Article

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Research Paper

Xylella fastidiosa Dispersion on Vegetal Hosts on Demarcated Zones in the North Region of Portugal

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Abstract: The detection of *Xylella fastidiosa* in Portugal occurred through prospection, in 2019 in Vila Nova de Gaia, Porto, in plants *of Lavandula dentata* L.. Currently, in Portugal there are 18 *Xylella fastidiosa* demarcated zones (DZ). The main objective of this study is to gain a comprehensive understanding of this problem within the defined Demarcated Zones in the North Region of Portugal, since 2019 to June 2023. This work comprehended two phases: the prospection phase (inspecting plants, sampling and submission of samples to the laboratory) and the research phase (collecting and organizing data and statistical treatment). **Our findings provide essential insights, suggesting that the Northern region of Portugal is highly conducive to *Xylella fastidiosa*. O**ur country has *Xf*-preferred hosts such as the olive tree, orange tree, *Laurus nobilis*, *Rosa* spp., *Nerium oleander* L., *Pelargonium* sp., *Hedera helix* L. and *Lavandula dentata* L. **Our favorable environmental factors such as temperature and humidity can have an important role in influencing the interaction between bacteria and hosts.** Nevertheless, it is crucial to consider variations in the behavior of insect vectors, as these variations can limit the prevalence of the disease. **Activities like the transport of infected planting materials from the first Demarcated Zone of the Area Metropolitana do Porto could be associated with the introduction and spread of *Xylella fastidiosa*, potentially triggering new disease outbreaks in our country. Our findings confirm the alarming spread of *Xylella fastidiosa* across Portugal.** Factors such as the presence of insect vectors, abundance of host plants, and climate suitability play pivotal roles in *Xylella fastidiosa* dispersal. We recommend that countries identified with relatively high risk, like Portugal, undertake thorough individual risk analyses. The implementation of preventive measures and, if necessary, the enhancement of surveillance systems for early detection of *Xylella fastidiosa* in plants and insect vectors are crucial steps.

Keywords: *Philaenus spumarius*; olive quick decline syndrome; citrus variegated chlorosis; bacterial leaf scorch; pierce disease

1. Introduction

The introduction of invasive species can have severe effects on the economy and ecosystems, leading to significant financial burdens. This is especially noteworthy in the agricultural sector, which is highly susceptible to the infiltration of pathogenic organisms. These invasions can lead to a decline in food supply, increased prices, compromised food quality, and potential concerns regarding food safety and security [1]. It is crucial to evaluate the consequences of invasive plant pathogens in terms of their impact on agriculture and also by considering the broader range of services provided by the agroecological system in its landscape context [2]. Invasive plant pests and diseases, like *Xylella fastidiosa* (*Xf*), can have a profound impact on the ecosystems by significantly disrupting its flow.

Xf is an aerobic bacterium[3], transmitted through xylem-sucking insects [4], that multiplies in the xylem [5], causing occlusion of xylem vessels and water and nutrient stress. It has been considered a quarantine pest, for affecting economically important agricultural crops [6]. It is imperative to understand the real incidence of this bacterium and the corresponding risk factors, which are, to date, poorly understood.

This plant pathogen, originally from the Americas, has been detected in various European and Near Eastern countries [2]. The initial outbreak of this pathogen in the European Union was identified in olive trees in Italy's Apulia region, in 2013 [7,8]. Subsequent occurrences of the bacterium have been reported in France, starting with an outbreak in Corsica in 2015, followed by Provence in 2019 and Occitania in 2020 [9]. Those countries have reported infected coffee plants that came from Latin American countries such as Mexico, Ecuador, Costa Rica, and Honduras [10], indicating that the global spread of this pathogen was expanding due to the exchange of commodities and plant materials.

The initial outbreak of the plant pathogen in Spain occurred in the Balearic Islands in 2016 [11,12]. This outbreak involved three subspecies of the bacterium, affecting more than 20 plant species such as grapevine, almond, olive, and fig [13]. By December 2020, over 600 olive trees in the Balearic archipelago were confirmed to be infected. As the situation progressed into 2021, the affected area expanded, covering 2292 hectares, and resulting in the destruction of over 100,000 almond trees [6].

The detection of *Xylella fastidiosa* in Portugal occurred through prospection, in 2019 in Vila Nova de Gaia, Porto, in plants *of Lavandula dentata* L. [14]. Currently, in Portugal there are 18 *Xf* demarcated zones (DZ), as we can see in Figure 1, and 1 deleted DZ in Tavira in the Region of Algarve [15].

In Porto, once the presence of the bacterium was confirmed in 2019, measures were immediately taken to prevent its spread and ensure eradication [16]. Following the detection of *Xf*, a "Demarcated Zone" was promptly established. This zone encompasses the "Infected Zone", which includes all susceptible plants within a 50m radius around the contaminated plants. Additionally, there is a surrounding "Buffer Zone" extending to a radius of 2.5 km [14]. Subsequently a series of control or eradication measures were implemented. These measures included uprooting infected trees, prohibiting the planting of susceptible plant species, restricting the transportation of plants from the infected areas, as well as conducting vector control through vegetation removal and the application of chemical treatments [17].

North

☐ DZ Área Metropolitana do Porto
☐ DZ Sabrosa
☐ DZ Alijó
☐ DZ Baião
☐ DZ Mirandela
☐ DZ Mirandela II
☐ DZ Bougado

Center

☐ DZ de Castelo Novo
☐ DZ da Covilhã
☐ DZ do Fundão
☐ DZ Gândaras
☐ DZ Marrazes
☐ DZ of Monte Redondo
☐ DZ of Penamacor
☐ DZ of Póvoa de Midões

Lisbon an Tagus Valley

☐ DZ Área Metropolitana de Lisboa
☐ DZ of Colares
☐ DZ of Palmela

Figure 1. Demarcated zones (DZ) of *Xylella fastidiosa* in Portugal.

Following the guidelines stipulated in Implementing Regulation (EU) 2020/1201, all European countries must prospect for quarantine pests [16]. The objective of prospecting for *Xf* is primarily geared towards identifying the presence of this pest. This is achieved through the inspection and collection of samples, followed by the implementation of control or eradication measures [2].

The General Directorate of Food and Veterinary (DGAV), as the National Phytosanitary Authority, is tasked with defining and coordinating the nationwide survey for *Xf*. As part of the program, several key components are incorporated. These include identifying priority sites that require monitoring, establishing specific timelines for observations and sampling, compiling a comprehensive list of host plants that need to be monitored, describing the symptomatology associated with *Xf* infections, and establishing procedures for collecting samples for laboratory analysis [14,18]. This responsibility extends to various entities involved in the prospecting program. These entities include the Regional Directorates for Agriculture and Fisheries (DRAP), the Directorates of Agriculture of the Autonomous Regions of Madeira and Azores (DRA), and the Institute of Conservation of Nature and Forests (ICNF) [19]. Collaboratively, they work together to ensure the effective implementation of the survey across Portugal. The critical task of analyzing these samples in the laboratory is entrusted to the National Institute for Agrarian and Veterinary Research (INIAV). They play a vital role in providing accurate and reliable results for further assessment and action [19].

The main objective of this study is to gain a comprehensive understanding of this problem within the defined DZs in the North Region. The focus is on analyzing the available prospection data from DRAP North, since 2019 to June 2023, within these zones. By doing so, we aim to improve our knowledge about the host biology, dispersion of symptoms, control methods, and associated risks of those *Xf* outbreaks in our country. This information will serve as a foundation for formulating hypotheses for further investigation. The study findings will be valuable in assisting public authorities and private entities, in making informed decisions and taking decisive actions related to this issue. Moreover, this preliminary assessment will contribute to the current decision-making process regarding the planning and execution of control operations, providing valuable insights for future data-driven evaluations.

2. Materials and Methods

In this study we will analyze DRAP North prospection data, since 2019 to June of 2023, from DZ of Oporto Metropolitan Area; DZ Baião and DZ Bougado; and from several DZ of the interior North part of the country, including Sabrosa; Alijó; Mirandela; Mirandela II. This work comprehends two phases: the prospection phase on Figure 2 (inspecting plants, sampling and submission of samples to the laboratory) and the research phase (collecting and organizing data and statistical treatment).

