ACCADEMIA NAZIONALE DEI LINCEI

Xylella

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Abbreviations

EC	*European Community*
CNR	*National research Council*
CoDiRO	*Complesso del Disseccamento rapido dell'olivo*
OQDS	*Olive Quick Decline Syndrome*
COST	*European Cooperation in Science and Technology*
DiSSPA	*Department of Soil, of Plants and Food Sciences*
EFSA	*European Food Safety Authority*
EPPO	*European and Mediterranean Plant Protection Organization*
EPS	*Extracellular Polysaccharides*
GdF	*Financial police*
IAMB	*Mediterranean Agronomic Institute of Bari*
IPSP	*Institute for Sustainable Plant Protection*
ISI	*Institute of Scientific Information*
ISPA	*Institute of Sciences of Food Production*
LAIR	*Law and Agroecology - Ius et Rus*
MiPAAF	*Ministry of Agricultural Food and Forestry Policies*
NAC	*N-Acetylcysteine*
EPIU	*Environmental Police Investigation Unit*
PCR	*Polymerase Chain Reaction*
PD	*Pearce's disease of grapevines*
subsp.	*subspecies*
EU	*European Union*

1. Summary and Conclusions

1.1. *Summary*

The report of the Group constituted to study the decline of olive trees in Apulia starts by recognizing the current state of scientific recognition of the Xylella bacterium as the cause of the *OQDS* (*CoDiRO*) disease of the olive trees - as analyzed by EFSA, verified on site during meetings with scientists and technicians, and based on the data available in the literature. Furthermore, special attention is given to the different interaction levels between scientists and environmental associations. The Group refers to six annexes the titles of which can be found below. The Group was tasked to prepare an analytical report pertaining to the information gathered, the opinion of various scientists, specialists and expert in the field, the concauses, especially of agrotechnical nature, which contribute to the intensification or reduction of the bacterium's toxicity, as well as the social and ethical implications arising from the case. All of these aspects are considered, including the danger that the presence of the bacterium in Apulia represents for all of Europe.

- Annex A. Chronology of the events in the development of *Xylella* epidemic, including a list of regional, national and international legislations which regulate similar situations. Active actions by MiPAAF, Apulia, and the EU.
- Annex B. Schedule of visits and interviews. Annotations from the visits and interviews with scientists and agrotechnical operators.
- Annex C. *Xylella* epidemic in Apulia: genomic analysis and source of infection.
- Annex D. Annotations extracted from the literature.
- Annex E. Annotations from the European Food Safety Authority (EFSA).
- Annex F. Bibliography.

1.2. *Conclusions*

The causal agent of the disease is *Xylella fastidiosa*, a conclusion that is no longer questionable. All the *Xylella* isolates can be attributed to an identical genotype: *Xylella fastidiosa* subsp. *pauca* and sequence type ST53, called the CoDiRO strain to distinguish it from other *pauca* strains. Their molecular homogeneity supports the idea that the origin of the disease is a single and recent source of infection. The vectors of the disease are insects and plants which are carried from nurseries to new olive orchards.

The relevance of potential concauses associated to a primary role - or to a less direct role - of *Xylella* is difficult to assess, since available data on the effect of multiple agrotechnical interventions and environmental conditions are, at best, insufficient. In this context, the EFSA's negative response to the suggestion of primary infection causes different from the bacterial infection, either biotic or abiotic is justified.

Xylella causes new and important diseases, and the spread of its vectors makes *Xylella* relevant and interesting because of the damages it causes. The pathogenicity in areas newly colonized by exotic strains of *Xylella* is the cause of plant-pathogen associations not reported previously.

The consequences of the bacterium's presence in Apulia, as well as in Corsica and in Provence-Alps-Cote d'Azur region, constitute a serious danger for Europe. Several strains of *Xylella*, present in the same host, may recombine their genomes, creating hybrids which, by initiating new speciation cycles, may attack and destroy important trees and shrubs, both wild and of agricultural interest present in the European continent.

The lincean Group does not understand the contrast opposing scientists from Bari vs Lecce on the *Xylella* case. Above all, the radicalization of the contrast, which has been reported to be caused by the competition for access to research grants for studying the new disease, cannot be justified. Moreover, the presence of local ideological positions, also extended to the *Xylella* case, must be reported, which opposes to the importance of scientific knowledge and refuses technologically advanced agricultural models. Conversely, it is essential to reduce dialectic redundancy which hinders the possibility of performing feasible experiments and the reaching of scientific conclusions that are necessary and preliminary to setting procedures and operational decisions.

Actions based on experimental results rather than on intuition and prejudice must be implemented. In light of this consideration, the lincean Group is surprised by the fact that easy agronomic experiments have not been yet carried out or even planned, although these procedures were well defined based on previous cases of epidemics occurred in America over the last 40 years.

Considering the results obtained, the Group concludes that the funds allocated to the research institutions in Bari were put to good use. Nevertheless, the early assignment of research contracts for agronomic field experiments may well contribute to bring the discussion back on a scientific path.

The interventions proposed by the European regulation addressing infection containment include the removal of infected plants in the outer strip of the containment area bordering the *buffer* zone, and an efficient monitoring to prevent the spread of the pathogen. Reducing the vector population by applying chemical or biological methods or through mechanical approaches or other sustainable procedures may contribute to such containment. The report lists a long set of measures which, if justified on the basis of locally-assessed experimental data, may be effective in containing the bacterium and in preventing its further spread. The assessment of the program proposed for the containment and cure of the disease includes four components: *Infection containment and management of olive groves where the bacterium is present; Curing the diseased plants; Need for research; Interaction between Institutions and retreat of incorrect statements on the case.*

Following the block of eradications due to the requisition of olive trees by the Judicial Authority, Apulia has recently drafted a Plan containing new measures. The lincean Group notes that, while the various versions of the Plan are accurate in defining the measures to be adopted, also due to the European regulations involved, they devote little attention to justifying, on the basis of definite data extracted from experimental verification carried out in the Apulian territory, the reasons for the implementation of such measures. In this regard, the lincean Group points out that new and simple agronomic experiments to foster a better definition of the implementation of the infection containment measures and to contribute to the rescue of monumental olive trees have not been yet carried out or planned. At a European level, Yves Bot, Advocate General at the European Court of Justice, when drawing conclusions within his assistance to the Court itself, confirmed the accuracy of the prevention measures provided and the need for their application at the earliest.

The lincean Group shares Bari researchers' conclusions on the *Xylella* case, which are also the ground for the EFSA conclusions, for they rest on solid scientific procedures. Additionally, it points out that some researchers who have contributed to the proposal of alternative hypothesis in light of scientific elements emerged during the past year, have not retreated their statements despite the scientific evidence provided during the last year nor have dissociated themselves from groups supporting the disproved hypothesis.

2. Introduction

The case of the *Xylella*, the bacterium which threatens to destroy olive trees in Apulia, caused the development of heterogeneous opinions, at first related to the possibility of various and numerous causes of the epidemic outbreak (Annotation 1). Hence, the Lincean Academy begins its involvement, in view of the possibility for the practice of scientific research to be used instrumentally for purposes that differ from scientific practice and for reasons not compatible with it.

