Predisposing Factors for “Olive Quick Decline Syndrome” in Salento (Apulia, Italy)

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Abstract: Recently, a new severe disease has been reported in the Salento area (Apulia region, southern Italy) in the multimillennial olive agro-ecosystem, given the common name “olive quick decline syndrome” (OQDS). Together with *Xylella fastidiosa* subsp. *pauca*, some pathogenic fungi such as *Phaeoacremonium* spp. have been found associated with the disease. The main predisposing factors to the disease seem to be local cultivar susceptibility, depletion of some micronutrients in the soil that could be related to some agronomical practices favoring the depletion of soil fertility, an incorrect pruning cycle, climatic changes that result in increased soil waterlogging, and frost and drought events. The possible synergistic action of microorganisms other than *X. f.* subsp. *pauca* cannot be excluded. The features characterizing the areas where OQDS first appeared and subsequently spread, described and discussed here, would point to a rather fragile environment where one or more adverse climatic and/or edaphic factors could have acted together. The intrinsic peculiarities and management of the Salento olive agro-ecosystem could also have played a fundamental role in enhancing the virulence of *X. f.* subsp. *pauca* once introduced from abroad.

Keywords: *Xylella fastidiosa*; olive agro-ecosystem; cultivar susceptibility; soil fertility; agronomical practices; pruning; climate change; frost; drought

1. Introduction

For plant disease to develop, besides a susceptible host and a virulent pathogen, the environmental factors acting in the area where the initial disease outbreak is found are fundamental for the pathogen to settle and for the disease to spread [1]. Indeed, a number of environmental factors can interact with the host and the pathogen either to predispose and enhance host colonization and infection by the pathogen or to incite microorganism pathogenicity in terms of virulence and aggressiveness. Soil conditions in terms of texture, chemical composition, microbial fertility, and management as well as some climatic parameters (rain, humidity, drought, strong winds, adverse temperatures) can promote or reduce the disease progression in a particular area [1,2].

Recently, a new severe disease in olive (*Olea europea* L.) has been reported in the Salento area (Apulia region, southern Italy) [3,4], which has been given the common name “olive quick decline syndrome” (OQDS) [5]. Main symptoms include leaf and twig wilting, branch die-back, and plant death. Despite the indication given by the name, an olive tree can collapse within two to five years from the initial infection [6–8]. *Xylella fastidiosa* subsp. *pauca* is a plant quarantine bacterium according to the European and Mediterranean Plant Protection Organization (EPPO). Quarantine pathogens previously not found in the European territories are ruled out by strict phytosanitary laws aimed at impeding their further spread. *X. f.* subsp. *pauca*, a pathogen transmitted by sap-feeding insect vectors, has been frequently found associated with the syndrome, so it has been considered to be mainly responsible for the disease [5]. Some other phytopathogenic fungi were also found to be associated with the disease at the beginning of the outbreak [9].
Many studies have been performed on OQDS, aiming at elucidating the cultivar susceptibility, detection aspects, taxonomy, pathogen origin and vector spread in the area, economic implications, and eradication measures [6]. However, very little attention has been given to the predisposing factors that could have played a role in promoting the initial outbreak and further spread of the disease in the Salento area. In the present brief report, some environmental factors that may have interfered with the particular olive agro-ecosystem of Salento in enhancing the epidemic of OQDS are presented and discussed. They could serve as points for further insights.

