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Research Article

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Mitigation of Quick Decline Syndrome in Ancient and Monumental Olive Trees of Ostuni (Apulia, Italy) Positive to *Xylella fastidiosa* and other Phytopathogens

Giorgio Doveri[1], Giovanni Pergolese[2], Emanuela Sardella[3], Marco Scortichini[4], Giusto Giovannetti[5], Michele Saracino[3], Luigi Botrugno[6] and Marco Nuti[7*]

[1]*Pharmaceutical Technologist, Multidisciplinary Scientific Committee, Italy*

[2]*Chemist, Multidisciplinary Scientific Committee, Italy*

[3]*Agronomist, Multidisciplinary Scientific Committee, Italy*

[4]*Plant Pathologist, Multidisciplinary Scientific Committee, Italy*

[5]*Soil Biologist, Multidisciplinary Scientific Committee, Italy*

[6]*Chemist and Environmental Engineer, Multidisciplinary Scientific Committee, Italy*

[7]*Soil/Plant Microbiologist, Institute of Crop Sciences, School of Advanced Studies Sant'Anna – University of Pisa, Italy*

[*]Corresponding Author
Marco Nuti, Soil/Plant Microbiologist, Institute of Crop Sciences, School of Advanced Studies Sant'Anna – University of Pisa, Italy.

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Abstract

The Olive Quick Decline Syndrome (OQDS) in Apulia region, south of Italy, has affected over the last decade more than 6.5 million of olive trees. The syndemic nature of this syndrome, which includes the phytopathogenic bacterium Xylella fastidiosa (Xf), has caused great difficulties in the control of OQDS, and both the insect vector control of Xf, and eradication of olive trees have proven to be of limited efficacy. In this on-farm case study a co-existence and mitigation approach is described, applied to thirty-eight monumental, or having the traits of monumentality, olive trees of the municipality of Ostuni. They were PCR-positive to Xf in 2021, showing random symptoms of desiccation, and the symptoms of generalized OQDS. The trees were affected by different bacterial and fungal pathogens, nematodes, and pests. These same trees, treated during three consecutive years with an agronomic protocol (Good Agronomic Practices including microbial biostimulants), in 2025 are alive, productive, still PCR-positive to Xf except one, and having milder symptoms associated to the syndrome. The results suggest that mitigation measures, and co-existence with Xf, might represent an alternative to the generalized eradication of olive trees affected by OQDS.

Keywords: Olive Quick Decline Syndrome, Olive Desiccation, Olive Syndemic Outbreak, Xylella fastidiosa, Mitigation

1. Introduction

The Olive Quick Decline Syndrome (OQDS) in Apulia region, south of Italy, has affected over the last decade millions of trees. Although a precise estimate to date of the number of dead trees is lacking, the data obtained with the analysis of satellite images, indicate approximately 6.5 millions diseased trees at the end of 2017, with a rapidly growing trend [1,2]. Although initially the spread of the disease was called OQDS, contrasting views were issued to explain the syndrome, one view attributing the death of such an important number of trees solely to the causal agent *Xylella fastidiosa* (Regione Puglia, 2024 http://www.emergenzaxylella.it/portal/portale_gestione_agricoltura), another view being that OQDS in Apulia could be identified as a syndemic outbreak, due to concurrent pathological events of biological, agronomic, and societal nature, requiring the development and application of risk mitigation measures [3-7]. Initially the contrast approaches were developed, targeting mainly at one of the causes of OQDS, namely the phytopathogenic bacterium *Xylella fastidiosa* subsp. *pauca* (*Xfp*) [8,9]. These treatments were successfully applied to 20-years-old (cv. Ogliarola salentina) and to 60-75 years old olive trees (cv. Ogliarola salentina and Cellina di Nardò), respectively. However, it was progressively recognized that the eradication of the bacterium is impossible and that attempts to eradicate the *Xfp* insect vector are of limited efficacy [10,11]. Moreover, in 2021-2022 *Xfp* was detected only in 3.21% of the sampled plants affected by the OQDS [12]. Consequently, the pathogenic bacterium was moved from the A1 to the A2 EPPO list due to its establishment in southern Europe [13]. Phytopathogens other than *Xfp* are found in desiccating olive trees, namely the agents of cercosporiosis, of leprosy *Phlyctema vagabunda Desm*., of olive leaf spot, of root and crown rot, root nematodes (https://ipm.ucanr.edu/agriculture/olive/nematodes/#gsc.tab=0), of olive tree scab due to *Pseudomonas savastanoi* pv. *savastanoi,* of verticilliosis [14-20]. Recently *Neofusicoccum mediterraneum* has been described as associated to the OQDS [21]. Overall considered, mitigation and co-existence with *Xfp* are retained as possibly the only effective strategies to control OQDS, along with the control of the other phytopathogens, within a holistic approach [12]. Here we describe an agronomic approach, applied to thirty-eight olive trees, monumental or having the monumentality traits, of the Municipality of Ostuni, aiming at (a) developing a sustainable mitigation strategy of OQDS to olive trees PCR-positive to *Xylella fastidiosa*, and (b) verifying at field level whether the application of the protocol allows to avoid the death or mandatory eradication measures of these trees of historical and naturalistic interest.

