



Grower Summary

**Xylella fastidiosa: what are the factors
that make this bacterium pathogenic
and host-specific?**

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AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

Headline

*Fundamental knowledge of the bacterium *Xylella fastidiosa* – detected in over 350 different plant species in Europe alone and causing diseases in many crops – can lead to targeted treatment plans, instead of destroying acres of valuable crops.*

Background

What is *Xylella fastidiosa*?

Xylella fastidiosa (*Xf*) bacterium that lives in the foregut of insects and the xylem of plants and causes diseases in several economically significant crops, including Pierce's disease (PD) of grapevine, phony peach disease (PPD), oak leaf scorch (OLS) and olive quick-decline syndrome (OQDS). *Xf* has been detected in over 350 different plant species in Europe alone, but detection of the bacterium in a plant does not necessarily lead to disease. However, these asymptomatic hosts may however act as a reservoir for insect vectors to further spread the bacteria to susceptible plants. Very little is known about the mechanisms behind what makes *Xf* cause symptoms in some plants but not in others. This research project investigates what makes *Xf* host-specific and pathogenic by means of molecular and computational biology. More specifically, the genes that encode effector proteins. Effector proteins are secreted by bacteria and interact with a host plant's immune system, the importance of which is explained below.

Currently, there is no treatment available for diseases caused by *Xf*. Management measures are restricted to vector control, pruning of infected plant tissue and destruction of the infected host. All surrounding potential plant hosts in a 100 m radius are destroyed and a demarcation order of a 5 km radius is set up banning the movement of any plant material outside this area.

Plant hosts of *X. fastidiosa*

There are over 350 different potential host plants in Europe alone, many of which are economically important crops and could devastate a country's economy if affected. The bacterium has not yet been detected in the UK, but the threat is very high as many of its host plants are cultivated here. These include, but are not limited to economically significant plants grown in forest nurseries, as protected crops and ornamental garden plants. Some of the most significant crops are:

alfalfa, bay, blueberry, *Brassica*, *Cercis* (redbuds), *Chionanthus* (fringe tree), *Cytisus* (broom), elderberry, elm, fig, grapevine, *Hedera* (ivy), *Hypericum* (St. John's Wort), magnolia, maple, mulberry, *Nandina domestica* (sacred bamboo), lavender, oak, olive, pear, *Prunus* (e.g. apricot, cherry, plum), *Rubus* (e.g. raspberries, blackberries), *Rosa*, rosemary, strawberry, *Trifolium* (e.g. clover), walnut, willow.

Xf's large host range, its long incubation period, which may be up to six months in some plants, and rapid spread makes it a highly threatening pathogen. However, in order to come up with effective treatment plans for affected plants, it is important to understand the fundamental biology of the disease-causing bacteria.

How do plant bacteria cause disease?

Phytopathogens, or plant pathogens, have the ability to invade the host, evade host defence mechanisms and ensure disease progression by secreting virulence factors. Virulence factors are proteins, lipids and carbohydrates produced by the pathogen. One of the best-characterised virulence factors include effector proteins, which are secreted through secretion systems or channels in the bacterial cell. Those secreted through type 3 and type 4 secretion

systems (T3SS and T4SS, respectively) are among the most extensively studied in structure and function. The majority of bacterial phytopathogens have been found to secrete effectors through the T3SS, for example *Pseudomonas syringae*, *Erwinia* spp. and *Xanthomonas* spp. (Buttner, and He, 2009). However, *Xf* lacks the T3SS, meaning its strategy to cause disease may be quite different. Effectors may have several functions. In *Xf*, for example, a number of effector proteins are found to be involved in biofilm formation. Biofilm is an adhesive state of bacteria, where they aggregate in clusters. In the case of *Xf*, biofilm formation often leads to the blocking of the xylem, which stops the flow of water and minerals in the vessels and thus disease symptoms appear.

***X. fastidiosa* is an increasing threat in the European continent**

Xf is believed to be native to the Americas and outbreaks of diseases caused by the bacterium within Europe have only been discovered in 2013. The first outbreak of *Xf* in Europe was detected in Italy, followed by France and Spain, and isolated cases in the Netherlands, Belgium, Switzerland, Germany and Portugal. *Xf* spread has been connected with human-mediated movement of infected plants, resulting in distribution of *Xf* across large geographical distances. In Europe, four *Xf* subspecies have been identified: *fastidiosa*, *multiplex*, *pauca* and *sandyi*. Subspecies *fastidiosa* originated in Central America, *multiplex* in North America and *pauca* in South America (Sicard, et al., 2018). The origin of *sandyi* is still under debate (Almeida, and Nunney, 2015). A fifth subspecies, *tashke*, has only been found in the Americas (Randall, et al., 2009; Janse, and Obradovic, 2010). And a sixth subspecies (*morus*) has been proposed but is still under review (Nunney, et al., 2014a).

The importance of studying *X. fastidiosa*

Xf is an increasing threat to British agriculture. Climate change makes the environment more suitable for *Xf* which is known to favour warmer regions (Henneberger, 2003; Feil, and Purcell,

2007; Meyer, and Kirkpatrick, 2008), but also international plant trade is growing every year, meaning the bacteria have many ways to enter the country.