Following up on a letter dated May 6th, 2015 by Prof. Giovanni Martelli, a member of the Lincean Academy, President Lamberto Maffei on March 10th, 2016, appointed a working group coordinated by the lincean member Francesco Salamini and composed of the same and of members Roberto Bassi and Giorgio Morelli to investigate the issue. There is a significant complication: the Apulian social world holds varied opinions on the case, which are frequently opposite to the ones held by those who experimentally addressed the research of the epidemic causal agent (b16).

Meanwhile, the data recently published (summarized in Annex C) provide that the causative agent of the *Olive Quick Decline Syndrome (OQDS, also known as CoDiRO)* is *Xylella fastidiosa* bacterium, subspecies *pauca*. The present report recognizes the scientific state of research on *Xylella* occurrence in Apulia, as duly analyzed by EFSA, verified on site during meetings with scientists and technicians, and by considering the data available in the literature. Furthermore, attention is given to the different interaction levels between scientists, farming, and environmental concerns. Conclusions by EFSA affirm the identification of *Xylella* as causal agent of the disease, and deny other possible primary infective causes, both abiotic and biotic, different from bacterial infection (EFSA 2015a; 2015b; 2015d; 2016a; 2016b; 2016c; 2016d). EFSA was also asked to (i) assess the risk associated to *Xylella* and to its vector for Europe; (ii) identify options for risk reduction and evaluate their efficacy; (iii) evaluate the phytosanitary practices against the disease pursuant to Directive 2000/29/EC; and (iv) act on the basis of data generated in investigations carried out in the infected Apulian area (Bragard, 2016).

In the face of European serious concerns for the potential establishment of the bacterium in the continent, we must report skepticism on the part of several local researchers and specialists regarding the causal agent of the disease affecting olive trees. Already in 2000, at the time of the Brazilian researchers' publication on the *Xylella* genome, the report should have warned about the danger represented by the bacterium. In their work, Simpson and collaborators (2000) commented that *Xylella* genes are similar to the *rpf* gene cluster 21 of *Xanthomonas* (*Rpf, regulation of pathogenicity factors*), which encodes regulatory proteins for the synthesis of enzymes involved in the virulence activity. One should have inquired, already then, why a bacterium still pretty unknown at the time had its genome sequenced, being the first plant pathogenic bacterium to have it so (Almeida and Nunney, 2015). De facto, *Xylella* causes new and important diseases and the dissemination of its vectors makes *Xylella* significantly interesting from an economic point of view for the damages it causes (Chatterjee *et al.*, 2008). For some of these diseases, the pathogenicity within areas newly colonized by exotic strains of *Xylella* results in the host-pathogen combinations not reported previously (Hopkins and Purcell, 2002).

The report contains a critical consideration of the feasible options to contain the bacterium. However, the authors are pessimistic about the possibility of infection containment. Researchers from Naples University claim the bacterium could spread in the entire Mediterranean basin, affecting local plants and olive trees in Portugal, Spain, Italy, Corsica, Albania, Montenegro, Greece, and Turkey as well as in all African and Middle East countries. In Italy the bacterium may spread beyond the north of Apulia to Calabria, Basilicata, Sicily, Sardinia, Campania, Lazio, and south Tuscany (Bosso *et al.*, 2016). Moreover, the EFSA evaluation on the *Xylella* risk concludes that the vectors of the disease are insects and plants which spread due to transport in olive orchards, and that the potential consequences of the bacterium's presence in Apulia is rated as seriously dangerous for European agriculture and environment (Stancarelli *et al.*, 2015).

3. *Xylella* and the role of "good agronomical practice"

In August 2013 in Traviano (Lecce) olive trees were signaled to show leaf desiccation symptoms. The case was subject to investigations by the University of Bari (DiSSPA) and the Bari CNR (IPSP). A few months earlier, during a conference, the same symptoms were attributed to the presence of the *Zeuzera pyrina* insect. Saponari, from CNR, ruled out this possibility and advised to search for other etiological agents. Martelli, recalling grapevine desiccation observed in California, hypothesized *Xylella* as the possible causative agent. Saponari, through the PCR and sequencing, proved that *Xylella* was present on 10 olive trees as well as on almond and oleander trees. On October 13th, 2013, Saponari, Martelli, Boscia and Nigro reported the case to the regional phytosanitary service, which reported it to the national service and to Bruxelles (reconstruction in b1, Annex B).

The occurrence of *Xylella* reported at the end of 2013 did not demonstrate that the bacterium was the only causal agent. In those months, other additional hypotheses were proposed besides *Xylella*. The University of Lecce and environmental and farmer's associations developed an alternative hypothesis based on the role of fungi and insects, as well as on bad conditions of the Salento olive trees, arising from environmental pollution and unsuitable or missing agronomic practices. It also rested upon the notion that different *Xylella* strains were present in the Salento area; at present this hypothesis is rejected through experimental evidence (Annex C). Multiple strains would have given a hint that the bacterium is a usual host in the plant, including the CoDiRO strain, to be thus considered as a secondary agent of the disease insurgence. The hypothesis is consistent with local environmental associations' and some farmers' claims (b16). Therefore, two conflicting ideological groups were created: the one based in Lecce opposing the one of Bari (still in May 2016, Luigi De Bellis, Director of the Department of Sciences and Biological and Environmental Technologies, DiSTeBA at the University of Lecce doubted of the identification of *Xylella* as causal agent). It should be noted that in 2013 the Bari researchers examined the possible role of fungi and insects in the disease etiology (Nigro *et al.*, 2013; Martelli 2013; EFSA 2015b) excluding this hypothesis.

As of the completion date of this Report, the situation is clear: *Xylella* subsp. *pauca* is the etiological agent, a conclusion which is to be considered no longer questionable (cited from the introduction; Annex C). Nonetheless, various etiological positions focused on possible concauses and on factors affecting the infection symptoms still persist today. The opinion group in Lecce (b23; b26; b30; b31) believes in the existence of concauses as in the Gallipoli area olive groves, both damaged and not damaged closely located, were identified, an evidence not fully explained in the context of a bacterial epidemic. Unsuitable agronomical practice, fungi, *Zeuzera pyrina*, an insect, and poor management of sane and infected olive groves might be involved. Poor agronomical practices, such as pruning errors or excessive pruning, field working, treatments with herbicides and in particular the use of glyphosate might make plants weaker and more sensitive to pathogen attack or even harms them. Contamination with industrial powders, cement, ashes-derived fertilizer, and the use of water from contaminated are suggested to be the underground reason for desiccation.

Researchers from Bari have a different appreciation of these factors. Guario *et al.* (2013) synthetically clarify that: "*Groundwater contamination and pollution of irrigation waters or toxic substances introduction in the soil are not enumerated among the causes of desiccation for the reason that the symptomatology affected exclusively olive trees and not other crops (citrus and other orchards including grapevine), ornamental plants, or plant species commonly present in the same area, also in subsidiary form, which did not show any symptom of vegetative decline, although identified as sensitive to xenobiotics*". Saldarelli, of CNR (b24), on causes and concauses mentions a visit to the Gallipoli fields in October 2013 where the olive groves showed bacterial symptoms. The symptoms were present in well managed plots, irrigated and with appropriate pruning. The same applies to organic olive growing, which is not significantly different from those subjected to conventional farming in terms of epidemic spread levels. The interviewees, whose opinions are provided in Annotations b1, b10, b11, b24, maintain similar opinions.