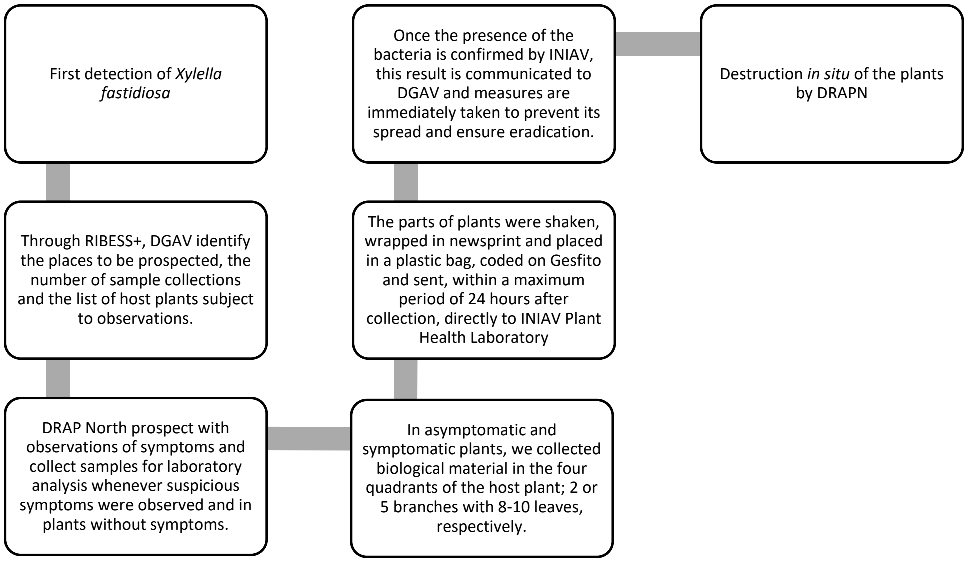


Figure 2. Resume for prospection phase.

2.1. Prospection Phase

Prospecting for *Xf* includes, inspecting and carrying out sample collection and in the event of their presence, implement control or eradication measures. The first step includes identifying the places to be prospected, the times when observations and sample collections are carried out, the list of host plants subject to observation, the description of symptoms and the establishment of sample collection procedures for analysis. This procedure is subject to detailed instructions prepared by DGAV. Following the EFSA guidelines for territorial prospecting based on statistical data (Guidelines for statistically sound and risk-based surveys of *Xf*), the number of plants to be sampled is determined for each area with homogeneous characteristics, using the statistical tool RIBESS+. Thus, resorting to the land use classification of the Corine Land Cover database, the territory is subdivided into four classes with epidemiological significance regarding *Xf*- agricultural, urban, forestry and semi-natural - to obtain areas with homogeneous characteristics. For each of these areas, the number of host plants *per* hectare is estimated to calculate the size of the target population subject to prospecting, from which the number of plants to be sampled through RIBESS+ is determined.

After DGAV determined the places to be prospected and the number of sample collections, DRAP North can implement the reinforcement. DRAP North prospect with observations of symptoms and collect samples for laboratory analysis whenever suspicious symptoms were observed and in plants without symptoms. Then all prospected plants and insects must be georeferenced and registered by each prospector on the GESFITO platform for coding.

2.1.1. Sample Collection

In asymptomatic and symptomatic plants, we collected all biological material in the four quadrants of the host plant; 2 branches or 5 branches with 8-10 leaves, respectively. The parts of plants that make up the sample were previously shaken, wrapped in newsprint paper and placed in a plastic bag, carefully closed to protect the dispersion of possible insect vectors during transport.

Then, after sample coding, the samples were sent, within a maximum period of 24 hours after collection, directly to INIAV Plant Health Laboratory.

2.1.2. Results Reception

Once the presence of the bacteria is confirmed by INIAV this result is communicated to DGAV and measures are immediately taken to prevent its spread and ensure eradication. To ensure the enforcement and adherence to these measures, the national phytosanitary authority (DGAV), pursuant to Decree-Law No. 67/2020 of 15 September, designates the Demarcated Zone (DZ), outlining the eradication measures for the bacteria and the restrictions on the movement of plants intended for planting within the Infected Zone and Buffer Zone [18,19]. Within this DZ, destruction measures are subsequently established and executed by DRAPN (Figure 3):

Figure 3. Destruction of positive plants (author's image).

- In situ destruction of infected plants and other plants of the same species.

- In situ destruction of all plants listed in Annexes I and II of Implementing Regulation (EU) 2020/1201.

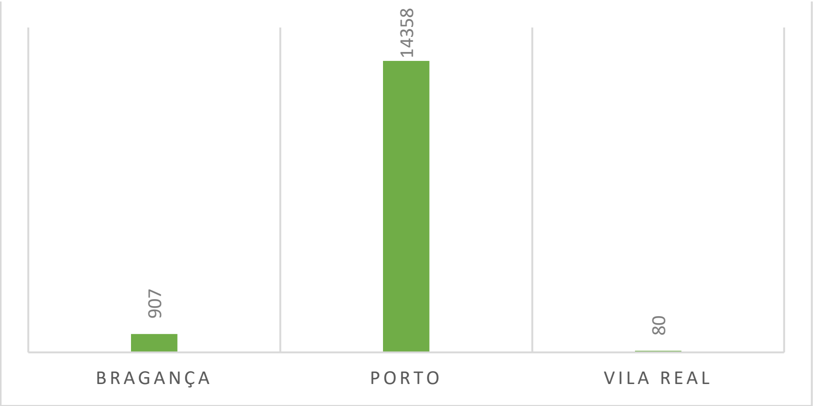
2.2. Collecting, Organizing and Statistical Treatment

Following the laboratory results, data from DZ in Área Metropolitana do Porto, DZ Sabrosa, DZ Alijó, DZ Baião, DZ Mirandela, DZ Mirandela II, and DZ Bougado were compiled. Subsequently, we selected variables for statistical analysis, including the number of locations observed *per* district, types of locations observed, presence of symptoms, and observed plant hosts. All selected variables underwent statistical analysis using the JMP statistical program.

3. Results and Discussion

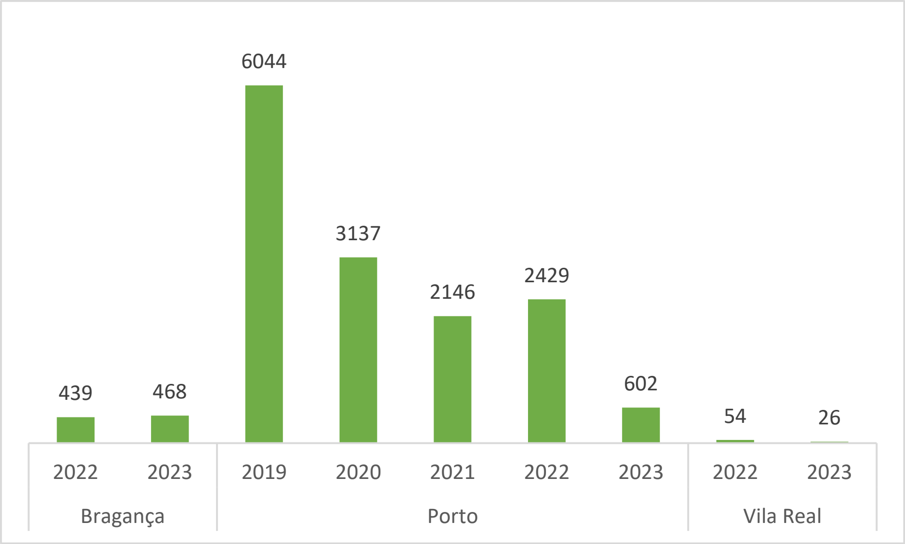
3.1. Number of Locations Observed

Bar Scheme 1 presents the count of locations surveyed from 2019 to June 2023. In the Porto district, there are the DZ of Área Metropolitana do Porto, DZ Baião, and DZ Bougado. In the Bragança district, you'll find DZ Mirandela and DZ Mirandela II. Lastly, in the Vila Real district, there are DZ Sabrosa and DZ Alijó. Notably, 14,358 out of 15,345 locations were examined in Porto (93.6%). This discrepancy can be attributed to the establishment of the first DZ of Porto, in 2019. The regions of Bragança (5.9%) and Vila Real (0.5%) began sample observations in 2022, reflecting a lower number of observations.



Scheme 1. Locations observed on North Districts of Portugal: Porto- 14358 observations (93.6%); Bragança– 907 observations (5.9%); Vila Real – 80 observations (0.5%).

