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The Olive Quick Decline Syndrome: a Syndemic Outbreak in the Apulia Region, Southern Italy

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Abstract

Since a decade in Apulia, south-east of Italy, an increasing number of olive trees developed the quick decline syndrome (OQDS) leading to partial or total dessication of the canopy and subsequent death. Currently six million of olive trees show the symptoms of the decline, despite the mitigation measures which were undertaken to contrast the progression of the dessication. Associated with the syndrome, several phytopathogenic fungi were detected in the rhizosphere, endosphere and phyllosphere of the trees, along with the phytopathogenic bacterium *Xylella fastidiosa* subsp. *pauca*. Alongside, other pathogenic events were clearly identified, mostly defeating soil resilience: salinization, pollution, erosion, decline of biodiversity. Further events include delays in the adoption of appropriate mitigation measures not directed to challenge solely a bacterial pathogen, misuse of the territory, erratic agronomic management practices. The OQDS impacted also societal aspects. All the above concurrent causes strongly suggest that (1) the olive quick decline in Apulia is not a too symplistic epidemic outbreak due to a bacterium, but rather a syndemic outbreak formed by several diverse biotic and abiotic pathologies and (2) only a more holistic approach can help coping with the uncertainties and difficulties of an enduring co- existance with this syndemic events.

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Introduction

An increasing number of cases of olive tree decline in Apulia, an area located at the south-east of Italy, extending for 19,350 km2 with a perimeter of 1,260 km and an overall coastal development of 784 km, the largest in mainland Italy [1], were observed during the years 2008-2009 by farmers and regional technicians. The extent of this event had never been noticed in the years before. The presence of the olive quick decline syndrome (OQDS) was reported since 2013 over an area of 10.000 hectares, and firstly described as the Complex of the Rapid Olive Dessication (Co.Di.R.O.) in Salento, a sub-regional area of Puglia [2-5]. Within this complex the quarantine phytopathogenic bacterium *Xylella fastidiosa* was identified, requiring eradication measures [6] [7]. However, in the case of *Xylella fastidiosa* subsp. *pauca* the asymptomatic period for olive was then estimated up to 390 days [8], thus delaying the implementation of the mitigation measures.During seven years the OQDS outbreak expanded, even in the presence of partial eradication measures, and in 2020 about 6 million olive trees in Puglia showed the symptoms of complete or partial decline including dessication over an area of 350.000 hectares, mainly northward from the Province of Lecce towards the Provinces of Brindisi, Taranto and Bari. Unfortunately the symptoms of OQDS were inferred, soon after the first reports, exclusively to *Xylella fastidiosa* subsp. *pauca,* overlooking any other possible co-factor: the environment, the agronomic management practices, the climate, the soil, the aquifers, the social and cultural aspects. This resulted in a quite reductive bi-univocal vision plant/pathogen and viceversa. Therefore, a critical retrospective view of the outbreak seems appropriate, considering that the eradication of the plant pathogen is not feasible [9-11] and that the co-existance with the OQDS encompasses new approches [11, 12]. The aim of this paper is to discuss the progressive deterioration of the soil health, the progressive salinization and contamination of soil and aquifers, the presence of different concurrent phytopathogens, the loss of plants resilience in the area where the olive decline outbreak had occurred, along with the mitigation measures undertaken to defeat this syndemia.