The relevance of potential concauses associated to a primary role - or less direct role - of *Xylella* is difficult to assess since available data on the effect of multiple agrotechnical treatments and of environmental conditions on containment and spread of the disease are insufficient at best. The lack of knowledge on potential concauses is also accepted by some of the advocates of the statements from Lecce opinion groups on the quick desiccation etiology (b23; b27; b30). The discussion on the concauses is summarized in a thorough document by EFSA (2016d) produced by Panel PLH, EFSA Panel on Plant Health. The Panel is composed of 21 high-rank European scientists, expert in plant disease. Recent statements questioning the EU control strategy against *Xylella* (EU Decision 2015/7893) have been used as the grounds for appeals to the European Court of Justice; so, the Panel was solicited to issue advisory opinions. The opposing statements concern: 1) the population heterogeneity of *Xylella fastidiosa* subsp. *pauca* in Apulia; 2) the possible concauses of the disease; 3) the role of *Xylella fastidiosa* in plants' death; 4) the efficacy of the infected plants' removal, as provided by the national and international regulation, on the spreading of the bacterium in different areas; 5) the methods to cure the diseased plants; 6) the application of insecticides against the bacterium. The Panel replied to points 2, 3, 4 and 6 in the cited document (the request for further clarifications of point 1 is dealt in a second document, EFSA, 2016a).

3.1. *Panel PLH's Replies*

Reply to point 2: *Xylella* subsp. *pauca* is the causal agent of the *OQDS* disease in olive trees. Reply to point 3: The symptoms of infected olive trees are due to *stress* from lack of water induced by the bacterium clogging of the xylematic vessels. All the interventions that improve the health of the plant prolong its productive phase and extend the symptomless phase of the disease. Reply to point 4: The Panel considers the removal of infected plants, operated under clear rules, as the only feasible option to prevent further incidence of the pathogen in new areas. In the outer strip of the *containment* area bordering the *buffer* zone (Annotation 2), the removal of infected plants and efficient monitoring may prevent the spread of the pathogen. In new outbreaks, the radical removal of *Xylella* host plants, whether infected or not infected, within the radius prescribed by the EU regulation may be effective in containing the pathogen. Reply to point 6: The reduction of vector populations by the application of chemical or biological methods, mechanical treatments, or other sustainable methods may well be effective in slowing down the spreading of the pathogen. There is currently no evidence of negative effects of pesticides on the interaction of *Xylella* with infected olive trees, the severity of the symptoms or the outcome of the infection.

In the context of the OQDS concauses discussion, and accepting the EFSA position conveyed in point 1 of its report, the reply to point 3 is favorable to good agronomical practices as a relevant instrument to strengthen plant resilience (vectors, however, might be even more attracted to plants showing optimal vegetative conditions; Daugherty *et al.*, 2011). EFSA approves some of the positions of those suggesting we should live with the disease through the implementation of good agronomic practices as the reduction and aeration of tall foliage, regular pruning and suckering, good management of soil fertility, techniques for the improvement and conservation of organic compounds in the soil, the use of *compost* and water, the control of fungi which negatively affect the quality of soil, tolerant variety, and superficial and minimal soil tillage (b23; Perrino, 2015). The EFSA Panel is harsh, instead, with those suggesting a role for significant agronomical concauses, and indicates plant removal to be the main containment method of the epidemic and states the need for targeted interventions in the different areas.

To summarize, unfortunately the situation is still open and allows for differing, and even imaginative, visions on the cause of the epidemic. Over time the number of contrasting issues has increased and positions have become multifaceted: there is a yes/no debate on the causes of *Xylella*, on the effect of good and poor agronomy, on the eradication of olive trees, with the implications on whether to conform or not to the national and European regulation. Conversely, it is compelling to reduce dialectic redundancy in favor of the definition of scientific conclusions, which are necessary and preliminary to the definition of rules and operational decisions. The issues to be taken into account and that are marked in this chapter of the Report examine: the cure of plants' bacterial attacks (as tested in grapevines in California); the accurate definition of *stress* conditions (e.g. drought) which may accentuate symptoms, especially wilting (Hearon *et al.*, 1980; yet the local available data is insufficient); pruning effects which are sometimes described positively but also negatively. Pruning was effective in the *Citrus variegated chlorosis* of the sweet orange of Brazil (do Amaral *et* *al*., 1994; Della Colletta Filho, 2016), yet, in that case, it was rigorously done at the onset of the infection and supported by containment measures. One out of 21 attempts of transmission by pruning induced the *Xylella* infection, an irrelevant value in percentage terms which, nevertheless, needs further investigation as a potential mechanism of new infections (Krell *et al*., 2007; b28). Pruning does not reduce bacterial concentration in symptomatic plants (Holland *et al*., 2014); rather, it might favor the growth of more vigorous and infected shoots which attract vectors (Marucci *et al*., 2014); the use of pesticides to limit the transmission of the bacterium through vectors. In their immature life stages, vector insects live in herbaceous weeds (Table 2 in EFSA, 2015a), and this is also true for *P. spumarius* in the Apulia olive groves (Cornara and Porcelli, 2014). Thus, pest control may reduce insect vector population (Purcell, 1979; Purcell and Sauders, 1999). As shown for *Pierce's disease* in grapevines (Black and Kamas, 2007), soil management may affect the composition of plant communities suitable for *Xylella* and its vectors, and it may affect the practicality of olive trees removal provided for in national and European regulations. Control is also applied to host plants for the bacterium, ornamental species or species growing among crops (polygala, oleander, etc.), even if asymptomatic. Effective solutions of low environmental impact for the containment of *Xylella* consist in the use of herbaceous vegetation strips non-attackable by *Xylella* and with allelopathic characteristics. The allelopathic effect allows for the natural avoidance of the growth of herbaceous species favoring the vector.

As regards to the agronomic and environmental management of the *Xylella* case, the expressed willingness to know more and to act on the basis of certain results, and not merely on the basis of intuitions and prejudices is a condition that cannot be delayed. In light of this consideration, the lincean Group elucidates that agronomic experiments, also easy to perform, have not been yet carried out or even planned, which is what was instead done in Brazil and California (examples in Della Colletta Filho, 2014; Almeida, 2016). An example concerns the evaluative effect of host plants removal, a measure approved to contain the pathogen, but which is hard to assess when applied to a new case of *Xylella* infection, a condition which requires prompt experimental verification (Annotation 3). The consequences of delay defining agronomic operative protocol are relevant:

the production loss is potentially high and additional effects also concern economic activities depending on farming, as well as the impact on cultural heritage. The effective use of herbicides is difficult when the effects on trophic chains are ascertained (Bragard, 2016).

4. Operational Plan of Apulia

The European Commission's decisions establishing phytosanitary practices to eradicate the pathogens are addressed to member States. Italian administration implements them through ministerial decrees while the phytosanitary Services later issue ordinances in their own area of jurisdiction for the stakeholders involved. A few days after the Italian authorities' notification of the presence of *Xylella* in Salento (10/21/2013), the Apulia Regional Council approved an emergency Plan for the prevention, control and eradication of the *Xylella* bacterium, strain CoDiRO (10/29/2013; a3). The rapidity with which the Plan was drafted is justified by the fact that the *OQDS* spread may compromise the economic development of factory farms in Salento, where olive growing is essential (Annotation 4). Even then, the spread of olive groves desiccation appeared to progress in the territory of the province of Lecce.

