REVIEW

Pest risk analysis on Xylella fastidiosa in Morocco

Mohamed Afechtal^{1*}, Antonio Vicent², Maria Saponari³, Anna Maria D'Onghia⁴

¹National Institute for Agricultural Research (INRA), Regional Center of Kénitra, Laboratory of Virology, Rue Ibn Temmam, P.B. 257. Kénitra, Morocco

² Istituto Valenciano di Investigaciones Agrarias (IVIA), CV-315, Km. 10,7-4613 Moncada, Spain

³ Istituto per la Protezione Sostenibile delle Piante, UOS Bari, Consiglio Nazionale delle Ricerche, I-70126 Bari, Italy

⁴ Centre International de Hautes Etudes Agronomiques Méditerranéenes, Istituto Agronomico Mediterraneo di Bari (CIHEAM/MAIB), Via Ceglie 9, 70010 Valenzano (BA), Italy

Vol. 58, No. 3: 215-219, 2018

DOI: 10.24425/jppr.2018.124640

Received: April 11, 2018 Accepted: August 24, 2018

*Corresponding address: mohamedafechtal.inra@gmail.com

Abstract

Morocco is basically an agricultural country; almost 40% of the workforce is employed in this sector. *Xylella fastidiosa* is a xylem-inhabiting pathogen which can infect more than 300 plant species, although most host species are symptomless. Until relatively recently, *X. fastidiosa* was primarily limited to North and South America, but in 2013 a widespread epidemic of olive quick decline syndrome caused by this fastidious pathogen appeared in southeastern Italy, and later several cases of *X. fastidiosa* outbreaks have been reported in other European countries (France, Germany and Spain). Following these recently confirmed findings of *X. fastidiosa* in the European Union, this bacterium has become a serious threat to the Moroccan flora. The national phytosanitary authorities have adopted several measures to prevent the introduction of *X. fastidiosa* into the national territory by deciding, inter alia, to suspend importation of host plant species to the bacterium from infected areas. This paper presents the phytosanitary risk of this bacterium in Morocco.

Keywords: Xylella fastidiosa, pest risk analysis

Pest risk analysis

Historically Morocco's economy has been based on agriculture and it still accounts for 40% employment and 14% of the gross domestic product. Morocco has semiarid Mediterranean climates and a wide topographical variation, which permit a high biodiversity and abundant natural resources. The country's agricultural productivity is often affected by droughts which regularly hit the country. Agriculturally usable land represents up to 13% of the total land area of the country; pastures and forests cover 30 and 8%, respectively. Some 8.7 million ha are arable of which 63% is devoted to cereals, 10% to tree crops (olives, almonds, citrus, and fruit trees) and 4% to vegetables. About 85% of the agricultural area is rain-fed, while the other 15% is irrigated (MAPM 2016). *Xylella fastidiosa*, a gram negative, devastating plant pathogenic bacterium and the causal agent of a number of severe diseases, including olive quick decline syndrome, Pierce's disease of grapevine, leaf scorch of almond, oleander and coffee, citrus variegated chlorosis, and other disorders of perennial crops and landscape plants (Hopkins and Purcell 2002; Janse and Obradovic 2010; Purcell 2013; Saponari *et al.* 2013). Many native plants (grasses, sedges and trees) in North and South America may carry the pathogen, often without showing visible symptoms (Hopkins and Purcell 2002; Janse and Obradovic 2010; Purcell 2013). *Xylella fastidiosa* is a genetically diverse species subdivided into six subspecies, and numerous sequence types, each one being more or less specific to a particular host range and a native zone in the American continents. The four most frequently reported subspecies are: ssp. fastidiosa, ssp. pauca, ssp. multiplex and ssp. sandyi (Schaad et al. 2004; Schuenzel et al. 2005). Randal et al. (2009) proposed a fifth one, ssp. tashke, that can be differentiated by DNA : DNA hybridization (Schaad et al. 2004) and multi-locus sequence typing (Scally et al. 2005). More recently, a sixth subspecies, ssp. morus, has been proposed (Nunney et al. 2014). Xylella fastidiosa causes various diseases on more than 350 plant hosts, inducing various symptoms: marginal leaf scorching, wilting of foliage and withering of branches, dieback and stunting with eventual plant death in susceptible hosts (Janse and Obradovic 2010). Xylella fastidiosa is transmitted by several species of sharpshooter leafhoppers (Hemiptera: Cicadellidae: Cicadellinae) and spittle bugs or froghoppers (Hemiptera: Cercopoidea), which are xylem-fluid feeders (Redak et al. 2004). There is also evidence that cicadas (Hemiptera: Cicadoidea), another group of xylemfluid feeders, transmit X. fastidiosa in grape (Krell et al. 2007). A search for vectors in the recent outbreak of X. fastidiosa in Apulia, Italy, detected the pathogen at high frequency in the spittle bugs Philaneus spumarius and Neophilaneus campestris as well as the leaf hopper Euscelis lineolatus (Elbeaino et al. 2014). Adults of N. campestris and P. italosignus have been used in experimental transmission tests to assess their ability to acquire and transmit the bacterium, with consistent successful transmissions for N. campestris and P. italosignus, confirming that they are competent vectors of the strain associated with the epidemic in southern Italy (F. Valentini from CIHEAM/IAM-Bari, personal communication).

