Xylella fastidiosa and the Olive Quick Decline Syndrome in Southern Italy

Giovanni P. Martelli
Department of Soil, Plant and Food Sciences, University Aldo Moro, Bari, Italy, Email: giovanni.martelli@uniba.it

Abstract

Martelli, G.P. 2018. *Xylella fastidiosa* and the Olive Quick Decline Syndrome in Southern Italy. Arab Journal of Plant Protection, 36(1): 57-63.

The olive quick decline syndrome (OQDS) is a devastating disease that occurs in the Salento peninsula of Apulia (south-east Italy). In autumn 2103 the xylem-limited Gram-negative bacterium *Xylella fastidiosa* was detected by laboratory assays in symptomatic plants, and studies for determining its epidemiology and involvement in the OQDS genesis were intiated. The bacterium was found in all diseased olive trees sampled in different and geographically separated infection foci, and culturing of over 50 isolates, each from a distinct OQDS focus, was accomplished. A representative bacterial isolate from a pure culture, identified by multilocus sequence typing (MLST) as belonging to sequence type 53 (ST53) of *X. fastidiosa* subsp. *pauca,* was used for needle-inoculation of pot-grown olive and other hosts, reproducing the field symptomatology. The spittlebug *Philaenus spumarious* was experimentally proven to be the vector of the olive-infecting bacterial strain. There is no cure for *Xylella* infections and effective methods for inoculum reduction in the field are few. Thus, combating *X. fastidiosa*-induced diseases relies primarily on the identification and use of resistant germplasm, as well as surveillance for restraining disease spreading through extensive field monitoring, uprooting infected and neighbouring healthy plants in newly detected foci, and vector control. The latter is being attempted in Apulia through mechanical weeding in late winter-early spring to kill juveniles that thrive on weeds, followed by chemical treatments of olive trees in late spring-early summer to control the adults.

The Disease

The quick decline syndrome of olive (OQDS) is a devastating disease that appeared around 2008-2010 in an olive orchard in the countryside of Gallipoli, a small town on the Ionian coast of the Salento peninsula (Apulia region, south-east Italy) and began spreading to neighbouring groves. Symptoms begin with leaf scorching that starts at the tip of the blade and progresses towards the petiole (Figure 1, inset). Dead leaves remain attached to the twigs, which also desiccate, and are shed in the rainy season. Desiccation starts in the upper part of the crown, from where it extends to the whole canopy, conferring upon it a burned-out look (Figure 1). Affected trees, regardless of whether they are young (20-30-year-old), centuries-old, or thousands of years old, decline and die within a few years from the onset of symptoms. Heavy rejuvenation pruning does not help in extending their life. OQDS has been spreading at a rate of about 30 Km/year in a northbound direction. By summer 2017 it was estimated that, in an area of over 5,000 ha, some 10 million trees had contracted the disease, were killed or are bound to die, and that the economic damage amounts to over one billion Euros.

Olive diseases strickingly resembling OQDS have been reported from Argentina and Brazil (7, 15) and, more recently, from the Balearic Islands (1), where symptomatic plants are infected by *X. fastidiosa* strains of the subspecies *pauca.*

The agent

The investigations carried out at Bari (southern Italy) by researchers of: (i) the local University; (ii) a phytopathological outfit of the National Research Council of Italy (CNR); (iii) the Mediteranean Agronomic Institute of Bari (IAMB); (iv) the Centro di Ricerca, Sperimentazione e Formazione in Agricoltura Basile Caramia at Locorotondo (Bari), disclosed that OQDS-affected trees consistently hosted *Xylella fastidiosa* (*Xf*). This finding created much concern in Italy and the European Union (EU) for *Xf*, a most feared quarantinable pathogen, had never been detected for certain in the EU territory, since an earlier record of *Xf* in grapevines in Kosovo (2) is retained as unconfirmed by the Europen and Mediterranean Plant Protection Organization (EPPO).