In the Plan, *Xylella* is considered the main agent of desiccation. Since *Xylella* is a quarantine bacterium inserted into the A1 list by EPPO (*European and Mediterranean Plant Protection Organization*) (Annotation 5) registered as absent in Europe, Art. 16.1 of the Dir 2000/29/EC imposes the adoption in the national territory of immediate measures of eradication and containment of the quarantine organisms (a1). Moreover, it asserts that there are no means to cure plants infected by *Xylella*; so, it is essential to implement preventive interventions to allow its eradication or prevent its diffusion.

The Plan deems it necessary to thoroughly monitor the province of Lecce, and also to inspect other provinces in the region, in order to determine:

- the *breeding ground area*: area or site where the presence of the pathogen is officially confirmed and its eradication is considered technically possible.

- the *colony area*: area where spread of the harmful organism makes its eradication impossible, and therefore the area where the implementation of containment actions is necessary.

- the *buffer area*: perimeter strip bordering the breeding ground or colony area, where the pathogen presence has not been identified yet.

- the *safety area*: perimeter strip bordering the buffer area as further guarantee of the pathogen's containment.

In order to verify the presence of *Xylella* both in olive trees and in the species which may host the pathogen, including spontaneous species, a sampling activity and analysis to be carried out in laboratories accredited pursuant to DD.MM 04/14/1997 and DM 08/09/2000 have begun. The need to start research programs is clear and such programs should focus on: deepening the analysis on all causal agents of *OQDS*; confirming the presence of *Xylella* in accordance to the provision of EPPO's official Protocol; establishing the genetic correlation between the Apulian bacterial strain and the subspecies of *Xylella* which cause the disease in other plants; determining the pathogenicity and virulence of *Xylella* strain(s) isolated from infected plants; determining the bacterium epidemiology, verifying its transmission by vector insects; developing molecular and serological diagnostic techniques of *Xylella*, and finalizing the database of host plants; starting the monitoring of vector insects (Leafhoppers) present within the area and subject them to experimental transmission tests; starting additional research aimed at opposing the spread of the quarantine pathogen.

The Plan so tackles the emergency, both in the management of the territory and in responding to the information requests by the European Commission, an information which will be used to establish more accurate emergency interventions against the spread of *Xylella* in Europe (a7). At the end of 2014, Apulia published a booklet titled GUIDELINES FOR THE CONTAINMENT OF THE SPREAD OF "*XYLELLA FASTIDIOSA*" subspecies *pauca* strain CoDiRO, which contains the actions to be deployed in the Salento territory (Annotation 6).

In the booklet it is claimed that: *Because of the detection of many other breeding grounds in several areas of the province of Lecce, it is particularly complex to distinguish, in the territory, the single breeding grounds and the buffer areas, 2 km large, and to keep them separate from pest-free areas. Therefore, on the basis of the latest surveys and laboratory analysis, the strategy of containment of the Xylella infections was defined, as cartographically represented in Figure 1.*

The guidelines, while leaving the freedom to decide whether to remove the plants infected by *Xylella* in the infected area or not, calls for tough eradication in the buffer zone, 1 km large, and in the possible breeding ground points in proximity to the same which are deemed to be a risk for

Fig. 1

the buffer zone, for the purpose of avoiding the progress of the infection toward pest-free territories.

4.1. *The state of emergency and the Silletti Plan*

In September 2014, the Ministry of Agriculture organized a scientific Committee to support the national phytosanitary Committee to examine in-depth the technical-scientific problems of the *Xylella* emergency. Boscia, D'Onghia, Nigro, Porcelli, Saponari, Savino, Surico and others took part in this Committee which also benefited of the expert advice of Almeida and Purcell (a8). The Cabinet declared the state of emergency (a12), and appointed Giuseppe Siletti as Delegate Commissioner to cope with it (a13). A month later, the Delegate Commissioner's Plan of action (known as

Siletti Plan) was published; in addition to the phytosanitary actions for the Lecce province, the Plan consists of the allocation of the required economic resources (a18).

The objectives of the Plan are to prevent a further broadening of the infections to other Apulian provinces north of Lecce, where the number of olive trees is 5 times higher than in Salento (Annotation 7), and to preserve as many monumental olive trees as possible; there are approximately 6 million of them in Apulia, of which a significant number is in the province of Brindisi and in the Gargano area. Hence, the "infected zone" is enlarged by including most of the Lecce province; a new and unique "buffer zone", of at least 2 Km in width, is identified, placed north of the infected area and made up of a continuous strip which transversally cuts the Salento Peninsula from the Adriatic to the Ionian. A "phytosanitary cordon" is defined north of the buffer zone and at appropriate distance from it, with a width of around 2 Km, where high phytosanitary monitoring is to be exercised with the aim to constitute an additional safety barrier. Finally, an eradication strip is identified in the buffer zone, with a width of 1 Km, where all the infected plants must be removed (Annotation 8).

The Plan provides for the elimination of host plants present along roads, streams, ditches, green areas (Measure A.1); the control of vectors present in herbaceous weeds of olive and fruits orchards (A.2); the treatment to control adult insect vectors (A.3); the eradication of infected plants (A.4); and the destruction of *Xylella* host plants present in nurseries (A.5).

A new decision of the European Community rethinks the boundaries of the various areas imposing the enlargement of the buffer zone to 10 Km. It also imposes that, in the containment area and within a radius of 100 m around the reportedly infected plants, infected plants and other plants showing symptoms indicating possible infection or suspected to be infected must be removed as well as host plants, regardless of their health status (a24).

The Silletti Plan, which includes the decisions taken in Europe, was presented in July 2015 (a29). It states that monitoring must be rearranged in the buffer and containment zones in the infected area, in the surveillance area, and that, in the presence of new breeding grounds, all the symptomatic and asymptomatic host plant within a radius of 200 meters must be identified and geo-referenced. The new boundaries of the buffer zone, which is moved north absorbing the Oria infection site, are defined while the area which comprises the Municipal Authorities of Torchiarolo, S. Pietro V., and Cellino S. Marco in the province of Brindisi is declared infected, as cartographically represented in Figure 2.

The plan of intervention is implemented through phases (Annotation 9). The first one concerns the Municipal Authority of Oria (BR). A distribution analysis of the olive trees present in this infection site clarifies the complexity of the issue, and explains perhaps the opposition to the eradication plan. In fact, 234 out of 918 analyzed plants were symptomatic, but only 47 of them were found positive to *Xylella*, and other 5 positive were asymptomatic. In the lincean Group's meeting with IAM of Bari (b19) it emerged: i) that the opponent to the intervention plan mentioned the low incidence of asymptomatic olive trees in Oria to deny the role of *Xylella* as causal agent in the epidemic; and (ii) that, however, the Oria's report, where at first only a limited number of the symptomatic plants resulted infected by *Xylella*, was clarified by a second sampling where 100% of the symptomatic plants resulted to carry the bacterium. This finding was also confirmed by an analysis of symptomatic plants in other areas of Salento (Annotation 10). In Torchiarolo, a town 20 km north of Lecce where the outbreak was found, positive symptomatic olive trees were absent two years prior, whereas now the disease is fairly widespread.

