

Dispersion of the Bacterium *Xylella fastidiosa* in Portugal

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Abstract: After the first detection of the gram-negative bacterium, *Xylella fastidiosa multiplex* in Portugal, in January 2019, a “Demarcated Area” was delimited and an “Action Plan” to control the pathogen has been developed. It is considered one of the greatest threats to plant health worldwide and leads to loss of forests vitality and productivity. The current study was developed till June 2020, with the objective to understand the dimension of the issue worldwide and in Portugal, analyzing the state-of-the-art on the biology, dispersion, symptoms, control and risks associated with this bacterium (Research Phase). In the Prospecting Phase, we collected 5 branches with 5-8 leaves, from the four quadrants of the tree, wrapping and labeling them—so 2,261 samples were collected and sent to the laboratory for analysis. It was possible to determine that the two initial outbreaks were not an isolated case, as 107 additional outbreaks were detected in other places, revealing a much more worrying panorama, requiring further analysis on the real impact of this bacterial strain on the natural environment. By the end of this study the demarcated area covered about 62,000 ha. The most affected species was lavender with 30% of positive outbreaks, followed by gorse with 22%, and rosemary with 11%. Spread of *Phyllaenus spumarius* and *X. fastidiosa* indicates that insect vector and transport links appear to be dispersal facilitators together with Douro River. As eradication is unfeasible to achieve in the coming years, containment will be the most appropriate strategy.

Key words: Phytopathogen, forest plant health, xylem-limited bacteria, *Xylella fastidiosa*, *multiplex*, biotic stressors, Demarcated Area.

1. Introduction

Forests play a vital role in responding to current global challenges, and as renewable resource contributes to improving the quality of life, providing multiple benefits to society and the economy while fulfilling the environmental, cultural, tourist and social functions [1, 2]. On the other hand, globalization puts growing pressure on natural resources, facilitating the spread of forest pests [3, 4]. The current climate change context significantly increases the likelihood of new and more aggressive pests in regions where, until recently, they were highly unlikely [5].

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ecosystems, due to harmful biotic agents, cause physiological imbalances leading to loss of vitality and decrease of net primary production [3]. This generates increased costs to control harmful biotic agents, negatively impacting on the integrated management programs of these biotic agents and causing environmental damage due to frequent use of chemicals or massive logging [6, 7]. The proclamation of the year 2020 as the International Year of Plant Health, by the International Plant Protection Convention (IPPC), meets precisely the concerns mentioned [8].

The gram-negative bacterium, *Xylella fastidiosa* [9], considered one of the greatest threats to plant health worldwide, is characterized by its slow-growing rod shape [9-18]. In plants, presence is restricted to xylem

vessels. It has great genetic plasticity, with four subspecies (*fastidiosa*, *multiplex*, *pauca* and *sandyi*) currently recognized and studied [9, 17, 19-22]. More recently, two new subspecies (*tashke* [23] and *morus* [24]) have been proposed. It is transmitted by insect vectors from *Hemiptera* order, more specifically *Aphrophoridae* family, which feed on xylemic sap [14, 25]. The most common is *Phyllaenus spumarius* [26, 27].

After the detection of the first outbreaks of infection, in Vila Nova de Gaia, on January 3, 2019, Portugal had to quickly delimit a “Demarcated Area” (comprising the “Infected Zone” (ZI), including all plants that are within a radius of 100 m around the contaminated plants surrounded by “Buffer Zone” (ZT) with a 5 km radius) and develop an “Action Plan” to avoid the dispersion of this pathogen [28].

With this study we intended to understand the current dimension of the problem worldwide, focusing, in particular, the study in Portugal. The goal was also to compile and analyze the up-to-date knowledge about the biology, dispersion, symptoms, control and

risks associated with this bacterium, identifying the ongoing risk factors and threats in Portugal.

