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Managing *Xylella fastidiosa* in Europe: Exploring Biocontrol Strategies

Xylella fastidiosa is a bacterial pathogen affecting many plants, causing diseases like Pierce's disease, citrus variegated chlorosis, and olive quick decline syndrome. This study examines its impact in Europe, current management strategies, and potential new approaches, including interactions with xylem endophytes, to contain its spread.

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Abstract

X. fastidiosa is a plant pathogenic bacterium classified as a quarantine organism in Europe, transmitted by insect vectors and capable of colonizing the xylem of various plant species, either as a pathogen or an endophyte. It has caused severe outbreaks globally and has recently emerged as a threat in several Mediterranean regions, raising concerns about its potential spread into northern Europe. A key challenge with *X. fastidiosa* is its adaptability to diverse pathosystems, enabling it to thrive in a wide range of environmental conditions and more than 600 plant hosts worldwide, making its spread highly unpredictable. This complexity makes it more challenging to assess its potential impact in areas where it has yet to be detected. However, identifying potential pathosystems in these regions is crucial for developing prevention and early detection strategies, as there are no effective curative treatments currently available. This work describes the pathogen and investigates its impact in Europe and the effectiveness of the current management practices. These include quarantine measures, control of insect vectors, or use of tolerant cultivars, and new leads to several studies beginning to characterize the microbiome diversity of crops infected by *X. fastidiosa*.

Learning Outcomes

1. Describe *X. fastidiosa* biology, host colonization process, and symptoms development.
2. Assess the impact of *X. fastidiosa* in Europe.
3. Evaluate the effectiveness of current management practices and propose new research areas for future control methods.

Introduction

X. fastidiosa is an emerging bacterial pathogen that poses a significant threat to agriculture across multiple regions globally ranking it in the "list of priority pests" for Europe. Since its discovery, *X. fastidiosa* has spread rapidly in various parts of the world, including North and South America, Europe, and Asia, impacting a wide range of crops such as grapevines, citrus, almond trees, peach trees, and olive trees. The bacteria are transmitted by sap-feeding insects of the Cicadellidae family and superfamily Cercopoidea, which complicates control efforts. In the affected areas, *X. fastidiosa* has led to substantial yield losses, tree mortality, and economic decline. The bacteria are able to thrive in the xylem vessels by moving actively with twitching motility due to Type IV pili and passively along the xylem flow. Large cell aggregates of *X. fastidiosa* in xylem vessels cause a reduced water and mineral transport sometimes causing symptoms resembling water deficits such as leaf scorching, browning of leaf tissues, or twig dieback. This case study explores the biology of *X. fastidiosa*, its transmission, the symptoms that it causes, and current management strategies. Accurate detection and identification of *X. fastidiosa* is crucial not only because of its quarantine status in Europe but also due to the significant differences in host range among its subspecies, which impact the severity of plant diseases. Another important factor is that *X. fastidiosa* can infect a wide variety of plant species without causing visible symptoms. Symptom development is influenced by the interaction between the plant species and the specific *X. fastidiosa* genotype and is typically associated with high bacterial populations within the plant. Since the bacterial population levels affect how efficiently vectors can acquire the pathogen, plants with lower bacterial loads may act as less effective reservoirs for vector transmission.

Causal Agent

Description of the causal agent

Xylella fastidiosa is a Gram-negative, catalase positive, oxidase negative bacterium which can sometimes appear as filamentous, non-motile rods, measuring 0.25 to 0.35 µm in width and 0.9 to 3.5 µm in length. *X. fastidiosa* gets its name from the fact that it colonizes the xylem and from the difficulty to cultivate it. *X. fastidiosa* is a bacterium belonging to the Proteobacteria phylum and the *Xanthomonadaceae* family. This family includes two xylem-limited phytopathogenic bacterial species: *Xanthomonas albilineans* and *X. fastidiosa*. The genus *Xylella* comprises two species: *X. fastidiosa* and *X. taiwanensis*. This second species, previously related to the *X. fastidiosa* species, has recently been proposed as a species in its own right, as it shows significant variations (significant single nucleotide polymorphisms at 18 randomly selected housekeeping genes loci, different observable phenotypes such as different susceptibility to antibiotics). *X. fastidiosa* species counts three official subspecies: *fastidiosa*, *pauca*, and *multiplex*. Simultaneously, Scally *et al*. (2005) proposed a multilocus sequence typing (MLST) approach based on the analysis of seven housekeeping genes distributed across the bacterial genome to classify detected strains within the different subspecies into several groups according to their allelic profiles, referred to as sequence types (STs). Thus, a subspecies contains various strains belonging to different STs. By 2022, 156 distinct STs had been reported (Available at: http://pubmlst.org/xfastidiosa/, accessed 15 September 2024). While there is not always a strict one-to-one correlation between ST and host range, certain STs show strong host specificity, helping researchers understand which crops or trees are at risk. If you are analysing an outbreak, MLST can thus be crucial for assessing pathogen spread and host adaptation (e.g.: ST53 (subsp. *pauca*) is strongly associated with OQDS, ST1 (subsp. *fastidiosa*) is primarily linked to PD in grapevines, ST6 and ST7 (subsp. *multiplex*) are often found in hardwood trees as oaks, elms, and sycamores, and ST73 (subsp. *pauca*) often causes CVC).

- Domain: Bacteria
- Phylum: Pseudomonadota
- Class: Gammaproteobacteria
- Order: Xanthomonadales
- Family: *Xanthomonadaceae*
- Genus: *Xylella*
- Species: *X. fastidiosa*

Current worldwide distribution

After 150 years, *X. fastidiosa* is widespread across the Americas and is expanding its range in Europe and other regions (Fig. 1). The first detection outside the Americas occurred in Taiwan in 1993, on Nashi pear (*Pyrus pyrifolia*) and grapevines in 2002. Initially attributed to *X. fastidiosa*, it was later identified as a different species, *X. taiwanensis*. The bacterium was subsequently reported on grapevines and almond trees in Iran and on almonds in Israel (subsp. *fastidiosa*). Reports from Turkey, Lebanon, Kosovo, and India remain unconfirmed, as further investigation is needed.

