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Long-Distance Finding of AOD-Related Bacteria in the Natural Environment: Risks to *Quercus ilex* (L.) in Italy

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Abstract: Acute Oak Decline (AOD), a bacterial disease previously known in Northern and Central Europe, has recently been reported in Salento (a Mediterranean coastal region of Southern Italy), where holm oak trees exhibiting AOD-like symptoms have tested positive for infection with AOD-related bacteria such as *Brenneria goodwinii* and *Gibbsiella quercinecans*. Sampling symptomatic trees, strains BLEC23 (*B. goodwinii*) and GSAC47 (*G. quercinecans*) were isolated and identified by partial 16S rRNA and *gyrB* gene sequencing. Pathogenicity tests demonstrate that these bacteria induce wood necrosis when inoculated in excised branches, providing details for the etiology of AOD in Italy. Phylogenetic analysis indicated a substantial genetic similarity between the Italian strains and those found in various European and non-European countries. These findings leave a space open to the possibility that the bacteria involved in AOD are much more widespread in Europe than the findings indicate, but that their presence is frequently hidden.

Keywords: Acute Oak Decline; *Brenneria goodwinii*; *Gibbsiella quercinecans*; *Quercus ilex*; Italy

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1. Introduction

Acute Oak Decline (AOD) is a recently described disease affecting oak trees that can lead to the death of the tree 4 to 6 years after the symptoms appear [1–6] and represents a new ecological challenge for European forests; indeed, oaks represent a key species in ecosystems, providing numerous ecological services and sustaining wildlife [7–9].

In past times in Europe, episodes of oak decline have been observed, but these episodes differ in the symptoms and in the rapidity with which the tree dies; in fact, there are various causes of episodes of oak decline, due to specific interactions between biotic and abiotic factors. Given the complexity of the phenomenon, there have been attempts to classify the various types of oak decline [10].

The symptoms of AOD are distinctive compared to others, for example, compared to Chronic Oak Decline, where there is no rarefaction of the foliage, but drying out, and the leaves, although dry, do not fall from the tree [11–13]. Alternately, it is distinguished by the lack of other symptoms which on the contrary are specific to other pathologies, as in the case of infection by *Armillaria* spp., in which the presence of fruiting bodies and a characteristic white mycelium under the bark is highlighted. [14].

AOD, described for the first time in the UK in 2014 on *Quercus robur* (L.) and *Quercus petraea* (Matt.) [15,16], has raised significant concerns due to the debilitating symptoms shown by affected plants, namely, thinning of the canopy, epicormic shoots, and dark-amber

fluid bleeding from vertical bark cracks; larval galleries can also be observed by removing the bark, and insect exit holes on the trunk attributed to insects belonging to the Bupestridae family [12,17]. AOD is a critical issue, as the exact etiology and chronology of events are still not known. However, it is thought that its complex origins involve biotic factors, like bacterial infection and insect attacks, and abiotic factors such as drought and poor soil conditions [18–21]. The combined effects of all these factors pose a substantial challenge in the identification, understanding, and management of AOD, so urgent attention is directed towards unravelling its complex origins, understanding its progression, and assessing its impact on individual trees and the broader ecosystem.

Regarding biotic factors, research has identified specific bacteria linked to AOD that are commonly isolated from the trunk exudates and necrotic wood: *Brenneria goodwinii*, *Gibbsiella quercinecans, Rahnella victoriana*, and *Lonsdalea britannica* [22]. Although all four bacteria have a necrotic attitude [23], *B. goodwinii* and *G. quercinecans* play a central role in the progression of the disease, and in wood inoculation tests they showed a strong ability to form wood necrosis, unlike *R. victoriana* [24]. As for *L. britannica*, there is less information about it, as it is less often associated with AOD trees [23].

Brenneria goodwinii and *Gibbsiella quercinecans* are Gram-negative bacteria belonging to the Enterobacterales order and the families Pectobacteriaceae and Enterobacteriaceae, respectively; these bacteria are facultatively anaerobic, oxidase negative, and catalase positive, and both grow in a temperature range of 10–40 °C. *G. quercinecans* has fimbriae but no flagella, unlike *B. goodwinii*, which also proves to be motile thanks to its peritrichous flagella [15,16].

Since the first description of this disease, the association between *B. goodwinii*, *G. quercinecans*, and several oak species has been reported in several European countries, specifically Poland, Latvia, Switzerland, Portugal, Spain, Croatia, and Slovakia [1,3,4,6,25–27], revealing a wide distribution of those pathogens in European forests, particularly in Northern and Central Europe.

In a previous study conducted in seven holm oak (*Quercus ilex* L.) forests in Salento, a peninsula located in southern Italy, 21 trees representative of the health status of the holm oak stands were found to have symptoms related to AOD. These symptoms were evaluated through Finch's index and the plants were tested for infection by AOD-related bacteria. Finch's index was developed to evaluate the health status of oaks and is made up of two parameters: the Phenotypic Decline Index (PDI), which measures the severity of the decline; and the Decline Acuteness Index (DAI), which distinguishes between different types of decline (acute and chronic). Finch's index, developed specifically for oaks, combines 31 parameters based on various visible symptoms, such as lesions, dark exudates on the trunk, crown decay, and branch mortality, to quantify the severity of the infection. After sampling wood fragments, necrotic bark, and exudates, the trees tested positive for *B. goodwinii*, *G. quercinecans*, and *R. victoriana* [11]. This finding indicates that the bacteria can establish in warmer and barren environments, represented by the Mediterranean basin. Furthermore, such data raise many questions regarding the epidemiological aspects, as Salento is very distant from other European bacteria outbreaks. Moreover, the findings do not concern plants of nursery origin, making long-range transmission via vector or marketing of infected plants unlikely.

The aims of this work, focusing on *B. goodwinii* and *G. quercinecans* as the main pathogens involved in AOD [24,28,29], were to isolate bacteria associated with AOD in Italy from infected trees, test their pathogenicity to understand the causes of the decline in holm oak in Salento, and study their phylogeny with respect to other strains isolated worldwide to understand how such pathogens can be found in Salento. Finally, the epidemiological status of the bacteria was analyzed in relation to the known infection sites, vectors, and secondary host plants, to formulate a hypothesis about the introduction of the pathogens in Southern Italy. The data could highlight the transmission and dispersal strategies of the bacteria involved in AOD, and this could be helpful in managing this emerging oak disease [23].

2. Materials and Methods

2.1. Sampling and Bacteria Isolation

In the autumn of 2023, holm oaks were sampled in the Cervalora (40°25′19.966″ N, 18°12′53.873″ E) and Spaccato (40°14′48.073″ N, 18°18′ 33.818″ E) forests in Salento peninsula (Southern Italy). Sampled trees exhibited the distinct symptoms associated with AOD, such as thinning of the canopy, epicormic shoots, and brownish exudates all over the trunk.

During sampling, five holm oaks were considered for the site. The trees identified and used for isolation were first diagnosed for AOD-related bacteria to verify the presence of *B. goodwinii* and *G. quercinecans*, as described by Crampton et al. (2020) [30]. At least three panels (about 3 × 4 cm) were obtained for each tree using a sterile chisel at the level of bark bleeding wounds. Each panel was composed of outer bark, phloem, and sapwood. During transport, the collected material was placed in a sterile tube, labeled and kept at 4 °C in an iced airtight container. Samples were immediately processed upon arrival at the laboratory.

