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Xylella fastidiosa – a new menace for Portuguese agriculture and forestry

Xylella fastidiosa: uma nova ameaça para a agricultura e floresta portuguesas

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ABSTRACT

Xylella fastidiosa is a gram-negative bacterium classified as a quarantine pest by EPPO (A1 list), since 1981. Is a xylem-limited bacteria, with a wide host range (more than 300 species), that affects economically important agricultural crops. It is a challenge of almost 30 years in America for grapevine (Pierce's disease) and citrus (Citrus Variegated Chlorosis) farmers. The first outbreak in Europe was reported in October 2013 in southern Italy, in Puglia region, Salento area. The CoDiRO strain (*Xylella fastidiosa* subsp. *pauca)* was responsible for the named – Complesso del disseccamento rapido dell'olivo (CoDiRO). More than 8000 ha of olive trees have been destroyed and nearly 21 000 ha are in quarantine, which counts for more than 53 million euros in losses. It was confirmed, in January 2015, by the European Food Safety Authority, that, the *X. fastidiosa* spreading is far from being contained. EFSA experts have alerted European countries that *X. fastidiosa* is now "very likely" to spread, posing a major risk to European olive trees and have warned that the disease is a "very serious threat" to European agriculture. There is no record of successful eradication of *X. fastidiosa* once it has been established. A wide-ranging research is necessary because *X. fastidiosa* has a remarkable power of adaptation. It has been proven that introgression events allowed *X. fastidiosa* to access new hosts. Its natural competence to acquire new genes turns it into a phytopatogen, with all the necessary conditions to adapt almost to any ecological niche.

Keywords: *Xylella fastidiosa*, olive tree, xylem fluid-feeding insects

RESUMO

Xylella fastidiosa é uma bactéria Gram-negativa classificada como organismo de quarentena pela EPPO (Lista A1), desde 1981. É uma bactéria restrita ao xilema, com uma ampla gama de hospedeiros (mais de 300 espécies), que afeta as culturas agrícolas e espécies florestais de grande importância económica. É um desafio de quase 30 anos na América para os produtores de videira (doença de Pierce) e citrinos (clorose variegada dos citrinos). O primeiro surto na Europa foi reportado em outubro de 2013, no sul da Itália, na região de Apúlia, área de Salento. A estirpe CoDiRO (*X. fastidiosa* subsp. *pauca)* foi responsável pela doença – "Complesso del disseccamento rapido dell'olivo" (CoDiRO). Mais de 8000 ha de oliveiras foram destruídas, e quase 21 000 ha estão em quarentena, o que equivale a mais de 53 milhões de euros em prejuízos. É confirmado pela EFSA, que emitiu um alerta em janeiro de 2015, que a disseminação de *X. fastidiosa* está longe de estar controlada. Especialistas da EFSA alertaram os países europeus para o facto de ser "muito provável" que *X. fastidiosa* se dissemine, o que representa um grande risco para olivais europeus, e advertiram que a doença é uma "ameaça muito séria" para a agricultura europeia. Não há registo de planos de erradicação bem-sucedidos, assim uma estratégia de investigação abrangente e multidisciplinar, é necessária porque *X. fastidiosa* tem um notável poder de adaptação. Está provado que eventos de introgressão têm permitido que *X. fastidiosa* angarie novos hospedeiros. A sua competência natural de adquirir novos genes transforma-a num agente patogénico, com todas as condições necessárias para se adaptar a quase qualquer nicho ecológico.

Palavras-chave: *Xylella fastidiosa*, oliveira, insetos picadores-sugadores de fluido xilémico

Introduction

Xylella fastidiosa Wells *et al.* (1987) is in the EPPO A1 List quarantine pest, since 1981. It is a gram-negative bacterium, xylem-limited, with a wide host range (more than 300 species), that affects economically important agricultural crops (EFSA, 2015(1)). According to serological and phylogenetic studies, its strains were divided into four subspecies: *X. fastidiosa* subsp. *fastidiosa* Wells *et al.*, *X. fastidiosa* subsp. *sandyi* Schuenzel *et al.*, *X. fastidiosa* subsp. *multiplex* Schaad *et al.* and *X. fastidiosa* subsp. *pauca* Schaad *et al.* (Schaad *et al.*, 2004; Schuenzel *et al.*, 2005). Edaphic conditions and climate change have profound impacts on the distribution, abundance and ecology of plant species, and affect directly plant-insect-pathogen communities. Main symptoms of *X. fastidiosa* disease are leaf scorching, leaf blade drop, marginal leaf necrosis and dieback (Figures 1, 2 and 3).

Figura 1 - Symptoms of *Xylella fastidiosa* infection in olive trees, with apical and complete leaf scorch (Photo: P. Sá-Pereira, 2014).

Figura 2 - Symptoms of *Xylella fastidiosa* infection in olive trees, with complete branch desiccation (A and B) (Photo: P. Sá-Pereira, 2014).

Figura 3 - Symptoms of *Xylella fastidiosa* infection in *Nerium oleander*, with marginal leaf scorch (Photo: P. Sá-Pereira, 2014).