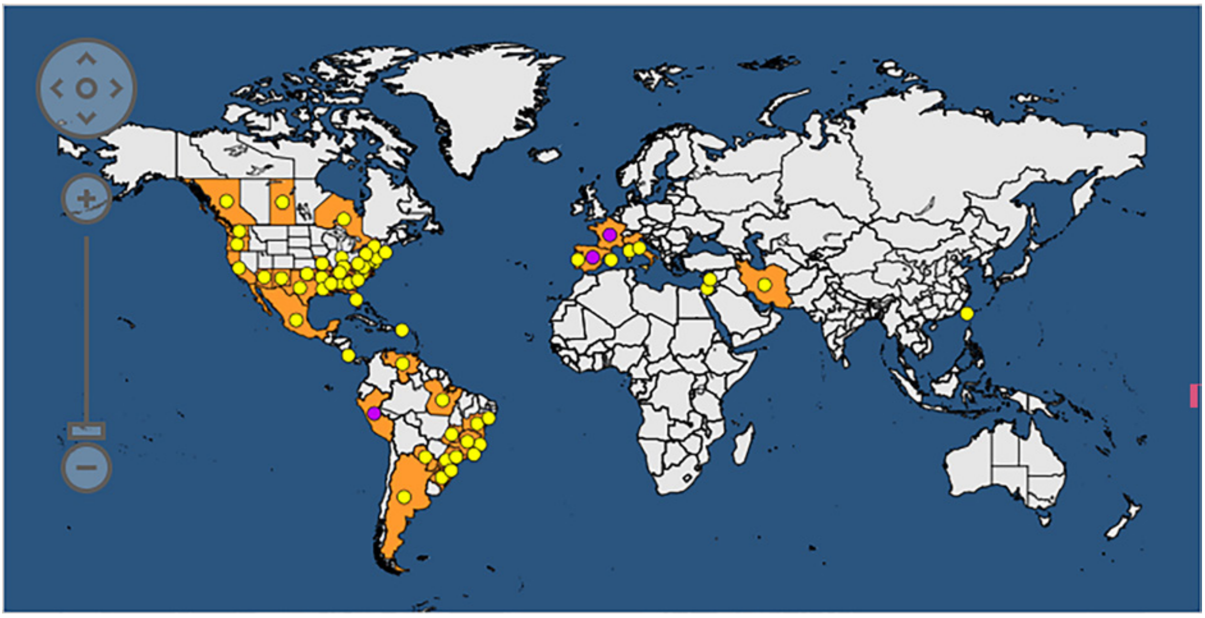

Fig. 1. World distribution of *X. fastidiosa* (Map generated by European and Mediterranean Plant Protection Organization (EPPO) last updated on 08 October 2024 and accessed on 11 October 2024).

Estimated impact

The damage caused by *Xylella fastidiosa* is vast, affecting multiple sectors. Its impact is not limited to economic and environmental consequences but extends to social and cultural realms as well. As a result, accurately assessing the full extent of the damage is challenging and likely underestimated. However, estimates for the major outbreaks do exist.

- In California, 104 million $US are annually lost to PD in efforts to breed healthy plants, the cost of research, production loss with more than 35,000 acres of vineyards destroyed between its introduction and 2010, and vectors control.
- In Brazil, 120 million $US were estimated to be lost annually to CVC in 2007. Progression in the implementation of control measures has dropped the incidence of the disease.
- In Apulia, OQDS has devastated around 4 million olive trees, causing a significant economic crisis, especially in olive oil production, and affecting nurseries due to strict European restrictions. If no replanting with tolerant cultivars occurs, projected losses could reach 5.2 billion euros, while replanting could reduce losses between 0.6 and 1.6 billion euros. The epidemic has also impacted the region's cultural heritage, as many of the olive trees are centuries old, and has altered the landscape. Environmentally, the loss of olive trees is expected to reduce ecosystem services and biodiversity by up to 30%.
- In Majorca, from 2010 to 2019, it has been estimated that the almond orchard surface has decreased from 29.789 to 11.814 ha.

Host plant colonization

X. fastidiosa is capable of infecting over 600 plant species from more than 80 families, but disease symptoms only manifest in some hosts. EFSA regularly updates a database reporting the host range of *X. fastidiosa* (EFSA Plant Health Panel, 2015). However, in many cases, *X. fastidiosa* sometimes colonizes plants without causing visible harm, while it acts as a pathogen in a smaller subset of species, responsible for a few but economically significant plant diseases (Landa *et al*., 2022). This suggests potential host adaptation in disease development, as observed in symptomatic plants in natural settings. Plants exhibiting severe symptoms often carry bacterial loads that are 100 to 1000 times higher than those found in asymptomatic plants. However, symptom expression can also depend on the plant's physiological and developmental stages. Key questions remain regarding the relationships between specific plant (sub) species and pathogen strains and what are the host range determinants. In addition, *X. fastidiosa* is mainly considered a threat to agriculture and horticulture due to the serious diseases it causes on fruit crops and, to a lesser extent, on ornamental plants and trees. No serious impacts of X. *fastidiosa* have been reported in forests in its native range. However, the impact of different X. *fastidiosa* strains introduced into new areas cannot be predicted from what is known in other areas, as illustrated by the case of the emergence of OQDS in Italy when little damage had been reported on olive trees in California. The introduction and establishment of X. *fastidiosa* in certain areas in Europe therefore raises the question of its potential future impact on European forest species.

In addition to the biological differences between the subspecies, work has also indicated that they represent allopatric populations with evidence of recent long-distance dispersal. Each of these subspecies has a specific host spectrum, the main ones of which are listed in Table 1.

Table 1. Important hosts of the three principal subspecies. For more information about subspecies – host plant spectrum, see EFSA *et al*. (2024).

Subspecies	**Hosts spectrum**
X. *fastidiosa* subsp. *fastidiosa*	Grapevine (also found in almond, coffee)
X. *fastidiosa* subsp. *multiplex*	hardwoods (ornamental hardwoods, *Prunus spp*.)
X. *fastidiosa* subsp. *pauca*	citrus (also found in coffee, olive tree)

The colonization of plants by X. *fastidiosa* is a gradual process, where the growth of bacterial populations, along with plant responses such as tylose formation and the spread of bacteria between vessels through pit membranes, progressively reduces sap flow in the xylem. This blockage disrupts the plant's ability to transport water and nutrients, leading to water stress. The density of X. *fastidiosa* cells plays a critical role in its colonization and movement within the plant. At low cell densities, processes that promote plant colonization are activated, while at high densities, the bacterial population shifts to a phenotype that facilitates insect acquisition and colonization, limiting further plant colonization. This molecular switch likely reflects an adaptation of X. *fastidiosa* biology. Water stress in plants, triggered by X. *fastidiosa* infection, increases xylem water column tension, negatively affecting xylem sap-feeding insects. Furthermore, plants under water stress, including those with leaf scorch symptoms common to X. *fastidiosa* infections, are visually avoided by sharpshooter vectors, whether or not the plants are infected. This density-dependent behaviour suggests that X. *fastidiosa* balances colonization with limiting excessive multiplication to prevent severe symptoms that would reduce the attractiveness of host plants to insect vectors. Research shows that X. *fastidiosa* spread peaks before full symptom development, a stage when bacterial populations within the plant are large enough to maximize acquisition by insect vectors, but before the vectors strongly avoid diseased plants. Although X. *fastidiosa* may adversely impact plants in various ways, there is limited evidence suggesting negative effects on insect vectors (Fig. 2).