In October 2013, an outbreak of X. fastidiosa was found in Apulia (southeastern Italy) in olive trees affected by olive quick decline syndrome (Saponari et al. 2013). In July 2015, the bacterium X. fastidiosa was detected on the island of Corsica (France) affecting ornamental plants of Polygala myrtifolia (EPPO 2015a). Later, in October 2015, it was detected for the first time in Alpes-Maritimes department (southern France) (EPPO 2015b). In July 2016, the bacterium was reported for the first time in Germany on a single potted oleander plant (Nerium oleander) in a small nursery producing young vegetable and ornamental plants in Saxony (EPPO 2016a). In November 2016, the presence of X. fastidiosa was confirmed in Islas Baleares, Spain. The bacterium was detected in sweet cherry (Prunus avium) in a garden center in Porto Cristo, municipality of Manacor, on the island of Mallorca (EPPO 2016b). In May 2017, X. fastidiosa was detected in Mallorca, Ibiza and Menorca on 12 plant species including grapevine (EPPO 2017a). More recently, in June 2017, X. fastidiosa was detected for the first time in mainland Spain (Alicante), affecting almonds (*P. dulcis*) (EPPO 2017b).

The world map of the Köppen-Geiger climate classification (Peel et al. 2007) shows a similarity between the climates of the affected areas in the European Union (EU) (Puglia, Corsica, Alpes-Maritimes, Islas Baleares and Alicante) and those of Morocco. Consequently, the Moroccan climate might not be a limiting factor for the establishment of X. fastidiosa and its vectors, especially in the coastal areas of northern Morocco (Mediterranean and Atlantic seas), thus representing a potential risk for the national plant heritage (cultivated, ornamental and forest plants). The geographical position of Morocco, at just 13 km away from Spain, makes this risk even bigger especially with the high flow of tourists and Moroccans traveling abroad. Indeed, Morocco has an important commercial exchange with several European countries, in particular France, Spain and Italy, which may contribute to the introduction of X. fastidiosa directly in infected plant material or indirectly via insect vectors.

Across the country a preliminary survey of olive, citrus and grapevine growing areas (Azilal, Gharb, Haouz, Loukkos, Meknès and Souss) for the presence of X. fastidiosa was undertaken by the Laboratory of Virology of the Regional Center of Agricultural Research at Kénitra (INRA). A total of 900 samples were collected: 220 olive trees (cv. Picholine Marocaine), 410 citrus trees belonging to seven different cultivars and 270 grapevine plants belonging to six different cultivars. All these samples were tested for the presence of X. fastidiosa by using an enzyme-linked immunosorbent assay (ELISA) commercial kit and polymerase chain reaction (PCR). Fortunately, no positive samples have been detected so far. These preliminary results are taken as a good indication, considering that X. fastidiosa was not found in Morocco (Afechtal et al. 2018). However, more extensive surveys on different plant hosts in different regions are needed in order to prevent its entrance into the country. Research and investigations on the Auchenorrhyncha species, potential vectors of X. fastidiosa, need to be conducted to determine their distribution and population density in the country.

Pest risk assessment

Probability of entry

Considering the factors cited below, the probability of entry of *X. fastidiosa* into Morocco is very likely because:

X. fastidiosa was primarily limited to North and South America, but in 2013 a widespread epidemic of olive quick decline syndrome caused by this fastidious pathogen appeared in southeastern Italy, and several cases of *X. fastidiosa* infections have been reported in other European countries (France, Germany and Spain). It is possible that the pathogen has spread to a greater extent than is currently known;

- Between 2013 and 2015 Morocco imported almost 8.5 million host plants susceptible to *X. fastidiosa*, especially grapevines, stone fruits, olives and blueberries. Imports of Italian, French and Spanish origin, countries infested by the bacterium, amounted to approximately 8 million plants, which represents about 94% of the total imported plants during this period, while ornamental host plants represented 0.5%;
- The difficulty of post-planting phytosanitary controls, on planting sites, of host plants that were imported in recent years;
- The movement of people and vehicles is very intense between Morocco and the EU countries where the disease has been reported. Most people are not aware that the introduction of this bacterium represents a serious threat to the national plant heritage;
- Infected vectors can be introduced by various means (in plant material, agricultural equipment, vehicles, etc.);
- Illegal introduction of plant material may be a source of entry of the bacterium and/or its insect vectors.