X. fastidiosa modus operandi

Xylem fluid-feeding insects convey the bacterium. For Europe, Aphrophoridae, Cercopidae, Cicadellidae subfamily Cicadellinae, Cicadidae and Tibicinidae are pointed as the potential vectors of disease dissemination. However, Purcell (1989) noted that any xylem fluid-feeding hemipteran should be regarded as a potential vector of the bacterium.

The development of the disease in plants depends mainly on the bacterium's ability to move from the inoculation point and to develop a systemic population on infected plants. After inoculation, the bacterial cells, adhered to xylem vessel walls, multiply, forming a biofilm of adherent colonies which can completely plug the xylem vessels, blocking the transport of water and salts. The formation of tylosis leads to the disease symptoms, leaf scorch and to a progressive death from the apical organs to the roots, depending on the host susceptibility.

The bacterium has a reduced growth rate and the symptoms, sometimes, are only evident too late, limiting a preventive diagnosis.

Recent outbreaks

In 2013, there was a *X. fastidiosa* outbreak in Apulia region (Italy) whereas *X. fastidiosa* subsp. *pauca* was associated with the quick decline symptoms on olive trees (Complesso del disseccamento rapido dell'olivo – CoDiRo). *X. fastidiosa* was also detected in oleander, almond, cherry, rosemary, myrtle-leaf milkwort, *Spartium junceum* L. and *Acacia saligna* (Labill.) H.L.Wendl. in that region (Saponari *et al*., 2014). After the CoDiRo outbreak, an EU decision No. 2014/497/EU was published, with specific measures applied to plants for planting, with the exception of the seeds of *Olea*, *Catharanthus*, *Nerium*, *Prunus*, *Vinca*, *Malva*, *Portulaca*, *Quercus* and *Sorghum*. However, important cultures such grapevine and citrus (namely *Citrus sinensis* (L.) Osbeck) are not included in the decision.

There are at least four factors that increase the risk of introduction of *X. fastidiosa* in Portugal: a) a very particular geographical position in Europe and in the world of global trade; b) the mild climatic conditions in the mainland and in the Atlantic islands (Azores and Madeira); c) the presence of insect vectors in Portugal as *Philaenus spumarius* L. (Hemiptera: Aphrophoridae), *Cicadella viridis* L. (Hemiptera: Cicadellidae) and others, as well as in Spain, France and Italy, and d) the presence of preferred hosts such as olive trees, grapevines, citrus, almond and plum trees, oleanders, and *Quercus* sp. which are crops of great economic importance for Portuguese agriculture.

Field surveys and transmission experiments have been carried out in Italy to identify the vector(s) and to describe the epidemiology of the infections. The *X. fastidiosa* vector identified in Italy was *Philaenus spumarius*, which also occurs in Portugal, but, so far, *X. fastidiosa* was not detected and several situations are being surveyed. Despite the legislation issued by the European Commission, which operates in a preventive sense, and after the evaluation of *X. fastidiosa* dissemination, knowledge of the insect-vector behaviour and the economic impact of *X. fastidiosa* infection on Italian olive oil production, it was launched an European alert. EFSA emitted a scientific opinion (EFSA, 2015(2)) where is considered very high the risk to plant health posed by *X. fastidiosa* in the EU territory, confirmed that the spread of *X. fastidiosa* in Italy, is far from being under control. EFSA stated that it is now ..."very likely"... that *X. fastidiosa* will spread to other European countries, one ..."very serious threat..." to European agriculture, because there is no record of successful eradication processes, since all xylem sap-feeding insects, such as Aphrophoridae (true spittlebugs), Cercopidae (false spittlebugs), Cicadellidae subfamily Cicadellinae (sharpshooters), Cicadidae and Tibicinidae (cicadas), are potential vectors also in Europe. Also refers that has not been identified the potential host range of *X. fastidiosa* in the European flora. EFSA concludes that the identification and evaluation of risk reduction options for each country are crucial.

In Portugal, the survey is being developed, to identify other potential vectors, and in Spain, the potential *X. fastidiosa* vectors were identified by Lopes *et al*. (2014) as the Typhlocybinae leafhopper, *Austroasca* (=Jacobiasca) *lybica* (Hemiptera: Cicadellidae) that were the most abundant species in vineyards and citrus orchards. However, spittlebugs already associated with susceptible crops in Spain, may allow fast spread of *X. fastidiosa* in case this pathogen is introduced.

How to act to prevent *X. fastidiosa* infection

As a result of *X. fastidiosa* plasticity, many hosts can be infected without showing any infection symptoms, making it a silent disease, but very deadly. The early detection of the presence of this pathogen and characterization of the pathosystem will allow drawing timely prevention plans, and effective contingency and eradication plans. In Portugal, the olive culture is extremely important, not only because it is a significant source of income, but also because olive trees are a national icon, along with *Quercus suber* L., being part of the Mediterranean landscape for over 8000 years.