2. Resilience of Olive Agro-Ecosystem of Salento

The occurrence of olive in Salento (Apulia, Italy) dates back to the Bronze Age [10], and over the subsequent millennia always represented a fundamental component of the local agriculture and landscape. Before the initial outbreak of OQDS, the total amount of olive groves located in the three provinces of Salento (Brindisi, Taranto, and Lecce) was about 200,000 ha [11]. In most cases the groves were obtained by grafting wild olive plants with scions of local cultivars, namely Ogliarola salentina and Cellina di Nardò. Considering the economic importance of the crop, the golden age was reached during the 19th century, when lamp oil produced in the area was considered to be the best oil for lighting in all major capitals of Europe and North America [12], and most of the oil from the area was intended for this purpose. During this time, this agro-ecosystem demonstrated its resilience to some abiotic and biotic dangers that sometimes severely threatened the crop. Two episodes of severe leaf scorch and twig withering, locally called *brusca*, threatened part of the olive groves of Salento at the end of the 18th and the beginning of the 20th centuries. In both cases, mainly in the eastern areas in Lecce province, trees of the cultivars Ogliarola salentina and, to a lesser extent, Cellina di Nardò were severely affected during autumn and early spring by unknown foliar disease that caused extensive twig die-back, defoliation, and associated economic losses [13]. Studies performed during the 20th century allowed researchers to distinguish two kinds of *brusca*: an abiotic *brusca* incited by physical causes that affect the water supply to the tree (i.e., insufficient water in soil, damage to the root system, hot and dry winds, salty winds, nutrient deficiency), and a biotic *brusca* caused by a weak foliar pathogen, *Phyllosticta panizzei* [13]. Lionello Petri, the pathologist who described the cycle of disease of the biotic *brusca*, described the phenomenon as “a specific marker of an internal alteration of the physiological processes of the leaf, alteration which in the absence of a parasite could remain without external visible symptoms” [14,15]. However, with both kinds of *brusca*, olive trees returned to being productive in a short time, indicating a remarkable equilibrium between the agro-ecosystem of Salento and the environmental threats occurring in the area in those times.

3. Current Characteristics of the Olive Groves of Salento

Ogliarola salentina and Cellina di Nardò are still the main cultivars of the Salento olive groves and yield an oil characterized by a very high polyphenol content [16] when trained with modern methods of cultivation and harvest. Their production is regulated by the European Commission according to the Protected Geographical Indication (PGI) rules. Besides orchards of the same age and the same cultivar obtained by clonal propagation and trained with regular spaces, there are many examples of olive groves where trees of different ages coexist and the two cultivars are intermingled. In addition, the occurrence of centennial trees, usually obtained by grafting wild plants scattered in the area, is quite frequent. Traditionally, the number of trees per hectare in such groves ranges between 80 and 120 [17], and the tree is trained as a “vase” with two or three main branches. All the soils are of calcareous origin with a sandy texture, with the frequent occurrence of rocks at ground level. The agronomic techniques usually applied in the area concerned the maintenance of a regular shape of the tree crown through pruning performed on an irregular timeframe and the spraying of agrochemicals to control the main pests and pathogens (*Bactrocera oleae*, *Spilócea oleaginea*, *Colletotrichum gloeosporioides*). In general, applying soil fertilizers is quite rare, whereas using herbicides to control weeds and prepare the ground
for harvest is quite frequent. In the past, the latter practice also induced some problems in the area where OQDS was subsequently found for the first time [18].

The introduction of the Statutory Management Requirements by the European Commission in 2004 for maintaining the land in good agricultural and environmental conditions (GAEC) ruled by standard 4.3, “Maintenance of olive groves in good vegetative conditions,” and the single payment scheme influenced olive farmers in the application of basic agronomic techniques [19]. The GAEC standards were intended to ensure a minimum level of land maintenance and avoid the deterioration of habitats. This should be obtained through (a) pruning olive trees at least every five years, and (b) removing suckers, brambles, weeds, and vegetation between the rows every three years. However, each region modified the timeframe of the conditions, and in the Salento area, tree pruning been generally been performed every four to five years, whereas weed control, mainly with herbicides, is applied several times per year, resulting in a strong increase of their utilization in the whole area during a prolonged timeframe [20].

4. Occurrence of Olive Quick Decline Syndrome in the Salento Olive Agro-Ecosystem

The first symptoms of an olive disease of unknown origin were observed in the Gallipoli area (Lecce province) around 2007–2008 when extensive leaf scorching and twig and branch die-back were observed on both young and centennial trees [21]. Considering that the time between the pathogen’s colonizing of the tree and the appearance of the first visible symptoms can be more than one year, as observed with artificially inoculated young olive plants [22] and epidemiological parameters [8], the initial infection could have taken place a few years before. Subsequently, the disease spread in the area surrounding Gallipoli to the point where, at the time of the first official record (October 2013) the number of hectares showing symptoms of OQDS ranged between 8000 and 10,000 [4,21], corresponding to about 800,000–1,000,000 olive trees. At Oria (Brindisi province), another OQDS outbreak was found a few months later. The plant quarantine bacterium X. f. subsp. pauca was found associated with the disease [3,4] and the rules established by the EPPO to try to eliminate it from the area had to be applied. However, according to the criteria elucidated by Sosnowski et al. [23], successful pathogen eradication in a certain area requires: (a) prompt identification of the causal agent found in a new site, (b) a restricted and limited infected area, (c) effective local organization for crop uprooting, and (d) biological features of the micro-organism that guarantee its complete elimination. Such a huge infected area and the fact that X. f. subsp. pauca can survive on many wild plant species [24,25] would not have facilitated the reliable elimination of the microorganism from the area, so it was retained as “infected” and the eradication measures were applied northward.