Probability of establishment

Xylella fastidiosa has never been recorded or intercepted from Morocco. However, under the current situation, the probability of *X. fastidiosa* to be established in Morocco is considered very likely with a low uncertainty for the following reasons:

- Susceptibility of potential Moroccan host plant species, especially cultivated crops (olive, citrus, grapevine, almond, alfalfa, etc.), forest and spontaneous plants (oak, oleander, etc.), ornamental plants (rosemary, polygala, etc.) and weeds (quack--grass, yellow-fruit nightshade, etc.);
- Due to the non-specific nature of the disease symptoms, outbreaks may remain undetected until the disease becomes established which increases establishment risks;
- Xylem feeding insects, especially sharp shooters/ leafhoppers (Cicadellidae, subfamily Cicadellinae) and spittle bugs (family Cercopidae) are the most important known vectors of *X. fastidiosa* and these potential insect vectors are present in Morocco (McKamey 2001). In the USA the spittle bug *P. spumarius*, which is common in Europe, has been

shown to efficiently vector *X. fastidiosa* in almond in experimental transmission studies (Purcell 1980). There is also the possibility that the pathogen could be introduced into Morocco with a new vector. The current *X. fastidiosa* outbreak in Apulia detected the pathogen at high frequency in the spittle bugs *P. spumarius* and *N. campestris* as well as the leaf hopper *E. lineolatus* (Elbeaino *et al.* 2014);

- The Moroccan climatic conditions are favorable for the multiplication of the bacterium, for the development of the disease, and for the intense activity of the insect vectors. Climatic suitability will most likely favor the impact of X. fastidiosa infections in Morocco. Bosso et al. (2016) developed a 'Maxent model' to estimate the potential geographic distribution of X. fastidiosa in the Mediterranean basin under current climatic conditions and different climate change scenarios. Presently, the potential geographic distribution of X. fastidiosa obtained for the current climatic conditions includes Portugal, Spain, Italy, Corsica, Albania, Montenegro, Greece and Turkey as well as all countries of northern Africa and the Middle East. Indeed, X. fastidiosa is not predicted to change its area of potential distribution very much in the Mediterranean basin due to climate change, while increasing its probability of establishment owing to higher temperatures (Bosso et al. 2016);
- The absence of effective control measures for this disease and its insect vectors at the international level increases the probability of its establishment in Morocco.

Probability of spread

It is very likely with low uncertainty that *X. fastidiosa* will spread for the following reasons:

- the majority of susceptible host plants of the bacterium are found throughout the Moroccan territory;
- the potential insect vectors of *X. fastidiosa* are present in Morocco (McKamey 2001);
- climatic conditions are favorable for the disease and its insect vectors;
- lack of effective control methods for this disease and its vectors;
- human intervention (cultural practices, plant movements, etc.).

Assessment of potential economic consequences

Citrus variegated chlorosis affects approximately 40% of citrus plants in Brazil and losses have been estimated

to be approximately 120 million US dollars to the Brazilian economy (Bove and Ayres 2007). *Xylella fastidiosa* was also responsible for significant economic losses in grape and almond production in the USA, as well as olive trees in Italy. It has also a strong impact on many ornamental plants such as oleander in the USA and Italy. In addition, this bacterium has a very wide host range.

In Morocco, many susceptible host plants and potential insect vector species are present. Introduction (entry + spread) and spread of this bacterium can lead to significant economic, social and environmental losses.

The overall potential impact is considered of great importance for Morocco. For agricultural production, the disease could lead to yield losses with additional costs for disease control. It also has a negative social impact through job losses. *Xylella fastidiosa* will affect susceptible ornamental and forest host plants with a negative impact on the country's landscape and forest biodiversity. Other potential environmental effects may be due to the increased use of insecticides and herbicides that can cause ecological disturbances.

Pest risk management

Control of diseases caused by *X. fastidiosa* should be based on the sanitary selection of plants, the elimination of infection reservoirs and the control of insect vectors.