Symptoms

Symptoms of X. *fastidiosa* infection often include leaf scorching, desiccation of branches, and gradual dieback of the canopy. Infected trees can decline rapidly, particularly in regions with hot and dry climates, leading to significant reductions in yield and tree death within a few years.

Symptom development is generally slow. Several typical symptoms can be observed such as leaf chlorosis appearing on the edge of the leaves, followed by leaf scorch. The latter can lead to the drying of the branches and even to the death of the subject in the most severe cases. These symptoms are randomly distributed in the canopy (Janse and Obradovic, 2010). Other biotic and abiotic factors can cause symptoms similar to those involved in X. *fastidiosa* infection. Proper identification of the causative agents of symptoms is paramount (Fig. 3).

The type of symptoms presented will depend mainly on the host species and the bacterial strain:

- On alfalfa, infected plants may exhibit stunted growth, known as dwarfism, which can impact yield and quality. Another symptom of X. *fastidiosa* infection on alfalfa is leaf discolouration, which typically starts as yellowing and progresses to brown or necrotic (dead) areas on the leaves. If left untreated, the disease can progress, leading to eventual plant death. It is important to monitor alfalfa crops for symptoms of X. *fastidiosa* infection and take prompt action if detected, including implementing appropriate management strategies and possibly removing infected plants to prevent further spread of the disease (Fig. 3A).

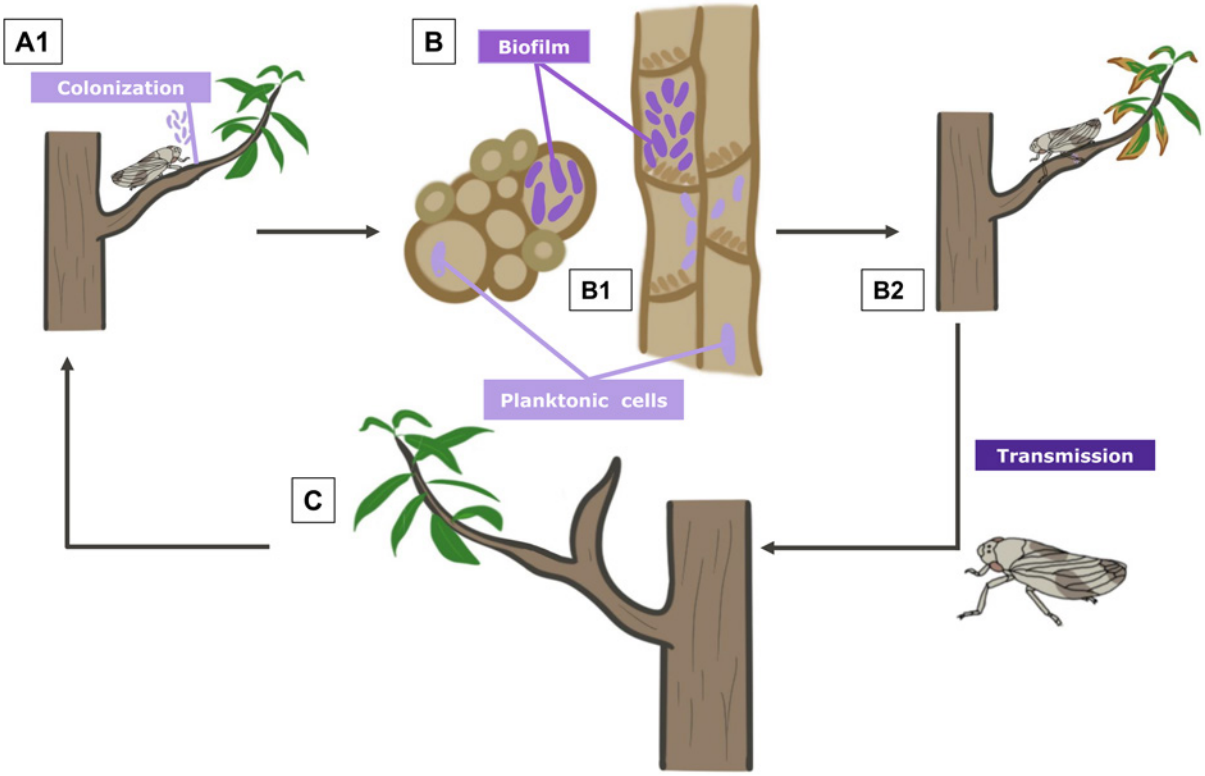

Fig. 2. Disease cycle.

- In almond trees, once the infection is in place, the leaves become discoloured and the edges are necrotic. Symptoms first appear on an isolated branch before spreading throughout the canopy over a period of about three to four years (Fig. 3B).
- On citrus, symptomatic plants usually do not die. They show small chlorotic spots between the veins on some leaves. These symptoms are similar to those caused by zinc deficiency. In the later stages of infection, the spots become necrotic on the underside of the leaf. Fruit production is reduced, and fruits remain small. Unlike most diseases caused by *X. fastidiosa*, symptomatic leaves affected by CVC do not develop a necrotic appearance. Leaves turn yellow between the veins on the upper surface. On the side, a brownish gum develops (Fig. 3C).
- On coffee trees, the first symptoms appear on young shoots. The tops of mature leaves appear scorched. Shoots are dwarfed and new leaves become pale green to yellow in colour. Finally, the shoots stun and die back. Production yield and fruit size are reduced (Fig. 3D).
- Oleander infected by *X. fastidiosa* can show various symptoms depending on the strain of the bacterium and the environmental conditions. Some common symptoms include leaf scorching, where the leaf margins dry out and turn brown or black, wilting, and defoliation. In addition, the disease can cause twig dieback and plant death. Infected oleander plants may also exhibit yellowing of the leaves, a reduction in leaf size, and overall stunted growth (Fig. 3E).
- In olive trees, the infection starts by reaching the upper part of the crown and then spreads throughout the canopy, which then takes on a withered appearance. The disease leads to tree death within a few years (Fig. 3F).
- On grapevines, infection begins with browning of the edges of some leaves. This necrosis is surrounded by a yellow to red halo. Symptomatic leaves usually fall from the basal, not distal, end of the petiole leading to its persistence after leaf fall. In the later stages of the disease, plant defoliation is very extensive. The plants are dwarfed and stunted. In addition, fruit production is low. These symptoms are similar to those caused by salt stress (Fig. 3G).
- On peach trees, young shoots are stunted and their leaves are darker than normal and show earlier flowering. The twigs of diseased trees are shortened and show significant lateral branching. Peach production is significantly lower than that of a healthy tree (Fig. 3H).
- Symptoms of *X. fastidiosa* infection in myrtle trees can include leaf scorching, twig dieback, and defoliation, which can possibly lead to the death of the entire tree (Fig. 3I).