The samples were processed for isolation according to Moradi-Amirabad et al. (2019) [31]. Wood panels were surface-sterilized and rinsed with sterile distilled water (DSW) three times. Then, wood fragments were excised from the sapwood's innermost layers at the interface between healthy and diseased wood and the outer bark. The wood chips were then placed in 0.5 mL of DSW and manually fragmented thoroughly with a sterile scalpel. After incubating for 30 min at room temperature, the suspension was scooped using a sterile loop and plated onto nutrient agar (NA) prepared using 15 g of agar powder (Merck, Darmstadt, Germany), 5 g of Bacto Peptone (Merck), and 5 g of Beef Extract powder (Merck) in 1 L of sterile double-distilled water, then incubated at 28 °C for five days with daily inspections.

Colonies were then subjected to an initial screening through qPCR [30] subsequent to DNA extraction [32]; qPCR was performed with a QuantStudio™ 3 Real-Time PCR thermocycler (Thermo Fisher, Waltham, MA, USA), following Crampton et al. (2020) [30]. The qPCR mixture for *G. quercinecans* consisted of 5 µL of TaqMan™ Environmental Master Mix 2.0 2× (Thermo Fisher) and 0.1 µM of each primer and probe (Table 1). For the screening of *B. goodwinii*, 0.25 µM of each primer was used.

Colonies testing positive for *B. goodwinii* and *G. quercinecans* were then plated separately on NA media.

Table 1. Primers and probes used for screening colonies.

Bacteria	Primers and Probes	Sequence (5′–3′)
Brenneria goodwinii	Bg99F	CTGGCCGAGCCTGGAAAC
	Bg179R	AGTTCAGGAAGGAGAGTTCGC
	Bg179P	CCAGAATCTCATATTCGAACTCCACCATGTT
Gibsiella quercinecans	Gq284F	GGCTTTGATAGTGGTGGCC
	Gq418R	CGTTCCGTTATCACCGTGG
	Gq342P	AACAGTTCCAGCGCCATTTTCTTCG

2.2. Sample Sequencing and Phylogeny

Data from qPCR initial screening were validated by 16S rRNA [33] and housekeeping gene *gyrB* [34] (Table 1) gene sequencing, based on Ruffner et al. (2020) and Fernandes et al. (2022) [1,3]. PCR was performed with a SimpliAmp™ Thermal Cycler (Thermo Fisher); for 16S rRNA PCR, the reaction mixture contained 12.5 µL of EmeraldAmp® MAX PCR Master Mix 2× (Takara Bio, Kusatsu, Japan), 0.5 µL of each primer (10 µM) (Table 2), and 5 µL of template, consisting of a bacterial colony resuspended in 10 µL of DSW, for a final volume of 25 µL. PCR conditions were: 95 °C for 2 min, 95 °C for 1 min, followed by 35 cycles of 53 °C for 1 min, 68 °C for 90 s, and 68 °C for 5 min; while for *gyrB* PCR conditions were as described by Brady et al. (2008) [34], with modifications: the PCR mixture consisted of

12.5 µL of EmeraldAmp® MAX PCR Master Mix 2× (Takara Bio), 0.5 µL of each primer (10 µM), and 7 µL of template for a final volume equal to 25 µL.

The amplification condition was as follows: an initial denaturation at 95 °C for 5 min; 3 cycles of denaturation at 95 °C for 1 min, annealing at 58.6 °C for 2 min 15 s and elongation at 72 °C for 1 min 15 s; followed by 40 cycles of denaturation at 95 °C for 35 s, annealing at 58.6 °C for 1 min 15 s, and elongation at 72 °C for 1 min 15 s and a final elongation of 7 min at 72 °C.

Table 2. Primer pairs used in this study for sequencing and phylogenetic analysis of *B. goodwinii* and *G. quercinecans* isolates. Some nucleotides of those primers are degenerate, so M stands for A/C, Y stands for C/T, and R stands for A/G [35].

Gene	Primers	Sequence (5′–3′)
16S	EUB9F	GAGTTTGATCMTGGCTCAG
	EUB1492R	ACGGYTACCTTGTTACGACTT
gyrB	gyrB 01-F	TAARTTYGAYGAYAACTCYTAYAAAGT
	gyrB 02-R	CMCCYTCCACCARGTAMAGTT

The amplicons obtained were sequenced using Sanger sequencing (Eurofins, Ebersberg), aligned with Clustal W [36] and compared on the NCBI (National Center for Biotechnology Information) database using the Basic Local Alignment Search Tool for nucleotides (BLASTn) [37]. Phylogenetic relationships were than established with partial 16S rRNA gene sequences via MEGA 11 software [38]. For phylogenetic trees, maximum likelihood based on the Tamura–Nei model was used with a number of bootstrap replications equal to 1000 [5,22,39].

2.3. Pathogenicity Test

Pathogenicity tests based on Denman et al. (2018) and Eichenlaub et al. (2024) [24,26] were conducted on holm oak excised branches, two for bacterium, collected from healthy trees; each branch was 20 cm long and had a 2–3 cm diameter. Excised branches were collected from holm oak trees that have not shown, from repeated observations in the last 12 months, symptoms attributable to AOD or other diseases such as Botryosphaeriaceae or *Armillaria* spp. infection. Excised branches were exposed for 2 h to UV light for surface sterilizing. With a cork borer, bark discs (1 cm in diameter) were removed from four points along the length of each segment to expose the xylem; then, for each wound, inoculation was performed by taking a day-old bacterial colony with a sterile loop and distributing it evenly over the entire inoculation site, while a mock inoculation was performed on the fourth area by using DSW. After repositioning the bark discs, a plastic film was applied on each inoculum point to preserve moisture. The excised branches were incubated at 27 °C with a 12 h photoperiod in a thermostatic chamber with light control, and checked weekly to prevent mold development.

After two months, the bark was removed using a sterile chisel, and wood chips were collected from both the inoculation points and mock inoculation point. These chips were superficially sterilized with 70% (*v*/*v*) ethanol and rinsed with sterile double-distilled water. They were immersed separately in 0.5 mL of DSW and further fragmented. After incubation at room temperature for 30 min, an aliquot of the suspension was collected with a sterile loop and plated onto NA. The plates were incubated at 27 °C for five days with daily inspections. After three to four days, the bacterial colonies that developed on NA were analyzed by qPCR screening with *B. goodwinii* and *G. quercinecans* primers; this process was carried out to confirm that the inoculated bacteria were indeed re-isolated.

3. Results

3.1. Sampling

The woodlands selected for sampling were mainly made up of pure holm oak forests, characterized by the dominant presence of holm oaks, with a limited presence of Mediterranean shrubs. In these areas, symptoms associated with AOD were widespread and easily seen. The holm oak specimens used for isolation and subjected to sampling were all found to be infected with the bacteria *Brenneria goodwinii* and *Gibbsiella quercinecans*.

3.2. Isolation, Sequencing and Phylogeny

On the third day of incubation, circular, mucoid and pale, white-colored colonies developed on NA. Colonies were than tested for *B. goodwinii* and *G. quercinecans;* colonies that tested positive were then plated separately onto NA to obtain pure culture.