Together with X. f. subsp. pauca, some pathogenic fungi such as Phaeoacremonium parasiticum, P. rubrigenum, P. aleophilum, P. alvesi, and Neofusicoccum sp. and the leopard moth Zeuzera pyrina were frequently found associated with OQDS [4,9,26]. However, due to the relevance to plant quarantine pathogens, most of the studies concerned X. f. subsp. pauca and its vectors. Studies on the vectors established that the meadow spittlebug Philaenus spumarius acts as an efficient vector for the bacterium [27], and it has been estimated that such a vector can spread the pathogen, starting at one site, up to a distance of 20 km per year [7]. The continuous occurrence of olive trees over many kilometers in Salento has largely facilitated the expansion of the pathogen in the area [28].

5. Predisposing Factors for Olive Quick Decline Syndrome Acting on the Salento Olive Agro-Ecosystem

The introduction of X. f. subsp. pauca in Salento has been linked with the import of infected ornamental coffee plants (Coffee arabica L.) from Central America [29,30]. Subsequent to their introduction in Apulia, Xylella-infected coffee plants from Central America have been repeatedly intercepted in northern Italy [31] and Europe [32]. In addition, the interception of other X. f. subsp. pauca strains associated with ornamental coffee plants has also occurred in France [33]. In Italy and Europe, ornamental coffee plants are utilized for indoor ambience. Considering the huge amount of ornamental
plants that are globally marketed from Central America every year (more than 43,000,000 plants just from Costa Rica during 2012; https://www.focus.it/ambiente/natura/xylella-il-batterio-killer-degli-olivi), the possibility that, over many years of commerce, one or few coffee plants infected by X. f. subsp. pauca reached only two nurseries in Gallipoli and Oria appears rather small, since this ornamental plant has been utilized quite a bit in Italy. In addition, the cultivation of olive is widespread almost everywhere, except in the most northern areas of the country. Similarly, the meadow spittlebug is widespread in Italy and in all Mediterranean countries [34]. The possibility that some particular factors predisposed the olive groves of Salento to the OQDS outbreaks and spread cannot be excluded and deserves examination.

5.1. Cultivar Susceptibility

The cultivar susceptibility to pathogens is an important factor that can deeply influence the outcome of a disease in a territory. In the case of OQDS, the local olive cultivars Ogliarola salentina and Cellina di Nardò are very susceptible to attack by X. f. subsp. pauca [5]. X. fastidiosa proliferates within the xylem vessels of the plant, thus impeding the normal water translocation from the root to the crown, which subsequently leads to plant death [35]. There are also indications that the plant reacts to xylem colonization by the pathogen by inciting cavitation and embolism, followed by the production of tyloses containing gums, pectin gel, crystals, resins, and phenolic compounds, which causes vessel occlusion and twig die-back [36]. Generally, the most susceptible cultivars show more vessel occlusions [37]. A similar situation was observed for olive cultivars with symptoms of OQDS that showed many occlusions and relatively few X. f. subsp. pauca cells in the xylem of the branches [38]. However, within the leaf petiole of the same cultivar a very high number of X. f. subsp. pauca cells was observed, causing xylem vessel blockage that led to leaf and twig die-back [39]. It would seem that, in any case, the local cultivars suffer from bacterium attack by producing tyloses in the branches or by being directly occluded in the leaf petiole xylem vessels.