Probability of continuing to exclude *Xylella fastidiosa* from Morocco

As far as we know, Morocco is still free from *X. fastidiosa*. The measures taken against this bacterium since 2002 have reduced its probability of entering Morocco, despite its strong presence in the Americas for several years. However, the large number of potential hosts that could harbor *X. fastidiosa*, the common occurrence of potential insect vectors in Morocco, the spread of the disease in Italy, France and Spain, the high risk of its extension to other European countries, and the flow of trade between Morocco and European countries via the importation of host plants, all indicate that the exclusion of this bacterium from Morocco is very unlikely.

Probability of eradication of outbreaks if *Xylella fastidiosa* is introduced into Morocco

If *X. fastidiosa* is introduced into Morocco, it will be very unlikely possible to eradicate it, considering:

- the presence of a wide range of susceptible host plant species at the national level;
- the presence of many potential insect vectors;
- the presence of asymptomatic host plants and latent infections, difficult to detect, which may serve as infection reservoirs of the bacterium;
- the presence of favorable climatic conditions for *X. fastidiosa* establishment and spread;
- the lack of effective control measures against *X. fastidiosa* and its vectors.

Measures taken by Morocco to prevent the introduction and spread of *Xylella fastidiosa*

Following the first outbreak of *X. fastidiosa* in Italy and in order to face the threat posed by this bacterium, the national phytosanitary authority has implemented several measures:

- suspension of the importation of host plant species to *X. fastidiosa* (citrus, grapevine, olive tree, stone fruits, oleander and oak) from infected areas;
- extension activities and awareness campaigns for the concerned parties (regional directorates of the national phytosanitary authorities, plant protection services, the Moroccan Freight Forwarders Association, the inter-professional federations of citrus, olive and stone fruit trees, and the General Administration of Customs);
- strengthening vigilance, control and surveillance of the disease at the national level.

Conclusions

Xylella fastidiosa is a regulated quarantine pest whose introduction and spread in Morocco is banned. Given the recently confirmed findings of X. fastidiosa in the EU (Italy, France, Germany and Spain); this bacterium has become a serious threat to the Moroccan plant heritage. In this context, the national phytosanitary authorities adopted measures to prevent the introduction of X. fastidiosa into the national territory by deciding to suspend importation of host plant species from infested areas. Pest risk analysis showed clearly that the total probability of the introduction and spread of X. fastidiosa in Morocco is considered very likely with low uncertainty. Therefore, it is of utmost importance to consider appropriate and preventive phytosanitary measures to avoid its introduction, especially through continuous extensive surveys on the pathogen and its potential insect vectors.

References

- Afechtal M., Ait Friha A., Bibi I. 2018. A preliminary survey on the presence of *Xylella fastidiosa* in olive, citrus and grapevine groves in Morocco. Revue Marocaine des Sciences Agronomiques et Vétérinaires 6 (1): 6–9. Available on: https://agrimaroc.org/index.php/Actes_IAVH2/article/ view/544/562
- Bosso L., Di Febbraro M., Cristinzio G., Zoina A., Russo D. 2016. Shedding light on the effects of climate change on the potential distribution of *Xylella fastidiosa* in the Mediterranean basin. Biological Invasions 18: 1759–1768. DOI: https://doi.org/10.1007/s10530-016-1118-1
- Bove J. M., Ayres A.J. 2007. Etiology of three recent diseases of citrus in Sao Paulo State: Sudden death, variegated chlorosis and huanglongbing. International Union of Biochemistry and Molecular Biology Life 59: 346–354. DOI: https://doi. org/10.1080/15216540701299326
- 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. Phytopathologica Mediterranea 53: 328–332. DOI: https://doi. org/10.14601/Phytopathol_Mediterr-14113
- EPPO. 2015a. European and Mediterranean Plant Protection Organization Reporting Service no. 08. Nr. 2015/144. Available on: https://gd.eppo.int/reporting/article-4942
- EPPO. 2015b. European and Mediterranean Plant Protection Organization Reporting Service no. 10. Nr. 2015/180. Available on: https://gd.eppo.int/reporting/article-5127
- EPPO. 2016a. European and Mediterranean Plant Protection Organization Reporting Service no. 07. Nr. 2016/133. Available on: https://gd.eppo.int/reporting/article-5878
- EPPO. 2016b. European and Mediterranean Plant Protection Organization Reporting Service no. 11. Nr. 2016/213. Available on: https:// gd.eppo.int/reporting/article-5958
- EPPO. 2017a. European and Mediterranean Plant Protection Organization Reporting Service no. 05. Nr. 2016/102. Available on: https://gd.eppo.int/reporting/article-6070
- EPPO. 2017b. European and Mediterranean Plant Protection Organization Reporting Service no. 07. Nr. 2017/133. Available on: https://gd.eppo.int/reporting/article-6101
- Hopkins D.L., Purcell A.H. 2002. *Xylella fastidiosa*: cause of Pierce's disease of grapevine and other emergent diseases. Plant Disease 86: 1056–1066. DOI: https://doi.org/10.1094/ PDIS.2002.86.10.1056
- Janse J.D., Obradovic A. 2010. *Xylella fastidiosa*: its biology, diagnosis, control and risks. Journal of Plant Pathology 92: S1.35–S1.48. DOI: https://doi.org/10.4454/jpp. v92i1sup.2504
- Krell R.K., Boyd E.A., Nay J.E., Park Y.L., Perring T.M. 2007. Mechanical and insect transmission of *Xylella fastidiosa* to *Vitis vinifera*. American Journal of Enology and Viticulture 58: 211–216. Available on: http://www.ajevonline.org/ content/58/2/211
- MAPM (Ministère de l'Agriculture et de la Pêche Maritime). 2016. Ressources hydriques. Available on: http://www.agriculture.gov.ma/pages/lirrigation-au-maroc
- McKamey S.H. 2001. Checklist of Leafhopper Species, 1758– 1955 (Hemiptera: Membracoidea: Cicadellidae and My-