The sequences of partial 16S rRNA and *gyrB* for the two strains showed 99.50% and 100% (GenBank Accession Nos. KY231164, KY321538) identities and 99.86% and 99.53% identities (GenBank Accession Nos. MZ895009, GU562334) with the reference strain, respectively, for *B. goodwinii* and *G. quercinecans*, confirming qPCR results. Nucleotide sequences of the isolated strains were deposited on GenBank with the following accession numbers: PP647363 and PQ449433 for 16S rRNA and *gyrB* of *B. goodwinii* (strain BLEC23), and PP647364 and PQ449434 for 16S rRNA and *gyrB* for *G. quercinecans* (strain GSAC47). Based on 16S rRNA sequences, phylogenetic trees were assessed (Figures 1 and 2) for each bacterium. Phylogenetic trees show how the strain BLEC23 of *B. goodwinii* isolated in Italy clusters with the French strains recovered on *Q. petraea* (Table S1), and how the strain GSAC47 of *G. quercinecans* clusters with the one recovered in Iran on *Morus* sp. (Table S1). It also should be noted that two other isolates of *B. goodwinii* found in Salento have been previously reported and sequenced (P2AC, P1BW) [40,41], but these are shorter than the sequences here reported; the information on *gyrB* is not included and they are not associated with *G. quercinecans*. However, strains P2AC and P1BW are 98.71% and 98.85% similar to the strain BLEC23 for the portion of the over-lapping sequence.

The evolutionary history was inferred by using the maximum likelihood method and the Tamura–Nei model [39]. The tree with the highest log likelihood (−3981.05) is shown. The percentage of trees in which the associated taxa are clustered is displayed next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura–Nei model and selecting the topology with the superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved eight nucleotide sequences. There were a total of 2386 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 [38].

3.3. Pathogenicity Tests

The bacteria isolated were used for pathogenicity tests to fully satisfy Koch's postulates. Two months after the inoculation of excised branches, at the level of the inoculation points, but not at the mock inoculation points, the wood developed a black discoloration with defined margins compared to the surrounding wood, which appeared lighter in color. Despite its moist appearance, the wood maintained a solid texture. Furthermore, one month after inoculation, the bark appeared moist and had brown exudates (Figure 3). The condition of the infected wood after two months, and the presence of exudates, reflected the key symptoms attributed to AOD and that can be observed on naturally affected oaks. Bacteria from necrotic bark and xylem were successfully re-isolated onto NA.

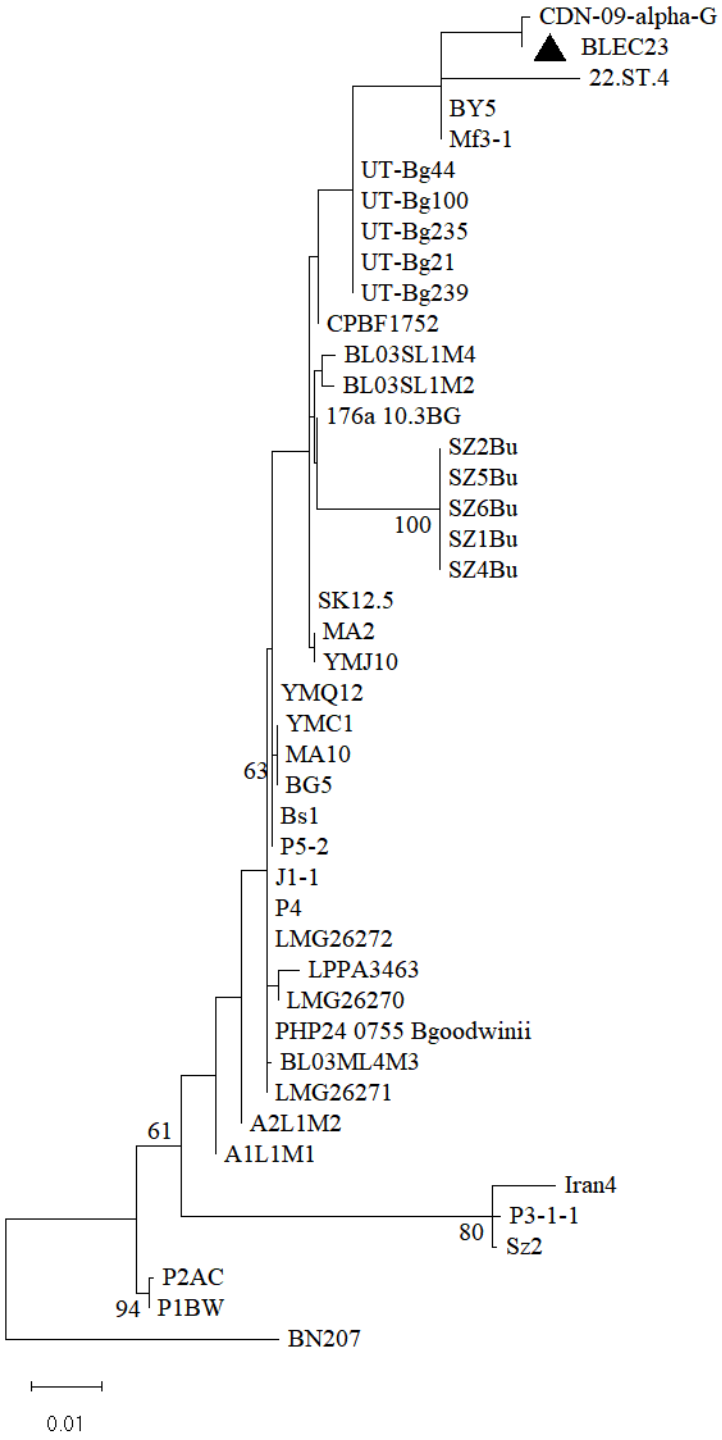

Figure 1. Phylogenetic tree based on 16S gene sequencing of *B. goodwinii* strain BLEC23. From the phylogenetic tree, it can be noted how the strain isolated in Italy BLEC23 (▲) of *B. goodwinii* shows more similarities with the strains (CDN-09-alpha-G) isolated in France on *Q. petraea*., while *B. nigrifluens* (BN207), representing the outer layer, is the most genetically distant strain.

