transmission by an efficient vector. *Phytopathology*, 87, 1197–1201.

Hopkins, D.L. (1989) *Xylella fastidiosa*: xylem-limited bacterial pathogen of plants. *Annual Review of Phytopathology*, 27, 271–290.

Ingel, B., Jeske, D.R., Sun, Q., Grosskopf, J. & Roper, M.C. (2019) *Xylella fastidiosa* endoglucanases mediate the rate of Pierce's disease development in *Vitis vinifera* in a cultivar-dependent manner. *Molecular Plant-Microbe Interactions*, 32, 1402–1414.

Ingel, B., Reyes, C., Massonnet, M., Boudreau, B., Sun, Y., Sun, Q. et al. (2021) *Xylella fastidiosa* causes transcriptional shifts that precede tylose formation and starch depletion in xylem. *Molecular Plant Pathology*, 22, 175–188.

Li, W.B., Pria, W.D., Jr., Teixeira, D.C., Miranda, V.S., Ayres, A.J., Franco, C.F. et al. (2007) Coffee leaf scorch caused by a strain of *Xylella fastidiosa* from citrus. *Plant Disease*, 85, 501–505.

Martelli, G.P. (2013) Disseccamento rapido dell'olivo. *Georgofili INFO*, 30 October 2013. Available at: https://www.georgofili.info/contenuti/disseccamento-rapido-dellolivo/1510 [Accessed 20 September 2022].

Martelli, G.P., Boscia, D., Porcelli, F. & Saponari, M. (2016) The olive quick decline syndrome in south-east Italy: a threatening phytosanitary emergency. *European Journal of Plant Pathology*, 144, 235–243.

Martelli, G.P., Di Franco, A. & Russo, M. (1984) The origin of multivesicular bodies in tomato bushy stunt virus-infected *Gomphrena globosa* plants. *Journal of Ultrastructure Research*, 88, 275–281.

Mircetich, S.M., Lowe, S.K., Moller, W.J. & Nyland, G. (1976) Etiology of almond leaf scorch disease and transmission of the causal agent. *Phytopathology*, 66, 17–24.

Pérez-Donoso, A.G., Sun, Q., Roper, C.M., Greve, C.L., Kirkpatrick, B. & Labavitch, J.M. (2010) Cell wall-degrading enzymes enlarge the pore size of intervessel pit membranes in healthy and *Xylella fastidiosa*-infected grapevines. *Plant Physiology*, 152, 1748–1759.

Petit, G., Bleve, G., Gallo, A., Mita, G., Montanaro, G., Nuzzo, V. et al. (2021) Susceptibility to *Xylella fastidiosa* and functional xylem anatomy in *Olea europaea*: revisiting a tale of plant–pathogen interaction. *AoB Plants*, 13, plab027.

Purcell, A.H. (2013) Paradigms: examples from the bacterium *Xylella fastidiosa*. *Annual Review of Phytopathology*, 51, 339–356.

Purcell, A.H. & Hopkins, D.L. (1996) Fastidious xylem-limited bacterial plant pathogens. *Annual Review of Phytopathology*, 34, 131–151.

Raju, B.C., Wells, J.M., Nyland, G., Brlansky, R.H. & Lowe, S.K. (1982) Plum leaf scald: isolation, culture, and pathogenicity of the causal agent. *Phytopathology*, 72, 1460–1466.

Rapicavoli, J., Ingel, B., Blanco-Ulate, B., Cantu, D. & Roper, M.C. (2018) *Xylella fastidiosa*: an examination of a re-emerging plant pathogen. *Molecular Plant Pathology*, 19, 786–800.

Roper, M.C., Greve, L.C., Warren, J.G., Labavitch, J.M. & Kirkpatrick, B.C. (2007) *Xylella fastidiosa* requires polygalacturonase for colonization and pathogenicity in *Vitis vinifera* grapevines. *Molecular Plant-Microbe Interactions*, 20, 411–419.

Sabella, E., Aprile, A., Genga, A., Siciliano, T., Nutricati, E., Nicolì, F. et al. (2019) Xylem cavitation susceptibility and refilling mechanisms in olive trees infected by *Xylella fastidiosa*. *Scientific Reports*, 9, 9602.

Saponari, M., Boscia, D., Altamura, G., D'Attoma, G., Cavalieri, V., Zicca, S. et al. (2016) Pilot project on *Xylella fastidiosa* to reduce risk assessment uncertainties. *EFSA Supporting Publications*, 13, 1013E.

Saponari, M., Boscia, D., Altamura, G., Loconsole, G., Zicca, S., D'Attoma, G. et al. (2017) Isolation and pathogenicity of *Xylella fastidiosa* associated to the olive quick decline syndrome in southern Italy. *Scientific Reports*, 7, 17723.

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, 668.

Saponari, M., Giampetruzzi, A., Loconsole, G., Boscia, D. & Saldarelli, P. (2019) *Xylella fastidiosa* in olive in Apulia: where we stand. *Phytopathology*, 109, 175–186.

da Silva, F.R., Vettore, A.L., Kemper, E.L., Leite, A. & Arruda, P. (2001) Fastidian gum: the *Xylella fastidiosa* exopolysaccharide possibly

involved in bacterial pathogenicity. *FEMS Microbiology Letters*, 203, 165–171.

Sun, Q., Greve, L.C. & Labavitch, J.M. (2011) Polysaccharide compositions of intervessel pit membranes contribute to Pierce's disease resistance of grapevines. *Plant Physiology*, 155, 1976–1987.

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 Physiology*, 161, 1529–1541.

Wells, J.M., Raju, B.C., Hung, H.Y., Weisburg, W.G., Mandelco-Paul, L. & Brenner, D.J. (1987) *Xylella fastidiosa* gen. nov., sp. nov: gram-negative, xylem-limited, fastidious plant bacteria related to *Xanthomonas* spp. *International Journal of Systematic and Evolutionary Microbiology*, 37, 136–143.

Wells, J.M., Raju, B.C. & Nyland, G. (1983) Isolation, culture and pathogenicity of the bacterium causing phony disease of peach. *Phytopathology*, 73, 859–862.

How to cite this article: Montilon, V., De Stradis, A., Saponari, M., Abou Kubaa, R., Giampetruzzi, A. & D'Attoma, G. et al. (2023) *Xylella fastidiosa* subsp. *pauca* ST53 exploits pit membranes of susceptible olive cultivars to spread systemically in the xylem. *Plant Pathology*, 72, 144–153. Available from: https://doi.org/10.1111/ppa.13646