The lincean Group is confident that the conclusions of the scientists from Bari University and CNR on the Xylella, on which the EFSA conclusions are based, rest on solid scientific foundations. Additionally, it is pointed out that some researchers who have supported alternative hypothesis have not retreated in their positions, and distanced themselves from opinion groups supporting them, despite of the clear evidences emerged in the last year.

Recently Apulia drew up a new Plan (a46) with new actions (Annotation 11). For eradication in new breeding grounds, the new guidelines state: *It is considered necessary to carry out all actions of eradication of the Xylella bacterium in new infection sites as provided for in DM 06/19/2015. However, pending the decision of the European Court of Justice, pursuant to Legislative Decree no. 214/05, the removal of infected plants must be compulsory, carried out by the owner/tenant.* For the management of the olive grove, the new guidelines add: *The prompt removal of plants infected*


Legend
Provincial boundaries
Municipal boundaries
Surveillance area
Buffer area
Infected area in the province of Lecce
Infected area in the province of Brindisi
Cellino S.Marco, San Pietro V., Torchiarolo
Outbreaks: Brindisi, San Donaci, S. Pancrazio S.
Oria: infected area of which DDS 195/2015 and breeding grounds
Infected area in the province of Taranto
Breeding grounds: Avetrana
0 5 10 20 Km

Fig. 2

by Xylella is compulsory. In the presence of infected monumental olive trees, it is compulsory to intervene ... with extraordinary conservative pruning of the precious structure of the plant ... and also with covering of the pruned plant with an anti-insect netting ... The suckers and possible new vegetation not protected by the netting will have to be continuously eliminated. It is compulsory to remove promptly all host species present within a radius of 100 m around the reportedly infected plants, with the exception of olive trees.

The difficulties of implementing the Plan because of the issues concerning the eradication and the use of chemicals due to the opposition of some researchers and of environmental groups lead the Commissioner to write the following note in the frontispiece of the intervention Plan - II version (09/30/2015; Annotation 12): *Xylella feeds and strengthen itself on the incomprehension among men*. The nature and cause of this incomprehension has already been partially discussed. If the etiology of the disease diminishes its relevance as a dialectical subject (in the Conference held in Lecce on May 18th, 2016, on *Xylella*, even those disagreeing on or doubting about *Xylella*'s causal agent agreed that the hypothesis of autumn 2013 was correct, and recommended, as a containment solution, the use of antibacterial treatments with a preparation containing lactoperoxidase, a compound evaluated only *in vitro* and hard to convey to the olive tree's xylematic vessels to get in contact with the bacterium), many more troubling issues remain. For instance, the lincean Group notes that, while the various versions of the Plan are accurate in defining the measures to be adopted, due also to the European regulations influencing them, they devote little attention to justifying the reasons for the implementation of such measures as based on verified experimental results. It is true that experimental data, which may help, are not available, but, this is exactly what the lincean Group wants to highlight: it is not known whether pruning transmits or reduces the infection; whether herbicides play a role; whether irrigation reduces symptoms; whether groundwater is polluted, and where the sources of contamination are located; whether relatively young plants are more resilient; whether winter temperatures play a role in in-plant containment; whether *stress* conditions plays a role; whether treatments with antibacterial solutions or with zinc and copper are effective; whether other cultivars in addition to Leccino are tolerant to the infection, (and one may continue with many other questions). In light of these considerations, the lincean Group repeats the recommendation of performing urgent and simple agronomic experiments in order to answer the above questions, with the aim to foster a better definition of the infection containment actions, and, especially, to contribute to the rescue of monumental olive trees.

5. Verification of the scientific value of the multiple hypothesis and proposals on causes, effects and diffusion of the *Xylella fastidiosa* subsp. *pauca CoDiRO* (*OQDS*) disease

The verification is based on researchers' depositions, notes extracted from the available bibliography, and on periodicals valued by the *Institute of Scientific Information*, ISI (analytical quotes on the topics used in the verification are reported in Annexes B, C, D, E, F).

a) In the conferences and courses held in Caserta and Valenzano in 2010, the *Xylella* issue was particularly discussed. Strains of *Xylella* were used for practicals, which may have been the cause of the bacterium's diffusion in Salento. There is no documental evidence that the experimentation was carried out in accordance with the required regulations.

b) *Xylella* is not the only etiological agent of *CoDiRO (OQDS)*, the other factors being infections caused by fungi and insects and poor agronomic practices. Only 2% of the samples analyzed (to April 2014) were positive to *Xylella*. Koch's postulate was not proven for the bacterium. It follows that its causal significance of the disease is low.

c) *Xylella* has been present in the territory since earlier times, as shown by the desiccations reported in 2009 and 2010 and by the existence of numerous strains of the bacterium.

d) Previous experiences in the USA, Brazil, and Taiwan show that the eradication of infected plants does not produce positive results. Therefore, eradication is useless.

e) The possible requirement for an environmental impact assessment before implementing actions for prevention against the spread of the disease. In particular those related to the use of insecticides to control the vector (*Philaenus spumarius* or spittlebug) and herbicides against the herbaceous vegetation on which the vector grows.

Hypothesis **a**) is difficult to interpret. From a scientific point of view, the interest of agronomists and pathologists for a bacterium which has influenced agriculture in California for 130 years and which is expanding both in Central and South America and in distant countries (Taiwan), constitutes an essential preventive work. Hypothesis **a**) contrasts with hypothesis **b**) which states that *Xylella* is only a "minor" contributing cause of *OQDS*, while fungi, insects larvae and poor agronomical practices are more relevant, as it is claimed to be demonstrated by the low incidence (later revealed high) of positive samples for the presence of *Xylella* in the plants sampled. The cause-effect relationship can be supported on the basis of a careful analysis of the geographical co-distribution of symptoms and causes. The purpose of sampling plants distributed throughout the territory (data analyzed in April 2014) was to determine, with an extensive analysis, the area of infection. Data show that in spring 2014 the infected plants were relatively few and that the infection could (possibly) be contained: however, all symptomatic plants were found positive to *Xylella*, a strong indication that *Xylella* was the cause of the symptoms. Positive and asymptomatic plants, frequently detected, reflect the long incubation period of the disease (6 months-1.5 years) and, being a reservoir for the pathogen, they should be subject to required actions just as symptomatic plants are.

Similar considerations apply to hypothesis **c**) where it is stated that *Xylella* has been present in Salento in times preceding 2010. This hypothesis is based on "desiccation" signals, a generic symptom that can be due to many causes. There is neither circumstantial nor analytical evidence that *Xylella* has been the cause of the desiccation phenomena which preceded 2013. In the absence of reliable data, the conclusion that olive trees live with the bacterium for a long time and succumb only in the presence of concauses, although not excludable at first, appears to be unfounded. Instead, it is certain that all the *Xylella* isolates on molecular analysis can be attributed to an identical genotype: *Xylella fastidiosa* subspecies *pauca* and sequence type ST53. Their molecular homogeneity supports the origin of the disease from a unique and recent source of infection. The authors of a recent article signed Bleve *et al.* (2016) confirm the data previously published by the IPSP-CNR researchers and by the University of Bari.