Xf is a Gram-negative bacterium of the family Xanthomonadaceae (30), that is inoculated in the xylem vessels of the hosts by xylem fluid-feeding insects and is transferred from plant to plant by the same vectors. Colonization of xylem vessels results in their clogging by bacterial colonies and biofilm, which impairs water uptake. This has already a major pathogenic effect, which is aggravated by virulence factors secreted by the bacteria (14, 20).

The *Xf* strain that infects Apulian olives was isolated in axenic culture from this host (4) and other naturally infected plant species, among which periwinkle, oleander, almond, cherry, *Polygala myrtifolia,* and *Westringia fruticosa.* In all cases, the shape and outward aspect of the colonies and

* This symposium was sponsored by the FAO Near East and North Africa Regional Office, and organized as part of the 12th Arab Congress of Plant Protection held in Hurghada, Egypt, 5-9 November 2017.

http://dx.doi.org/10.22268/AJPP-036.1.057063

bacterial cells conformed to those described in the literature.

The genus *Xylella* has two species: *Xf* and the recently described *Xylella taiwanensis* (28). Only the former, however, comprises subspecies four of which, *Xf fastidiosa*, *Xf multiplex*, *Xf pauca* and *Xf sandyi*, are currently retained as taxonomically valid and are differentiated by DNA:DNA hybridization (27) and multi-locus sequence typing (MLST), based on the amplification of seven house-keeping genes (26). These subspecies have a different geographic distribution in the American continent, the site of origin of *Xf*, and an extremely wide host range: 75 botanical families, 204 genera and 359 plant species (9), 22 of which out of more than 600 trees, shrubs and weeds analysed, including grapevines and citrus (22) proved to be hosts of the bacterial isolate present in Salento, which was denoted CoDiRO (abbreviation of Complesso del Disseccamento Rapido del'Olivo, the Italian name of OQDS).

The MLST of this isolate identified it as a divergent strain of *Xf* subsp. *pauca.* Molecular evidence was obtained of its identity with a bacterial isolate (ST53) of the same subspecies from Costa Rica, a country from which it may have landed in Salento with an unidentified ornamental plant (18). This identification was supported by sequencing of the whole genome, a DNA molecule of 2.46 MB (10, 12).

When *Xf* enters an environment whose climatic conditions are favourable, it becomes entrenched in the targeted crop(s) and the native flora and is no longer eradicable. Extensive surveys are therefore needed for delimiting the contaminated area and determining the progression of the infection, for which the use of dependable detection methods is desirable. Serological and molecular protocols were therefore developed and validated with an interlaboratory ring-test following which direct ELISA is now routinely applied (19) and, to a lesser extent, the direct tissue blot immunoassay (DTBIA) (8), utilizing commercial reagents by Loewe (Germany), Agdia (USA) and Agritest (Italy).

Standard, real time and quantitative PCR are employed for molecular testing, using Harper *et al.* (16) primers: forward XF-F:5′CACGGCTGGTAACGGAAGA-3′; reverse XF-R: 5′-GGGTTGCGTGGTGAAATCAAG-3′; Probe XF-P: 5′-6-FAM -TCG CAT CCC GTG GCT CAG TCC-BHQ-1-3′

An on-site version of real-time LAMP (Loop-mediated isothermal amplification) is also used, though to a lesser extent (29).

The validated ELISA and PCR protocols are both employed in the region-wide monitoring programme under way in Apulia under the responsibility of the regional phytosanitery service for the timely identification of novel infection foci. In this survey, some 300,000 olive trees have already been analysed.

Figure 1. Progressive stages of the quick decline syndrome in olive trees infected by Xylella fastidiosa in Salento (Apulia region, south-east Italy).

Although the comparative laboratory analysis of symptomatic and symptomless olive trees had disclosed a complete correlation between the presence of OQDS and *Xf* in declining olives and in other naturally infected and symptomatic hosts, pathogenicity tests were required for the ultimate assessment of the cause-effect relationship between the bacterium and the disease. This relationship was established by fulfilling Koch's postulates through the artificial inoculation by needle puncture of healthy pot-grown plantlets of olive and other hosts (e.g. myrtle-leaf milkwort, oleander) with bacteria from pure *Xf* cultures that reproduced the field symptomatology (25). These experiments provided ultimate evidence that the CoDiRO strain of *Xf* subsp. *pauca* is the agent of OQDS.