Soil Degradation and the Complex of Olive Tree Decline

Soil deterioration includes both natural and man-made events, the latter usually known as "soil degradation". This process causes a decrease of the actual or potential soil capacity to give rise to products or services. As much as 40 years ago, due to increase in salinity and alkalization, 200-300.000 ha/y were lost in industrialized countries. Twenty years later, the European Environmental Agency [13] listed as european soils affected by degradation (in million ha): 115 due to erosion, 42 due to wind, 85 due to acidification, 180 due to pesticide pollution, 170 due to nitrates and phosphates pollution, 33 for compaction, 3.2 for organic matter loss, 3.8 for salinization, 0.8 for waterlogging/anoxia. A few years later, aiming at contrasting progressive soil losses, the European Commission [14,15] officially listed the following causes of soil degradation: erosion, pollution (localized and diffuse), salinization/alkalization, decrease of organic matter content. Currently 84% of agricultural soils in the central and southern EU are below the threshold of 3.5% of organic matter; in Italy, the average is 2% and in Apulia, in the area of OQDS, is of about 0.8-1.3%. In addition cementification, overbuilding, flooding, compaction, and loss of soil biodiversity are among other factors that depauperize the soil. The treshold of 3.5 % of organic matter is the minimum level to maintain the soil functional biodiversity, i.e. the correct functioning of biogenic cycles [16]. Although the decline of organic matter content in Apulia follows the same trend as in the central and southern Europe, the damages deriving from soil erosion and salinization can be higher, particularly for the decrease of soil biodiversity and fertility [17]. The survey carried out by the Competent Authorities in Apulia has shown that soil deterioration is relevant, and the ESAs (Environmentally Sensitive Areas) index, i.e. sensitivity of a given area to desertification, is highly endangered since many years throughout the whole territory from northern Tavoliere to lower Salento. This vulnerability may be due to morphological and geografical reasons as well as to anthropogenic activities, the latter leading to the loss of sustainability of the resources since more than a decade [18] (Fig 1).

The anthropogenic causes include the overuse of pesticides, the presence of abandoned pieces of land