In addition to X. f. subsp. pauca, Ogliarola salentina and Cellina di Nardò can also be damaged by some fungi and bacteria that can contribute to OQDS, such as Phaeoacremonium spp. [9,26] and Pseudomonas savastanoi pv. savastanoi, the causal agent of olive knot. In Salento, the latter have affected both cultivars since ancient times [40]. It should also be noted that this bacterium, during its colonization and multiplication stages, can contribute to the overall decrease of plant defense substances. Indeed, a reduction of phenolic compounds released from plant cell vacuoles during the induction of cavity formation incited by the pathogen’s pectolytic and hemicellulolytic enzymatic activity has been observed (Figure 1) [41].

![Figure 1. Symptoms on trunk and twigs of olive trees induced by Pseudomonas savastanoi pv. savastanoi. The infection causes a decrease of compounds related to the tree defense, such as phenolic compounds.](image_url)
Consequently, if there are frequent attacks, the tree could be depleted of such compounds. However, the possible synergistic effect of X. f. subsp. pauca, fungi, and P. s. pv. savastanoi in inciting and/or increasing the symptoms of OQDS has not been investigated and would deserve particular attention. It should be noted, indeed, that there can be a long period of time between the initial observation of OQDS symptoms and the final collapse of the tree, and during this period detrimental pathogens could interact.

5.2. Soil Ionome Characteristics

Similar to Salento, the areas of northern Apulia are considered suitable for the survival and spread of X. f. subsp. pauca in olive trees [42]. However, there are some epidemiological features of OQDS that should be carefully analyzed. Taking into account that the initial outbreaks of OQDS occurred 15 years ago and the estimated spread of the vector is 20 km per year [7], the expected arrival of the pathogen in the northern areas of Apulia or the neighboring region (Basilicata) has not occurred, so far. It should also be added that the vector could spread the bacterium in the olive groves as hitchhikers or by feeding on wild plant species [25]. The bacterium has spread from the initial outbreak area to a distance of about 100 km [8]. Consequently, it would be important to investigate how it has been possible to arrive at such a different situation considering that the origin of soils in both areas is quite similar. Apulia soils, even though they have different geological ages, are all of calcareous origin [43].

The soil environmental conditions can also deeply influence the plant when facing disease, and soil management is critical in order to apply effective pathogen control strategies [1,44]. A study carried out by comparing the ionome composition of soil and leaves taken from olive groves of distinct areas of Apulia characterized by the presence or absence of OQDS (Lecce, Taranto, and Brindisi groves with OQDS and areas north of Bari without) revealed a marked difference between the two areas [45]. In particular, a higher content of zinc, copper, and manganese in both soil and leaves was found in areas north of Bari still free from OQDS with respect to southern Salento areas (Figure 2).

*Figure 2.* Soil ionome comparison between areas of Apulia and Basilicata (BAT-PZ) not affected by olive quick decline syndrome (OQDS) and areas of Salento (BR, LE, TA) characterized by the occurrence of OQDS revealed different zinc (Zn) and copper (Cu) content despite a similar calcareous origin. Areas where OQDS is present show a marked reduction of both ions.

These ions are micronutrients essential for plant growth: zinc is involved as a cofactor in many enzymes, specifically alcohol dehydrogenase, carbonic anhydrase, and RNA polymerase [46], whereas
copper is essential for the formation of chlorophyll [47]. These ions are also deeply involved in the plant defense mechanisms [48]. *Capsicum annuum* Zinc Finger Protein 1 (CAZFP1), a zinc-finger protein gene, encodes a transcription factor that is accumulated in the early phase of the infection caused by *Xanthomonas campestris* pv. *vesicatoria* in pepper fruits [49]. In addition, the zinc finger binding domains are related to the effector-triggered immune response [50]. Copper is linked in the receptor molecule for ethylene, an important signal for plant development and disease resistance [53]. In addition, high zinc concentrations can protect plants by direct toxicity and by zinc-triggered organic defenses [51,52].

*Manganese* is involved in the synthesis of phenolic compounds and terpenes [56] as well as salicylate and ethylene-dependent pathways [55]. Manganese is involved in the synthesis of defense-related molecules and is related to the interaction between *X. fastidiosa* and host plant. *X. fastidiosa* biofilm formation is inhibited by copper and zinc concentrations higher than 200 and 0.25 mM, respectively [57], and in planta zinc detoxification is required to trigger the full virulence of the pathogen [58]. Within this context, it has been observed that olive trees infected by *X. f. subsp. pauca* show a low copper content within the leaves [59].