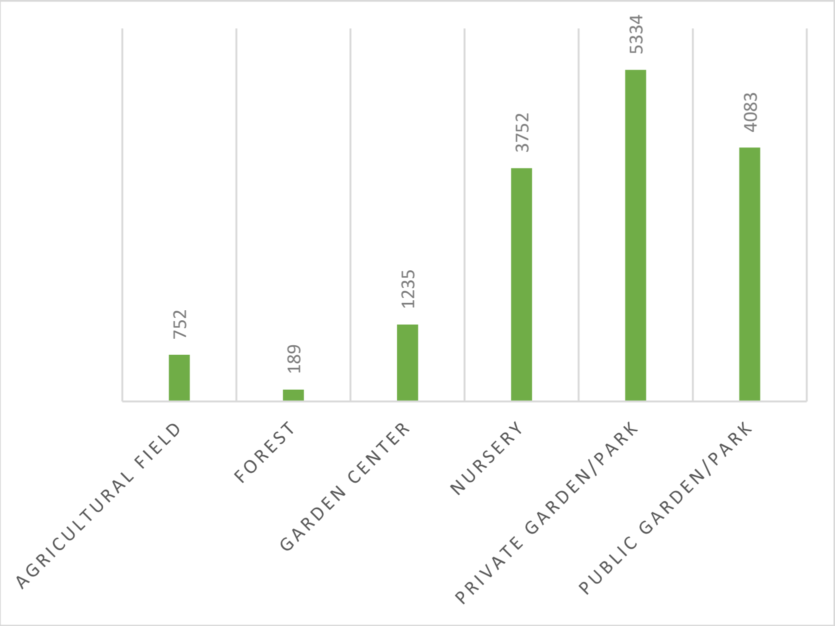
The bars of Scheme 2 illustrate a general decrease in the number of prospected points in the DZ over the years. This reduction can be attributed to several factors. In 2020, a weekly limit on the collection of plant material samples was imposed, leading to a consequently decrease in the number of observed locations and collected samples. Additionally, during the first quarter of 2021, in locations marked for prospecting, only georeferencing/survey of addresses was feasible due to restrictions on entering residents' gardens and backyards amid Covid-19 confinement. In 2023, the authorization for prospecting was granted late (in August), contributing to the diminished number of collected data.



Scheme 2. Locations observed on DZs of Bragança, Porto and Vila Real since 2019 to 2023.

3.2. Type of Locations Observed

Scheme 3 illustrates the various types of locations prospected in the North of Portugal since 2019. The majority of prospections were carried out in private gardens/parks (34.8%), followed by public gardens/parks (26.6%) and nurseries (24.5%). Garden centers, agricultural fields, and forests constituted a limited sample of observations. This distribution could be explained by the fact that since a DZ was stablished, it is necessary to identify the places to be prospected. This procedure is subject to detailed instructions prepared by DGAV. Following the EFSA guidelines for territorial prospecting based on statistical data (Guidelines for statistically sound and risk-based surveys of *Xf*), the number of plants to be sampled is determined for each area with homogeneous characteristics, using the statistical tool RIBESS+. Thus, resorting to the land use classification of the Corine Land Cover database, the territory is subdivided into four classes with epidemiological significance with regard to *Xf*- agricultural, urban, forestry and semi-natural - in order to obtain areas with homogeneous characteristics. For each of these areas, the number of host plants *per* hectare is estimated to calculate the size of the target population subject to prospecting, from which the number of plants to be sampled through RIBESS+ is determined [15]. Subsequently, DGAV identifies the locations designated for prospection, assigning priorities to areas such as nurseries, private gardens, and public gardens. Adhering to these guidelines, DRAP North implements reinforcement measures, resulting in the majority of prospected results in these locations.



Scheme 3. Type of local observed on North zone of Portugal. (1) - Private Garden /park, 5334 observations, 34.8%; (2) – Public Garden/park, 4083 observations, 26.6%; (3) – Nursery, 3752 observations, 24.5%; (4) – Garden center, 1235 observations, 8.1%; (5) – Agricultural field, 752 observations, 4.9%; (6) – Forest 189 observations, 1.2%.

Upon analyzing Scheme 4, it becomes apparent that public gardens (42.7%) accounted for the majority of positive plant results, followed by nurseries (37.1%) and private gardens (16.9%).

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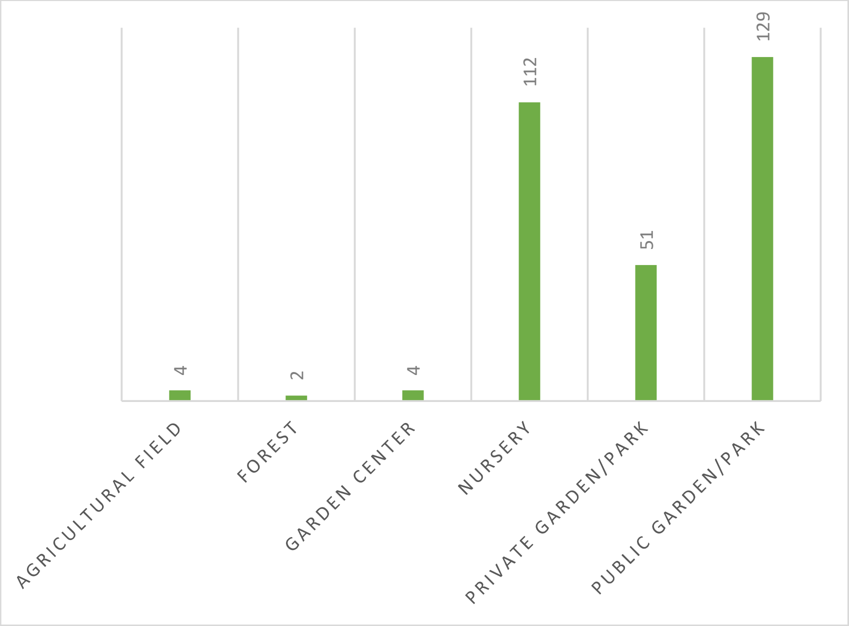


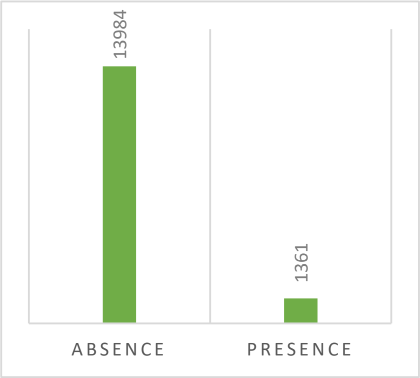
Scheme 4. Positive plants distributed by type of local observed on North zone of Portugal. (1) - Private garden/park, 51 observations,16.9%; (2) – Public Garden/park, 129 observations, 42.7%; (3) – Nursery, 112 observations, 37.1%; (4) – Garden center, 4 observations, 1.3%; (5) – Agricultural field, 4 observations=1.3%;(6) – Forest, 2 observations, 0.7%.

These observations can be attributed to two main factors: the higher number of observations (or samples) potentially leading to an increased number of positive results, and the fact that, when delineating DZ and prospecting plants within a 5km radius of positive findings, public garden/parks are commonly included in the DZ. This inclusion tends to result in a higher number of positive outcomes. Also, these locations often feature herbaceous vegetation, creating shade and humidity that serve as ideal conditions for the development of *Philaenus spumarius*, one of the main vectors of *Xf*. In nurseries, a notable occurrence of plants positive for *Xf* has been observed (37.1%). Nurseries engage in the buying and selling of annual plants, perennial plants and trees, and related products, encompassing activities like cultivating plants for sale and conducting transactions with customers. Due to their nature, nurseries can pose as potential sources for the dissemination of plant diseases, acting as reservoirs for pathogens such as *Xf*. The sale or distribution of infected plants within this context can significantly contribute to the spread of diseases. The current phytosanitary regulations and inspection systems in some parts of the region may have vulnerabilities, potentially allowing the transport of infected planting materials. This raises the possibility of *Xf* invading new districts, with the transport infrastructure inadvertently facilitating the movement of *Xf* insect vectors. Additionally, the skepticism surrounding the efficacy of eradication measures by Economic Operators, the financial burden of analysis costs on economic operators, and the delayed submission of analytical results to the Economic Operators (leading to economic impact) contribute to plant transactions occurring without official controls and the consequent positive results.

Consequently, a genuine risk exists that *Xf* could continue to breach geographical barriers and potentially triggering significant disease outbreaks [20].

3.3. Presence of Symptoms

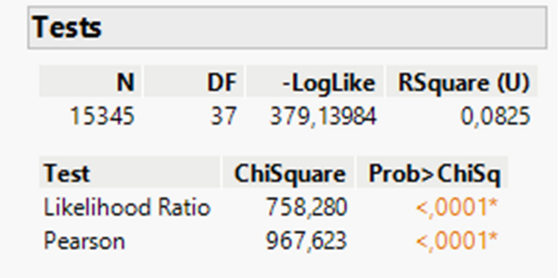
On Bar Scheme 5, the presence of symptoms in the prospected plants is depicted. It can be inferred that the majority of samples did not exhibit any symptoms (91.1%) of bacterial presence.