The vector

Xf differs from all known bacterial species because of the peculiarity of its way of spreading that relies on transmission by xylem sap-feeding leafhoppers of different families, which acquire the bacterium from the tracheids of the infected hosts while feeding. *Xf* localizes and multiplies in the anterior part of the vector's alimentary canal, where is retained for life, and is transferred to healthy hosts when the infective insect moves to and feeds on them (17, 24).

Among the putative vectors occurring in Salento, the attention was attracted by the meadow spittlebug, *Philaenus spumarious* (family Aphrophoridae), whose adults thrive in great numbers on the olive trees of the orchards of the infected area (5), and move within and between orchards with short flies of 100-120 mt. Because of the density and composition of host plants in the landscape, this behaviour plays a significant role in natural vector dispersal and disease spread from plant to plant.

P. spumarious has a single generation a year. Eggs hatch in winter, the juveniles feed on weeds within protective foam nests and the newly hatched adults, that emerge in late spring, move to olives where they feed on the tender vegetation of the growing shoots. In late summer-early autumn, when the vegetation has hardened, thus is no longer palatable, the adults descend to the ground, where they mate and lay eggs (Figure 2).

A comparative analysis of *P. spumarious* adults collected from infected and healthy olives showed that only those coming from diseased trees were PCR-positive for *Xf*, to a rate sometimes higher than 70-80%. Bacterial colonies were identified by transmission electron microscopy in the foregut of *Xf*-positive insects from populations used for transmission trials to field- and pot-grown olives. Plants exposed to infective spittlebugs reacted with leaf scorching and desiccation of the shoots that reproduced the field symptomatology, thus providing experimental evidence that *P. spumarious* is the vector of the CoDiRO strain of *Xf.* subsp. *pauca* (6, 25).