It is evident that Salento soils show a depletion of some important micronutrients (zinc, copper, manganese) and that such reduction can have direct negative consequences for the olive physiology and reaction to biotic stress.

5.3. Agronomical Practises

Over the long term, the agronomical practices applied to soil are strictly linked to its fertility and could have putatively played a role in augmenting the severity of OQDS in Salento. In the whole area, regular light tillage and/or harrowing of soil surface became rare during the last decades, whereas the utilization of herbicides, mainly glyphosate, to clean the soil and prepare the harvest is quite common [20,60]. This practice, when performed over a long period and more than once per year, can have negative effects on olive trees [61]. In the Salento area, herbicide is applied to the soil on average about three times per year [60]. Despite the advantage of obtaining a clean grove and eliminating weeds that compete with the trees for water resources, the overall availability and content of organic matter, nitrogen, and phosphorous in the soil decreases over the years [61]. In addition, over the long term there is a strong reduction of pseudomonads that control soil-borne pathogenic fungi and an increase of agrobacteria that act in manganese oxidation [62,63]. Prolonged utilization of glyphosate can also induce reduced zinc and copper assimilation in the plant [64]. The latter features could be related to the reduced manganese, copper, and zinc content found in Salento soils and olive leaves [45], as well as in Brazil in leaves of orange trees infected by *X. f. subsp. pauca* that were grown in glyphosate-treated soil [65]. A perturbation of metabolic pathways in the beneficial pseudomonads has been also observed in soils repeatedly treated with glyphosate [66], leading to a reduced capacity to face disease [67]. Impairment of the shikimic acid pathway due to the utilization of glyphosate has been observed to be strictly linked to crop vulnerability to disease [65,67]. It should be added that soil fertilization has been rarely performed on a regular basis in the Salento area. The agronomic practices for soil management over the last decades have mainly been based on the repeated utilization of glyphosate, which could have led to a relevant perturbation of the metabolic activity of the soil microflora and a depletion of some micronutrients, thus predisposing the trees to OQDS. These aspects deserve ad hoc studies to clarify all possible interrelationships between the different parameters to establish a direct link to OQDS, as recently observed for algae [68].

5.4. Tree Pruning

The pruning of olive trees has a direct influence on both the tree physiology and the quality of the oil [69]. It is worth noting that in adult trees, pruning is carried out to possibly maintain the
equilibrium between vegetative and reproductive functions in order to reduce the alternate bearing, and to facilitate pest and disease control as well as the harvest (Figure 3) [70,71].

Performing hard pruning on a four- to five-year basis, as usually done in many olive groves in Salento, has a negative influence on the trees, causing shading, an irrational spatial distribution of the canopy, shorter and compact vegetation, and deadwood in the inner part of the vegetation, resulting in lower tree productivity [72], and generally it is not considered a good practice for olive [71]. Further, hard pruning carried out in an attempt to reduce the X. f. subsp. pauca inoculum load within the tree results in subsequent further weakening and death (Figure 4) [22]. Excessive hard pruning, especially of centennial olives, could have weakened the trees and predisposed them to further biotic attacks.

Figure 3. An example of a centennial olive tree showing good pruning care. Note the reddish color of the trunk due to ferric sulfate.

Figure 4. Hard pruning performed on centennial trees to reduce Xylella fastidiosa subsp. pauca inoculums greatly worsens the symptoms of OQDS.
5.5. Climatic Changes

The climatic changes currently affecting Mediterranean areas could have played a role in enhancing the susceptibility of the local cultivars to OQDS. In recent years, extreme precipitation phenomena have dramatically increased in the Mediterranean Basin [73]. During the last years, the Salento area faced relatively more extreme precipitation events (a very large amount of rain in few hours on a relatively small area), sometimes accompanied by strong wind and hail. Apart from the direct damage to vegetation, there are also indirect negative consequences for the tree physiology due to prolonged water permanence on soils that in many cases are very compact due to the absence of proper and regular management or the presence of emerging rocks (Figure 5). When the soil is saturated with water for some days, anaerobic conditions prevail, inducing root hypoxia and leading to negative consequences for the olive trees [74,75].