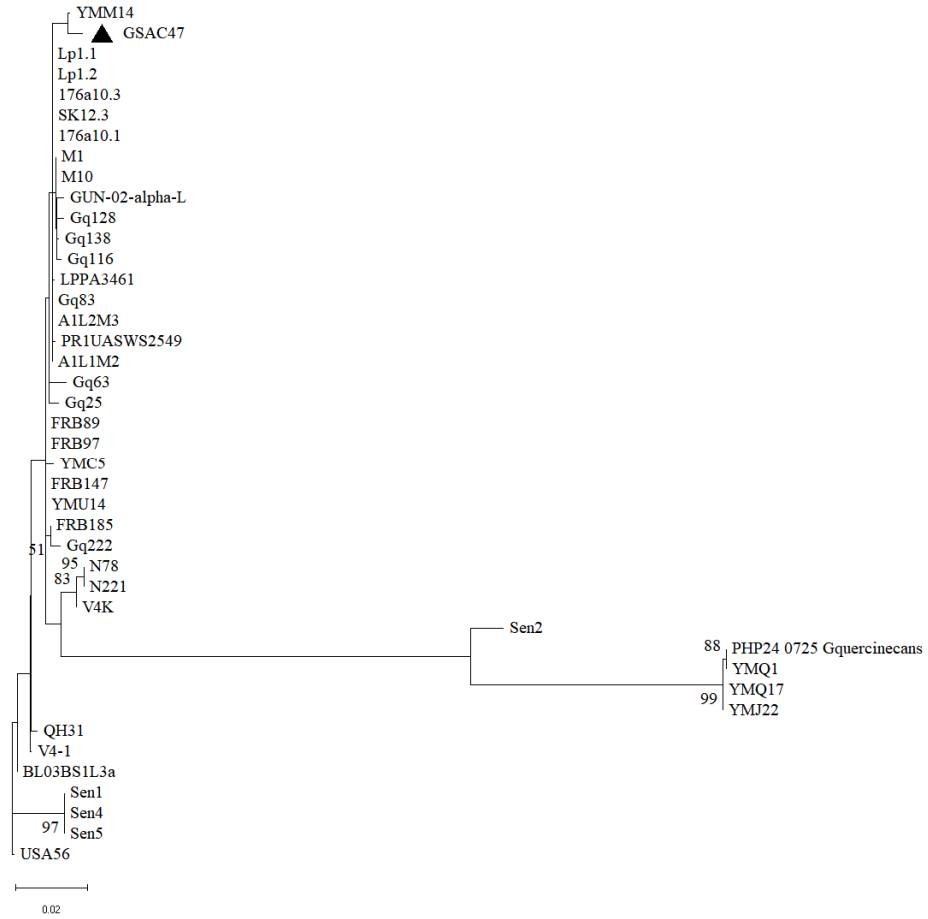

Figure 2. Phylogenetic tree based on 16S gene sequencing of *G. quercinecans* strain GSAC47. From the phylogenetic tree, it can be noted how the strain isolated in Italy GSAC47 (▲) of *G. quercinecans* shows more similarities with the YMM14 strain (from *Morus* sp. in Iran), while *G. greigii* (USA 56), representing the outer layer, is the most genetically distant strain.

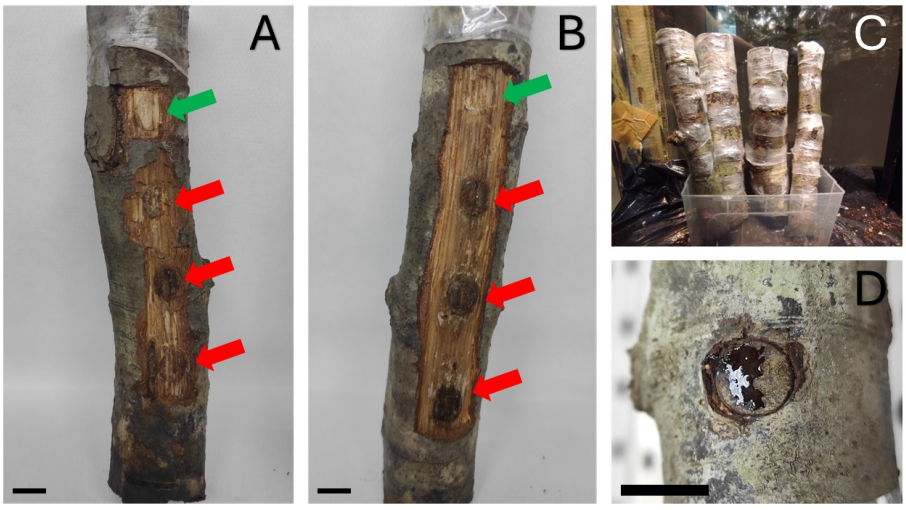

Figure 3. (**A**,**B**) Two excised branches inoculated with *B. goodwinii* and *G. quercinecans*, respectively. Red arrows indicate the inoculation points, while green arrows indicate the mock-inoculation points. Necrotic wood can be observed at the inoculation points. (**C**) The incubation conditions of the inoculated wood sections. (**D**) Brown exudate is observed outside the bark at the inoculation point. The reference bar is equal to 1 cm.

4. Discussion and Conclusions

In Italy, holm oak trees tested positive in 2023 for *B. goodwinii*, *G. quercinecans*, and *R. victoriana* [11], three bacteria that are considered to be the biotic cause of AOD [22]. *B. goodwinii* and *G. quercinecans* are thought to be the main causal agents for AOD, as their ability to cause wood tissue necrosis has been established [24,29]. In this study, from holm oak we successfully isolated the bacteria associated with AOD in Italy. These were confirmed to be *B. goodwinii* and *G. quercinecans* by sequencing the partial 16S rRNA and the *gyrB* genes. The choice of such genes was guided by the fact that, although the 16S gene is one of the most widely used markers for bacterial phylogenetic analyses and is known for its reliability [42,43], using it with other housekeeping genes such as *gyrB*, *rpoB*, *infB*, and *atpD* can result in more accurate genetic analysis [44,45], as is often done for bacteria associated with *Brenneria* sp. and *Gibbsiella* sp. [5,31,46–48] due to their polyphyletic nature, and thus permitting a differentiation of closely related strains [22,35,49].

Phylogenetic analysis revealed that the Italian strains of *B. goodwinii* and *G. quercinecans* share high genetic similarity with strains from Europe and Iran, thus suggesting that those pathogens may disperse over large distances and adapt to different climatic conditions. This finding is crucial for understanding the potential for the global spread and ecological adaptability of these bacteria. The clustering of the *B. goodwinii* strain with French (CDN-09-alpha-G) strains and *G. quercinecans* with strains from Iran (YMM14) highlights these pathogens' genetic relations and the possible transmission routes, underscoring the need for a comprehensive approach to monitoring and managing AOD. Moreover, although the dispersal of plant pathogens between distant locations can occur through the movement of goods [50,51], as far as the long-distance transmission strategies of these bacteria are concerned, nothing is known yet. Nonetheless, it has been observed that AOD-related bacteria can survive in soil, forest litter, and rain water, and are also associated with oaks' leaves, acorns, catkins [23,52,53], and wood borer insects [20], which, even if not empirically proven, could represent dispersal modes for these pathogens.

Pathogenicity tests also confirmed the virulence of the bacteria isolated, as inoculated excised branches developed moist-necrotic lesions, similar to those observed in naturally infected oaks, and from which the inoculated bacteria could be isolated again. This result fulfils Koch's postulates and conclusively demonstrates that *B. goodwinii* and *G. quercinecans* are causative agents of AOD on *Quercus ilex*. Those findings confirm the pathogenic nature of *B. goodwinii* and *G. quercinecans*, as determined also in other oak species such as *Quercus petraea* (Matt.), *Quercus robur* (L.), and *Quercus suber* (L.) [1,4]. In any case, it must be considered that AOD is defined as a 'complex disease', and other factors, such as drought and poor soil conditions, appear to be involved in the process leading to plant death; however, the relative weight of each factor on the exact chronology of the different stresses that lead to the onset of AOD symptoms is not known to date [12,14,47].

Furthermore, there could be the possibility that these pathogens have an endo/epiphytic lifestyle and that they can become pathogens following changes in the state of the plant [23]. This last possibility appears rather intriguing in light of (a) the large distance between the known outbreaks in Europe (both compared to the one in Salento and among others); (b) the absence of secondary/intermediate hosts of agricultural interest involved in nursery activities; and (c) the presence of insect vectors not yet confirmed but ubiquitously spread. These considerations leave a space open to the possibility that the bacteria involved in AOD are much more widespread in Europe than the findings indicate, but that their presence is hidden, perhaps due to their poor virulence in the absence of other concomitant factors (e.g., environment), or by limited attention paid to symptoms during forest monitoring activities.