2. Material and Methods

The action plan for the eradication of *Xylella fastidiosa* and control of its vectors was established. The foreseen survey protocol involved three defined zones within the Demarcated Area: the ZI, where samples were collected from all host plants in a buffer zone of 100 m around the focus; two ZTs, one in the first kilometer radius around the ZI (ZT1), where a sampling grid of 100×100 m was established, and the ZT2 from the first to the fifth kilometer radius, with a of 1×1 km sampling grid.

This study comprised three phases: the research phase (scientific research, state-of-the-art) and the survey phase (sampling and submission to laboratory for analysis) were carried out simultaneously, the analysis phase took place after the collection of field data, and encompassed geographic information system (GIS) map production and the analysis of the bacteria dispersal.

Xylella fastidiosa subsp.

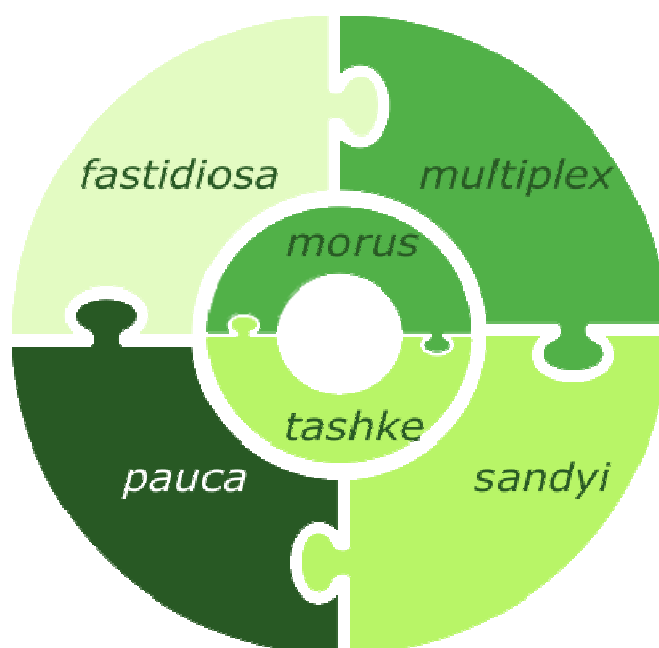


Fig. 1 *Xylella fastidiosa* subspecies.

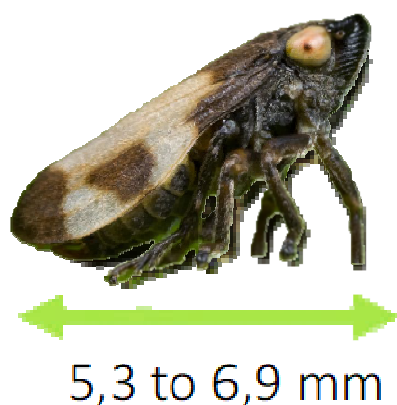


Fig. 2 Insect vector (*Phyllaenus spumarius*).

The sampling phase's steps included georeferencing the sample, collection of biological material in the four quadrants, the host species trees (5 or 10 branches, for individual or composite samples, respectively, with 5-8 leaves each), the disinfection of the pruning shears after collection from each tree, with 96% vol. alcohol (essential to avoid contamination). Afterwards, the sample was wrapped in newsprint, placed inside a plastic bag, labelled and wrapped in another stapled plastic bag to prevent the escape of insect vectors. At the end, the tree from which the sample was collected

was photographed (aspect and symptoms) and was marked with the sample reference by ink spray.

Samples were collected from all host forest tree species in the ZI, and at two or three sites respectively in ZT1 and ZT2, in case that visible symptoms were detected.

3. Results and Discussion

At the end of this study, the Demarcated Area covered about 62,000 ha. The Park Ranger teams prospected 5,885 points, collecting a total of 2,261 samples and revealing 16 new outbreaks. At the end of the first half of 2020, the number of ZIs increased fivefold, the grids to be prospected in ZT1 doubled and in ZT2 quadrupled when compared to 2019.