The diagnosis of *Xylella* required 130 years in California, 13 years in Brazil, and 22 years in Taiwan (point **d**). In Salento, within two years, researchers identified the bacterium, the vectors of the bacterium and its possible colonization of more than 20 different plant species (the confirmed host of the bacterium *Xylella* are 359 and belong to 75 different botanical families). Furthermore, it was possible to reconstruct with phylogenetic methods the probable origin from Central America of the strain CoDiRO (Elbaino *et al.*, 2014a). After warning from the Bari group, species and subspecies of the bacterium outbreaks were identified in other European countries, especially following the check-ups carried out by customs officers on imported plants, mostly ornamental species. Recently, the same group confirmed Koch's postulate, with the reproduction of the disease from pure isolates of the bacterium (EFSA, 2016b). In the case of *OQDS* just as for other diseases caused by *Xylella*, a long incubation period following the inoculation is required. This would have led to a greater consideration by those who are skeptic about the etiology of *OQDS* caused by *Xylella* of the general principle that "*lack of evidence is not proof of absence*".

Therefore, point **d**) originates a particularly important consideration: for the first time in history the diagnosis of xylellosis was carried out in extremely short times. This, along with the geographical configuration of Salento, would have allowed to circumscribe the phenomenon before its further diffusion to the rest of the country. The chance could be thwarted by the lack of appropriate interventions. It is unclear whether the situation is recoverable. Now, it is also known that on May 12th, 2016, the Advocate General of the European Court of Justice, Yves Bot, who assists the Court with the task of formulating judicial advice in complete impartiality and independence, upheld the accuracy of the prevention measures provided and the need to apply them at the earliest (previewed in www.ansa.it, May 12th, 2016, then upheld in June 9th, 2016, with the publication of the sentence, a47). The European position, therefore, is different from local initiatives in Apulia (b6).

As for the possibility of an assessment of the environmental impact of preventive treatments referred to in point e), Apulia decided (May 6th, 2016) that such proceeding is not necessary in cases of phytosanitary emergencies (Annex A, Apulia, May 12th, 2016).

What are the consequences in the light of the many hypothesis on the *Xylella* case? To date, it is possible that the consequences might be extremely serious. This is because of the characteristics of the spittlebug's life cycle, the insect vector of *Xylella*: the larvae of this insect live on herbaceous dicotyledonous species (non-infected) at the base of trees and move to olive trees in their adult stage. Here they acquire the bacterium which they then spread. From May onwards, new infections have occurred in a quantity proportional to the number of adults that feed on infected plants. Decreasing the number of infected plants and insects is, hence, of critical importance to contain the infection. A second reason for urgency concerns the possibility of occurrence of multiple infections generated by different strains of *Xylella*. *Xylella* colonizes many plant species visited by vectors. After the warning concerning *Xylella* in Salento, other breeding grounds of infection were identified in Europe. These were caused by strains of yet different subspecies of the bacterium that are now frequently found on plants imported especially from Central America. This is the case of the multiplex strain, present in France both in Corsican and continental locations. Several strains of *Xylella*, present in the same host, may recombine their genomes creating hybrids (Nunney *et al*., 2014) which, in the presence of new vectors and of vegetative species not visited beforehand by the bacterium, may initiate new speciation cycles of *Xylella* in Europe, which may be followed by an enlargement of the pathogenicity towards new species. The species infected by *Xylella* include many ornamental plants, forest and fruit trees including grapevine. The prospect that, in the future, an imported strain or one of its products of recombination may bring *Pierce's disease* in Italy is not remote and evokes scenarios in which not only the Italian olive oil but also the Italian wine would be damaged. The *Cercis siliquastrum* (jude-tree), which, like other wild plant species known or yet to be discovered, can host both subspecies of *Xylella*, *multiplex* and *fastidiosa*, is a permissive incubation system of the bacterium for the formation of hybrids, and is also a typical component of the Mediterranean bush.

6. What actions should be adopted to contrast the disease and to end the current deadlock status?

In the first five chapters of this report, the analysis of the *Xylella* case, carried out by contemplating the various aspects of the problem, has disclosed some possible measures - mainly agro-technical and diagnostic ones but also organizational and social ones - which may contribute, at any rate, to contain the state of uncertainty which surrounds the decline of Apulian olive trees. Our assessment of what has been recommended to contain and cure the disease contemplates four different angles.

6.1. *Containment of the infection and management of olive groves where the bacterium is present*

Apulia's Plan clarifies that there are no curative methods for plants infected with *Xylella*. It is therefore essential to implement preventive interventions to allow either the eradication or the prevention of the bacterium's diffusion. The measures that should be put in place in the infected area around Lecce include i) insecticide treatments against vectors; ii) agronomical interventions against the vectors' early stages and against herbaceous weeds; iii) the elimination of host plants present along roads, streams, ditches, green areas; iv) monitoring the presence of *Xylella* on host plants (EFSA, 2016d; Hammers, 2015a). The PLH Panel also recommends (EFSA, 2015b) to choose prevention strategies based on the inspection of plants grown in nurseries and used for new installations, and to arrange the intervention in an integrated approach: supervision, certification and monitoring of transplants produced in areas where the bacterium is absent (possible thermotherapy or antibacterial treatments for small-size plants); and inspections and insecticide treatments. In the critical examination of the possible actions adopted, suspended, or to adopt, it may be useful to compare those implemented in countries which have been facing the *Xylella* problem for decades. Annotations 14 and 15 summarize the events and actions put in place in California and Brazil. In both countries, the interventions for bacterium control do not differ from those provided for in Apulia, considering that, however, in both cases in the Americas the local eradication of strains responsible for *Xylella* was not possible. This is the situation today even in southern Apulia, a conclusion that should not influence the decision to remove infected plants should infection sites appear in the containment area. Should this not be done, the bacterium would spread in the north of Apulia (in areas where the concentration of monumental olive trees is even higher than in Salento; Annotation 4), in Europe and in all the Mediterranean basin. The spreading of breeding ground is explained by the migration of infected ornamental plants (milkwort, at least other 15 ornamental species including myrtle-leaf milkwort, oleander, lavender, rosemary and acacia). In Corsica too the presence of independent outbreaks of *Xylella* (subspecies *multiplex*) has been attributed to ornamental plants (b11, b12). An important preventive action is to keep the soil under the orchards clean from weeds with mechanical treatment. In conditions of grave epidemic, from 250,000 to 500,000 insect vectors are present per hectare, a density correlated to the level of symptoms reported on the olive tree (b15). A delayed weeding treatment would be useful and would have a low environmental impact. Knowing that bees do not visit olive tree, the best outcomes for the control of insect vector have been accomplished by treating with neonicotinoid insecticides: acetamiprid and imidacloprid (Dongiovanni *et al.*, 2016a).

Preventing the introduction and, if this has already occurred, the diffusion of exotic strains of *Xylella* in Europe (as Hopkins and Purcell, 2002 define potentially pathogenic bacteria for vegetative species present in still-immune geographical areas) is strategically relevant. As already mentioned, strains of various subspecies of the bacterium present together in the same host with which they have never been in contact before may create hybrids and initiate a new European speciation cycle of *Xylella*.