Although it is reported in Asia that AOD-related bacteria can infect woodland trees belonging to genera such as *Fagus*, *Carpinus*, and *Elaeagnus* [31,46,54], indicating that these bacteria exhibit a certain degree of host suitability, in Europe, they have been found almost exclusively on plants of the genus *Quercus* [1,3–6,11,22,25,26], with few exceptions: there is one report in Poland, where also on the genus *Tilia* spp. was involved [55], on *Malus*

spp. species in Switzerland [56], and *Acer* spp. and *Ulmus* spp. in Hungary [57,58] (Table 3). It is interesting to note the absence of hosts among plants of agricultural interest, which are more interested in frequent and massive commercial exchanges, as well as the low probability of propagation through intermediate hosts. Therefore, if the nurseries represent a source of inoculum (a fact not yet ascertained), the impact should mainly affect areas subject to reforestation. Given the spontaneous nature of the forests from which the bacteria were isolated in Salento and the absence of significant reforestation plans in the proximal areas, it is believed that the probability that the infectious event comes from the nursery activity is low, although it cannot be excluded.

Table 3. The table presents the plant species on which AOD-related bacteria have been found in European and non-European Countries, along with the indication of the country where the discovery occurred.

	Host	Country	References
European Countries	*Q. robur*	Britain	[12]
		Spain	[5]
		Switzerland	[1]
		Poland	[6]
		Latvia	[4]
		Slovakia	[25]
		France	[26]
		Hungary	[59]
	Q. petraea	Britain	[12]
		Switzerland	[1]
		Hungary	[60]
		France	[26]
	Q. suber	Portugal	[3]
	Q. ilex	Spain	[5]
		Italy	[11]
	Q. cerris	Switzerland	[1]
		Hungary	[61]
	Q. pubescens	Switzerland	[1]
	Q. rubra	Switzerland	[1]
	Q. pyrenaica	Spain	[5]
	Q. ilex	Spain	[62]
	Q. pyrenaica	Spain	[62]
	Tilia cordata	Poland	[55]
	Ulmus sp.	Hungary	[57]
	Acer campestre	Hungary	[58]
	Malus sp.	Switzerland	[56]
Non-European Countries	*Carpinus* sp.	Iran	[31]
	Elaeagnus sp	Iran	[46]
	Fagus sp.	Turkmenistan	[54]

Among the European countries where these bacteria have been identified, Slovakia, Hungary, and Switzerland are approximately the closest to the Italian location where *B. goodwinii* and *G. quercinecans* have been detected. The estimated distances between the

areas where bacteria associated with AOD were found and the sampling sites in Italy are approximately 890 km for Slovakia (in 2024), 920 km for Hungary (in 2022), and 1,102 km for Switzerland (in 2020). These values are indicative and provide an estimate of the geographical spread as the crow flies of these bacteria across Europe.

It is noteworthy that throughout Europe and parts of Asia, the beetle *Agrilus biguttatus* (Fabricius) is common. This is a coleopteran species that uses Fagaceae as its host plant [63] and is a potential vector of the AOD bacteria [20]; *A. biguttatus* has been reported also in Italy [64–66], along with other oak-damaging Buprestidae [67–69], and although a specific association between *A. biguttatus* and AOD-related bacteria has not been definitively demonstrated, it is known that other Buprestidae may serve as vectors for various plant pathogens. For example, *Ceratocystis fagacearum* (J. Hunt) spores have been detected on *A. bilineatus* (Weber), and *Pseudomonas* spp. and *Aspergillus* spp. have been isolated from the mouthparts of *A. nubeculosus* (Fairmaire) [70,71]. While *A. biguttatus* may not act as a direct vector, there may be a contributing association in triggering disease, as a correlation has been observed between trees infested by *A. biguttatus* and infection by *Armillaria* spp. [72]. Along with the possibility that *A. biguttatus* could be the vector of AOD bacteria, it is also remarkable to consider the dispersal capabilities of this insect; indeed, although the adults can fly for several kilometers, the spread between nations can occur through human activities [73]. A case study is represented by *A. planipennis*, which, originating from China [74], was introduced into North America and Western Russia through the transport of goods, such as biomass, packaging materials, and wooden artifacts, especially if they have the bark, which is where *Agrilus* spp. lays the eggs [75], and host plants in particular of the genus *Fraxinus* spp. [76–78]. This insect not only directly damages ash trees through its phytophagous and xylophagous activity, but in the USA and Canada, an association has been observed between the larval galleries of *A. planipennis* and various pathogenic fungi of ash trees, agents of canker and wood decay [79]. Therefore, if it is confirmed that *A. biguttatus* or other buprestids can act as vectors for AOD-related bacteria, it is plausible that the long-distance movement of such bacteria could occur through the transnational movement of infected insects, accidentally transported in wooden artifacts and host plants, as in the case of *A. biguttatus* oak trees. A central issue concerns how *B. goodwinii* and *G. quercinecans* might be transported by *A. biguttatus* and whether or not it is an accidental vector; one possibility is that this insect, living on the trunks and in the phyllosphere of oaks, may carry the bacteria by coming into contact with bacterial exudates [20]. Furthermore, it should be noted that an association has been observed between AOD bacteria and the larvae of buprestids found on AOD-symptomatic oaks [11]. Finally, it is important to clarify to what extent, if at all, AOD-related bacteria can survive when associated with the insect rather than the tree. An indication in this regard may come from the fact that larvae and adults of *Anoplophora chinensis* (Forster), an invasive species in Europe and found in Italy, have been associated with some bacteria in their mouthparts, including members of the Enterobacteriaceae family, such as the *Gibbsiella* genus [80], which is thought to be a symbiont and may assist these phytophagous beetles in degrading wood [80–82].

Therefore, we cannot exclude the role of insect vectors in the transmission of bacteria in Salento. Still, this hypothesis seems in contrast with the lack of findings of AOD-related bacteria in contiguous territories, leading to the assumption of a more-or-less progressive spread of the disease in Europe.

In conclusion, this study has demonstrated that holm oaks in Salento that exhibit symptoms attributable to Acute Oak Decline [11] are indeed infected with *B. goodwinii* and *G. quercinecans*, as isolates of these pathogens could cause wood necrosis, and from which, as highlighted by qPCR analysis, they could be re-isolated during pathogenicity tests. Additionally, phylogenetic analysis revealed that these isolates are closely related to strains found in Europe and Iran, suggesting a potential for long-distance spread that warrants further investigations, or their hidden presence in many other parts of Europe, making their presence much more contiguous than it seems. Identifying *B. goodwinii* and *G. quercinecans* as pathogens affecting multiple oak species poses a significant threat to

biodiversity, as other oak species may also be vulnerable to these infections, potentially impacting European forests. Consequently, further research is necessary to elucidate the mechanisms of pathogen spread and the environmental conditions conducive to their establishment, particularly in the context of climate change [48], and to identify other forest species at risk.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f15122055/s1, Table S1. List of *Brenneria* sp. and *Gibbsiella* sp. strains used for constructing the phylogenetic trees. Strains isolated in the present work are marked in bold.