Scheme 5. Presence of symptoms on the positive prospected plants on North of Portugal. (Absence) – 13984 observations, 91.1%; (Presence) – 1361 observations, 8.9%.

After conducting the chi-square test to analyze the relationship between the species variable and the presence or absence of symptoms (Table 1), we determined a statistically significant association ($p < 0.0001$). This suggests that the occurrence and expression of symptoms are influenced by distinctive factors characteristic of the Portuguese Northern region hosts.

Table 1. Chi-square test of independence between species and symptoms.



Tests

N	DF	-LogLike	RSquare (U)
15345	37	379,13984	0,0825

Test	ChiSquare	Prob>ChiSq
Likelihood Ratio	758,280	<,0001*
Pearson	967,623	<,0001*

Although infection in certain host species leads to noticeable visual changes, there are several species in which these bacteria establish colonization without producing any symptoms [21]. In the context of North Portugal, it was observed that the majority of positive reports for Xf did not exhibit any symptoms (255 cases) on Citrus, Lavandula, Olea europaea and Prunus. In contrast, 45 positive reports did show symptoms on Lavandula dentata. It seems that Xf infection in perennial plants has the potential to evolve into a chronic state and endure over extended periods. The longevity of the bacterium within perennial plants is contingent upon factors such as the specific host species, cultivars [22–24], bacteria strain [25], time of infection [23,26] and winter temperatures.

Furthermore, the timing of infection can impact the presence or absence of symptoms. Plants infected early in the growing season were more likely to develop persistent infections and exhibit symptoms. This is because the pathogen had sufficient time to reproduce and spread within the plant's perennial tissues, allowing it to endure through the winter [23,26]. Xf was previously reported to translocate and colonize the roots of perennial plants such as olive [27,28] almond [29], peach [30,31] blueberry [32], sycamore [33] and citrus [34,35]. As trees transition into dormancy, there is a decrease in the presence and concentration of Xf within their leaves. This decline can be ascribed to leaf senescence, bacterial mortality, and the migration of viable cells into perennial tissues, where they endure the winter season [36]. Consequently, these factors likely contribute to the presence or absence of symptoms. These results are in accordance with the report of Amanifar et al. (2016) in almond leaves [29]. Upon the emergence of leaves in the spring, overwintering bacterial cells initiate their migration from perennial tissues towards the developing leaves. Substantial colonization of the leaves by Xf typically occurs approximately two months after leaf emergence, and the pathogen's concentration continues to rise over time, reaching its peak in mid to late summer, coinciding with the period when foliar symptoms become most pronounced.

This pattern may clarify the absence of symptoms in our research findings. Among the 255 positive reports of Xf that didn't exhibit any symptoms, 170 were collected in the first quarter of the year. According to the authors, Xf migrated into perennial tissues, causing the consequent absence of symptoms.

Additionally, Purcell (2013) suggested that infection or colonization by *Xf* does not invariably lead to disease development. *Xf* can effectively colonize its hosts to ensure its survival and facilitate transmission through vectors. This pathogen can also mitigate harm to the host by modulating its gene expression in response to external signals.[37]. Bragard et al. (2019) reported that the duration of the asymptomatic period following infection can differ significantly, spanning from 1 month in ornamental plants to as long as 3-4 years in other hosts. [38]. Queiroz-Voltan et al. (2004) observed that symptom severity varied even within the same variety grown under identical soil and climate conditions, cultural practices, and management [39]. Hopkins et al. (2007) and Kadel et al. (2017) discovered that less infectious subspecies of the bacteria provide vine protection against more aggressive subspecies, all the while exhibiting reduced symptom manifestation [40,41]. On a recent investigation on *Quercus pyrenaica*, the xylem vessels composed with intact pit membranes and fully coated with lignin, made them more restrictive to bacterial migration. Considering the oak's limited manifestation of symptoms and the identification of a solitary bacterium, the authors conclude that the formation of tyloses may hinder the pathogen's dissemination from the initial point(s) of introduction. Also, the presence of starch grains in stems may acted as a refilling mechanism, preventing the loss of plant hydraulic conductivity and potentially serving as a means to entrap the bacteria. All of these mechanisms can be considered examples of constitutive defense systems of the plant against *Xf*.

It's apparent that external symptoms aren't solely determined by the presence or absence of Xf. The way symptoms respond and manifest is shaped by a multitude of physiological and environmental factors. These include variances in plant resistance or tolerance, fluctuating levels of bacterial concentration, diverse transpiration rates, and the occlusion capacity across different plant subspecies. Nevertheless, the presence of perennial plants in the Northern region of Portugal may account for our findings, as *Xf* infection in perennial plants have the capacity to persist in a chronic state over prolonged periods without exhibiting symptoms.

3.4. Hosts Observed

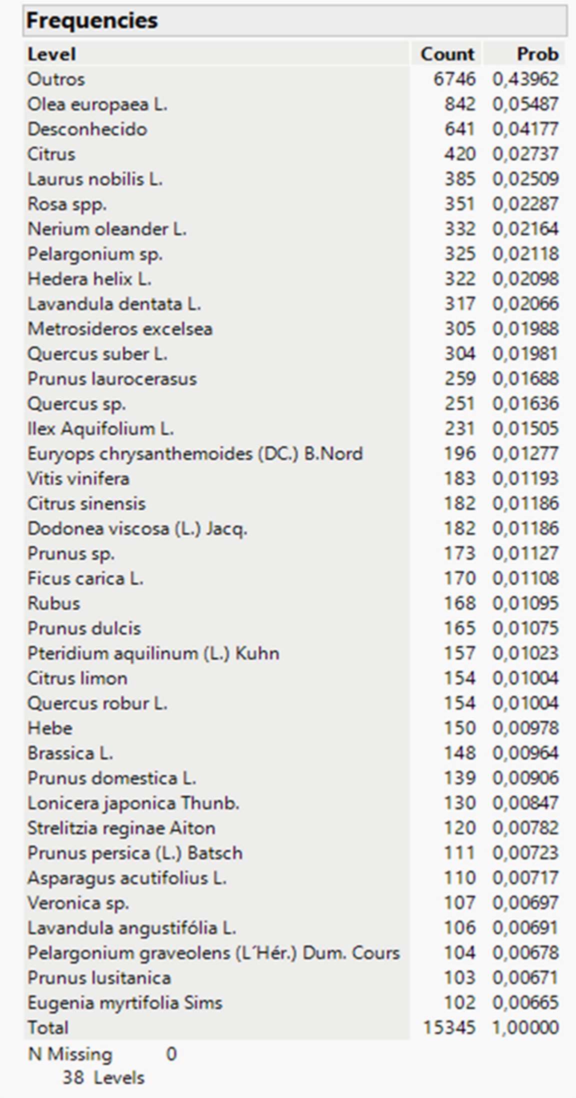
The initial step in organizing the observed host species and preparing the data for statistical analysis was to group all species with fewer than 10 observations into a single category, referred to others as "outros" (Table 2).

Our findings (listed on Table 2) indicated that, in a universe of 15345 samples, the most frequently prospected host genders or species included *Olea europeae* (5.5%), *Citrus* (2.7%), *Laurus nobilis* (2.5%), *Rosa* spp. (2.3%), *Nerium oleander* L. (2.2%), *Pelargonium* sp. (2.1%), *Hedera helix* L. (2.1%), and *Lavandula dentata* L. (2.1%). This observation aligns with the directives from DGAV, which emphasize the prospection of species listed in Annex I and II of Implementing Regulation (EU) 2020/1201, including the aforementioned species [16].

Analyzing the positive plant species in Table 3, it is evident that *Lavandula dentata* (18.9%), *Hebe* (2.0%), *Citrus* (1.7%), *Lavandula angustifolia* (1.7%), and *Olea europaea* L. (1.7%) were the most commonly observed species with positive results.

Our results are not surprising because all those species are vulnerable to infection and can develop diseases caused by these bacteria. The plant genera or species referred are identified as susceptible to subspecies of the bacteria in Annex I or II on Commission Implementing Regulation (EU) 2020/1201 [16].

Table 2. Most frequently prospected host species.