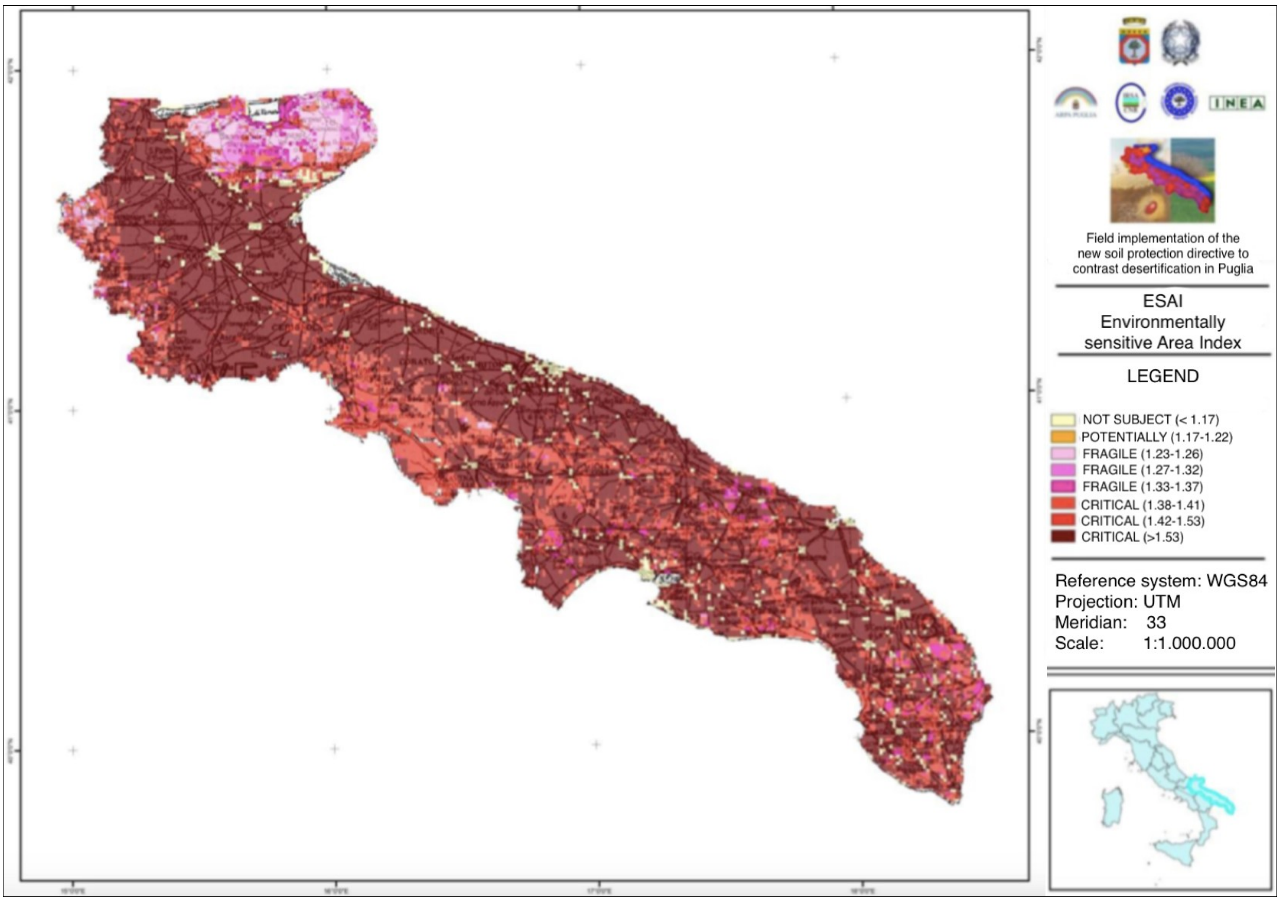

Figure 1. Map of the environmental sensitivity to desertification of Puglia (ARPA, 2010). [18]

(the so-called "amateurial properties", i.e. properties of people not resident in Apulia, visiting occasionally their property, with or without olive orchards completely overlooked and not agronomically managed), the overexploitation of water resources leading directly to progessive desertification. Although we know that this event is almost invariably irreversible, it seems that the agronomic management of the specific territory where the olive decline was started and then expanded has not changed much, mantaining therefore high the level of risk. This includes (a) the size of the olive trees, well above the average of the european olive either because of age and of absence of pruning, and (b) the overall number of olive trees, reaching about 65 million in Puglia of which 11 million only in the Salento peninsula. The agro- ecosystem has become at risk, despite the natural resilience to drought of the olive trees. Agronomists and regional technicians have begun since a decade to detect formerly undetected phytopathogens. It is well known that among the effects of desertification there is the depletion of soil life and functioning [19, 20].

Water Resources and Salinization

Throughout the Salento and coastal areas of Murgia the subterranean aquifer is of salted marine origin. Up to about thirty years ago the water wells were shallow, 3-10 meters deep. During more recent years the wells were digged through rock layer drilling to reach the deeper aquifer where salty and sweet waters co-exist, depending on the hydraulic loads of salty and sweet waters, respectively. Due to the karstic nature of the rock, along with unappropriate anthropic intervention among which numerous artesian wells, abusive (about 100.000 not registered) and overexploited, the balanced underground water resources have been compromised. Most of the surface aquifers are now empty, and their waters are called not a resource but "aquifer exudates" instead, the salty waters being recalled from the sea up to the plant roots.

The risks associated with the salinization and anthropic contamination have been shown by Fidelibus and Tulipano [21]. The same Authors predicted that the desertification of Apulia territory would have started from the Salento peninsula after 10-12 years, which then happened indeed, unfortunately. The University of Salento confirmed these serious difficulties [22]. The same conclusions were drawn by the Health and Environment Centre [23] and by the Regione Puglia [24]. The projects on this aspect, to mitigate the above risks, have not taken off yet. The survey of the quality of surface waters and of the aquifers [25] has shown a strong chemical impairment level of water resources in the areas of the QODS, i.e. a relevant anthropic impact with poor hydro-chemical traits, including excess of nitrates and a stress above the utilization potential of water resources. It is known that prolonged drought and soil salinization close to the root canopy can lead to growth reduction of the aboveground olive organs than of the underground counterparts [26], although the salt tolerance in olive may depend on the variety [27], being cultivars Leccino sensitive and Frantoio or Piqual relatively tolerant. However intrusion of seawater in the aquifers can threaten soil biodiversity along with crop yields and the survival of some soil communities can be threatened, leading to the loss of biodiversity of the biota [28].The same can occur for soil and rhizospheric microbes, including symbionts [29-31], and their biodiversity [32]. This, in turn, can expose the olive trees to the risk of disbioses and of a reduced resilience towards biotic and abiotic stresses.

Soil Pollution

Besides the risks associated to the intrusion of saline waters in the aquifers, hopefully not irreversible, in the last few decades there has been an increasing overuse of pesticides and misuse of chemical inorganic fertilizers leading to remarkable soil and aquifers stress.