2. Materials and Methods

2.1. Study Location Area

Ostuni, 218 m a.s.l. and 10 Km from the Adriatic Sea, is one of the Municipalities of the Province of Brindisi (Apulia). Its surface is 22,417 Ha including 7,037 Ha cultivated with olive trees, several of them being centenary or millenary in protected areas. The soil of the Municipality is of reduced depth, with a calcareous crust on the surface, remarkably stony, and has been subjected to anthropic pressure since the last 50 years; it has high to very high salinity (750 wells are present in the territory), with progressive salinization of the aquifer [5]. The soil erosion risk assessment, according to PESERA model (https://www.fao.org/land-water/land/land-governance/land-resources-planning-toolbox/category/details/en/c/1111230/), is 1-3 t/Ha/year. The residents in the Municipality at the date of January 31st, 2024 were 29,877 but thousands visitors during the year make it a very densely populated recreational area.

2.2. Olive Trees and Their Environment

Table 1 reports the geographic coordinates of each of the olive trees, monumental or having traits of monumentality (cv. Cellina di Nardò, Ogliarola, Toscanina, and Cima di Melfi Figure 1).

The date of first sampling was October 21st, 2021, the latter is reported in for all of them. The date of second sampling was October 21st, 2024, for all of them. The study ended in January 2025. The studied trees are within olive orchards, randomly spaced (Figure 2). Soil physicochemical characteristics of the orchards are: pH 7.8, EC 123 mS/cm^{-1}, organic carbon 0.8% w/w, organic matter 1.5% w/w. The trees were rainfed, and no irrigation was carried out. The main climatic conditions, i.e. temperature and rainfall 2021-2024 are reported in Figure 3.

Olive tree ID	Latitude	Longitude	Land Reg. sheet	Land Reg. parcel
1257489	40,72608697	17,50561063	134	215
1257485	40,72607236	17,50577193	134	215
1257442	40,72619162	17,5059434	134	215
1257471	40,72613572	1750588506	134	215
1249521	40,72652684	17,50593536	134	215
1249661	40,72682175	17,50591278	134	215
1251618	40,72691169	17,50641279	134	166
1251624	40,72687866	17,50642329	134	166
1251584	40,72683671	17,50648521	134	166
1251658	40,72678623	17,50656495	134	166
1253656	40,72685238	17,50672247	134	24
1251636	40,72678482	17,50649195	134	166
1251709	40,72672266	17,50657542	134	166
1251779	40,7267086	17,50645007	134	166
1251745	40,72667031	17,50657171	134	166
1251762	40,72665812	17,50651225	134	166
1251873	40,72665778	17,50645229	134	166
1253396	40,72660425	17,50650533	134	166
1253398	40,72659976	17,50657728	134	166
1252016	40,72654669	17,50651624	134	166
1253407	40,72655699	17,50658445	134	166
1251970	40,72649523	17,50647868	134	166

1253409	40,72649957	17,50658892	134	166
1253419	40,7265168	17,50672691	134	24
1253827	40,72637198	17,50667626	134	167
1253913	40,7263713	17,50686	134	25
-	40,726492	17,506554	134	167
1255513	40,72638439	17,50643447	134	167
1255567	40,72635841	17,50648618	134	167
1255636	40,726236	17,50670426	134	167
1255704	40,72618711	17,50659071	134	167
1255798	40,72614337	17,5064912	134	167
1255820	40,72606366	17,50655314	134	167
1254068	40,72626984	17,50728737	134	241
1259408	40,72626474	17,50728737	134	241
1254096	40,72637354	17,50725699	134	241
1259380	40,72625701	17,50712022	134	241
1223144	40,7376708	17,51586594	83	51
1233189	40,73771999	17,51613204	83	148