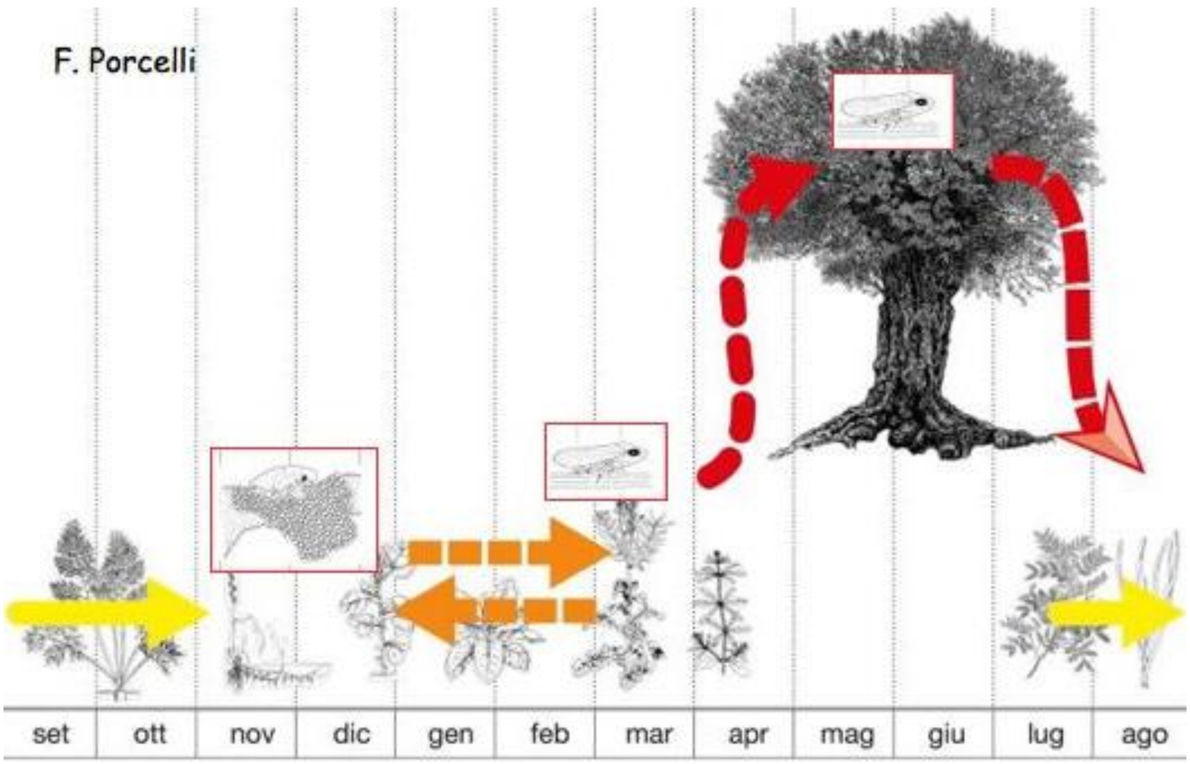

Figure 2. Biological cycle of *Philaenus spumarious* in the *Xylella fastidiosa*-infected area of Salento. Eggs hatch in winter, the juveniles feed on weeds within protective foam nests. The adults emerge in late spring, move to the olive canopy where they remain.

Although *P. spumarius* may not be the only responsible for OQDS spreading (a search for other putative vectors is under way) the size of its populations and the transmission efficiency is such, so as to justify the rate at which OQDS is expanding in the olive orchards of southern Apulia.

Other methods of *Xf* spreading exist that are human-mediated and instrumental for the establishment of novel infection foci in places out of vector reach. For example, vegetative propagation through grafting of *Xf*-susceptible plant species represents an insidious way for disease dissemination over medium and long distances, whereas transport of *Xf*-contaminated vectors by vehicles (cars, trucks, buses, agricultural implements) and humans is an additional method of disease dispersal. Several cases of passive transport of infective vectors that gave rise to novel infection foci several kilometers away from the front of the infection have been documened in Apulia. To avoid this kind of unwelcome events, a few simple rules must be observed after visiting an infected spot, e.g. (i) brush hair and clothes to which the insects adhere before boarding vehicles; (ii) keep windows closed during parking in, or driving through, infected areas; (iii) refrain from collecting weeds and bushy plants from infected and surrounding areas.

Xylella fastidiosa in Europe

The consequences that the arrival of *X. fastidiosa* would have represented for Europe and the Mediterranean basin in terms of territorial invasion by the bacterium have been illustrated by Purcell (23) and, more recently, by Bosso *et al*. (3), following the Apulian OQDS outbreak. These consequences, i.e. the unrestricted conquest over time of wider and wider agricultural areas, are even more incumbent considering that the EU countries have witnessed multiple newly documented *Xf* records from 2012 onwards (Table 1). There is no evidence that any of these new reports is originated by bacterial transfer from an EU country to another. Rather, they seem to derive from new introductions from abroad, most frequently mediated by the import of infected plants for planting. The lack of an EU-implemented quarantine system does not help reducing or suppressing these risks.

Control measures

As a disease agent included in the EPPO 1A list of quarantinable pathogens, *Xf* falls under the European Union Directive 2000/29/CE concerning the implementation of protective measures against the introduction and spreading in the EU territory of organisms harmful to crops. When the presence in any member State of such an organism is ascertained, the Directive compels this State to enforce all possible actions for its eradication or, should this be no longer feasible, as in the case of Italy, for containing its expansion. Thus, following the emergency raised by the Apulian OQDS outbreak, the EU Commission required the application of measures to prevent the spread of *Xf* through the promulgation of the Commission Implementing Decision (EU) 2015/789, amended by the Commission Implementing Decision (EU) 2016/764. Italy was thus requested to conceive and apply a strategy for confining OQDS and its agent within the boundaries of the infected area.

Table 1. Records of *Xylella fastidiosa* in the European Union territory.