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References

1. Ruffner, B.; Schneider, S.; Meyer, J.B.; Queloz, V.; Rigling, D. First Report of Acute Oak Decline Disease of Native and Non-native Oaks in Switzerland. *New Dis. Rep.* **2020**, *41*, 18. [CrossRef]
2. EPPO. Recent Studies on Acute Oak Decline, Involving Brenneria goodwinii and Other Bacteria. Available online: https://gd.eppo.int/reporting/article-7365 (accessed on 2 October 2024).
3. Fernandes, C.; Duarte, L.; Naves, P.; Sousa, E.; Cruz, L. First Report of *Brenneria goodwinii* Causing Acute Oak Decline on *Quercus suber* in Portugal. *J. Plant Pathol.* **2022**, *104*, 837–838. [CrossRef]
4. Zalkalns, O.; Celma, L. The Distribution of Bacteria *Gibbsiella quercinecans* and *Brenneria goodwinii* in Oak (*Quercus robur* L.) Stands in Latvia. *IOP Conf. Ser. Earth Environ. Sci.* **2021**, *875*, 012033. [CrossRef]
5. González, A.J.; Ciordia, M. *Brenneria goodwinii* and *Gibbsiella quercinecans* Isolated from Weeping Cankers on *Quercus robur* L. in Spain. *Eur. J. Plant Pathol.* **2020**, *156*, 965–969. [CrossRef]
6. Tkaczyk, M.; Celma, L.; Ruņģis, D.E.; Bokuma, G. First Report of *Brenneria goodwinii* and *Gibbsiella quercinecans* Bacteria, Detected on Weaken Oak Trees in Poland. *Balt. For.* **2021**, *27*, 166–169. [CrossRef]
7. Ostfeld, R.S.; Keesing, F.; Jones, C.G.; Canham, C.D.; Lovett, G.M. Integrative Ecology and the Dynamics of Species in Oak Forests. *Integr. Biol. Issues News Rev.* **1998**, *1*, 178–186. [CrossRef]
8. Parmain, G.; Bouget, C. Large Solitary Oaks as Keystone Structures for Saproxylic Beetles in European Agricultural Landscapes. *Insect Conserv. Divers.* **2018**, *11*, 100–115. [CrossRef]
9. Mölder, A.; Meyer, P.; Nagel, R.-V. Integrative Management to Sustain Biodiversity and Ecological Continuity in Central European Temperate Oak (*Quercus robur*, *Q. petraea*) Forests: An Overview. *For. Ecol. Manag.* **2019**, *437*, 324–339. [CrossRef]
10. Manion, P. *Tree Disease Concepts*, 2nd ed.; Prentice-Hall: Englewood Cliffs, NJ, USA, 1991.
11. Carluccio, G.; Sabella, E.; Greco, D.; Vergine, M.; Delle Donne, A.G.; Nutricati, E.; Aprile, A.; De Bellis, L.; Luvisi, A. Acute and Chronic Oak Decline in Urban and Forest Ecosystems in Southern Italy. *For. Int. J. For. Res.* **2024**, *97*, 739–749. [CrossRef]
12. Denman, S.; Brown, N.; Kirk, S.; Jeger, M.; Webber, J. A Description of the Symptoms of Acute Oak Decline in Britain and a Comparative Review on Causes of Similar Disorders on Oak in Europe. *Forestry* **2014**, *87*, 535–551. [CrossRef]
13. Strouts, G.R.; Winter, T.G. *Diagnosis of Ill-Health in Trees*; HMSO: London, UK, 1994.
14. Ragazzi, A.; Capretti, P.; Ghelardini, L.; Moricca, S. *Malattie Delle Piante in Bosco, in Vivaio e Delle Alberature*, 1st ed.; Patron: Bologna, Italy, 2020.
15. Denman, S.; Brady, C.; Kirk, S.; Cleenwerck, I.; Venter, S.; Coutinho, T.; De Vos, P. *Brenneria goodwinii* sp. nov., Associated with Acute Oak Decline in the UK. *Int. J. Syst. Evol. Microbiol.* **2012**, *62*, 2451–2456. [CrossRef] [PubMed]
16. Brady, C.; Denman, S.; Kirk, S.; Venter, S.; Rodríguez-Palenzuela, P.; Coutinho, T. Description of *Gibbsiella quercinecans* gen. nov., sp. nov., Associated with Acute Oak Decline. *Syst. Appl. Microbiol.* **2010**, *33*, 444–450. [CrossRef] [PubMed]
17. Denman, S. Symptoms and Identification of Acute Oak Decline. Available online: https://www.forestresearch.gov.uk/tools-and-resources/fthr/pest-and-disease-resources/acute-oak-decline/symptoms-and-identification-of-acute-oak-decline/#:~:text=Acute%20oak%20decline%20is%20a,and%20death%20in%20most%20cases (accessed on 26 June 2024).
18. Denman, S.; Kirk, S.; Webber, J. *Managing Acute Oak Decline*; Forestry Commission: Edinburgh, UK, 2010.
19. Vansteenkiste, D.; Tirry, L.; Van Acker, J.; Stevens, M. Predispositions and Symptoms of *Agrilus* Borer Attack in Declining Oak Trees. *Ann. For. Sci.* **2004**, *61*, 815–823. [CrossRef]