From the last international conferences on *X. fastidiosa*, (October 2014, in Gallipoli, Italy and February 2015, in Córdoba, Spain) and EFSA experts opinion (2015(1)), several indications were presented in order to restrict the dissemination of this bacteria since, probability models of introduction of *X. fastidiosa* in Iberian Peninsula, namely in Spain, are quite pessimistic because there are no effective methods of disease prevention. So, it is generally accepted that there are key strategies that have to be applied, such as: limit the mobility of host plants in the affected areas, being aware that a large number of host plants, *X. fastidiosa* symptomless, establish safety barriers, buffer areas, to fight the vectors and their shelters, especially leafhopper insects; mandatory certification of nurseries with the implementation of a phytosanitary passport for movements of plants (potential hosts) between internal borders.

It seems that *X. fastidiosa* have affected the weakest olive trees in Italy, with nutritional deficiencies, so Italian experts recommended application of insecticides in affected areas, weeding, pruning and other cultural practices, in order to improve general health of orchards.

Highlights for combating *X. fastidiosa*

The xylem is the battleground for plant hosts (Janse and Obradovic, 2010; Schuman and D'Arcy, 2010) and specifically for olives, since its sectored network architecture affects the transport of water, propagation of embolism, plant survival and growth (Redak *et al*., 2003). This sectored architecture, may explain the effect of the irregular distribution of *X. fastidiosa* symptoms on olive trees in Italy. Resistance or susceptibility may depend on *X. fastidiosa* ability to survive inside the xylem elements over the winter. Feil and Purcell (2001) found that populations of *X. fastidiosa* in grapevine seedlings declined 160-fold after 15 days at 5°C. Conversely, Meyer (2010), observed that *X. fastidiosa* could survive up to 8 weeks in artificial media at -5°C. These results suggest that some components of the xylem fluid in plants may be affecting *X. fastidiosa* survival. Additionally, *X. fastidiosa* growth rate has been correlated to the concentration of Cu, Mg, P and Zn, among other components such as amino acids and citric acid. Cobine *et al.* (2013) showed significant accumulations of copper (30-fold), manganese (6-fold), zinc (5-fold), calcium (2-fold) and potassium (2-fold) in *X. fastidiosa* cells grown in biofilm when compared to planktonic. The organic fraction of xylem-sap is responsible for many functions, and could be associated with oxidative stress to overcome the host defence mechanisms and insect-plant tropism. Other functionality of the xylem-sap is that it is a "culture media" for not just *X. fastidiosa*, but also for local endophytes. In all plants, endophytic communities play an important role in agriculture by improving plant performance and adaptation against biotic and abiotic stresses. The research approaches of endophytic communities are mainly associated with plant macerates, usually leaves. There are very few studies about the characterisation on xylem-sap endophytes. Bell *et al.* (1995) studied the xylem-sap endophytes grapevines, and concluded that there was no significant difference in the number of endophytic bacteria found in two grape varieties, but there was a significant difference between vineyards. These results show the importance of the biotic and abiotic factors over the genetics of each variety, and the evaluation of influence which olive grove location, soils etc., will have, for certain, a major contribution for gathering extra knowledge to be applied in *X. fastidiosa* infection. Also, the salivation-egestion feeding process in the insect vector may have a determinant role in *X. fastidiosa* infection establishment. *X. fastidiosa* is a non-circulative pathogen and immediate salivation of the insect-vector before feeding process is necessary for detachment of *X. fastidiosa* (Sharman *et al*., 2014). Saliva loosens the *X. fastidiosa* bacterial biofilm by enzymatically degrading the β-1,4 glucans that form the chemical backbone of the exopolysaccharide matrix binding bacteria to the insect's cuticle. The enzymatic cocktail that is injected on xylem vessels, polygalacturonases, β-glucosidases, ß-1,4-glucanases and endo-ß-xylanases are key enzymes related with *X. fastidiosa* spreading and biofilm formation (Killiny *et al.*, 2010; Backus *et al*., 2012). So, the identification of specific inhibitors for those enzymes can be a valuable tool to control the *X. fastidiosa* infection.

Conclusions

A comprehensive research is needed because *X. fastidiosa* has a remarkable ability to adapt to different conditions. There is evidence that introgression events allowed *X. fastidiosa* to infect new hosts. Its natural competence to acquire new genes turns it into a phytopathogen, with all the tools that are needed to adapt almost in any ecological niche. This flexibility to adaptation and climate change, leads us to predict that if we do not design a strong prevention plan based on local data, yield losses and other kind of damage, will reflect a great blow in the European economy, which will require extremely expensive control measures.

In order to be alert, it is essential to understand the disease and their vectors, and be aware of the symptoms in different hosts, because this is the only way to protect plants in each country. It is therefore crucial financing knowledge networks with technical skills and complementary expertise, including farmers, farmers' associations, research institutes with skills and authorisation to investigate quarantine organisms, the national plant protection authorities, and regional directorates of agriculture, in order to facilitate the transfer of knowledge and innovation, and reducing the negative impacts resulting from a deficient strategy, since there are no knowledge about susceptibility and resistance of indigenous hosts and their cultivars to infection by *X. fastidiosa* .

Strategic knowledge of the action plans implemented in countries that are struggling with *X. fastidiosa*, is crucial for other *X. fastidiosa*-free countries to draw preventive, contingency and eradication plans, supported by critical success criteria, allowing the resources optimisation.

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