Country and year	*Xylella* subspecies	Host	Field outbreak
Kosovo 1998	*X. fastidiosa*	Grapevine	Yes (unconfirmed record according to EPPO)
France 2012	*X. fastidiosa*, *X. sandyi*, *X. pauca*	Interception in imported plants	No
Italy 2013	*X. pauca*	Olive and other hosts	Yes
The Netherlands 2014	Unidentified	Interception in imported plants	No
France (Corsica and mainland) 2015	*X. fastidiosa*, *X. muliplex*, *X. pauca*, recombinant *X. multiplex/X. sandyi*	*Polygala myrtifolia*, *Lavandula* sp., *Pelargonium* sp. and other hosts	Yes
Switzerland 2015	*X. sandyi*, *X. pauca*	Imported coffee plants	No
Germany 2016	*X. fastidiosa*	Oleander	No
Spain (Balearic islands and mainland) 2016	*X. fastidiosa*, *X. multiplex*, *X. pauca*	Olive, almond, cherry	Yes

The information gathered with the studies carried out in Apulia has laid the bases for a OQDS containment plan designed in 2015 and approved by by the Italian Ministry of Agriculture. This plan identified demarcated areas, whose size was extended in 2016 by the the EU Standing Committee on Plants, Animals, Food and Feed (PAFF), that consisted of a "containment area" ajacent to the infected zone, and a "buffer zone" immediately north of it, both of which are several kilometers wide and extend across the Salento peninsula, from the Adriatic to the Ionan coast. The "buffer zone" and "containment area" are the object of: (i) extensive monitoring for the presence of *X. fastidiosa* and its vector in primary (olive) and secondary (various species of woody and bushy plants) hosts; (ii) immediate uprooting of infected olive trees and those bordering them within a 100 mt radius in novel infection foci; (ii) mechanical elimination of weeds and shrubs on which vector juveniles thrive; (iii) chemical treatments of olives against adult vector populations; (iv) avoidance of passive transport of vectors and pathogen across and outside the delimited areas.

Whether or not this earnest attempt to restrain OQSD within its current boundaries could succeed will very much depend on the rigorous and continuous surveillance of the territory and the stringent enforcement of the envisaged actions.

While hopes are nursed for confining OQDS within its current boundaries to save from disaster the olive groves of the Italian regions neighbouring Apulia, the problem remains of what kind of action should be taken to reclaim the infected area, where the disease is out control and no ways are known to save an olive industry largely based on a couple of cultivars ('Cellina di Nardò' and 'Ogliarola salentina') highly susceptible to *Xf*.

The imput of local growers corroborated by extensive field surveys has allowed the identification of a set of less grown cultivars, among which cv. Leccino, that survive *Xf* infection, show very mild symptoms and diaplay a vegetation that can be defined as luxuriant when compared with that of the two above-mentioned major cultivars (Figure 3). Artificially inoculated young plants of cv. Leccino were infected but displayed very mild symptoms, whereas the analysis of Leccino's transcriptome (13) revealed: (i) an up-regulation of the genes encoding receptor-like kinases (RLK) and receptor-like proteins (RLP), which are involved in resistance mechanisms and are missing in cv. Ogliarola salentina; (ii) the bacterial concentration is much lower in cv. Leccino (133,267 CFU/ml of tissue extract) than in cv. Ogliarola salentina (2,094,000 CFU/ml of tissue extract) and in cv. Cellina di Nardò (1,060,000 CFU/ml).

Therefore, looking for and multiplying *Xf*-resistant germaplasm appears at the moment a most promising option, and the one that would allow a prompt reconversion of the Salentinian olive indusry.

Figure 3. Different reaction to Xylella fastidiosa infection of adjacent rows of cv. Leccino and Ogliarola salentina growing in a heavily infected area. The resistant cv. Leccino grows much more vigorously than the susceptible and strongly symptomatic cv. Ogliarol

الملخص

مارتللي، جيوفاني. 2018. بكتيريا *Xylella fastidiosa* ومتلازمة التدهور السريع للزيتون في جنوب إيطاليا. مجلة وقاية النبات العربية، 36(1): 57-63.