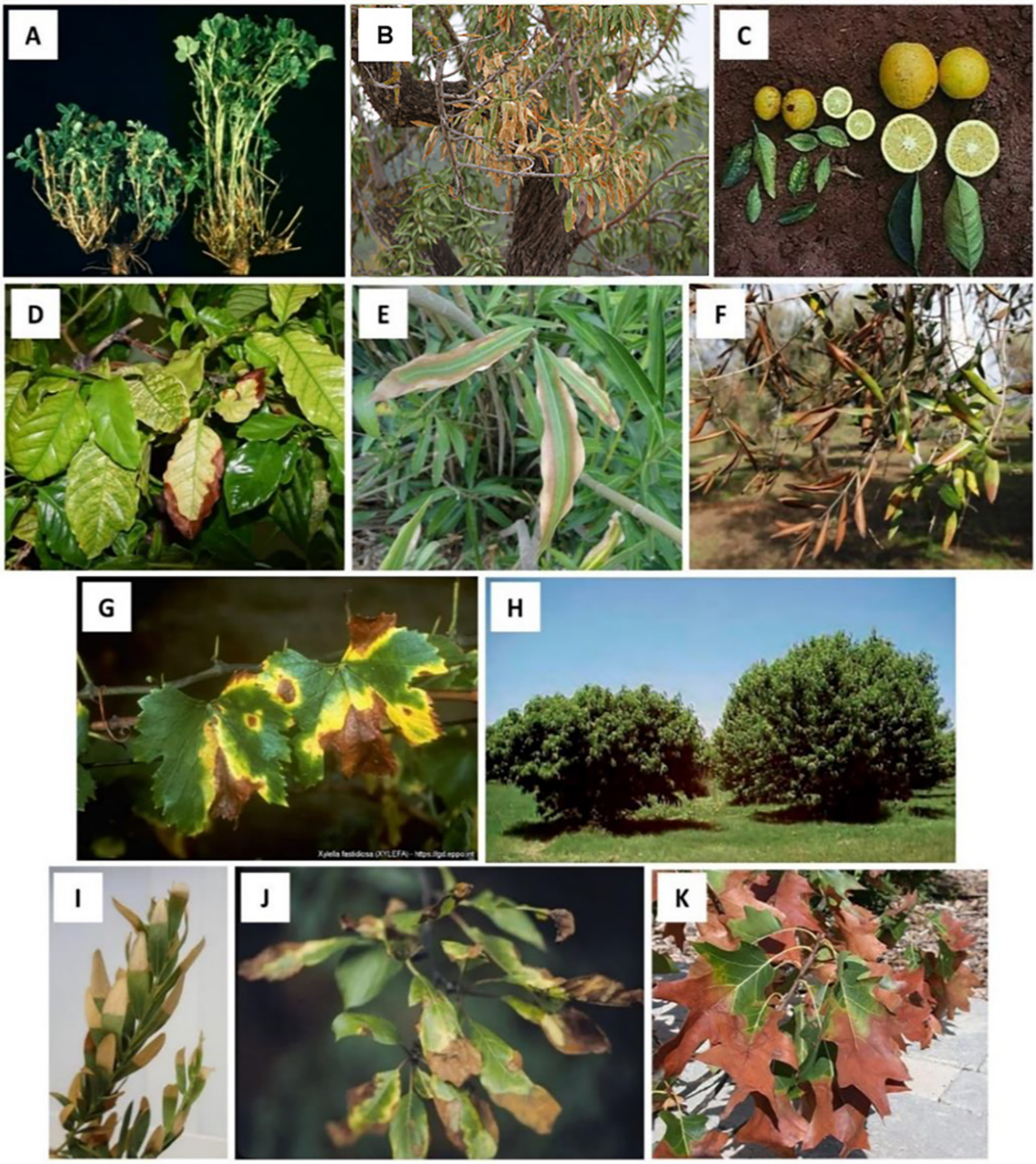

Fig. 3. Symptoms of the diseases caused by *Xylella fastidiosa* on several host plants: (A) Alfalfa dwarf (R.M. Davis in Available at: https://www.plantdiseases.org/alfalfa-dwarf-alfalfa, accessed 14 October 2024); (B) Almond leaf scorch (Courtesy of J. A. Navas-Cortés); (C) Citrus variegated chlorosis (A. H. Purcell in Bugwood.org); (D) Coffee leaf scorch (B. Legendre in Available at: https://gd.eppo.int/taxon/XYLEFA/photos, accessed 14 October 2024); (E) Oleander leaf scorch (D. Boscia in Available at: https://gd.eppo.int/taxon/XYLEFA/photos, accessed 14 October 2024); (F) Olive quick decline (C. Picard in Available at: https://gd.eppo.int/taxon/XYLEFA/photos, accessed 14 October 2024); (G) Pierce's disease (J. Clark in Available at: https://gd.eppo.int/taxon/XYLEFA/photos, accessed 14 October 2024); (H) Phony peach disease (Janse and Obradovic, 2010 in Available at: http://www.aces.edu/, accessed 14 October 2024);(I) Symptoms on *P. myrtifolia* (D. Boscia in https://gd.eppo.int/taxon/XYLEFA/photos, accessed 14 October 2024); (J) Plum leaf scald (C.-J. Chang in Overall and Rebek, 2015); (K) Bacterial leaf scorch (A.B. Gould in Gould and Lashomb, 2005).

- Symptoms of *X. fastidiosa* infection in plum trees can include leaf scorching, wilting, defoliation, twig dieback, and fruit drops, which can impact crop yield (Fig. 3J).
- Symptoms of *X. fastidiosa* infection in shade trees (e.g. *Quercus* sp., *Platanus* sp.) can include yellowing of the leaves, browning of leaf veins, stunted growth, twig dieback, and possibly death. This kind of disease is known as bacterial leaf scorch (Fig. 3K).