serslopiidae) with Synonymy and Distribution [Catalogue of the Homoptera, Fascicle 6, Abridged]. USDA, Agricultural Research Service, Systematic Entomology Laboratory; Washington, D.C.: National Museum of Natural History, Smithsonian Institution, 516 pp. Available on: http://www. bio-nica.info/biblioteca/McKameyCicadellidae.pdf

- Nunney L., Schuenzel E.L., Scally M., Bromley R.E., Stouthamer R. 2014. Large-scale intersubspecific recombination in the plant-pathogenic bacterium *Xylella fastidiosa* is associated with the host shift to mulberry. Applied and Environmental Microbiology 80: 3025–3033. DOI: https://doi.org/10.1128/ AEM.04112-13
- Peel M.C., Finlayson B.L., McMahon T.A. 2007. Updated world map of the Köppen-Geiger climate classification. Hydrology and Earth System Sciences Discussions 4: 439–473. DOI: https://doi.org/10.5194/hess-11-1633-2007
- Purcell A.H. 2013. Paradigms: examples from the bacterium Xylella fastidiosa. Annual Review of Phytopathology 51: 229–356. DOI: https://doi.org/10.1146/annurev-phyto-082712-102325
- Purcell A.H. 1980. Almond leaf scorch: leafhopper and spittlebug vectors. Journal of Economic Entomology 73: 834–838. DOI: https://doi.org/10.1093/jee/73.6.834
- Randal J.J., Golberg N.P., Kemp J.P., Radionenko M., French J.M., Olsen M.W., Hanson S.F. 2009. Genetic analysis of a novel *Xylella fastidiosa* subspecies found in the southwestern United States. Applied and Environmental Microbiology 75: 5631–5638. DOI: https://doi.org/10.1128/ AEM.00609-09
- Redak R.A., Purcell A.H., Lopes J.R.S., Blua M.J., Mizell III R.F., Andersen P.C. 2004. The biology of xylem fluid-feeding insect vectors of *Xylella fastidiosa* and their relation to disease epidemiology. Annual Review of Entomology 49: 243–270. DOI: https://doi.org/10.1146/annurev. ento.49.061802.123403
- 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. DOI: https://doi.org/10.4454/JPP.V9513.035
- Scally M., Schuenzel E.L., Stouthamer R., Nunney L. 2005. Multilocus sequence type system for the plant pathogen *Xy-lella fastidiosa* and relative contributions of recombination and point mutation to clonal diversity. Applied and Environmental Microbiology 71: 8491–8499. DOI: https://doi. org/10.1128/AEM.71.12.8491-8499.2005
- Schaad N.W., Postnikova E., Lacy G., Fatmi M., Chang C.J. 2004. Xylella fastidiosa subspecies: X. fastidiosa subsp. piercei, subsp. nov., X. fastidiosa subsp. multiplex, subsp. nov., X. fastidiosa subsp. pauca, subsp. nov. Systematic and Applied Microbiology 27: 290–300. DOI: https://doi. org/10.1078/0723-2020-00263
- Schuenzel E.L., Scally M., Stouthamer R., Nunney L. 2005. A multigene phylogenetic study of clonal diversity and divergence in North American strains of the plant pathogen *Xylella fastidiosa*. Applied Environmental Microbiology 71: 3832–3839. DOI: https://doi.org/10.1128/AEM.71.7.3832-3839.2005