The latter consist of nitrates, iron, manganese, ammonium ions, electric conducibility, clorine, sulfates which resulted almost invariably above the permitted concentrations. The causes were inferred to the misuse of nitrogen fertilizers, the overuse of zootechnical waste disposal, bad sludge management, dispersion of sewer networks, misfunctioning of disposal installations [23] [33]. As far as pesticides are concerned, in Apulia 11.500 tonnes of phytopharmaceuticals were used in 2013, including chlorpyrifos which was later prohibited because residues had been found in olive oil. This amount corresponds to 5.500 tonnes of active substances. In one soil sample the simultaneous presence of fifteen different pesticides was detected [34]. More recently, having in mind the sole biunivocal relationship OQDS/bacterium, the use of pesticides such as neonicotinoids (acetamiprid and imidacloprid) and pyretroids (deltamethrin and lambda-cyalotrin) was authorized by the national Competent Authorities to challenge the insect vector of *Xylella fastidiosa,* i.e. *Phylaenus spumarius* [9][34-36]. Among herbicides, there has been an increasing usage of glyphosate as an effective aid to control weeds also in olive orchards. Although it has become the best-selling herbicide used in agriculture, horticulture, silviculture, and urban environments, it has been argued that its residues may interfere with resilience of crops to pests and phytopathogens, and with both fungal and insect biological control, which is especially relevant considering the global call for integrated pest management strategies [37]. A study has shown that this broad- spectrum, post-emergence herbicide alters the soil texture and microbial diversity by reducing the microbial richness and increasing the population of phytopathogenic fungi [38]. In temperate climate, glyphosate-based herbicides significantly decrease root mycorrhization, soil arbuscular mycorrhizal fungi (AMF) spore biomass, vesicles and propagules [39-41]. Studies testing glyphosate effects on AMF show an inhibition of arbuscular mycorrhizae fungal spore germination and germ tube growth or reduced mycorrhizae in soil, though only at concentrations greater than those recommended for field use. A cumulative risk assessment, never done yet, for repeated use, overusage or misuse of glyphosate in olive orchards could clarify its impact, also in connection with drainage waters, rainfalls and irrigation. In fact leaching and soil erosion by water can transport glyphosate from land to water environments [42], while mycorrhizae could lead to stronger absorption of glyphosate by binding and enmeshing soil particles into larger aggregates [43]. Leaching of glyphosate after a simulated rainfall was substantial and altered by earthworms and AMF [44] indicating an impairment of the soil biota. An extensive utilization of glyphosate can also determine a reduction of some soil micronutrients such as zinc, copper and manganese [45]. A depletion of such ions was found in olive groves of Salento area showing severe OQDS sympr*o*ms [46].

Climate

The climatic suitability for the potential establishment of X. fastidiosa is highly variable throughout within the EU individual MSs [8]. However, the south of the EU has been evaluated as being the most at risk. There is considerable uncertainty about recent estimates of climatic suitability, particularly considering the subspecies or sequence type level. This is due to lack of data and a potential bias in reported cases, and in particular at northern european latitudes where symptoms expression of *X. fastidiosa* is lacking, may bias current climatic suitability estimates. Nonetheless, variability of climatic suitability within EU individual Member States may be accounted for when designing a survey [47], taking into consideration that the resilience and crop and olive yield may depend on climatic factors [48, 49], and that the symptoms of the dessiccation can be confused with with the symptoms of water stress and drought [47] [50].

Phytopathogenic Microorganisms

During the first surveys after the occurrence of the OQDS several phytopathogens were identified in the rhizosphere, phyllosphere or as endophytic of olive trees: (i) among fungi, *Phaeoacremonium aleophilum, Phaeoacremonium alvesii, Phaeoacremonium parasiticum, Phaeoacremonium italicum, Phaeoacremonium sicilianum, Phaeoacremonium scolyti, Neofusicoccum parvum, Pleurostomophora richardsiae*, *Pleurostomophora richardsiae* [34] [51,53],

Verticillium dahliae [2] [54]; (ii) among bacteria *Xylella fastidiosa* subsp. *pauca* [55,56]. In addition *Phylaenus spumarius,* and other insect vectors of Xylella were found [57,58], and *Zeuzera pyrina* L. a Lepidopteran of the Family Cossidae; among macrobiota, nematodes were sporadically detected in olive root canopy (Figure 2). The latter, at the larval stage, start digging tiny tunnels at the apex of the smaller olive branches 1-2 years old, and then they dig larger tunnels on the main branches and finally on the trunk.

During a subsequent survey in the buffer area, 41.520 samples were analyzed of which 1.518 symptomatic. *Xylella fastidiosa* was identified in only one case [5]. Another survey showed that the bacterium could be detected, using the biochemical (ELISA) test and the molecular (PCR) reaction, in 1.8% of the tested samples [59]. A positive test is given by alive and also by dead cells of the bacterium [60].