6.2. *Cure of the diseased plant*

It is extremely important to have treatment protocols for infected olive groves to hope to keep alive the over six million monumental olive trees of Apulia. The topic was examined in-depth by the PLH Panel (2016a) on the basis of experiments on the olive tree of Scortichini and of Carlucci and Lops. Both groups believe that their data requires at least another year of confirmation. Under field conditions, different concentrations of copper and zinc are employed, while the use of bioactive substances is at an experimental stage. The treatments, combined with appropriate agronomic care, translate into a greater vegetative vigour of infected olive trees. However, both the cited groups report that their experiments are aimed at eliminating the symptoms and not the bacteria which persist in the plant even after the treatments. Although currently there is no treatment able to eliminate the bacterium from the plants' tissues, the Panel acknowledges that this approach may extend the productive life of the infected plants.

In this context, methods and molecules recommended against bacterial infections may be generally discussed and possibly experimented. Such methods and molecules include: i) antibiotics, ii) bio-activators of endogenous resistance, iii) compounds which stimulate plant growth, iv) antagonist bacteria and fungi, v) products which inhibit the transport of bacteria by insect vectors. Antibiotic treatments against *Xylella* (the PLH Panel cites a long series of publications) do not eliminate the bacterium. For the xylellosis of citrus fruits, the effect of N-Acetylcysteine (NAC) was analyzed. Above 1 mg/mL concentration, the compound reduces the formation of biofilm and EPS, extracellular polysaccharides, and orange plants show a remission of the symptoms, which is associated with a reduction in the bacterial concentration. A slow-release fertilizer was also developed to apply the NAC compound to diseased plants (Muranaka *et al.*, 2013; De Souza *et al.*, 2014). The use of attenuated strains of the bacterium which does not cause the disease insurgence can also limit the effect of virulent strains; however, this is a hazardous approach for it allows the initiation of new strains (Hopkins and Purcell, 2002).

Temperature alternation in various seasons seems to have reduced the concentration of the inoculated bacterium in olive trees. The same has occurred for oleanders and myrtle-leaf milkwort trees (Saponari *et al.*, 2016). Hill and Purcell too (1995) report that the permanence of the bacterium in grapevines may depend on the infection period: in case of summer infections, the shoot may recover over the winter and free itself of the bacterium in the following season, a result which is compatible with that of the Carlucci Group for CoDiRO, which needs to be further investigated. The PLH Panel ends this discussion by reporting that grapevine treatments, similar to the ones evaluated in Apulia, do not eradicate the disease but may protect the plant from other fungi. Furthermore, the Panel states that, *in vitro*, some of the compounds mentioned are effective, but evidence from treatments of complete plants in open fields are lacking. Zinc and antibiotics require continual injections in order to be brought into contact with the bacterium (it is the already mentioned objection for the lactoperoxidase formulation reported as resolved in the meeting "*Xylella Roundtable* - integrated approach and synergy between Apulian, Italian and International Universities" held in March 18th, 2016). Moreover, the Panel states that now antibacterial antibiotics are not allowed for plant treatments in the field. Finally, it recommends to consider the effect in the long run of repeated copper and zinc treatments, which is potentially harmful.

6.3. *Need for research*

EFSA's PLH Panel (2015b), repeating what already specified in the 2013 intervention Plan of Apulia (a3), broadly recommends to intensify the research on host plants, epidemiology, and on bacterium control and local confinement; and to perform comparative analyses of similar situations taking place in California, Brazil, Corsica, etc. Potential research contributions should involve epidemiology, bacterial transmission, check-ups and, above all, the genetic of tolerances and resistances and its application to the constitution of varieties able to live with the bacterium. This has already emerged as a point of contrast between scientists and environmental groups who denounce this intervention as being a "Trojan Horse" directed to transform Salento olive tree farming from extensive to intensive.

Because *Xylella* infects a high number of plant species, at present there is great uncertainty on which host species should be eliminated in order to include them in the monitoring plan. The Plan of Apulia essentially provides for researches on the nature of causal agent and vectors, neglecting, however, the need to gain greater knowledge about the concauses of the spreading on the infection.

With respect to the insect vector, it would be necessary to research on phenology, ecology and preferences of *P. spumarius* on olive groves and vineyards (also where the olive grove is not monoculture); on *P. spumarius* population typing; on the vector's response to plants' volatile compounds; on the use of insecticides for conventional and organic agricultures; on the vector transmissibility of the bacterium; and on temporal dynamics of plant-host association (Bosco, 2016; the author concludes: "*Little information at this time! A lot of research work is needed*").

6.4. *Interaction between Scientific institutions and their possible retreat on incorrect positions on the case*

Undoubtedly, differences of opinion on the *Xylella* case exist among active research groups: on the one hand there are the Universities, the IPSP-CNR and the Bari IAMB, and on the other there are the ISPA-CNR of Lecce and the University of Lecce, Matera and Foggia. Those differences of opinion (example b27) would require an ultimate synthesis to allow for a homogenous approach to the issue (an example is the meeting held on May 18th, 2016 titled "*Xylella Roundtable*. Integrated approach and synergy between Apulian, Italian and International Universities", where the causal role of the strain *pauca* ST53 was unreservedly approved, although in the complete absence of researches from Bari who discovered the disease's etiology in 2013). It is also significant to observe that, among two CNR laboratories, the laboratory in Lecce produced sequences of bacterial strain isolated from diseased trees and found them 100% identical to the ones published by the CNR laboratory in Bari. This Report has already commented on a radicalization of the debate; in this respect, it helps to recall that, EFSA (2015b) recommends the use of common sense to avoid the insurgence of contrasts between scientists - unless on the basis of confirmed data (b20) - in one of its Reports. It should be noted that the funding sources for the research on *Xylella* are regional, national and European (Annotation 15), while the first investigations to determine the causes of *OQDS* were entrusted to accredited laboratories, agreed upon with the Phytosanitary Observatory of Apulia (Annotation 16).

Another aspect of differences of opinions that exists in the scientific world of Apulia concerns the connection between the research Group LAIR (Law and Archeology - Ius et Rus, which was born within the University of Salento, and with which Luigi De Bellis, Director of the Department of Environmental and Biological Sciences and Technologies of the University of Lecce, collaborates for scientific aspects on the *Xylella* case) and several environmental groups which operate in Apulia, also including WWF. De Bellis and Massimo Monteduro (Associate Professor of Administrative Law) coordinated an inter-disciplinary study on the *Xylella* problem with researchers and students belonging to LIAR, which led to the writing of the academic paper "*The Xylella fastidiosa emergency: why the idea of* *eradicating all uninfected olive trees (those lacking symptoms indicating possible infections and not suspected to be infected) within a radius of 100 meters from the infected ones is a measure both legally and scientifically contestable*" (Annotation 14). Given the new intervention Plan of Apulia (a46), it would be necessary to assess whether a common understanding with the authors of the cited document has been reached.