Table 1: Geographic coordinates of the 39 olive trees under study (ID = identifier)

Figure 1: One monumental olive tree of this study (see Table 1 for coordinates of ID 1233189; see also the aerial map reported in Figure 2b, the monumental olive tree is on the right). The monumental olive tree before (left), and after (right) the mandatory pollarding, and the application of GAP, including the treatment with microbial biostimulants. The symptom severity (desiccation scale 0-4) was 0 in 2022 and remains 0 in 2025

Figure 2: Aerial map: the olive orchards (a) and (b) are distant ca.1 km from each other; the trees of this study are the ones marked with the white placeholder icons

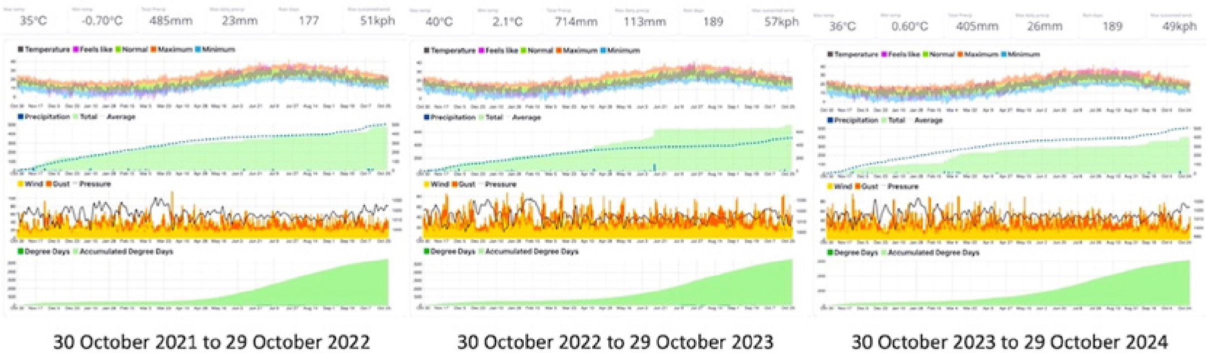

Figure 3: Climatic data throughout the study period. The upper line represents the maximum temperature, the total precipitation, the maximum daily precipitation, the rain days, and the maximum sustained wind, respectively, from October 30th, 2021 to October 29th, 2024

2.3. Agronomic Management

The studied trees were managed according to the cultivation techniques of the area, i.e. no regular pruning, no soil fertilization, no control of main pests and diseases, no herbicide treatment for most of them. According to the Regional Authorities provisions at the beginning of 2022, the trees were pollarded and grafted with the aim of minimizing the risk of diffusion of Xf. The tree 1255513, positive to Xf in 2021, within the same orchard and left without the treatment described in this case report during the entire period, was considered as a control. The trees were rainfed, and no irrigation was carried out. The first agronomic treatment was carried out in April, 2022 by spraying 150 L/ha (per 100 L water: 500 ml industrial bleach, 400 g wettable sulphur). Five days after the treatment, both the upper canopy and the tree base were sprayed with a biostimulant microbial consortium (containing Bacillus sp., Pseudomonas sp., and Trichoderma sp.) purchased from BEA, Galazzano, Republic of San Marino. A week after the olive trees had been pollarded and grafted between the end of May and June 2022, according to the provisions established by the Regional Administrative Court of Bari, a second treatment was carried out with 150 L/ha of a solution/suspension (per 100 L water: 100 g citric acid, 300 g Ergofito shark, 200 g Nemacontrol, 300 g technical urea, 200 g calcium nitrate, 100 g Ergofito boron) [22]. A third treatment was carried out in June 2023 with a solution/suspension [per 100 L water: 100 g citric acid, 250 g of ternary fertilizer 30-10-10, 300 g Ergofert start plus Bio, 200 g Nemacontrol 100 g Ergofito (Fe-Mg-Cu-Mo), 100 g Ergofito (Zn-Mn-Mg-Mo)]. The products for the second and third treatment were purchased from BEA, Galazzano, Republic of San Marino. In March 2024 the olive trees were sprayed with a solution of lime and wettable sulphur, 4-10 L per tree, followed by foliar zeolite suspension (350-400 L/ha). In June 2024 the pollards and cuts were disinfected where needed, and the tree were cleaned from occasional dry twigs. The severity of the desiccation symptom is expressed according to the scale 0-4 proposed by Scholten et al. [1].