Vectors transmission and epidemiology

X. fastidiosa is a bacterium limited to the xylem and is exclusively transmitted by xylem sap-feeding insects from the order Hemiptera, suborder Auchenorrhyncha. In total, 52 different species were reported worldwide as *Xylella* spp. vectors, including 42 Cicadellidae, 9 Aphrophoridae, and one Membracidae (Fig. 4). In Europe, until now, three spittlebugs species have been identified as vectors: *Philaenus* spumarius, *P. italosignus,* and *Neophilaenus campestris*. *P. spumarius* is considered the more significant

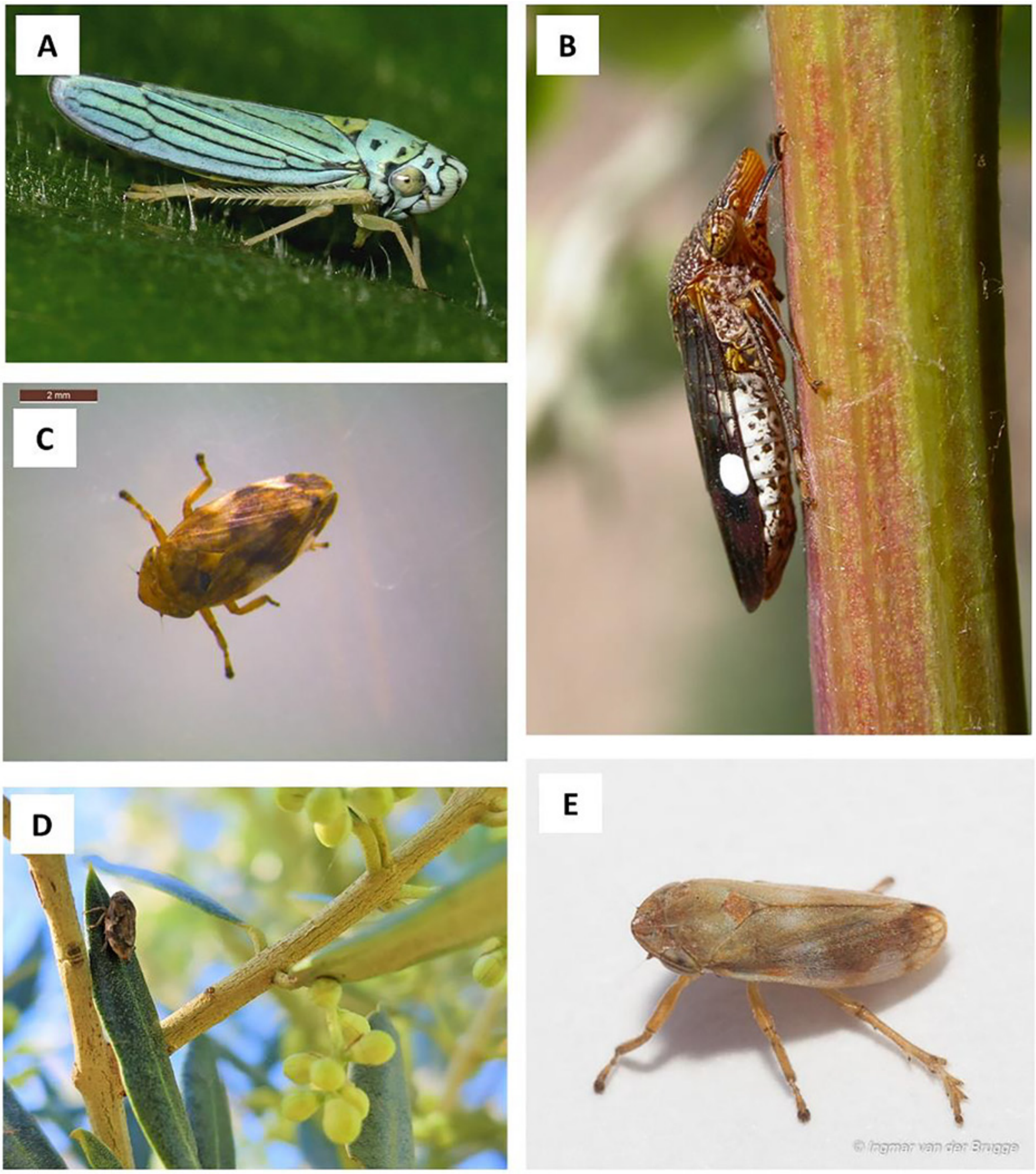

Fig. 4. Examples of vectors of *Xylella fastidiosa* worldwide: (A) *Graphocephala atropunctata* (L.J. Friesen, 2004; in Available at: https://calphotos.berkeley.edu/cgi/img_query?enlarge=0000+0000+0804+0127, accessed 14 October 2024); (B) *Homalodisca vitripennis* (in Haviland *et al*., 2021); (C) *Philaenus spumarius* (Hasbroucq, 2017, personal communication; Courtesy of S. Hasbroucq); (D) *Philaenus spumarius* on olive leaf (López-Mercadal; Courtesy of J. López-Mercadal); (E) *Neophilaenus campestris* (I. van der Brugge in Available at: https://www.gbif.org/fr/occurrence/3723035777, accessed 14 October 2024).

current vector in Europe. Unlike many plant pathogens, *X. fastidiosa* does not require a latent period before transmission; it is persistently transmitted by both nymphs and adult insects. Vectors acquire the bacterium by feeding on the xylem of infected plants and can immediately transmit it to healthy plants after acquisition. The bacteria remain confined to the insect's alimentary canal, specifically adhering to and multiplying in the foregut (pre-cibarium and cibarium), without spreading systemically within the insect's body. Once infected, adult insects can transmit the bacterium throughout their entire lifespan, as *X. fastidiosa* persists and multiplies in their foregut. The bacterium is not passed down to the progeny through eggs, meaning it is not transovarially transmitted. The spread of *X. fastidiosa* is primarily driven by winged adult vectors, which have higher mobility.

While *X. fastidiosa* transmission is limited to xylem sap-feeding insects, the process is not species-specific, meaning that all insects feeding on xylem are considered potential vectors. Insect vectors thus have a major role in shaping the epidemiology of the disease, including its spread and the plant infection. However, the efficiency of transmission varies greatly depending on the insect species, host plant, and the specific genotype of *X. fastidiosa*.

Once it has entered the xylem, the bacterium spreads through the plant. Once part of the xylem vessels is colonized, *X. fastidiosa* can degrade the plant wall to disperse into neighbouring vessels. It can also move up the sap stream in a process called twitching motility. Finally, the bacterium can form a biofilm inside the vessels when the infection state is more advanced. This formation is possible thanks to type I pili which allow the bacteria to adhere to plant surfaces. The switch between these different types of colonization is regulated by quorum sensing.

Sampling and Detection

According to EPPO (2019), there is a procedure for diagnosing *X. fastidiosa* in samples from plants or insect vectors. EPPO describes various screening tests: isolation screening, serological tests, molecular tests, PCR, and microscopy. Samples are considered infected by *X. fastidiosa* if at least two of these tests, based on different biological principles or targeting different parts of the genome, are positive. Once samples are confirmed as infected, subspecies can be determined using additional molecular tests.