Leaves of 84 species belonging to 48 genera were sampled. The most frequent are eucalyptus (*Eucalyptus* spp.), oak (*Quercus* spp.) and acacia (*Acacia* spp.), with only 33 of the referred species set affected by the disease. The most affected was undoubtedly lavender (*Lavandula* spp.), with 30% of positive outbreaks, followed by gorse (*Ulex* spp.), with 22%, and rosemary (*Rosmarinus officinalis*), with 11%.

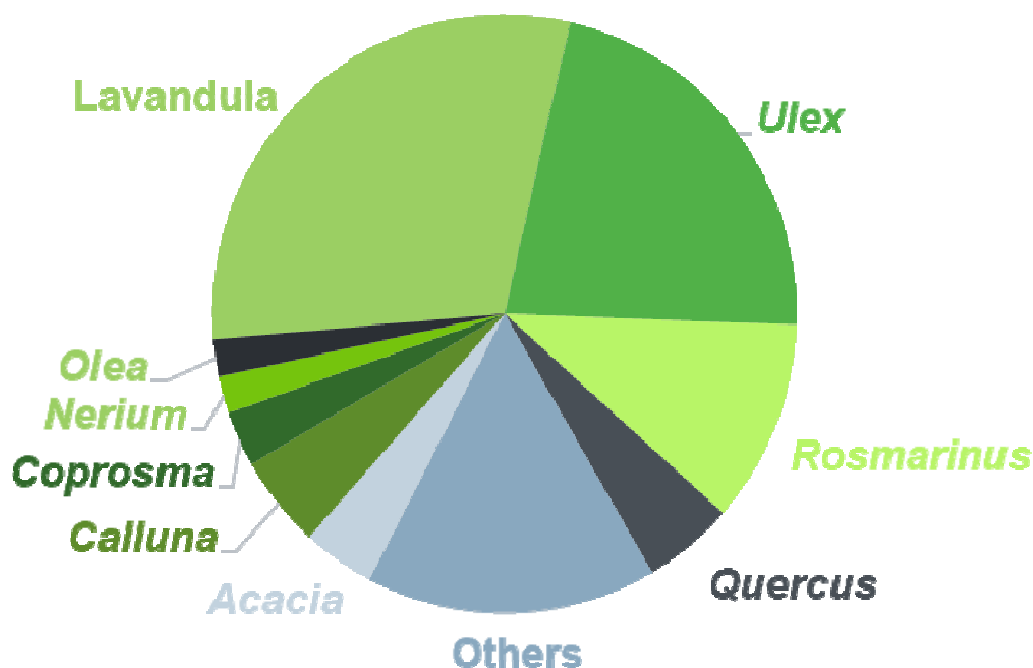
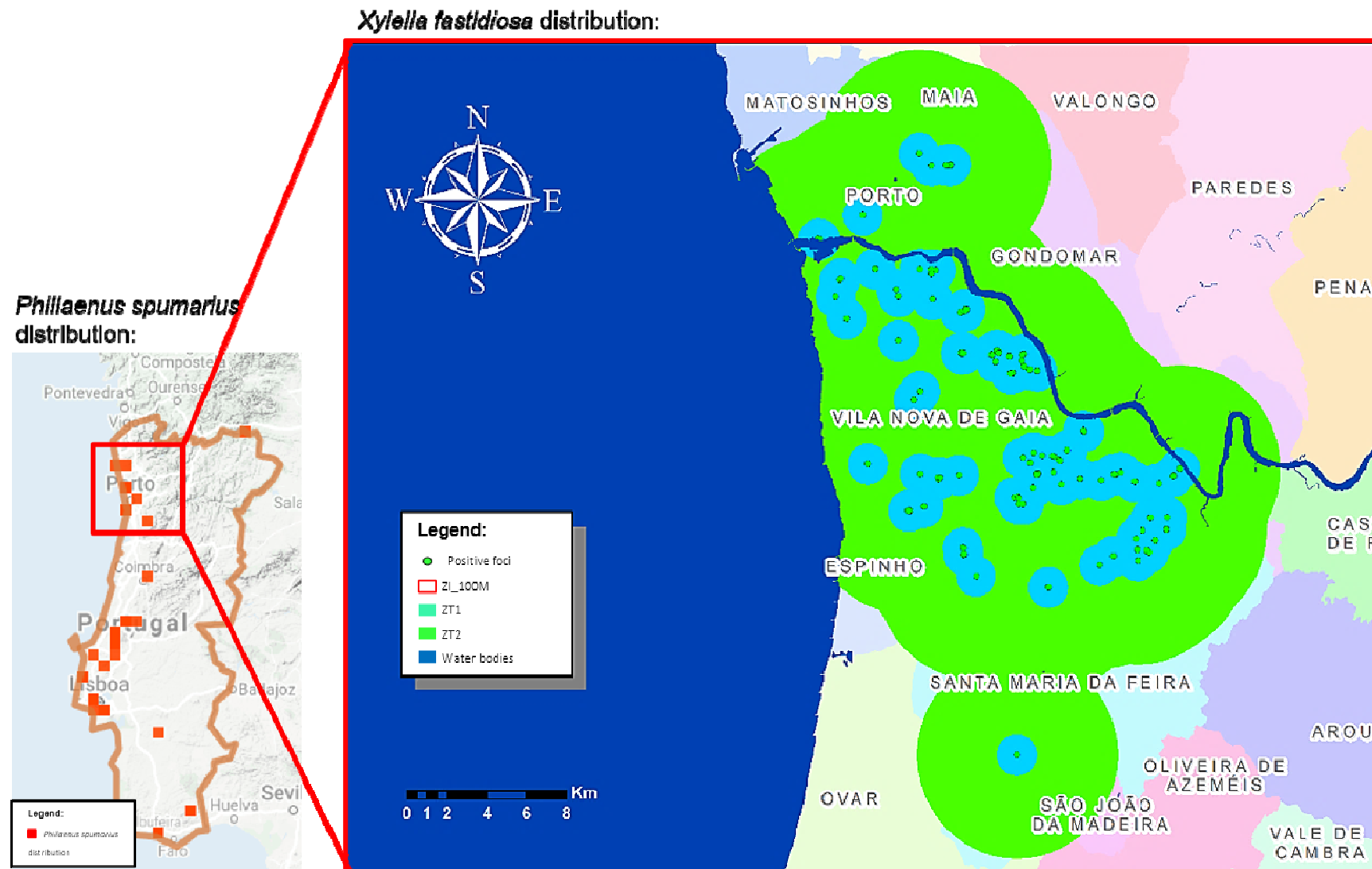