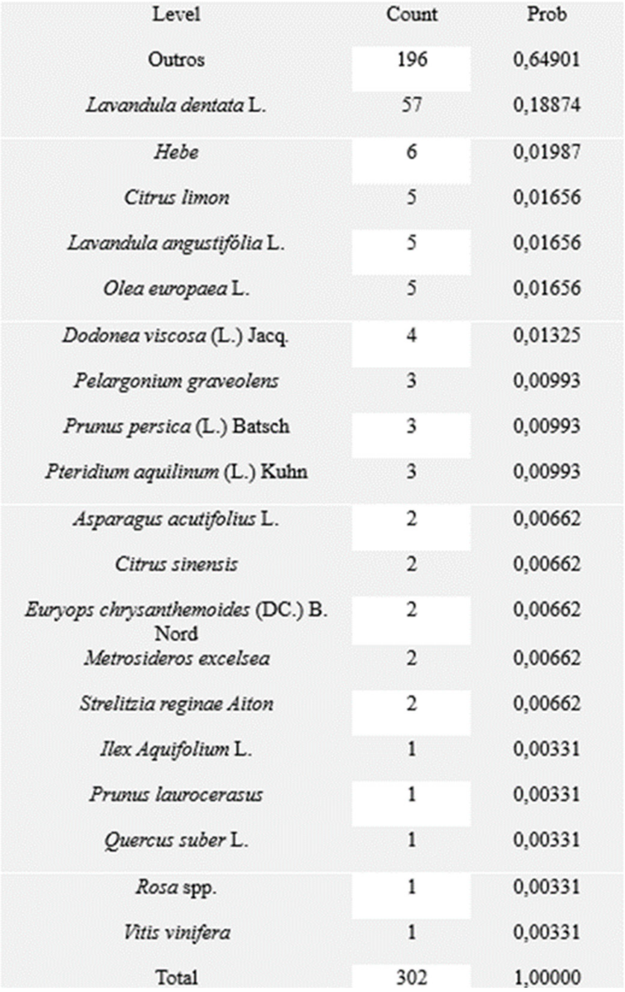


Frequencies

Level	Count	Prob
Outros	6746	0,43962
Olea europaea L.	842	0,05487
Desconhecido	641	0,04177
Citrus	420	0,02737
Laurus nobilis L.	385	0,02509
Rosa spp.	351	0,02287
Nerium oleander L.	332	0,02164
Pelargonium sp.	325	0,02118
Hedera helix L.	322	0,02098
Lavandula dentata L.	317	0,02066
Metrosideros excelsea	305	0,01988
Quercus suber L.	304	0,01981
Prunus laurocerasus	259	0,01688
Quercus sp.	251	0,01636
Ilex Aquifolium L.	231	0,01505
Euryops chrysanthemoides (DC.) B.Nord	196	0,01277
Vitis vinifera	183	0,01193
Citrus sinensis	182	0,01186
Dodonea viscosa (L.) Jacq.	182	0,01186
Prunus sp.	173	0,01127
Ficus carica L.	170	0,01108
Rubus	168	0,01095
Prunus dulcis	165	0,01075
Pteridium aquilinum (L.) Kuhn	157	0,01023
Citrus limon	154	0,01004
Quercus robur L.	154	0,01004
Hebe	150	0,00978
Brassica L.	148	0,00964
Prunus domestica L.	139	0,00906
Lonicera japonica Thunb.	130	0,00847
Strelitzia reginae Aiton	120	0,00782
Prunus persica (L.) Batsch	111	0,00723
Asparagus acutifolius L.	110	0,00717
Veronica sp.	107	0,00697
Lavandula angustifólia L.	106	0,00691
Pelargonium graveolens (L´Hér.) Dum. Cours	104	0,00678
Prunus lusitanica	103	0,00671
Eugenia myrtifolia Sims	102	0,00665
Total	15345	1,00000

N Missing 0
38 Levels

Table 3. Positive Plant Species.



Level	Count	Prob
Outros	196	0,64901
Lavandula dentata L.	57	0,18874
Hebe	6	0,01987
Citrus limon	5	0,01656
Lavandula angustifólia L.	5	0,01656
Olea europaea L.	5	0,01656
Dodonea viscosa (L.) Jacq.	4	0,01325
Pelargonium graveolens	3	0,00993
Prunus persica (L.) Batsch	3	0,00993
Pteridium aquilinum (L.) Kuhn	3	0,00993
Asparagus acutifolius L.	2	0,00662
Citrus sinensis	2	0,00662
Euryops chrysanthemoides (DC.) B. Nord	2	0,00662
Metrosideros excelsea	2	0,00662
Strelitzia reginae Aiton	2	0,00662
Ilex Aquifolium L.	1	0,00331
Prunus laurocerasus	1	0,00331
Quercus suber L.	1	0,00331
Rosa spp.	1	0,00331
Vitis vinifera	1	0,00331
Total	302	1,00000

On the other hand, *Citrus sinensis* and *Vitis vinifera* exhibited limited positive results, despite being also susceptible species. This outcome can be attributed to the genetic characteristics of the identified subspecies. In Portugal is commonly found the prevailing Sequence Type 7 of *Xf* subsp. *multiplex* (in *Santolina chamaecyparissus, Quercus suber, Lavandula dentata, Hebe* sp., *Coprosma repens, Cytisus scoparius*) [42]. The Sequence Type with a closer affinity to *Citrus* belongs to *Xf* subsp. *pauca* [43] never found in our country, while for *Vitis vinifera*, belongs to *Xf* subsp. *fastidiosa* [44].

According to data from EFSA's 2023 report, there are currently 690 plant species that have been identified as susceptible to the bacteria, corresponding to 306 genus and 88 families [42]. The most common host species are *Olea europaea, Vitis vinifera, Prunus dulcis, Citrus sinensis, Nerium oleander* and *Polygala myrtifolia*. In comparison with the previous database, published in December 2022, 12 new species (and two genera) have been identified as *Xf* hosts, 9 of them were identified naturally infected in Portugal (*Castanea sativa, Genista triacanthos, Grevillea rosmarinifolia, Lonicera periclymenum, Mentha suaveolens, Platanus hispanica, Pyracantha coccínea, Quercus pyrenaica, Ulex micranthus*) [6,42].

The list of all known plant species that have been identified in Portugal are explained on Table 4 [45].

Table 4. Plant species identified in Portugal 2019-2023 [45.].

1. *Acacia longifolia* (Andrews) Wild.	41. *Lagerstroemia indica* L.	38. *Hypericum androsaemum* L.
2. *Acacia melanoxylon* R. Br.	42. *Laurus nobilis*	39. *Hypericum perforatum* L.

3. *Acacia dealbata* Link.	43. *Lavandula angustifólia* L.	40. *Ilex aquifolium* L.
4. *Adenocarpus lainzii* (Castrov.)	44. *Lavandula dentata* L.	78. *Vinca*
5. *Argyranthemum frutescens* L.	45. *Lavandula stoechas* L.	79. *Vitis* spp.
6. *Artemisia arborescens* L.	46. *Lavatera cretica* L.	
7. *Asparagus acutifolius* L.	47. *Liquidambar styraciflua* L.	
8. *Athyrium filix-femina* (L.) Roth.	48. *Lonicera periclymenum* L.	
9. *Berberis thunbergii* DC.	49. *Magnolia grandiflora* L.	
10. *Calluna vulgaris* (L.) Hull.	50. *Magnolia x soulangeana* Soul.-Bod.	
11. *Castanea sativa* Mill.	51. *Mentha suaveolens* Ehrh.	
12. *Cistus psilosepalus* Sweet.	52. *Medicago sativa* L.	
13. *Cistus salviifolius* L.	53. *Metrosideros excelsea* Sol. Ex Gaertn.	
14. *Citrus limon* (L.) N. Burman	54. *Myrtus communis* L.	
15. *Citrus paradisii* Macfadyen	55. *Nerium oleander* L.	
16. *Citrus reticulata* Blanco	56. *Olea europaea* L.	
17. *Citrus sinensis* (L.) Osbeck	57. *Pelargonium graveolens* (L´Hér.) Dum.	
18. *Coprosma repens* A. Rich.	58. *Pyracantha coccinea* M. Römer	
19. *Cortaderia selloana*	59. *Plantago lanceolata* L.	
20. *Cytisus scoparius* (L.) Link.	60. *Platanus x hispanica*	
21. *Dimorphoteca ecklonis* (DC.) Norl.	61. *Prunus laurocerasus* L.	
22. *Dodonea viscosa* (L.) Jacq.	62. *Prunus persica* (L.) Batsch	
23. *Echium plantagineum* L.	63. *Prunus cerasifera* Ehrh.	
24. *Elaeagnus x submacrophylla*	64. *Pteridium aquilinum* (L.) Kuhn	
25. *Erica cinerea* L.	65. *Quercus coccinea* Münchh.	
26. *Erigeron canadensis* (L.)	66. *Quercus robur* L.	
27. *Erodium moschatum* (L.) L. Her.	67. *Quercus rubra* L.	
28. *Euryops chrysanthemoides* (DC.) B. Nord.	68. *Quercus suber* L.	
29. *Frangula alnus* Mill.	69. *Rosa*	
30. *Gazania rigens* (L.) Gaertn.	70. *Rubus idaeus* L.	
31. *Genista triacanthos* Brot.	71. *Rubus ulmifolius* Schott.	
32. *Genista tridentata* (L.)	72. *Ruta graveolans* L.	
33. *Gleditsia triacanthos* L.	73. *Salvia rosmarinus* Spenn.	
34. *Grevillea rosmarinifolia*	74. *Sambucus nigra* L.	
35. *Hebe*	75. *Santolina chamaecyparissus* L.	
36. *Helichrysum italicum* (Roth) G.Don	76. *Strelitzia reginae* Ait.	
37. *Hibiscus syriacus* L.	77. *Ulex* spp.	