Screening by isolation

Unlike with other bacteria, isolation is not recommended by EPPO for detecting *X. fastidiosa*, as it is very difficult to isolate in culture. While isolation is not recommended as a standard diagnostic tool by EPPO, obtaining isolates remains valuable, even though it is laborious. Isolates allow for detailed genotyping, making it possible to study the diversity of strains more comprehensively. This deeper insight into strain variation can significantly enhance our understanding of the pathogen's epidemiology and behaviour.

Serological tests

Several serological tests can detect the pathogen: ELISA, membrane trapping immunofluorescence (MEIF), dot-immunobinding assay (DIBA), Western blot, indirect immunofluorescence, and more recently, the direct tissue transfer immunoassay (DTBIA).

Molecular detection

Conventional qualitative PCR can be performed after DNA extraction using CTAB or kits from Quickpick, BioNobile, or Qiagen. The PCR protocol was initially developed by Minsavage, with additional protocols available for amplifying bacterial DNA. Some PCR tests can also be performed in real time, such as the one developed by Harper. Several PCR methods are available for identifying *X. fastidiosa* subspecies and STs. Specific PCR tests can differentiate subspecies by amplicon size and detect mixed infections. Additionally, MLST, using seven housekeeping genes, determines the ST by comparing sequences in the PubMLST database, though sequencing just two genes (cysG and malF) can indicate subspecies.

Isothermal amplification tests and kits

Harper *et al.* (2010, erratum 2013 cited in EPPO, 2019) developed primers for the LAMP method, though it is not yet widely used in the EPPO region. Li *et al.* (2013 cited in EPPO, 2019) also created kits that can be applied to plant extracts.

Microscopy

Various types of microscopy can be employed to observe bacteria in plants and insects, but effective use typically demands specialized professional training. Light microscopy techniques, such as dark field and fluorescence microscopy, are available, along with electron microscopy methods, which include transmission and scanning microscopy.

Current Management Practices – Prevention and Control

Currently, no control measures exist to completely eliminate *X. fastidiosa* from infected plants in the field. However, several promising preliminary studies have demonstrated a reduction in symptoms and bacterial populations within plants. It is important to emphasize that each pathosystem requires a tailored control strategy. Therefore, preventing the bacterium's establishment and lowering inoculum pressure in the field are critical components in managing *X. fastidiosa*.

Controlling its spread relies on interrupting interactions between host plants and vectors. Reducing insect vector populations helps to limit primary spread (from alternative host to host) and secondary spread (from host to host). Another solution is to apply quarantine measures and control host plants population (alternative host and host).

Quarantine measures and prevention

In Europe, strict phytosanitary and quarantine regulations are enforced to limit the spread of *X. fastidiosa* between regions. Infected plants and those within a buffer zone around the infection site are removed and destroyed. Restrictions on the movement of potentially infected plant material are essential to prevent new outbreaks.

X. fastidiosa is recognized as belonging to the A2 list of the EPPO, i.e. it is categorized as a locally present pest in the EPPO region. According to the European Commission (EC), *X. fastidiosa* is recognized as a quarantine pest within the European Union (EU) in accordance with Directive 2000/29/EC. This is a Council Directive on protective measures against the introduction into the Community of organisms harmful to plants or plant products, and against their spread within the Community. As such, it is forbidden to import the bacterium into the territory of member states in order to contain its spread. In addition, this directive imposes very strict rules on member states where the bacterium is already present regarding the management of this pathogen. Several emergency measures have already been put in place in the EU to combat this organism. These measures are based on EFSA's comprehensive plant health risk assessment and on Directive 2015/789. This directive was subsequently amended and modified by new directives (2015/2417 and 2016/764). Three types of measures are defined: those concerning the categorization of plants, those concerning the limitation of propagation in an already infected territory, and finally those concerning the prevention of introduction. Two categories of plant species are regulated under this directive: host plants and specified plants. Measures are also listed to limit the spread within the EU. Delimited zones (infected zones and buffer zones) will be defined as soon as the presence of *X. fastidiosa* is confirmed. The movement of specified plants within the demarcated areas is regulated. Measures to eradicate contaminated material will also be put in place. These include the destruction of all host plants within a 100-meter radius of infected plants. In addition, intensive surveillance of crops and plantations will be carried out. Finally, measures have been taken to prevent the pathogen from being introduced into non-EU countries, such as a ban on imports of coffee plants from Honduras and Costa Rica. Every year, member states carry out surveys to determine the presence of *X. fastidiosa*. Within this framework, a list of specified plants on which surveys are carried out is available in the annex to Decision 2015/789. Any detection of the bacterium must be reported to the EC and other member states within eight working days of detection at the latest. Specific procedural guidelines have been published by the EC in order to detect outbreaks as early as possible.

Vectors control

Controlling the insect vectors that transmit *X. fastidiosa* is a critical component of managing the disease. This is achieved through the use of insecticides, habitat modification, and use of natural enemies of *X. fastidiosa* vectors.

- *Insecticide*: In field trials conducted in Italy and Spain, the most effective active substances against *P. spumarius* are neonicotinoids (imidacloprid, acetamiprid, thiamethoxan) and pyrethroids (deltamethrin and lambda-cyhalothrin). In USA, the neonicotinoids (imidacloprid and dinotefuran) are also widely used against *H. vitripennis*. However, widespread insecticide use has raised concerns about environmental sustainability and insect resistance so, in organic farming, citrus essential oil is more effective than pyrethrins. In addition, some of those pesticide have been banned for outdoor use in the EU since then (imidacloprid, thiamethoxam, dinotefuran).
- *Habitat modification*: The life of vectors is closely linked to that of their herbaceous and weed hosts with tillage in Italy, planting vineyards more than 100 m from riparian zones harbouring alternative hosts and insect vectors in California, and placing screen barrier around vineyards to prevent the entry of insect vectors. In the case of *P. spumarius*, removing weeds and straw from the ground in olive groves helps to regulate the vector population. Another practice is to apply kaolin to the foliage of host plants to change their colour and prevent insect vectors from feeding on them.
- *Predation*: Very few natural enemies of *X. fastidiosa* vectors have been identified. Some Hymenoptera, such as Eulophidae and Mymaridae, parasitize eggs of *P. spumarius* in Corsica and *H. vitripennis* in USA. Some Diptera, such as Pipunculidae, parasitize nymphs and adults *P. spumarius* in Italy. There are also entomopathogenic fungi capable of infecting the nymphs of insect vectors. Additional biological control studies target entomopathogenic fungi capable of infecting the nymphs of insect vectors. Symbiotic control approaches have also been explored by introducing a genetically modified symbiont bacterium, *Alcaligenes xylosoxidans* var. *denitrificans*, into the insect's mouthparts. This bacterium has been engineered to produce antibiotics that specifically disrupt the pathogenicity of *Xylella fastidiosa*. However, to date, studies on these parasites have been scarce. As a result, little is known about their natural parasitism rates and how they would be effective in reducing pest populations. Moreover, in the context of integrated pest management, the introduction of non-native species presents several constraints and regulations.