تعد متلازمة التدهور السريع للزيتون مرضاً خطيراً منتشر في شبه جزيرة سالنتو في منطقة أبوليا التي تقع في جنوب شرق إيطاليا. تم في خريف العام 2013 الكشف عن وجود بكتيريا *X. fastidiosa* وهي سالبة لجرام ومحددة في الأوعية الخشبية بوساطة فحوصات مختبرية على النباتات التي ظهر عليها أعراض الإصابة، وبناء عليه تم البدء بدراسات حول بيئيات مرض التدهور السريع للزيتون وإسهام البكتيريا في نشوء متلازمة التدهور السريع للزيتون. لقد تم تأكيد وجود البكتيريا في جميع أشجار الزيتون المصابة والتي جمعت عينات منها من جميع مواقع الإصابة، وتم زرع 51 عزلة بكتيرية، واحدة من كل بؤرة إصابة مميزة.استخدمت عزلة ممثلة من هذه البكتيريا، تم تحديدها بوساطة نمذجة التسلسل النيوكليوتيدي متعدد المواقع (MLST) بأنها تتبع التسلسل 53 للبكتيريا *X. fastidiosa* subsp. *pauca*، من أجل إلقاح الزيتون وعوائل أخرى بها بواسطة الإبرة، لإعادة إظهار الأعراض نفسها التي شوهدت في الحقول المصابة. كما أكدت التجارب بأن الناقل الحشري لهذه البكتيريا هو نطاط الضفادع *Philaenus spumarious*. لا يوجد علاج ناجح للأشجار المصابة كما أن الطرائق الكفيلة للتقليل من مصدر العدوى محدودة. لذلك فإن الطريقة الفضلى لمكافحة هذه البكتيريا هو من خلال تحديد واستعمال الأصناف المقاومة، بالإضافة إلى المراقبة الموسعة لمناطق الإصابة وتحديد النباتات المصابة ومن ثم إزالتها مع تلك السليمة المجاورة لها في البؤر المكتشفة حديثاً، وكذلك مكافحة الحشرة الناقلة. وقد تم استخدام هذه الطريقة الاخيرة في منطقة أبوليا من خلال الإزالة الميكانيكية للأعشاب الضارة في أواخر الشتاء وأوائل الربيع لقتل الأعمار اليرقية للناقل الحشري التي تعيش على هذه الأعشاب، يتبع ذلك رش أشجار الزيتون، في أواخر الربيع وأوائل الصيف، بالمبيدات الحشرية لمكافحة الطور الكامل للناقل الحشري.

عنوان المراسلة: جيوفاني مارتللي، قسم التربة والنبات والعلوم الغذائية، جامعة ألدو مورو، باري، إيطاليا، البريد الإلكتروني: giovanni.martelli@uniba.it