The above data cast some doubts on the axiom of a unique responsible of the OQDS and the consequent mitigation and curative approaches. The concurrent presence of so many and diverse mycetes in adult olive trees is by someone considered as disease aggravators [61]. On the other hand the estimated

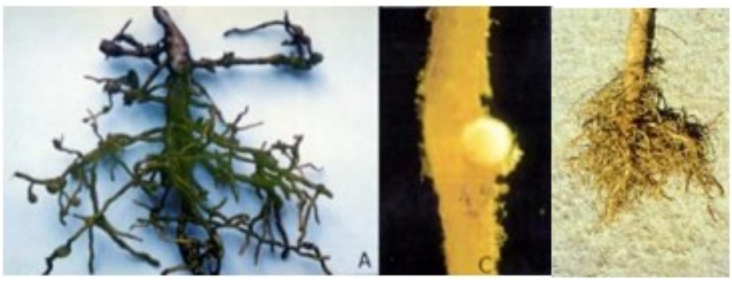

Figure 2. Symptoms of damaged olive roots by Nematodes (*Pratylencus vulnus*) present in Apulia.

probability of the most representative genera becoming infected by *Xylella fastidiosa* according to the current EU *Xylella* outbreak information places the genus *Olea* in the penultimate position after (in brackets the probability, 1 being equivalent to 100%): *Polygala* (0.551), *Helichrysum* (0.511), *Euryops* (0.471), *Calicotome* (0.452), *Genista* (0.315), *Spartium* (0.161), *Lavandula* (0.152), *Cistus* (0.126), *Prunus* (0.093), *Olea* (0.076), and before *Vitis* (0.057) [47]. All the remaining biotic and abiotic factors which can contribute to the increasing disappearence of the olive trees resilience are neglected, when designing risk mitigation measures.

Risk Mitigation Measures

Several attempts have been carried out to overcome the OQDS in Apulia, taking into consideration that the eradication of *X. f.* subsp. *pauca* and its insect vectors was not feasible and already in 2015 EFSA quoted [9] that other elements should be taken into account, since the establishment and spread of the pathogen have a high probability of finding a climatically suitable environment with few adverse abiotic factors. In addition the systematic use of insecticides for vector control may create environmental impacts. At that time, with regard to risk reduction options, strategies were envisaged for the prevention of introduction and for the containment of outbreaks, focusing on the two main pathways (plants for planting and infectious insect vectors) and combine the most effective options in an integrated approach. But soon it appeared that it was not enough, and that co-existance needed something more, including appropriate surveys according to different guidelines [47]. We should not forget that the severity of the outbreak is unprecedented in EU, despite the current presence of *Xylella fastidiosa* and its vectors in many European countries. Among the possible interventions Xiloyannis [10] enlisted the following targets (i) the content of soil organic carbon and the carbon stocks in the olive orchards, (ii) soil water retention potential, (iii) plant and soil microbiome (iv) plant management, i.e. pruning and nutrition. For the first time the dramatic situation of the olive plants underground was called into question along with a more appropriate plant management. On the other hand it is known that both crop resilience and yields depend on climatic factors [62], biodiversity and ability to stimulate the ecosystem functions, as well as an adequate information system of farmers [63, 64]. Other Aa. and International Organizations have stressed that it is essential to strenghten the binomial healthy soil - healthy and resilient plant [41][65,66]. The literature on olive microbiome is scanty. However, in a study aiming at revealing the influences on the tree microbiome but also to develop successful biocontrol strategie sagainst *Verticillium* wilt in olives [67], the olive leaf endosphere was shown to harbor mostly Proteobacteria, followed by Firmicutes, Actinobacteria, and Bacteroidetes. In addtion, a high portion of archaeal phyla Thaumarchaeota, Crenarchaeota, and Euryarchaeota were identified. All isolates with antagonistic activity against the olive-pathogenic fungus *Verticillium dahliae* Kleb. belong to *Bacillus amyloliquefaciens.* The presence of endophytes in the olive trees could be particularly relevant because it has been shown in *Citrus* that *X. fastidiosa* interacts in the xylematic channels with bacterial endophytes mainly with *Methylobacteriurn mesophilicum* and *Curtobaterium flaccumfaciens*. The latter species has been found at high frequency in asymptomatic plants, suggesting a role of this bacterium in the resistance of the plant to variegated chlorosis of agrumes caused by *X. fastidiosa* [68] . It is surprising that almost no data are available on the use of microbial biopesticides on olive trees as biocontrol agents for *X. fastidiosa,* fungi and nematodes. The same applies for the use of microbial biostimulants in olive orchards, although both categories of products are well described [69]. Preliminary results on the use of microbial consortia, containing mycorrhizal fungi and bacteria, as biofertilizers in olive orchards showing heavy symptoms of decline [70] indicate that the treatment stimulates the microbial activity at the root canopy level and that the plant can react at the leaf canopy level on both primary and secondary branches (Figure 3).