Control of host plants

Removal of infected plants

Continuous monitoring programmes are crucial for the early detection of *X. fastidiosa*. Surveillance efforts include visual inspections, the use of molecular diagnostic tools to identify the bacterium in symptomatic and asymptomatic plants, and monitoring insect vector populations. Recommendations to control the disease include maintaining the overall health of plants and removing infected trees. Early detection and removal can help reduce *X. fastidiosa* inoculum, although this can be challenging due to latent infections. In northern Georgia, removing infected trees and nearby reservoir hosts, such as wild plum trees, has proven effective. However, this approach was less successful in southern Georgia, where disease incidence was higher. Severe pruning and roguing are also commonly used to reduce PD inoculum in the USA. In Italy, particularly in regions like Puglia where *X. fastidiosa* has severely affected olive groves, the removal of infected olive trees has been a key control measure. Authorities implemented a strict eradication strategy, which involved the felling and destruction of infected trees to contain the spread of the bacterium. Buffer zones were established, where both symptomatic and asymptomatic trees within a certain radius of an infected tree were removed to prevent further transmission.

External application of compounds

Studies on *X. fastidiosa* subsp. *fastidiosa* in the USA revealed that altering mineral homeostasis, such as zinc, copper, and calcium ions, can impact the bacterium's pathogenicity. A 3-year study on Dentamet® spray (a complex of zinc, copper, and citric acid) on olive trees in Italy showed reduced disease severity, though it did not provide full control. However, further trials are needed to confirm these results. N-acetylcysteine (NAC), a cysteine analog, has also shown promise in controlling *X. fastidiosa* in sweet orange plants under controlled conditions, leading to symptom remission and reduced bacterial growth. However, symptoms reappeared in some plants after treatment was stopped. When NAC was tested on infected olive trees in Italy, no significant reduction in bacterial population was observed, suggesting the need for more research to evaluate its effectiveness. Additionally, research on diffusible signal factor (DSF)-producing transgenic plants inspired studies on external applications of DSF homologues. Palmitoleic acid, C16-cis, and similar compounds were sprayed on diseased grapevines, showing a reduction in symptom severity. Applications of macadamia oil also demonstrated similar effects, indicating the potential of DSF-related treatments in managing the disease. In other studies, the peptide BP178 has shown a strong bactericidal activity in vitro against *X. fastidiosa* and other plant pathogens, and has triggered defence responses in tomato plants. Priming plants with BP178 triggered defence responses primarily via the salicylic acid pathway, while also upregulating genes in jasmonic acid and ethylene pathways. This bifunctional peptide shows great potential as a candidate for further development in managing *X. fastidiosa*-induced disease.

Resistant varieties

There is ongoing research into developing olive tree varieties that are resistant to *X. fastidiosa*. Some cultivars have shown promise in laboratory settings, but widespread adoption of resistant varieties is still in the research phase. A key strategy for preventing *X. fastidiosa* damage is cultivating resistant or tolerant plant varieties, as there is significant variation in symptom expression and transmissibility. Resistant cultivars have been identified in crops such as citrus, grapevines, and olives. For olives, the cultivars Leccino and FS17® have shown reduced symptoms and lower bacterial populations. Additionally, these cultivars have been linked to decreased acquisition and transmission of the bacterium by *P. spumarius*, making them preferable for cultivation. In the southeastern USA, grapevine breeding programmes found no resistant *Vitis vinifera* genotypes, but resistance was discovered in wild native *Vitis* species. Researchers successfully introduced resistance genes from these wild grapevines into economically important *V. vinifera* cultivars, and after more than 20 years of development, five resistant varieties were recently released. Additionally, transgenic grapevines were developed to produce diffusible signal factor (DSF), which reduced symptom expression and the systemic spread of the bacterium. In the presence of high levels of DSF, *X. fastidiosa* adheres quickly to xylem walls, limiting its virulence.

Exploring the interactions with the endophytome

The investigation of the xylem microbiome reveals notable differences in microorganism populations between susceptible and tolerant plant varieties. The study of microbiomes is relatively recent, and significant advancements have been made through next-generation sequencing (NGS) technologies. However, many tests involving endophytes against *X. fastidiosa* are still in the in vitro stage. For example, some *Bacillus* species have a potential to inhibit the growth of *Xylella fastidiosa* subsp. *pauca*. Some *B. velezensis* strains produced culture filtrates with inhibitory effects against *X. fastidiosa* ST53. Additionally, two of these *B. velezensis* strains, specifically D747 and QST713, are already registered and commercially available as biocontrol agents. In another study, 12 out of 200 isolates identified various species belonging to the genera *Paenibacillus*, *Bacillus*, *Pantoea*, *Microbacterium*, *Stenotrophomonas*, *Delftia*, and *Pseudomonas, which* were effective in inhibiting the growth of *X. fastidiosa in vitro*, with clear zones ranging from 4.0 to 38.6 mm. Additionally, an evaluation of antimicrobial activity revealed that five of the twelve antagonistic bacteria—*P. rigui*, *B. subtilis*, *B. pumilus*, *M. oxydans*, *and S. rhizophila*—produced culture filtrates with inhibitory properties. Those kinds of *in vitro* results could pave the way for further studies aimed at developing a sustainable biocontrol strategy for *Xylella*-related diseases. Other studies showed the impact of endophytes *in vivo*. One endophytic bacterium, *Curtobacterium flaccumfaciens*, is commonly found in asymptomatic *Citrus* trees. When periwinkles were co-inoculated with this bacterium alongside *X. fastidiosa*, the interaction resulted in a reduction of symptom severity. Another endophytic bacterium, *Paraburkholderia phytofirmans*, has been shown to have beneficial effects when co-inoculated with *X. fastidiosa* subsp. *fastidiosa* in grapevines, significantly reducing symptoms and nearly eradicating the plant. It is suggested that this bacterium may activate plant resistance pathways. However, preliminary trials in Italy involving olive trees and a local strain indicated no significant biocontrol effect, though these findings were limited to a single growing season. In a study comparing resistant and susceptible olive cultivars in Italy, two noteworthy bacteria for biocontrol were identified: *Methylobacterium mesophilicum* and *M. radiotolerans*. These bacteria produce extracellular siderophores, which may contribute to biocontrol efficacy. Competition assays with a specific strain are currently underway (Fig. 5) (reviewed in Landa *et al*., 2022). The findings on endophytic bacteria and their inhibitory effects on *X. fastidiosa* create opportunities for introducing specific microbiome members or communities to help mitigate diseases caused by *X. fastidiosa*.