It seems that introgression events and its inherent ability to assimilate new genetic material have enabled Xf to expand its host range in our country [42,46]. However, also trophic interactions between insect vectors and their host plants can shape the epidemiology of Xf in North of Portugal. To understand the increase of Xf one must consider the behavior of the insect vectors.

Xf spreads naturally among plants through insect vectors from the order Hemiptera, primarily involving cycads, aphrophids, and cercopids [15]. Among the key European insect vectors are members of the *Aphrophoridae* family, such as *Philaenus spumarius (Ps)*, *Philaenus italosignus*, and *Neophilaenus campestris* [5,47]. These insects are widely distributed in tropical and temperate climates, inhabiting forests, meadows, and grasslands [48–50]. They acquire the bacteria while feeding on

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infected plants, becoming hosts themselves. Subsequently, they transmit the pathogen into the vascular system of host plants by piercing the leaf petiole with their stylets [46], facilitating the spread of bacteria into the xylem of branches and stems [5].

These insects undergo a biological cycle wherein they overwinter as eggs. Following a dormancy period of around 100 days, the eggs hatch in early spring. The nymphs then progress through five developmental stages over 5-8 weeks, all the while enveloped in a protective mucilaginous foam [51]. Adults usually emerge in April or May and engage in mating during early summer, after which they tend to linger in the nearby vegetation [48]. During spring, nymphs are typically found in weeds, while adults predominantly inhabit the canopy from May throughout the summer months. Come autumn, the adults retreat to the weeds within the plot and surrounding areas, or migrate to other nearby plants [4]. According to Drosopoulos and Asche (1991) in Greece [52], Godefroid and Durán (2022) [53] in southern Spain, and Karban and Strauss (2004) in California [54] humidity and temperature exerts a significant influence on the developmental duration of immature *Philaenus spumarius* stages [55]. Cooler and humid conditions seem to be more advantageous for egg hatching and larval development with the temperature ranging between 4 and 10°C [55,56]. Adequate humidity is conducive to both hatching and the survival of nymphs, as they rely on an ample sap supply for nourishment. Consequently, severe or prolonged summer droughts can significantly diminish *Philaenus spumarius* populations [54,57]. This phenomenon may account for the transition in host plants, as the insect shifts from arid vegetation to shrubs and trees that are less water-stressed or migrates to cooler locales, such as areas near rivers. This underscores the significant influence of humidity and temperature in the ecological dynamics of this insect [57].

In southern Italy, for instance, the summer migration of adult insects from ground-level vegetation to the canopy has been noted in conjunction with the desiccation of herbaceous plants [49,58]. In particular, adults of *Philaenus spumarius* were observed from May to August in the vicinity of olive orchards, and on groves of *Quercus ilex*, *Quercus crenata*, *Pistacia lentiscus*, *P. terebinthus*, *Hedera helix* and *Myrtus communis* [49,59]. In the Apulia region, *Philaenus spumarius* adults have also been found on *Pinus halepensis* from May to September and have been reported once on *Ulmus minor* in October [59].

The nymphal stages of *Philaenus* spumarius exhibits a preference for specific plant species, primarily those belonging to families such as Asteraceae (Sonchus, Crepis, Picris), Fabaceae (Medicago, Vicia, Lathyrus), Rubiaceae (Galium), and Apiaceae (Daucus, Foeniculum), with G. album and F. vulgare being the favored species. On the other hand, certain species were categorized as “non-preferred” or actively avoided, including those from the Poaceae family (Avena, Lolium, Hordeum), Brassicaceae (Raphanus), Papaveraceae (Papaver), Fumariaceae (Fumaria), and Oxalidaceae (Oxalis) [60]. On the other hand, in Belgium, Hasbroucq et al. (2020), reported adults of Philaenus spumarius on Ranunculaceae, Prunus sp., Rubus sp., Crataegus sp., Alnus sp., Picea sp., Quercus sp., Salix sp., Carex sp. and Urtica sp. [61]. Recent studies performed in Corsica, France, suggest that Philaenus spumarius, at least locally, has affinity with Cistus monspeliensis [62,63]. Chatois et al. (2003), highlighted a positive relationship between Ps abundance and the density of Cistus monspeliensis. They also found larger populations of Ps in cooler and moister plots [58]. Lester et al. observed that among the various botanical families, those with the highest occurrence of host plants harboring Philaenus spumarius nymphs included Lamiaceae (10%), Rosaceae (10%), Onagraceae (9%), and Poaceae (9%). Lavender (Lavandula sp.) emerged as the most frequently encountered host plant in gardens, while in unmanaged areas like waste grounds, parks, and natural areas, grasses (Poaceae) were the predominant hosts. Additionally, adult Philaenus spumarius were also collected from trees and hedges, such as birch (Betula sp.), hawthorn (Crataegus sp.), and willow (Salix sp.) during the month of August [64].

Several studies have explored host associations of the insect vector in olive groves in Italy [65,66], Portugal [67], Spain[68] and Greece [69].

Philaenus spumarius may have limited flight ranges, confining the spread of the disease to a localized area as well as specific host preferences for feeding. Variations in these preferences can impact the range of plant species that may serve as hosts for *Xf*, influencing the types of ecosystems and agricultural crops that are at greater risk of infection. If a vector has a limited flight range and prefers specific host plants, the disease may be prevalent in certain localized areas but constrained from spreading to distant regions. The interplay of these vector behaviors directly impacts the geographic range of *Xf*.

In addition to the insect vector, it is recognized that each subspecies of the bacteria also exhibits a preference for a specific group of hosts. However, their genetic plasticity allows them to infect other plant species to some extent [14]. The taxonomic classification of Xf strains is a crucial step for identifying susceptible hosts. Additionally, in Europe, it holds practical significance as the management strategies mandated by the European Commission are tailored to the specific subspecies present in each outbreak.

The techniques employed for detecting the bacterium and determining its sequence type (ST) in plants align with the guidelines outlined in the EPPO protocol PM7/244 [10]. Briefly, the detection is performed using the real-time PCR and the identification of the ST by applying an Multilocus Sequence Typing (MLST) scheme of seven housekeeping genes and using the PubMLST database to identify allele numbers and ST [10,70].

Europe has reported four subspecies of the pathogen (Xyllela fastidiosa subsp. fastidiosa, multiplex, sandyi, and pauca) [42]. The initial outbreak in the European Union was detected in 2013, affecting olive trees near Gallipoli, located in the Lecce province of Italy's Puglia region [7,8]. Genetic analysis of the subspecies prevalent in this area suggests a strong correlation with the subspecies found in Costa Rica. Nunney et al. (2012) conducted an analysis using MLST on isolates from grapevines across the United States and identified ST2, which differed from ST1 by a single base pair. This distinct pattern strongly suggests a recent common ancestry and challenges the previous assumption that the bacterial pathogen responsible for Pierce's disease of grapevine (PD) is native to the United States. Instead, it indicates that the pathogen descended from a single genotype introduced from Central America [71]. Their invasion hypothesis is supported by five pieces of compelling evidence: a genome-wide lack of genetic diversity in Xf subsp. fastidiosa in the U.S., which is consistent with a recent common ancestor; evidence suggesting historical separation (allopatry) of the North American subspecies Xf subsp. multiplex and Xf subsp. fastidiosa; indications that Xf subsp. fastidiosa evolved in a more tropical climate compared to Xf subsp. multiplex; a considerably higher level of genetic variability in the proposed source population in Central America, within which the U.S. genotypes are phylogenetically nested; circumstantial evidence of the importation of known hosts (coffee plants) from Central America directly into southern California, just prior to the first documented outbreak of the disease [71,72]. Also, Yuan et al. (2010), using MLST scheme for the bacterial plant pathogen had consistent results with the hypothesis that Xf subsp. fastidiosa were introduced into the United States [70].