Based on these results, it can be assumed a dual activity of the microbial consortia used, a recovery of the soil vitality and the presence of endophytic microorganisms. A soil vitality recovering generally plays a key role in all crop systems, particularly for the plant growth, health and resilience against biotic and abiotic stressors. By contrast, a specific use of endophytic strains, counteracts the negative effects of pathogens, in which a potential plant-growth-promoting strain can

Figure 3. (left) Old olive tree heavily compromised which, after the first cycle of treatments with microbial consortia, has shown a clearly improved resilience and re-vegetation – (right) Younger tree showing and improved resilience to decline after pruning and treatment with consortia containing mycorrhizal fungi and bacteria (see also https://youtu.be/AXdbb4xrImQ).

have a certain contrast activity against endo-pathogens. Several other different experimentations on the use of microbial consortia on main crops, such as tomato, potato and mais [71] [72] [73], lead to the same assumptions, indicating that the role of a certain microbial consortium is firstly the recovering of the soil vitality, as well as the presence of endophytic strains for contrasting biotic stressors. Beneficial effects were noticed also using a mixture of copper and zinc to form a hydroxyacidic complex with citric acid [74].

The treatment of the soil with microbial consortia as biofertilizers does not exclude the agronomic treatments on leaves, if the tree is symptomatic because of other fungi. The latter, if not entering the xylematic vessels, can be treated by pruning. Overall, the soil health and olive resilience can be improved by adopting good agronomic practices and sustainable management systems [75, 76]. It has been recognized that relinquishing such practices may have facilitated the extension of the OQDS [61]. These practices include grafting, soil tillage, protection measures against insect vectors, pruning, fertilization with organic-based fertilizers and irrigation, soil management practices, careful monitoring and assessment of the healthy status of the environment.

Conclusive Remarks

There are currently 595 potential host species reported in the scientific literature [47], 221 of which have been found infected by *X. fastidiosa* in natural conditions, therefore suggesting the putative endemic presence of this bacterium in the central and southern EU. According to EFSA [47], the species *Prunus dulcis*, *Prunus avium*, *Polygala myrtifolia*, *Spartium junceum*, *Nerium oleander*, *Rhamnus alaternus* and *Rosmarinus officinalis* have been reported as being susceptible to at least three subspecies of *X. fastidiosa*. However, the decline events including the dessication in Europe is not even comparable to the ones occurring for olive trees in Apulia, the latter being even more intense in the coastal areas. Therefore, the bacterium cannot be considered the unique cause of the outbreak, although requiring by itself adequate mitigation measures. We believe that there is a need for a substantial shift of mitigation measures and of the field approach to co-existance with the multiple causes of the OQDS. In other terms we should cure the sick, not only the disease, and in this case the sick is the territory including the olive trees.

The progressive soil desertification and aquifer salinization, the progressive decrease of soil organic matter and increased (mis)use of pesticides and herbicides, the spread of phytopathogenic microorganisms including the quarantine bacterium *Xylella fastidiosa* subsp. *pauca* on commonly present host plants including the highly dense olive tree plantations, the increasing vulnerability of the territories involved in the OQDS, along with the lack of appropriate agricultural management practices and mitigation measures point clearly at a syndemic nature of the outbreak, rather than a too simplistic epidemic outbreak due to a sole, though harmful, phytopathogenic bacterium.

The syndemic nature of the threat we face in Apulia means that a more innovative and multifactorial approach is needed if we are to protect the soil health, the resilience of olive groves, the quality of the food produced and the health of the human communities.

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