20. Brown, N.; Inward, D.J.G.; Jeger, M.; Denman, S. A Review of *Agrilus biguttatus* in UK Forests and Its Relationship with Acute Oak Decline. *Forestry* **2015**, *88*, 53–63. [CrossRef]
21. Brown, N.; Vanguelova, E.; Parnell, S.; Broadmeadow, S.; Denman, S. Predisposition of Forests to Biotic Disturbance: Predicting the Distribution of Acute Oak Decline Using Environmental Factors. *For. Ecol. Manag.* **2018**, *407*, 145–154. [CrossRef]
22. Brady, C.; Arnold, D.; McDonald, J.; Denman, S. Taxonomy and Identification of Bacteria Associated with Acute Oak Decline. *World J. Microbiol. Biotechnol.* **2017**, *33*, 143. [CrossRef]
23. Maddock, D.; Brady, C.; Denman, S.; Arnold, D. Bacteria Associated with Acute Oak Decline: Where Did They Come from? We Know Where They Go. *Microorganisms* **2023**, *11*, 2789. [CrossRef]
24. Denman, S.; Doonan, J.; Ransom-Jones, E.; Broberg, M.; Plummer, S.; Kirk, S.; Scarlett, K.; Griffiths, A.R.; Kaczmarek, M.; Forster, J.; et al. Microbiome and Infectivity Studies Reveal Complex Polyspecies Tree Disease in Acute Oak Decline. *ISME J.* **2018**, *12*, 386–399. [CrossRef]
25. Tkaczyk, M.; Sikora, K.; Galko, J. First Report of Bacteria Causing Acute Oak Decline on *Quercus robur* in Slovakia. *Eur. J. Plant Pathol.* **2024**, *169*, 113–120. [CrossRef]
26. Eichenlaub, L.; Denman, S.; Brady, C.; Maddock, D.; Robledo-Garcia, F.; Aubert, A.; Husson, C.; Robin, C. First Report of *Brenneria goodwinii*, *Gibbsiella quercinecans* and *Rahnella victoriana* in Declining Oaks in France. *New Dis. Rep.* **2024**, *49*, e12264. [CrossRef]
27. Pernek, M.; Brady, C.; Lacković, N.; Dubravac, T.; Jukić, A.; Kovač, M. Acute Oak Decline (AOD) New Complex Disese on Holm Oak (*Quercus ilex* L.) and Possibilities of Spread on Other Oak Species in Croatia. *Sumar. List* **2022**, *146*, 439–445. [CrossRef]
28. Doonan, J.; Denman, S.; Pachebat, J.A.; McDonald, J.E. Genomic Analysis of Bacteria in the Acute Oak Decline Pathobiome. *Microb. Genom.* **2019**, *5*, e000240. [CrossRef] [PubMed]
29. Denman, S.; Plummer, S.; Kirk, S.; Peace, A.; McDonald, J.E. Isolation Studies Reveal a Shift in the Cultivable Microbiome of Oak Affected with Acute Oak Decline. *Syst. Appl. Microbiol.* **2016**, *39*, 484–490. [CrossRef] [PubMed]
30. Crampton, B.G.; Plummer, S.J.; Kaczmarek, M.; McDonald, J.E.; Denman, S. A Multiplex Real-time PCR Assay Enables Simultaneous Rapid Detection and Quantification of Bacteria Associated with Acute Oak Decline. *Plant Pathol.* **2020**, *69*, 1301–1310. [CrossRef]
31. Moradi-Amirabad, Y.; Rahimian, H.; Babaeizad, V.; Denman, S. *Brenneria* spp. and *Rahnella victoriana* Associated with Acute Oak Decline Symptoms on Oak and Hornbeam in Iran. *For. Pathol.* **2019**, *49*, e12535. [CrossRef]
32. Pettifor, B. Survival of *Brenneria goodwinii* and *Gibbsiella quercinecans*, Associated with Lesion Formation in Acute Oak Decline, in Rainwater and Forest Soil. Master's Thesis, Bangor University, Bangor, UK, 2019.
33. Lane, D.J. 16S/23S RRNA Sequencing. In *Nucleic Acid Techniques in Bacterial Systematic*; Wiley: New York, NY, USA, 1991; pp. 115–175.
34. Brady, C.; Cleenwerck, I.; Venter, S.; Vancanneyt, M.; Swings, J.; Coutinho, T. Phylogeny and Identification of Pantoea Species Associated with Plants, Humans and the Natural Environment Based on Multilocus Sequence Analysis (MLSA). *Syst. Appl. Microbiol.* **2008**, *31*, 447–460. [CrossRef]
35. Guhathakurta, D.; Stromo, G.D. Finding Regulatory Elements in DNA Sequence. In *Bioinformatics: Methods Express*; Scion Publishing Ltd.: Rotorua, New Zealand, 2007; pp. 117–139.
36. Thompson, J.D.; Higgins, D.G.; Gibson, T.J. CLUSTAL W: Improving the Sensitivity of Progressive Multiple Sequence Alignment through Sequence Weighting, Position-Specific Gap Penalties and Weight Matrix Choice. *Nucleic Acids Res.* **1994**, *22*, 4673–4680. [CrossRef] [PubMed]
37. Lobo, I. Basic Local Alignment Search Tool (BLAST). *Nat. Educ.* **2008**, *1*, 215–399.
38. Tamura, K.; Stecher, G.; Kumar, S. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Mol. Biol. Evol.* **2021**, *38*, 3022–3027. [CrossRef]
39. Tamura, K.; Nei, M. Estimation of the Number of Nucleotide Substitutions in the Control Region of Mitochondrial DNA in Humans and Chimpanzees. *Mol. Biol. Evol.* **1993**, *10*, 512–526. [CrossRef]
40. National Center for Biotechnology Information (NCBI)[Internet] Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information. Available online: https://www.ncbi.nlm.nih.gov/nuccore/OR907136.1 (accessed on 9 September 2024).
41. National Center for Biotechnology Information (NCBI)[Internet] Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information. Available online: https://www.ncbi.nlm.nih.gov/nuccore/OR907048.1 (accessed on 9 September 2024).
42. Tremblay, J.; Singh, K.; Fern, A.; Kirton, E.S.; He, S.; Woyke, T.; Lee, J.; Chen, F.; Dangl, J.L.; Tringe, S.G. Primer and Platform Effects on 16S rRNA Tag Sequencing. *Front. Microbiol.* **2015**, *6*, 771. [CrossRef] [PubMed]
43. Peplies, J.; Kottmann, R.; Ludwig, W.; Glöckner, F.O. A Standard Operating Procedure for Phylogenetic Inference (SOPPI) Using (RRNA) Marker Genes. *Syst. Appl. Microbiol.* **2008**, *31*, 251–257. [CrossRef] [PubMed]
44. Wang, L.-T.; Lee, F.-L.; Tai, C.-J.; Kasai, H. Comparison of *gyrB* Gene Sequences, 16S rRNA Gene Sequences and DNA–DNA Hybridization in the *Bacillus subtilis* Group. *Int. J. Syst. Evol. Microbiol.* **2007**, *57*, 1846–1850. [CrossRef] [PubMed]
45. Delmas, J.; Breysse, F.; Devulder, G.; Flandrois, J.-P.; Chomarat, M. Rapid Identification of *Enterobacteriaceae* by Sequencing DNA Gyrase Subunit B Encoding Gene. *Diagn. Microbiol. Infect. Dis.* **2006**, *55*, 263–268. [CrossRef]
46. Basavand, E.; Khodaygan, P.; Doonan, J.M.; Rahimian, H. *Gibbsiella quercinecans* as New Pathogen Involved in Bacterial Canker of Russian Olive. *3Biotech* **2021**, *11*, 286. [CrossRef]