2.4. Sampling and Analysis of Xfp by qPCR

The sampling was carried out in agreement with the European Union's guidelines [23]. After visual inspection, sampling was made on branches at least one year old and according to the cardinal points plus between cardinal points. The twigs were mainly the symptomatic ones, 6-8 for each tree, cut by using a telescopic lopper. The latter was disinfected with hypochlorite at each sampling. Each twig was placed in a sterile plastic bag, numbered according to the tree identifier, and brought to the laboratory in a refrigerated portable container within 3 hours. The twigs from each of the plants reported in Table 1 were collected on October 21st, 2021, and October 21st, 2024, respectively. The presence of Xfp was determined by Polymerase Chain Reaction (PCR) confirmed by quantitative PCR (qPCR). Samples were finely chopped and then sonicated for 1 min, then incubated 15 min at room temperature before DNA extraction following the procedure described by Dupas et al. [24]. The occurrence of phytopathogenic fungi and nematodes was monitored on all the studied trees.

3. Results

3.1. Visual Inspection

The grafts obligatorily made at the beginning of 2022, according to the provisions of the Regional Authorities, were unsuccessful since 2023. In spring 2023 the new twigs and old branches emerging from the pollarded trees were vegetated. At the beginning of this case study in 2022 some olive trees showed random symptoms of desiccation (Figure 4a).The symptoms of generalized OQDS were visually present in all trees: scattered leaf scorching and twig death throughout the upper part of the canopy, or occasionally along with signs of olive tree scab (caused by Pseudomonas savastanoi; Figure 4b), caries (caused by Fomitiporia punctata; Figure 4c), root rot (caused by pathogenic oomycetes; Figure 4d), distorted roots (caused by nematodes; Figure 4e), internal galleries in the branches (caused by Zeuzera pyrina, the common yellow woodworm; Figure 4f), peacok eye (caused by Spilocaea oleaginea; Figure 4g), sooty mold and cochineal (Figure 4h). At the end of the study case in 2025, all the trees showed symptoms of agronomically not relevant desiccation (scale 0-2; usually the desiccated twigs are removed by pruning) except the trees ID 1249521, ID 1255567, and the untreated tree 1255513, which were at scale 3-4 (Figure 5).

Figure 4: The OQDS symptoms were exhibited by the olive trees, besides being positive to Xfp, at the start of the study in 2022. Left to right (upper part): 4a – random symptoms of desiccation; 4b - olive tree scab; 4c - caries; 4d – root rot; left to right (lower part): 4e – distorted roots; 4f – internal galleries; 4g – peacock eye; 4h - sooty mold and cochineal

Figure 5: Olive trees showing different levels of desiccation on the canopy (from left to right: ID 1259408 level 0, ID 1249661 level 1, ID 1253398 level 2, ID 1249521 level 3, and the untreated control tree ID 1255513 level 4) at the beginning of 2025. The frequency, i.e. the number of trees affected, of symptoms of desiccation is reported in Table 2

3.2. OQDS Monitoring and PCR Test

At the beginning of the case study, all the trees were PCR-positive to Xf. At the end of the case study they were still qRT-PCR-positive to Xf subsp. pauca, except one (ID 1223144, cv. Cima di Melfi).

3.3. Mitigation

After 3 years of agronomic management focusing on mitigation measures, all treated trees rebuilt their foliage and new vegetation, produced drupes, and were without or strongly reduced desiccation symptoms. They were visually healthy, but with signs of scattered OQDS symptoms. The level 0 desiccation affected 24 trees, level 1 affected 9 trees, level 2 affected 3 trees, level 3 affected 1 tree, and level 4 affected 2 trees. Table 2 enlists the symptoms for each individual tree affected to date by OQDS including desiccation symptoms, along with the productivity of drupes. The latter were in the majority within the range of 5-15 kg/tree. Figure 6 summarizes the symptomatology present on January 2025.