![Figure 5. Waterlogging in olive groves due to a relative excess of precipitation occurring over a short time. The soil of Salento where olive groves are cultivated can be very solid, which does not allow the water to infiltrate. Prolonged anaerobic conditions in the soil can occur, causing deleterious effects to the root system of the trees.](image)

As a consequence of waterlogging there is a reduction of the photosynthesis rate [76] and reduced absorption and transport of water and nutrients, negatively influencing growth, flowering, fruit set, and drupe development [77]. Recurrent instances of waterlogging can dramatically worsen the situation. In addition, strong wind and hail can cause direct damage to the trees and favor the colonization of other phytopathogens such as P. s. pv. savastanoi. In the area, there are also frequent hot winds that usually blow from the south, causing extensive leaf withering and defoliation during spring and autumn [13]. A warm wind blowing from the east in early May 2020 augmented the wilting of olive groves facing the Adriatic Sea that already showed OQDS.

In the Mediterranean Basin, frost and drought events are becoming less rare than in previous decades. Frost occurs not only in winter, but sometimes also in early spring. As an example, in the first decade of January 2017, in some areas of Lecce province, over one week the daily minimum temperature reached values below 0 °C and on some days it reached −4.0 and −5.1 °C (Figure 6) [78].

Such low temperatures can have direct negative effects on olive, like defoliation and bark fissuration [79]. By contrast, during July–August 2015, for more than 40 consecutive days the maximum temperature in areas of Lecce province was 1–4 °C higher than historical records for the area, whereas in summer 2017, in the same areas, the total rainfall was only 7.8 mm from 1 June to 30 August [78]. The frost events of January 2017 reduced the X. f. subsp. pauca cell density within the olive leaves, although, as the air temperature increased during spring, the bacterium proliferated again [78].
Figure 6. During 2017 the Salento area faced frost (first part of January) and drought (only 7.8 mm of rainfall from 1 June to 30 August). These severe phenomena have occurred more often in the last decades than in the past. Reproduced with permission from [78].
Drought can also cause harmful consequences for olive cultivation, particularly under rainfed conditions, and represents one of the most critical factors for crop maintenance in the Mediterranean Basin [80]. It causes a decrease in leaf water content, resulting in a loss of turgor and an associated reduction of photosynthesis [81]. In addition, since xylem potential decreases, there is an increase of xylem cavitation, which can cause embolism and the interruption of water transport from the root to the canopy [82]. Drought stress results in a marked reduction of nutrient uptake within the plant, resulting in reduced yield, fruit dry mass, and oil accumulation [83,84]. Ethylene might inhibit plant growth under drought conditions, as it is involved in leaf abscission [85]. It is evident that such adverse and recurrent climatic events could negatively predispose the trees to any kind of further biotic and abiotic perturbation, possibly including also OQDS.

6. Conclusions

The current olive agro-ecosystem of Salento appears to be managed differently now than in the past and seems to be more prone to damage by pests and pathogens introduced from abroad. The features that characterize the areas where OQDS first appeared and subsequently spread reveal a rather fragile environment where one or more adverse climatic and/or edaphic factors acting together with the peculiarities of the olive groves could have played a role in enhancing the virulence of X. f. subsp. pauca once introduced from abroad. The susceptibility of the local cultivars and some agronomic practices that have depleted the soil fertility over the long term could have further worsened the situation. The bacterium has also found a very efficient vector that favors tree colonization and further spread of the disease in the territory.

During the last decades, most of the olive groves received just basic care, mainly to maintain the shape of the crown and harvest what the tree yielded, with little attention paid to restoring or improving the soil fertility. In addition to climatic stress, such as excess precipitation, frost, and drought, the additional negative impact could have been due to the occurrence of other phytopathogenic microorganisms that, together with X. f. subsp. pauca, further weakened the tree [86], so that over a little more than a decade, a great part of the olive groves of Lecce province became totally withered.

The restoration of the Salento agro-ecosystem nowadays appears quite problematic, since many areas have been abandoned by farmers discouraged from the very beginning to apply any kind of control strategy that could have reduced the impact of X. f. subsp. pauca and OQDS [87]. This abandonment of any kind of agronomical practice largely contributed to augmenting the proliferation of the vector, and consequently further expansion of the pathogen in the area (Figure 7).

![Figure 7. Abandonment of olive groves led to further spread of Xylella fastidiosa subsp. pauca due to proliferation of the insect vector.](image-url)
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