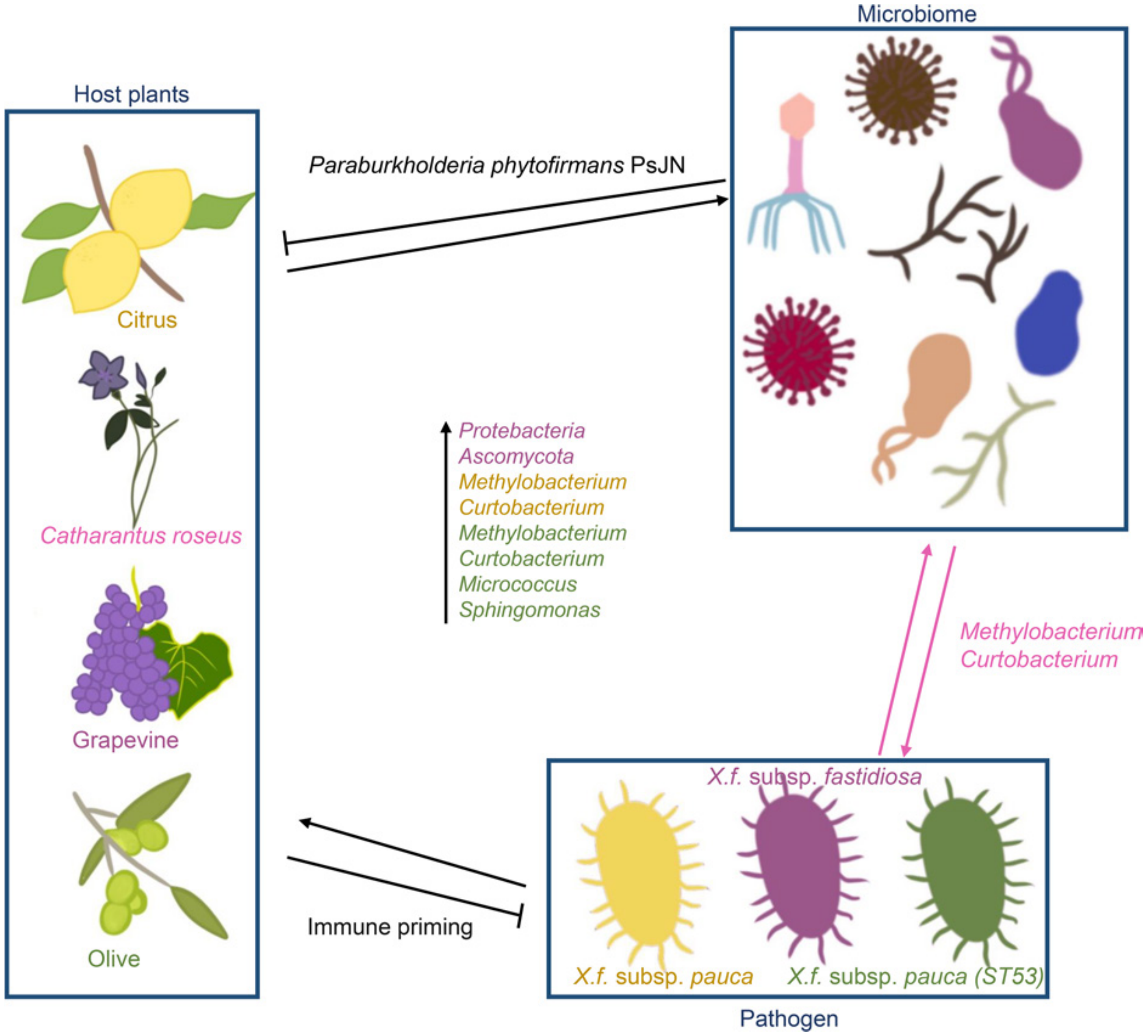

Fig. 5. The establishment of a pathogen's lifestyle is influenced by its interactions with both the host microbiome and immune system. Endophytes can suppress pathogen growth (indicated by blunt-ended arrows) through direct microbial interactions or by priming the host plant's immune response. For example, members of the microbiome from *Catharanthus roseus* and the introduction of the commensal *Paraburkholderia phytofirmans* PsJN have been shown to inhibit the growth of *X. fastidiosa*. If not controlled, pathogen growth can disrupt microbiome diversity and richness, ultimately impacting plant health. Infected grapevines, citrus, and olives with symptoms of *X. fastidiosa* display dysbiosis, characterized by an imbalance in their microbiome, with increased abundance of specific groups such as *Proteobacteria*, *Ascomycota*, *Methylobacterium*, *Curtobacterium*, *Micrococcus*, and *Sphingomonas*. The colour coding indicates the plant host's origin, while black arrows depict bidirectional interactions and coloured arrows represent increases in certain microbiome taxa. *P. phytofirmans* PsJN, was originally isolated from onions but can establish itself as an endophyte in grapevines (adapted from Landa *et al.*, 2022).

Future Research Area

Genetic resistance and harnessing plant microbiome

Developing genetically resistant olive tree cultivars is a key focus of current research. Advances in genome sequencing and gene editing technologies, such as CRISPR, could play a crucial role in enhancing plant resistance. While developing genetically resistant olive tree cultivars remains a primary focus, the implementation of genetically modified organisms (GMOs) in Europe presents significant challenges. Strict regulations and public sentiment surrounding GMOs often complicate their adoption in agricultural practices. Given these challenges, an alternative strategy worth pursuing is the enhancement of the plant microbiome. By promoting beneficial microbial communities, researchers can improve the overall health and resilience of olive trees without the complexities associated with genetic modifications.

Biological control

Investigating the use of natural predators and entomopathogenic fungi presents a promising avenue for reducing vector populations that transmit *X. fastidiosa*. Biological control strategies could offer a sustainable alternative to conventional chemical insecticides, minimizing environmental impact. Future research should focus on the efficacy of various biological agents in field conditions and their potential integration into existing pest management systems.

Advanced detection methods

Early and accurate detection of *X. fastidiosa* is crucial for effective disease management. Emerging technologies such as remote sensing, drone surveillance, and advanced molecular diagnostics are being explored to enhance detection capabilities. Future research could concentrate on refining these technologies to facilitate rapid, real-time monitoring of disease spread, as well as developing predictive models to anticipate outbreaks based on environmental factors.

Discussion Points

1. What are the main challenges in controlling the spread of *X. fastidiosa*?
2. How might genetic resistance alter the management strategies for *X. fastidiosa*?
3. What potential improvement for the sustainability of current vector control and plant host measures could be done?

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Conflict of interest

The author has no conflicts of interest to declare.

Further Reading

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