ID number	Desiccation severity (level 0 to 4)	Tree scab %	Caries	Root rot	Aspecific nematodes	Internal galleries	Production of drupes (Kg / Tree)	Additional symptoms/ Notes
1257489	1	10 - 30	Extended	Present	Present	0	B	
1257485	2	10 - 30	Extended	Present	0	0	B	
1257442	1	31 - 50	Extended	Present	Present	Present	B	Wood-eating insect, presumed Armillaria
1257471	1	10 - 30	Extended	Present	Present	0	B	Wood-eating insect

1249521	3	10 - 30	Extended	Extended	Present	Present	B	Phleotrib insect Mechanical damages on the roots
1249661	1	31 - 50	Extended	Extended	Present	Present	B	Sooty mold
Peacock eye								
1251618	0	31 - 50	Extended	Present	Present	Present	B	
1251624	0	10 - 30	Extended	Present	Present	Present	B	Cottony mealbug / leaf stress by heat
1251584	0	10 -30	Extended	Extended	Present	0	B	
1251658	0	10 - 30	Extended	Present	Present	0	B	
1253656	0	< 10	Absent	Present	Present	0	B	
1251636	0	0	Absent	Present	Present	0	B	
1251709	1	10 - 30	Limited	Present	Present	0	B	
1251779	0	10 - 30	Limited	Extended	Present	0	B	
1251745	1	10 - 30	Limited	Present	Present	0	B	Sooty mold
1251762	0	10 - 30	Extended	Extended	Present	0	B	
1251873	0	10 - 30	Extended	Present	Present	0	B	
1253396	0	10 - 30	Extended	Present	Present	Present	B	Sooty mold
1253398	2	10 - 30	Extended	Extended	Present	0	B	
1252016	0	0	Extended	Present	Present	Present	B	
1253407	1	10 - 30	Extended	Present	Present	0	B	Sooty mold and cochineal
1251970	0	10 - 30	Extended	Extended	Present	0	B	Sooty mold and cochineal
1253409	1	< 10	Extended	Present	Present	Present	B	
1253419	0	0	Extended	Present	Present	0	B	Sooty mold and cochineal
1253827	2	10 - 30	Extended	Extended	Extended	Si	B	Sooty mold and cochineal
1253913	1	0	Extended	Present	Present	Si	B	Bark rot symptoms
-								
	0	> 50	Extended	Present	Present	No	B	Sooty mold. Tree Xf-negative in 2021 to 2025
1255513	4	0	Extended	Present	Extended	Extended	A	Sooty mold. Heavily pollarded tree. Not treated comparator tree
1255567	4	0	Extended	Present	Present	Extended	A	Sooty mold, heavily pollarded (>50%) tree
1255636	0	< 10	Extended	Present	Present	Present	B	Heavily pollarded (>50%) tree
1255704	0	10 - 30	Extended	Extended	Extended	Present	B	
1255798	0	< 10	Extended	Present	Present	0	B	
1255820	0	< 10	Extended	Present	Present	Present	B	
1254068	0	0	Extended	Present	Present	Extended	B	
1259408	0	< 10	Extended	Present	Present	Extended	C	Sooty mold and heavy cochineal
1254096	0	< 10	Extended	Extended	Present	Present	B	Sooty mold and cochineal
1259380	0	< 10	Extended	Present	Present	Present	C	Sooty mold and cochineal

1223144	0	< 10	Extended	Present	Present	0	C	Leprosy. Tree Xf + in 2021 and negative in 2025
1233189	0	< 10	Extended	Present	Present	0	C	Peacock eye

Table 2: Symptoms of generalized OQDS and of desiccation of the trees at the date of january 2025. For productivity a = 0-5 kg/tree, b = 5-15 kg/tree, c = 15-25 kg/tree

Figure 6: At the end of the monitoring period (January 2025) the trees showed some OQDS symptoms (for the description see Table 2)