Fig. 3 Most affected host plants.



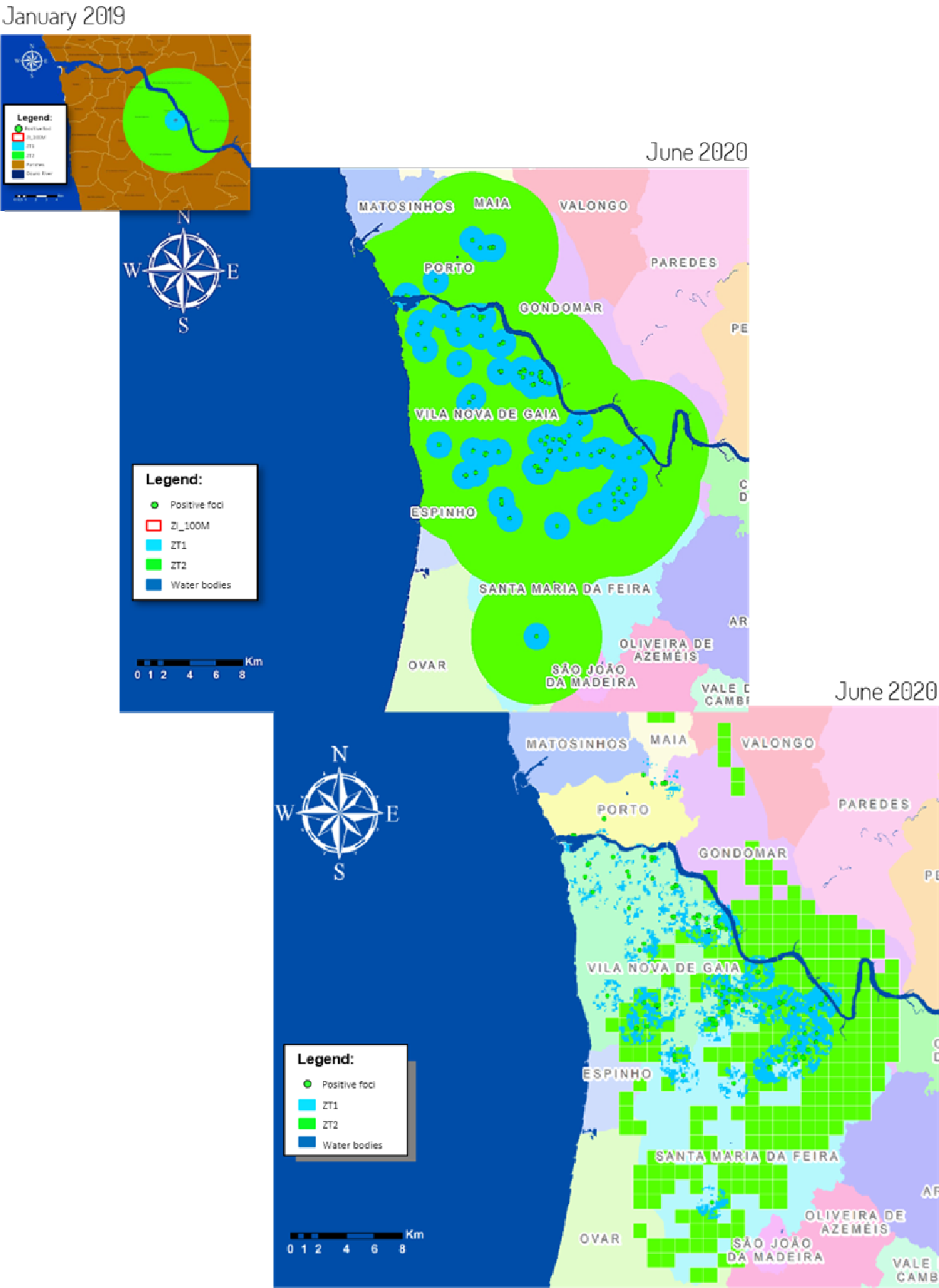


Fig. 5 Evolution of the *Xylella fastidiosa* demarcated area in Portugal.

In the vicinity of the Demarcated Area, two major entry points can be identified: Francisco Sá Carneiro Airport and the Port of Leixões, close to Porto city. The plant nurseries within the Demarcated Area are potential source of outbreaks.

It is possible to correlate the maps produced as they clearly show a similarity between the known distribution of the potential insect vector and the current dispersal of the *X. fastidiosa* bacterium. This indicates dispersal by the insect vector and allows the prediction of the risk of spreading the disease.

4. Conclusion

It was possible to determine that the two initial outbreaks were not isolated cases as 107 additional outbreaks were detected, revealing a much more worrying panorama in the 62,000 ha of Demarcated Area, thus requiring further analysis on the real impact of this bacterial strain on the natural environment.

The distribution map of outbreaks may be due both to a higher sampling rate in urban areas and to plants from local plant nurseries that revealed a higher number of positive foci.

The similarity between the known distribution of the potential insect vector *P. spumarius* and the current known dispersal of the *X. fastidiosa* bacterium indicates dispersal by insect vector and allows the prediction of the risk of spreading the disease. Roads seem to be facilitators of dispersal as well as the Douro River.

As eradication is unfeasible to achieve in the coming years, so containment will be the most appropriate strategy to implement.

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