In 2015, the subspecies multiplex ST6 and ST7 genotypes were detected in France and annual official surveys with plant and vector samplings confirmed the presence and dissemination of these STs in Corsica and PACA regions [73,74]. In 2020, a new region, Occitanic (Aude), was found to be impacted by the presence of this genotype. Landa et al. (2020) constructed a phylogenetic tree using the available genome sequences of Xf subsp. multiplex and incorporated Xf subsp. fastidiosa strains. They also conducted ancestral state reconstruction using the geographic location of the strains as a character. The findings suggest that the ancestral nodes within Xf subsp. multiplex originated in the southeastern United States, implying that isolates from California, Europe, and Brazil likely resulted from introductions [75].

Up to now, 89 sequence types (ST) of Xf have been reported all around the world [6]. The sequence types most found in the world are described on Table 5 [42].

Table 5. Sequence types of *Xf* most found in the world.

Sequence Types	*Subspecies*	*Country most frequently found*	*Number of records*
ST53	*pauca*	*Italy, France, Costa Rica*	*475*
ST11	*pauca*	*Brazil*	*52*
ST1	*fastidiosa*	*US, Spain, Mexico*	*201*

ST06	*multiplex*	*France, Spain, US*	*160*
ST07	*multiplex*	*Portugal, US, France*	*142*
ST81	*multiplex*	*Spain*	*100*
ST87	*multiplex*	*Italy*	*91*
ST29	*morus*	*US*	*10*
ST05	*sandyi*	*US*	*25*

In Europe, the most common sequence types are ST53 in Italy [3,7], ST06 found in Spain and France [73], sequence type 07 in Portugal and France [76]. All these records of different STs in distant areas are clear indications of various independent introductions of Xf strains. Most of these STs had previously been described in the Americas hence that represents their most probable source of introduction in Europe [75]. These hypotheses were tested and confirmed by a first analysis of a limited number of strains from most EU outbreaks [77].

In Portugal, until now, we can only find the subspecies multiplex ST7 [76], and subspecies fastidiosa ST2 [78]. We found majoritarian de ST7 (in Santolina chamaecyparissus, Quercus suber, Lavandula dentata, Hebe sp., Coprosma repens, Cytisus scoparius) [42]. This indicated the potential maintenance of this variant over time in our country. The ST7 is closely related to STs already found in Europe, such as ST6 (France, and Spain mainland), ST81 (Balearic Islands) and ST87 (Italy) [27,79]. We can find this ST also in France, Balearic Islands and US [42]. Moreover, five samples of ST2 were found to be infected. That means additionally, this variant was introduced. We interpret these results as a consequence of commercial trade of plant material infected with Xf. The first reason is due to Xf, as a species, infects hundreds of plants, initially symptomless, and simultaneously the insect vectors are unlikely to disseminate at continental scales. So, it is reasonable to assume that anthropogenic activities are linked to the introduction of these pathogen strains into Europe. According to Yoon et al. (2023), current phytosanitary regulations and airport inspection systems in some parts of the region may have vulnerabilities, potentially allowing passengers to unknowingly transport infected planting materials. This raises the possibility of Xf invading new countries, with the transport infrastructure inadvertently facilitating the movement of Xf insect vectors. In the vicinity of the Demarcated Area of Porto, two major entry points can be identified: Francisco Sá Carneiro Airport and the Harbor of Leixões, close to Porto city. The plant nurseries within this Demarcated Area could represent a potential source of outbreaks. Consequently, a genuine risk exists that Xf could continue to breach geographical barriers and potentially triggering significant disease outbreaks [20,50].

In their study, Godefroid et al. (2018) examined the potential spread of three Xf subspecies by utilizing datasets delineating both native and recently colonized territories alongside four distinct species distribution models. The findings suggest that the subspecies multiplex and fastidiosa pose a significant threat to vast swathes of Europe, whereas climatically suitable regions for the subspecies pauca are primarily confined to the Mediterranean basin. The potential distribution of French strains ST6 and ST7, belonging to subsp. multiplex, is further influenced by a gradual shift of suitable areas. These areas are transitioning from Southern France, Italy, and Portugal toward Northern France, Belgium, the Netherlands, and Southern England. The potential expansion of these strains, particularly in Corsica and Southern France, is substantial. However, the dynamics of their expansion rely more on plant exchanges and disease management than solely on climate suitability. Furthermore, Godefroid et al. (2018) have predicted a moderate to highly severe risk of Pierce's Disease (subspecies fastidiosa) in numerous lowlands and coastal regions of the Mediterranean areas, encompassing Spain, Italy, the Balearic Islands, and North Africa. This risk extends along the Atlantic coasts of France, Northern Spain, and Portugal [80]. Yoon et al. (2023) sought to determine the shared climatic traits of Xf. Furthermore, they calculated the spatiotemporal distances between occurrence areas and categorized them into either natural or anthropogenic spread. Among countries without ongoing outbreaks, those in the Mediterranean basin, notably Turkey at the highest level of risk, followed by Greece, Morocco, and Tunisia at a high-risk level, are most vulnerable to Xf's arrival and establishment. The ranking model also underscores the susceptibility of southern European countries (Italy, Portugal, and Spain), where the pathogen has previously been detected [50].

According to models' predictions, the currently reported distribution of *Xf* in Europe is small compared to the large extent of climatically suitable areas [80]. Species distribution models serve as valuable tools for forecasting the presence of species within a particular location and facilitating investigations related to fields such as biogeography, species preservation, invasive species ecology, and the management of natural resources [81]. These models empower researchers to untangle the influence of ecological and environmental factors on species distribution.

In their research, Hoddle et al. (2004) employed the CLIMEX algorithm to predict the global severity potential of PD. Their model indicated that while most Mediterranean regions are conducive to PD, the cold winter temperatures in certain areas might impede the expansion of *Xf* into several economically crucial wine-producing regions of France, as well as central and northern parts of Spain and Italy. Based on the model predictions, *Xf* had a high probability of colonizing areas characterized by low altitude (0–150 m a.s.l.), precipitations in the driest month < 10 mm, in the wettest month ranging between 80–110 mm and during the warmest quarter < 60 mm; mean temperature of coldest quarter ≥ 8°C; forest (essentially oak woodland) and Mediterranean shrubland [82].

The main reason for the exclusion of *Xf* from coldest and high altitudes is that cold stress on high altitudes can affect both, the bacterium and its vector [83]. The pathogen is probably less harmful where winter temperatures are colder, such as at higher altitudes, because freezing events can strongly decrease *Xf* infections [82,84]. European, and especially Euro-Mediterranean areas, are characterized by a broad spectrum of climatic conditions and plant species, providing the bacterium high chances to encounter hosts and environmental conditions favoring invasion and establishment. Conversely, the prospective behavior of *Xf* in regions characterized by exceptionally high summer temperatures, such as southern and central Spain, remains largely ambiguous due to the limited understanding of the effects of extreme heat on *Xf*. While elevated temperatures during spring and summer promote *Xf* proliferation in plants, research suggests that *Xf* populations decline in grapevines subjected to temperatures exceeding 37 °C [85]. According to the National Institute of Statistics, in 2022, the Northern region of Portugal encountered average temperatures of 9.8ºC in the coldest quarter and around 22°C in the hottest quarter, indicating favorable conditions for *Xf* [86].

Bosso et al**.** (2016), concluded that the analysis of single variable contribution showed that precipitation of the driest (40.3%) and wettest (30.4%) months were the main factors influencing model performance. Altitude, precipitation of warmest quarter, mean temperature of coldest quarter, and land cover provided a total contribution of 19.5% [87]. Yoon et al. (2023), reported that 90 % of precipitation density ranged from 19 to 112 mm, was suitable for *Xf* occurrence. Moreover, the average precipitation and high standard deviation suggested that the infections occurred easily in environments with regular precipitation with relatively large seasonal variations [50]. According to the National Institute of Statistics, in 2022, the average precipitation during the first and second quarters of the year was 37.2mm and 35.4mm respectively, creating favorable conditions for the establishment of *Xf* on North of Portugal.