4. Discussion

The olive trees monumental or having traits of monumentality of Ostuni offered the opportunity to confirm and expand our knowledge also considering the experimental approaches of other Authors on younger trees, because (a) these ancient trees were the rarest affected by OQDS, (b) they had never been approached to evaluate mitigation strategies, (c) they were present at the beginning of this case report in the former so-called "containment area", i.e. the area where containment legal provisions included the removal of infected plants (Regione Puglia, https://press.regione.puglia.it/-/xylella-fastidiosa-aggiornata-la-zona-di-contenimento, accessed 29.10.2024). The olive trees of this report were pollarded at least 50% and then subjected to the agronomic treatments. In this case study a co-existence and mitigation protocol was applied, based on good agronomic practices, including the treatment with microbial biostimulants. Although some difficulties were encountered in field-testing new protocols due to stringent, and sometimes not well aligned, provisions established, Apulian Regional Authority, Regional Administrative Court of Bari, and international Authorities, the results obtained in the field for three consecutive years indicate that the protocol can be effective by mitigating the olive quick decline syndrome [11,22,25-29]. The olive trees were without or strongly reduced OQDS symptoms, resulting visually healthy, and PCR-positive to *Xfp* (except one, cv. Cima di Melfi, ID 1223144) which was *Xfp*-negative. Clearly, further investigation would be required for the trees *Xfp*-positive to understand whether they contain Xfp cells alive, since the PCR test only says that there is the DNA of *Xfp*. However, this finding confirms and extends the observation of other Aa. that OQDS symptomatology can occur also in the absence of *Xfp*. Up to 97% of *Xfp* PCR-negative vs. OQDS-positive trees was found in the areas monitored to reveal olive trees infected by *Xfp*. Contrary to expectations, there is a not significant effect of cultivar and interaction treatment x cultivar [12]. In our case, it was interesting to note that another olive tree (latitude 40,726492 longitude 17,506554), five meters from the tree ID 1255567 and negative to *Xf*, remained PCR-negative to *Xf* from 2021 to 2024, without being affected by OQDS. Most reports are focused on the inhibition of just one of the components of OQDS, namely the bacterium *Xfp*, and the conclusion by EFSA was that *"based on the reviewed results, although several published experiments show some effects in reducing symptoms development, the tested control measures are not able to completely eliminate Xf from diseased plants, and that there is currently no control measure available to eliminate Xf from a diseased plant in open field conditions"* [11]. Genomic analysis suggests that the pathogen arrived in southern Italy in 2008 on a coffee plant from Costa Rica [30]. It is interesting to note that previous studies on another crop, i.e. coffee, indicated that the deterioration of soil properties, damage of root-knot nematodes, and accumulation of soil fungi may exacerbate the coffee plants diseases, and that the gradual decline in rhizosphere microorganism diversity and imbalanced community structure, which enriches harmful bacteria, directly contributes to coffee diseases in long-standing continuous plantations [31]. Unfortunately measures to contrast the various components of OQDS in Apulian olive plantations (e.g. erratic agronomic management practices, salinization, pollution, erosion, decline of organic matter and biodiversity, misuse of the territory) have not been adopted on a scale larger than small field experiments. The organic carbon in Ostuni is actually 0.8%, while in the neighbouring Salento sub-region where severe desiccations have occurred, the organic carbon is 0.4%. Furthermore, to our knowledge, studies on how the olive rhizosphere and endophytic microbiome diversity and community structure can change in the presence of OQDS in Apulia are scanty. However, interest is recently growing on the relevance of olive microbiome for the olive tree biology, resilience, and health [32-35]. A pioneering work on the olive-*Xf* pathosystem has recently shown that in susceptible plants there is a significant change in the associated microbiota with a drastic loss of beneficial genera [36]. Studies aiming at inhibiting *in vitro* the growth of one component of OQDS, i.e. *Xf* and the application of olive tree endophytes and species of Bacillus were recently reviewed [4,9]. The use of silver ultrananoclusters, and the management of mineral composition of host plants have also been proposed as a control strategy of the bacterium [37,38]. A protocol which promotes, supports, and restores new vegetation, flowers, fruits, and oil production of the treated olive plants affected by OQDS without losing susceptible olive plants has been recently proposed [39]. In this study the productivity (Kg of drupes per tree) was in the average of 5-15, while a productivity of 0-5 was observed in the trees where level 4 desiccation was present. In the case of olive trees affected by OQDS but *Xfp*-negative, (as well as in the case of OQDS-positive and *Xfp*-positive) other phytopathologies can be associated to the olive quick decline syndrome, including olive cercosporiosis, leprosy, olive wilt, olive leaf spot, peacock eye, root and crown rot, root nematodes, tree scab, verticilliosis. All these symptoms can lead to desiccation, and this could help understanding why Xf was not found in most desiccated trees in the surveillance area and in many desiccated trees of the infected area.

5. Conclusive Remarks

Our field case study on ancient olive trees affected or not hit by OQDS, but positive to *Xfp*, indicates that the co-existence with microbial phytopathogens including *Xfp* is possible without losing productivity or, even worst, without the elimination of the tree or the forced adoption of other drastic measures such as heavy pollarding and grafting, provided that careful application of good agronomic practices is made. These findings might be relevant to those farmers who prefer to adopt alternative, more sustainable risk mitigation measures, leading to the rescue of such an important tree crop in Apulia.

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