References

1. **Anonymous.** 2017. Detectada en Ibiza la subespecie de *Xylella fastidiosa* causante de la muerte de miles de olivos en Italia. Phytoma España, 287: 2.
2. **Berisha, B., Y.D. Chen, G.Y. Zhang, B.Y. Xu and T.A. Chen.** 1998. Isolation of Pierce's disease bacteria from grapevines in Europe. European Journal of Plant Pathology, 104: 427-433.
3. **Bosso, L., M. Di Febbraro, G. Cristinzio, A. Zoina and D. Russo.** 2016. Shedding light on the effect of climate change on the potenetial distribution of *Xylella fastidiosa* in the Mediterranean basin. Biological Invasions, 18: 1759-1768.
4. **Cariddi, C., M. Saponari, D. Boscia, A. De Stradis, G. Loconsole, F. Nigro, F. Porcelli, O. Potere and G.P. Martelli.** 2014. Isolation of a *Xylella fastidiosa* strain infecting olive and oleander in Apulia, Italy. Journal of Plant Pathology, 96: 425-429.
5. **Cornara, D., G. Loconsole, D. Boscia, A. De Stradis, R.K. Yokomi, D. Bosco, F. Porcelli, G.P. Martelli and M. Saponari.** 2014. Survey of *Auchenorrhyncha* in the *Salento penisnsula* in search of putative vectors of *Xylella fastidiosa* subsp. *pauca* CoDiRO strain. Journal of Plant Pathology, 96: S4.97-S4.104.
6. **Cornara, D., M. Saponari, A.R. Zeilinger, A. de Stradis, D. Boscia, G. Loconsole, D. Bosco, G.P. Martelli, R.P.P. Almeida and F. Porcelli.** 2017. Spittlebugs as vectors of *Xylella fastidiosa* in olive orchards in Italy. Journal of Pest Science, 90: 521-530.
7. **Della Coletta-Filho, H., C.S. Francisco, J.R. De Oliveria and L.F. de Oliveira Da Silva.** 2016. First report of olive leaf scorch in Brazil, associated with *Xylella fastidiosa* subsp. *pauca*. Phytopathologia Mediterranea, 55:130-135.
8. **Djelouah, K., D. Frasheri, F. Valentini, A.M. D'Onghia and M. Digiaro.** 2014. Use of tissues blot immunassay for detection of *Xylella fastidiosa* in olive trees. Phytopathologia Mediterranea, 53: 559-564
9. **EFSA.** 2016. Update of a database of host plants of *Xylella fastidiosa*: 20 November 2015. EFSA Journal, 14: 4378
10. **Giampetruzzi, A., M. Chiumenti, M. Saponari, G. Donvito, A. Italiano, G. Loconsole, D. Boscia, C. Cariddi, G.P. Martelli and P. Saldarelli.** 2015. Draft genome sequence of the *Xylella fastidiosa* CoDiRO strain. Genome Announcements, 3: e01538-14.
11. **Giampetruzzi, A., M. Morelli, M. Saponari, G. Loconsole, M. Chiumenti, D. Boscia, V. Savino, G.P. Martelli and P. Saldarelli.** 2016. Transcriptome profiling of two olive cultivars in response to infection by the CoDiRO strain of *Xylella fastidiosa* subsp. *pauca*. BMC Genomics, 17: 475.
12. **Giampetruzzi, A., M. Saponari, R.P.P. Almeida, S. Essakhi, D. Boscia, G. Loconsole and P. Saldarelli.** 2017a. Complete genome sequence of the olive-infecting strain *Xylella fastidiosa* subs. *pauca* De Donno. Genome Announcements, 5: e00569-17.
13. **Giampetruzzi, A., M. Saponari, G. Loconsole, D. Boscia, V.N. Savino, R.P.P. Almeida, S. Zicca, B.B. Landa, C. Chacón-Diaz and P. Saldarelli.** 2017b. Genome-wide analysis provides evidence on the genetic relatedness of the emergent *Xylella fastidiosa* genotype in Italy to isolates from Central America. Phytopathology, 107: 816-827.
14. **Gouran, H., H. Gillespie, F. Nascimiento, S. Chakraborty, P.A. Zaini, A. Jacobson, B.S. Phinney, D. Dolan, B.P. Durbin-Johnson, E.S.**

Antonova, S.E. Lindow, M.S. Mellema, L.R. Goulart and A.M. Dandekar. 2016. The secreted protease PrtA controls cell growth, biofilm formation and pathogenicity in *Xylella fastidiosa*. Scientific Reports, 6: 31098 DOI:10.1038/srep31098