Forest coverage or Mediterranean shrubland was associated to a higher probability of detecting the insect vector of *Xf*. This habitat likely provides a shelter for *Ps*, especially adults, which may move into woodland areas when the conditions in olive orchards or vineyards are less favorable (i.e., during hot days or crop management practices). Moreover, forests can provide nutritious hosts such as actinorhizal plants, which are characterized by a reliable supply of xylem-borne organic nitrogen compounds and are exploitable by spittlebugs when plants within crops are under stress [88]. According to Hoddle et al. (2004), the oak woodland creates a high probability of *Xf* colonization. *Quercus pyrenaica* Willd dominates nearly 95% of its natural distribution area in Spain, covering approximately 600,000 hectares, and 62,000 hectares in Portugal. In Portugal, the primary concentration of this species is observed in the Bragança region, encompassing around 40% of the total rangeland area [89]. It stands out as one of the most prevalent and distinctive species in Northern Portugal, making it favorable for *Xf* infection**.**

Godefroid et al. (2018) meticulously documented the natural and invaded territories of *Xf* subspecies *fastidiosa*, *pauca,* and *multiplex*. They employed bioclimatic species distribution models (SDMs) to gauge the potential climatic adaptability of these pathogens across Europe. Their findings highlight regions at heightened risk, including the coastal Mediterranean zones of Spain, Greece, Italy, and France, along with the Atlantic coastal regions of France, Portugal, and Spain, as well as the southwestern areas of Spain and low-lying regions in southern Italy. The subspecies *multiplex*, and to some extent *fastidiosa*, pose significant threats to much of Europe, whereas areas climatically suitable for subspecies *pauca* are primarily confined to the Mediterranean basin [80]. Bosso et al**.** (2016), developed a Maxent model to detect the current and future potential distribution of *Xf* in the Mediterranean under climate change. The potential distribution obtained comprises Portugal, Spain, Italy, Corsica, Albania, Montenegro, Greece and Turkey as well as all countries of Northern Africa and the Middle East [90]. That means that the bacterium could colonize those areas whose climatic regime is especially favorable for it. In contrast, cold stress would not allow the bacterium to spread [82,91]. *Xf* was not predicted to change its distribution in the Basin in response to climate change. Also, Cardone et al**.** (2022), confirmed the vulnerability of Euro-Mediterranean countries in terms of *Xf* dispersal. In the MENA and Balkans regions, countries with fragmented and small farms are likely to face the worst social impacts, whereas the Euro-Mediterranean region runs the highest economic losses on the target crops [92].

It seems that this disease occurs in a wide range of climatic zones, including tropical, subtropical, and continental areas, but is concentrated in temperate climate zones [38]. Similar to the studies mentioned earlier, our findings provide essential insights, suggesting that the Northern region of Portugal is highly conducive to *Xf*. The presence of a bacterial species with highly infectious genetic capabilities, along with hosts that are ideal for the bacteria, is evident. External conditions affecting the interaction between bacteria and hosts, such as environmental factors (temperature, humidity), are also present. This, combined with anthropogenic activities like the transport of infected planting materials from the first DZ of the Area Metropolitana of Porto, could be linked to the introduction and spread of *Xf*, potentially triggering new disease outbreaks in our country. Also, different subspecies of *Xf* and insect vector distributions must be considered. Another significant area of uncertainty lies in the potential adaptation of *Xf* to novel environmental pressures within its invaded territories, possibly through mechanisms like genetic recombination. Additionally, microclimate variations, yearly climate fluctuations, host plant susceptibility, interactions between hosts and pathogens, landscape configurations, and the spatiotemporal distribution of potential vectors all play crucial roles in shaping the epidemiology of the bacteria.

4. Conclusions

Our findings provide essential insights, suggesting that the Northern region of Portugal is highly conducive to *Xf*.

Introgression events have facilitated the ability of *Xf* to enter and infect national hosts. Its highly infectious genetic characteristics and natural competence to acquire new genes position makes it as a phytopathogen equipped with the tools necessary for adaptation to a wide range of ecological niches. This adaptability to changing climates leads us to anticipate that, without the implementation of a robust prevention plan grounded in local data, the Portuguese economy may suffer significant setbacks, including yield losses and other forms of damage. Mitigating these potential impacts will likely be necessary the implementation of expensive control measures.

Additionally, our country has preferred hosts, with priority given to specific plant species due to their economic significance or the expansion of production areas. Notably, plants such as *Laurus nobilis*, *Rosa* spp., *Nerium oleander* L., *Pelargonium* sp., *Hedera helix* L. and *Lavandula dentata* L., are selected for sampling. Olive trees, almond trees, vine and Citrus crops are also selected for prospecting, given their economic significance and uncertainties regarding their susceptibility to the subspecies of *Xf* present in the EU. Considering these factors, prospecting efforts are concentrated on monitoring and sampling these priority plant species. Nevertheless, it is crucial to consider variations in the behavior of insect vectors, as these variations can limit the prevalence of the disease. *Ps* may have limited flight ranges, confining the spread of the disease to a localized area. Also has specific host preferences for feeding. Variations in these preferences can impact the range of plant species that may serve as hosts for *Xf*, influencing the types of ecosystems and agricultural crops that are at greater risk of infection. The interplay of these vector behaviors directly impacts the geographic range of *Xf* impact.

Our adequate environmental factors such as temperature and humidity play a role in influencing the interaction between bacteria and hosts. The climatic conditions suitable for plant cultivation align with the climatic preferences of *Xf* outbreaks and insect vectors, posing a high risk of spread. Historical patterns of disease and insect vector spread indicate the potential for dispersion to various regions with favorable climatic conditions, facilitated by both anthropogenic and natural factors. Activities like the transport of infected planting materials from the first DZ of the Area Metropolitana do Porto could be associated with the introduction and spread of *Xf*, potentially triggering new disease outbreaks in our country. Trade networks are implicated in the increasing rates of spread of plant diseases through the transportation of live plants and other plant-related products. However, the risk of significant outbreaks is contingent on the presence of a suitable climate and land use to support the establishment and spread. Finally, it is worth noting that microclimate conditions, inter-annual climate variability, host-plant sensitivity, host-pathogen interactions, landscape structure and the spatiotemporal structure of the community of potential vectors can also affect the bacteria epidemiology.

Also, w**e** can conclude that the presence of perennial plants in the Northern region of Portugal may account for absence of symptoms, as *Xf* infection in perennial plants has the capacity to persist in a chronic state over prolonged periods without exhibiting symptoms. The expression and appearance of symptoms are shaped by a myriad of physiological and environmental factors, encompassing variations in plant resilience or tolerance, diverse rates of transpiration, fluctuating levels of bacterial concentration, and differences in occlusion abilities among plant subspecies.

Our findings affirm the alarming spread of *Xf* across Portugal. Moreover, existing evidence suggests that the conditions in central and southern Europe are conducive to the introduction and further dissemination of *Xf*. Emphasizing the importance of proactive risk prediction for early detection to minimize damage, predictive studies are essential to establish fundamental data for monitoring and controlling *Xf* infections in our country. This entails analyzing the climatic characteristics and distribution patterns of *Xf* and its associated insect vectors.

As there are currently no effective control measures targeting the bacterium itself, this remains an open area of research. Limited knowledge exists about how *Xf* interacts with the plant host's immune system, leaving numerous unanswered questions regarding *Xf* biology and its interactions with national plant hosts. Key inquiries include understanding how the plant immune system interfaces with *Xf* in the xylem and identifying the specific or shared mechanisms underlying the susceptibility of different hosts. Additionally, given that many Portuguese *Xf* plant hosts are non-symptomatic, there is potential to glean insights from these interactions to enhance the tolerance of other susceptible hosts.

This study introduces a straightforward statistical method to analyze the dispersion characteristics of *Xf* in the Northern region of Portugal, coupled with spatial data selection and extraction. This foundational data is essential for informing strategies to prevent the spread of *Xf*. Recognizing the interconnected nature of our world, where international plant trade and human travel act as potential pathways for *Xf* introduction into new areas, it becomes imperative to adopt comprehensive approaches considering multiple risk drivers. Factors such as the presence of insect vectors, abundance of host plants, and climate suitability play pivotal roles in *Xf* dispersal. We recommend that countries, especially those identified with relatively high risk, like Portugal, undertake thorough individual risk analyses. The implementation of preventive measures and, if necessary, the enhancement of surveillance systems for early detection of *Xf* in plants and insect vectors are crucial steps. Additionally, raising awareness is of equal importance to mitigate socioeconomic and ecological impacts on their ecosystems.

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