47. Tóth, T.; Lakatos, T. *Brenneria bubanii* sp. nov., Isolated from Decaying Plant Tissues. *Int. J. Syst. Evol. Microbiol.* **2023**, *73*. [CrossRef]
48. Li, Y.; Fang, W.; Xue, H.; Liang, W.; Wang, L.; Tian, G.; Wang, X.; Lin, C.; Li, X.; Piao, C. *Brenneria populi* sp. nov., Isolated from Symptomatic Bark of *Populus*×*euramericana* Canker. *Int. J. Syst. Evol. Microbiol.* **2015**, *65*, 432–437. [CrossRef]
49. Sapp, M.; Lewis, E.; Moss, S.; Barrett, B.; Kirk, S.; Elphinstone, J.; Denman, S. Metabarcoding of Bacteria Associated with the Acute Oak Decline Syndrome in England. *Forests* **2016**, *7*, 95. [CrossRef]
50. Karnosky, D.F. Dutch Elm Disease: A Review of the History, Environmental Implications, Control, and Research Needs. *Environ. Conserv.* **1979**, *6*, 311–322. [CrossRef]
51. Sicard, A.; Saponari, M.; Vanhove, M.; Castillo, A.I.; Giampetruzzi, A.; Loconsole, G.; Saldarelli, P.; Boscia, D.; Neema, C.; Almeida, R.P.P. Introduction and Adaptation of an Emerging Pathogen to Olive Trees in Italy. *Microb. Genom.* **2021**, *7*, 000735. [CrossRef]
52. Bonham, E. Potential Transmission Pathways of Two Bacteria Associated with Acute Oak Decline *Brenneria goodwinii* and *Gibsiella quercinecans*. Ph.D. Thesis, Harper Adams University, Newport, UK, 2021.
53. Pettifor, B.J.; Doonan, J.; Denman, S.; McDonald, J.E. Survival of *Brenneria goodwinii* and *Gibbsiella quercinecans*, Associated with Acute Oak Decline, in Rainwater and Forest Soil. *Syst. Appl. Microbiol.* **2020**, *43*, 126052. [CrossRef] [PubMed]
54. Araeinejhad, M.-H.; Charkhabi, N.F.; Brady, C.; Rahimian, H. Reliable and Specific Detection and Identification of *Brenneria goodwinii*, the Causal Agent of Oak and Oriental Beech Decline. *Front. For. Glob. Chang.* **2024**, *7*, 1325897. [CrossRef]
55. Tkaczyk, M.; Sikora, K.; Plewa, R. Dieback of Small-leaved Lime Trees (*Tilia cordata* Mill.) Caused by *Gibsiella quercinecans* in Urban Areas in Poland. *For. Pathol.* **2024**, *54*, e12861. [CrossRef]
56. National Center for Biotechnology Information (NCBI)[Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information. Available online: https://www.ncbi.nlm.nih.gov/nuccore/MZ503699 (accessed on 9 October 2024).
57. National Center for Biotechnology Information (NCBI) Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information. Available online: https://www.ncbi.nlm.nih.gov/nuccore/LR735261.1 (accessed on 9 October 2024).
58. National Center for Biotechnology Information (NCBI)[Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information. Available online: https://www.ncbi.nlm.nih.gov/nuccore/OP586670 (accessed on 9 October 2024).
59. National Center for Biotechnology Information (NCBI)[Internet] Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information. Available online: https://www.ncbi.nlm.nih.gov/nuccore/OP616040.1/ (accessed on 9 September 2024).
60. National Center for Biotechnology Information (NCBI)[Internet] Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information. 2022. Available online: https://www.ncbi.nlm.nih.gov/nuccore/OP586671.1 (accessed on 9 October 2024).
61. National Center for Biotechnology Information (NCBI)[Internet] Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information. Available online: https://www.ncbi.nlm.nih.gov/nuccore/OP586675 (accessed on 9 September 2024).
62. Biosca, E.G.; González, R.; López-López, M.J.; Soria, S.; Montón, C.; Pérez-Laorga, E.; López, M.M. Isolation and Characterization of *Brenneria quercina*, Causal Agent for Bark Canker and Drippy Nut of *Quercus* spp. in Spain. *Phytopathology* **2003**, *93*, 485–492. [CrossRef] [PubMed]
63. Davis, E.E.; French, S.; Venette, R.C. Mini Risk Assessment Metallic Beetle: Agrilus biguttatus Fabricius [Coleoptera: Buprestidae]. 2004. Available online: https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=4d7415b17fa6c883e06e07d1abbc7136d8625f73 (accessed on 26 September 2024).
64. Riziero, T.; Ragazzi, A.; Marianelli, L.; Sabbatini, P.; Roversi, P.F. Insects and Fungi Involved in Oak Decline in Italy. *Int. Organ. Biol. Integr. Control.* **2002**, *25*, 67–74.
65. Curletti, G. L'entomocenosi Xilofaga Del Parco Della Mandria (Piemonte, Italy). *Riv. Piem. St. Nat.* **1996**, *17*, 151–165.
66. Curletti, G. Considerazioni Su Alcune Specie Di *Agrilus curtis* Presenti in Italia e Su Alcuni Sottogeneri Proposti Di Recente. *Fragmentaentomologica* **2013**, *45*, 71–82.
67. Solinas, M. *Coroebus Florentinus* (Herbst) (Coleoptera, Buprestidae) Biologia, danni, lotta. *Entomologica* **1974**, *10*, 141–193.
68. Solinas, M. Considerazioni Ecologiche Sul Preoccupante Sviluppo Di *Coroebus florentinus* (Herbst) Nelle Leccete Del Gargano. *Entomologica* **1971**, *7*, 115–122.
69. Curletti, G. Coleotteri Bupestridi Del Piemonte e Valle d'Aosta. *Riv. Piem. St. Nat.* **1980**, *1*, 69–104.
70. Kalil, S.A.A.; Atta, H.A.E.; Aref, L.M. Increased Gum Arabic Production after Infestation of *Acacia senegal* with *Aspergillus flavus* and *Pseudomonas pseudoalcaligenes* Transmitted by *Agrilus nubeculosus*. *Afr. J. Biotechnol.* **2011**, *10*, 7166–7173.
71. Rexrode, C.O. Tree-Wounding Insects as Vectors of the Oak Wilt Fungus. *Forest science* **1968**, *14*, 181–189.
72. Mezencev, A.I. Swolovye Vrediteti i Openok v Ochagakh Usykhanija Duba. izv. *Khar'k. Entom* **1993**, *1*, 193–198.
73. Dueñas-López, M.A. *Agrilus biguttatus (Oak Splendour Beetle)*; CABI Compendium: Wallingford, UK, 2022.
74. Poland, T.M.; McCullough, D.G. Emerald Ash Borer: Invasion of the Urban Forest and the Threat to North America's Ash Resource. *J. For.* **2006**, *104*, 118–124. [CrossRef]
75. Schans, J.; Schrader, G.; Delbianco, A.; Graziosi, I.; Vos, S. Pest Survey Card on *Agrilus planipennis*. *EFSA Support. Publ.* **2020**, *17*, 1945E. [CrossRef]

76. EPPO. *Pest Risk Analysis for Agrilus*; EPPO: Paris, France, 2013.
77. Flø, D.; Krokene, P.; Økland, B. Invasion Potential of *Agrilus planipennis* and Other *Agrilus* Beetles in Europe: Import Pathways of Deciduous Wood Chips and MaxEnt Analyses of Potential Distribution Areas. *EPPO Bull.* **2015**, *45*, 259–268. [CrossRef]
78. Evans, H.F.; Williams, D.; Hoch, G.; Loomans, A.; Marzano, M. Developing a European Toolbox to Manage Potential Invasion by Emerald Ash Borer (*Agrilus planipennis*) and Bronze Birch Borer (*Agrilus anxius*), Important Pests of Ash and Birch. *For. Int. J. For. Res.* **2020**, *93*, 187–196. [CrossRef]
79. Held, B.W.; Simeto, S.; Rajtar, N.N.; Cotton, A.J.; Showalter, D.N.; Bushley, K.E.; Blanchette, R.A. Fungi Associated with Galleries of the Emerald Ash Borer. *Fungal Biol.* **2021**, *125*, 551–559. [CrossRef]
80. Rizzi, A.; Crotti, E.; Borruso, L.; Jucker, C.; Lupi, D.; Colombo, M.; Daffonchio, D. Characterization of the Bacterial Community Associated with Larvae and Adults of *Anoplophora chinensis* Collected in Italy by Culture and Culture-Independent Methods. *Biomed. Res. Int.* **2013**, *2013*, 420287. [CrossRef]
81. Colman, D.R.; Toolson, E.C.; Takacs-Vesbach, C.D. Do Diet and Taxonomy Influence Insect Gut Bacterial Communities? *Mol. Ecol.* **2012**, *21*, 5124–5137. [CrossRef]
82. Tkaczyk, M. Bioclimatic Variables and Their Impact on the Potential Distribution of *Brenneria goodwinii* in Europe. *For. Pathol.* **2023**, *53*, e12820. [CrossRef]