15. **Haelterman, R.M., P.A. Tolocka, M.E. Roca, F.D. Guzman, F.D. Fernandez and M.L. Otero.** 2015. First presumed diagnosis of *Xylella fastidiosa* causing olive scorch in Argentina. Journal of Plant Pathology, 97: 393.
16. **Harper, S.J., L.I. Ward and G.R.G. Clover.** 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
17. **Hopkins, D.L.** 1989. *Xylella fastidiosa*: xylem-limited bacterial pathogen of plants. Annual Review of Phytopathology, 27: 271-290
18. **Loconsole, G., R. Almeida, D. Boscia, G.P. Martelli and M. Saponari.** 2014a. Multilocus sequence typing reveals the distinctiveness of the *Xylella fastidiosa* strain CoDiRO. Journal of Plant Pathology, 96: S4.110.
19. **Loconsole, G., O. Potere, D. Boscia, G. Altamura, F. Palmisano, P. Pollastro, M.R. Silletti, N. Trisciuzzi, K. Djelouah, T. Elbeaino, D. Frasheri, D. Lorusso, F. Valentini, V. Savino and M. Saponari.** 2014b. Detection of *Xylella fastidiosa* in olive trees by molecular and serological methods. Journal of Plant Pathology, 96: 7-14.
20. **Nascimiento, R., H. Gouran, S. Chakraborty, H.W. Gillespie, H.O. Almeida-Souza, A. Tu, B.J. Rao, P.A. Feldstein, G. Bruening, L.R. Goulart and A.M. Dandekar.** 2016. The Type II secreted lipase/esterase LesA is a key virulence factor required for *Xylella fastidiosa* pathogenesis in grapevines. Scietific Reports, 6: 18598. DOI: 10.1038/srep18598
21. **Nigro, F., D. Boscia, I. Antelmi and A. Ippolito.** 2013. Fungal species associated with a severe decline of olive in southen Italy. Journal of Plant Pathology, 95: 668.
22. **Potere, O., L. Susca, G. Loconsole, M. Saponari, D. Boscia, V. Savino and G.P. Martelli**. 2015. Investigation on the presence of *Xylella fastidiosa* strain CoDiRO in some forestry and ornamental species in the Salento peninsula. Journal of Plant Pathology, 97: 373-376.
23. **Purcell, A.** 1997. *Xylella fastidiosa*, a regional problem or global threat? Journal of Plant Pathology 79: 99-105.
24. **Purcell, A.** 2013. Paradigms: exampls from the bacterium *Xylella fastidiosa*. Annual Review of Phytopathology, 51: 339-356.
25. **Saponari, M., D. Boscia, G. Altamura, G. Loconsole, S. Zicca, G. D'Attoma, M. Morelli, F. Palmisano, A. Saponari, D. Tavano, V.N. Savino and G.P. Martelli.** 2017. Isolation and pathogenicity of *Xylella fastidiosa* associated with the olive quick decline syndrome in southern Italy. Scientific Reports, (submitted)
26. **Scally, M., F.L. Schuenzell, R. Stouthamer and L. Nunney,** 2005. Multilocus sequence type system for the plant pathogen *Xylella fastidiosa* and relative contributions of recombination and point mutation to clonal diversity. Applied and Rnvironmental Microbiology, 71: 8491-8499.
27. **Schaad, N.W., E. Postikova, G. Lacy, M. Barek Fatmi and J.C. Chung.** 2004. *Xylella fastidiosa* subspecies: *X. fastidiosa* subsp *piercei*, subsp. nov., *X. fastidiosa* subsp. *multiplex* subsp. nov., and *X. fastidiosa* subsp. *pauca* subsp. nov. Systematic and Applied Microbiology, 27: 290-300
28. **Su, C.C., W.L. Deng, F.J. Jan, C.J. Chang, H. Huang, H.T. Shih and J. Chen.** 2016. *Xylella taiwanensis* sp. nov., causing pear leaf scorch disease. International Journal of Systematic and Evolutionary Microbiology, 66: 4766-4771.
29. **Yaseen, T., S. Drago, F. Valentini, T. Elbeaino, G. Stampone, M. Digiaro and A.M. D'Onghia.** 2015. On-site detection of *Xylella fastidiosa* in host plants and in "spy insects" using the real-time loop-mediated isothermal amplification method. Phytopathologia Mediterranea, 54: 488-496.
30. **Wells, J.M., B.C. Raju, H.Y. Hung, W.G. Weisburg L. Mandelco-Paul and D.J. Brenner.** 1987. *Xylella fastidiosa* gen. nov., sp. nov.: Gram-negative, xylem-limited fastidious plant bacteria related to *Xanthomonas* spp. International Journal of Systematic Bacteriology, 37: 136-143.