This research will provide a better understanding of the evolutionary history of *Xf* and the molecules involved in disease progression, which can ultimately help with the generation of targeted treatments for plants infected by *Xf*. There is currently no treatment solution for plants infected by *Xf*, with the only option being destruction of the host, its surroundings and a quarantine order. This research may gain more insight into the complex host range of the bacterium, its yet unknown mode of action within the plant, and determine why the pathogen causes disease in some hosts but remains asymptomatic in others. Ultimately, the outcome of the study could pave the way to implementing further control measures and creating diagnostic tools for the prevention of an outbreak. Genomics can create diagnostics, and understanding how the pathogen causes disease could lead to a potential treatment rather than having to destroy bacteria-carrying hosts and face huge economic loss.

Summary

Currently, the only control measure of *Xf* is prevention and destruction of plant hosts. Coming up with a targeted treatment plan could mean saving potentially millions of pounds. There are several reasons why there is still very little known about *Xf*, some of which include its long incubation period in the plant, difficulty to cultivate *in vitro* and thus study in the laboratory, and the many asymptomatic host plants where the bacterium lives in without causing any disease. The ability of computational methods to investigate an organism's genome has become very powerful, allowing a better understanding of the organism. Investigating genes that are involved in disease development will help with the understanding of the bacterium's molecular biology. Understanding how the bacterium works in a molecular level could bring us a step closer to establish a targeted treatment plan for this devastating bacterium.

A number of interesting putative effector proteins are promising – but as this is an on-going research, more analyses need to be carried out. Understanding the molecules involved in pathogenesis of *Xf* could help in the development of an efficient treatment plan for plants infected by the bacteria. Furthermore, the first detection of *Xf* in Colombia is described here. Twelve *Coffea arabica* samples of five cultivars and one *C. arabica* of unknown cultivar have tested positive for *Xf*. The detection of *Xf* in the country opens more questions about this fascinating bacterium. Colombia does not appear to have an outbreak of *Xf*, unlike its neighbouring country Brazil, where coffee leaf scorch due to *Xf* is rampant. It would be interesting to perform comparative analyses between Brazilian and Colombian *Xf* strains to understand why *Xf* appears to be more pathogenic in one country/host than another.

Financial Benefits

The financial impact of *Xf* is difficult to accurately estimate due to its large host range. However, it is clear that the arrival of *Xf* in a country has an enormous impact in many sectors, as the detection of *Xf* would not only farms, but also nurseries, retailers, and importers/exporters. According to Lindow (2019), PD had caused an annual loss of US\$ 104 million in California by 2014. Approximately US\$ 50 million is also spent on preventative measures every year. In Brazil, 40% of citrus plants are affected by CVC, which had caused an annual loss of US\$ 120 million by 2005 (Rapicavoli, et al., 2018a). In Europe, the first *Xf* epidemic was identified in Apulia in the South of Italy, where the bacterium was found to be the cause of OQDS. 40% of olive trees are grown in Apulia for the production of olive oil in Italy (Strona, Carstens, and Beck, 2017), and over 10 ha of olive trees have since been destroyed (Martelli, et al., 2016). Undeniably, the detection of *Xf* in a country has a significant economic impact. Prevention of the arrival of *Xf* in the UK is the only control measure currently available.

Action Points

As *Xf* has not yet been detected in the UK, there are no action points to be tackled for growers. However, it is advised to remain vigilant of symptoms and report any potential ones to the correct authority. If an outbreak is suspected, contact the APHA Plant Health and Seeds Inspector or PHSI Headquarters for England and Wales (planthealth.info@apha.gsi.gov.uk), the Scottish Government's Horticulture and Marketing Unit (hort.marketing@gov.scot), or the DAERA Plant Health Inspection Branch for Northern Ireland (planthealth@dardni.gov.uk). Be aware of *Xf* disease symptoms, these can vary between different plants. Visit the EPPO website (<https://gd.eppo.int/taxon/XYLEFA/photos>) for disease pictures and the European Commission website for an extensive list of susceptible *Xf* plant hosts (https://ec.europa.eu/food/plant/plant_health_biosecurity/legislation/emergency_measures/xyella-fastidiosa/susceptible_en). It is also advised to keep up-to-date with plant health news. And most importantly, to avoid importation of plants from areas affected by *Xf* (<https://www.cabi.org/isc/datasheet/57195#toDistributionDatabaseTable> and <https://www.cabi.org/isc/datasheet/57195#toDistributionMaps>) and/or ensure the imported material holds appropriate plant passports and phytosanitary certificates.

According to DEFRA, import requirements for *Coffea* sp. plants, plant produce and products from third countries are regulated, meaning that imports are only allowed if products are reported prior to entering the UK and are accompanied by phytosanitary certificates. *Coffea* imported particularly from Costa Rica and Honduras are prohibited unless a scientific research license or derogation has been procured. Import from these two countries has been added to DEFRA's "prohibited" list most likely because coffee plants imported from these countries into the Netherlands have tested positive for *Xf* (Bergsma-Vlami, et al., 2015).

The detection of *Xf* in Colombia reveals that the prevalence of the bacteria in the Americas is more common than previously assumed. This means that *Xf* could also be more prevalent in the European continent than currently known, as the pathogen is mainly only reported in countries with existing outbreaks. In general, it is advised to avoid direct coffee plant imports from countries where *Xf* has been detected, particularly Argentina, Brazil, Colombia, Costa Rica, Honduras, Paraguay, Puerto Rico and Venezuela.