Considering the obtained results, one must conclude that the funds allocated to the research Institutions in Bari have been put to good use. Nevertheless, the early assignment of research contracts for agronomic field experiments - a suggestion already made here and decisions to be prioritized - may contribute to restore useful and constructive scientific discussion. Such decisions, suggested by several parties, may contribute to encourage new collaborations and to tackle *Xylella* on the basis of shared scientific conclusions.

7. *Xylella* at the cross-road between science and society

The European Court of Justice confirmed the validity of the actions including the eradication of olive trees in the containment area around the infected plants present in the buffer zone, and in the presence of new infection points. This intervention is also suggested in the new phytosanitary actions planned by Apulian Regional government (Annotation 11). We hope that the requisition of infected olive trees will not persist, and that MiPAAF and the Italian Government will concretely strive with Apulia to safeguard the territory and the Italian productions which are located in the north of Salento. The example mentioned above is only one of the many examples, emerged from the *Xylella* case, which are intertwined in between science and Institutions, which, together, model and regulate society. In science as in society, the need for truth exists as a crucial component of the interaction between individuals. Scientists furthermore posses a particularly solid vision of truth - a scientific assertion which has undergone a methodological process confirming it to a certain level of confidence with a certain degree of trust - and do not tolerate ambiguities about reality. This claim to truth derives from their ability to ensure that matter and energy do what they expect (Dawkins, 2003), partly in contrast to the culture of our time that stems from centuries of philosophical and historical discussions. When science proposed its vision on the topic, a contrast between scientists and humanists arose (Sansavini, 2015). It is, however, a fact that science offers certainties (although these are approximated and can be improved; Blackburn, 2011).

We have verified that the Bari researchers' statements on the *Xylella* case have a sound scientific basis. Unfortunately, the social world holds varied opinions on the case. A hypothesis formulated by environmental groups at the first press conference where the identification of *Xylella* as cause of *OQDS* was announced (October 4th, 2014) suggested the bacterial strain was leaked from laboratories where it had been used in a 2010 COST refresher course.

Such hypothesis persists even today (b31; b32), despite knowing that no strain of *Xylella pauca* present in germplasm banks has the same DNA sequence of the CoDiRO strain (Elbaino *et al*., 2014a; Gianpetruzzi *et al*., 2015a; 2015b; Marcelletti and Scortichini, 2016). In addition, a marked skepticism regarding how much and what "scientists" do was noticed. This is an attitude that emerges at times from the proposals of both political parties and opinion movements (Annotation 19), often magnified by the media. However, it is surprising that the same proposals are shared even by University Professors, almost as if their alignment to particular beliefs or social events exonerates them from their deontological duties. The lincean Group reveals that some of the researchers that contributed to the support of alternative hypothesis, did not retreat their positions and distanced themselves from groups supporting them despite the clear scientific data emerged during the last year.

8. Annotations

Annotation 1. A European Community COST workshop devoted to pathogens of large crop plants was organized in 2010 by major international experts, among them P.H. Janssen and R. Almeida (researchers with extensive experience and fully aware of both the importance of containing the bacterium and not letting it out of laboratories as well as of containment methods). The practical exercises were carried out with the use of grapevine seedlings affected by *Pierce's disease* - caused by *Xylella fastidiosa* subsp. *multiplex* - which were destroyed at the end of the workshop. It is common practice to organize such conferences in an international context of intense circulation of goods and people, including plant material. Other COST conferences were organized to prevent the insurgence of epidemics and were held in quite a similar way, including the practicals, to those adopted in the course held in Bari.

Annotation 2. The containment plan launched by Gen. G. Silletti, Government Commissioner in charge of managing the *Xylella* emergency, provided for the consideration of four areas: infection, eradication, *buffer*, and prevention, and enumerated the actions to be implemented in each area.

Annotation 3. Internationally the issue is widely discussed. Please consult the academic works of Mumford, 2006; Thomson, 2006; Sosnowski *et al.*, 2009; Belasque *et al.*, 2010; de Boer and Boucher, 2011; Filipe *et al.*, 2012; Gordillo *et al.*, 2012; Palacio-Bielsa *et al.*, 2012; Sosnowski *et al.*, 2012; Bennett *et al.*, 2013; Su *et al.*, 2013; Behlau *et al.*, 2014; Cunniffe *et al.*, 2014 and 2015; Gottwald and Graham, 2014; MacMaster *et al.*, 2015; NTG, 2015; Rimbaud *et al.*, 2015, the full bibliographical quotes of who are reported in EFSA (2016d).

Annotation 4. Olive-growing heritage in Apulia by age classes

Annotation 5. List of pests recommended for regulation and listed as harmful organisms to be quarantined. `https://www.eppo.int/QUARANTINE/listA1.htm`

Annotation 6. Guideline 2014 `http://cartografia.sit.puglia.it/doc/LINEEGUIDA\_XYLELLAE\_CoDiRO.pdf`

Annotation 7. `http://cartografia.sit.puglia.it/doc/Piano\_operativo\_Xylella\_approvato18\_03\_2015.pdf`

Region	Provinces	no. TOTAL plants	of which			
			no. plants > 30 yeas	no. plants < 30 years	no. plants > 100 years	no. plants < 100 years
		(a)	(b)	(c = a – b)	(d)	(e = a – d)
Apulia	Bari	22.722.772	17.335.469	5.387.303	8.806.526	13.916.246
	Lecce	12.581.940	10.781.915	1.800.025	2.899.839	9.682.101
	Foggia	9.242.093	7.438.501	1.803.592	2.047.359	7.194.734
	Brindisi	7.891.935	5.405.353	2.486.582	2.225.116	5.666.819
	Taranto	5.667.873	5.202.558	465.315	1.009.723	4.658.150
TOTAL IN APULIA		**58.106.613**	**46.163.796**	**11.942.817**	**16.988.561**	**41.118.052**
% Apulia =100		*100,0*	*79,4*	*20,6*	*29,2*	*70,8*
TOTAL IN ITALY		**199.363.842**	**146.977.334**	**52.386.508**	**43.070.283**	**156.293.559**
% ITA =100		*100,0*	*73,7*	*26,3*	*21,6*	*78,4*
% Apulia / ITA		*29,1*	*31,4*	*22,8*	*39,4*	*26,3*

Table 1

Annotation 8. Definition of areas circumscribed for *Xylella* (03/13/2015) as cartographically represented in Figure 3.

Annotation 9. Implementation of the 2015 action Plan
First part of the Silletti Plan: `http://www.sit.puglia.it/portal/portale\_gestione\_agricoltura/Documenti/docCommissario/PortalXylellaDocCommissarioIstanceWindow?IDNEWS=70\&action=e\&windowstate=normal\&mode=view\&ACTION\_NEWS=DETAIL`
Second Part of the Silletti Plan: `http://www.sit.puglia.it/portal/portale\_gestione\_agricoltura/Documenti/docCommissario/PortalXylellaDocCommissarioIstanceWindow?IDNEWS=78\&action=e\&windowstate=normal\&mode=view\&ACTION\_NEWS=DETAIL`

Annotation 10. Analytical data on the presence of *Xylella* in Apulia. The official documentation related to the analysis carried out by IAM in 2015 is available at: `http://www.emergenzaxylella.it/portal/portale\_gestione\_agricoltura/Elenchi/risultati`

Annotation 11. Phytosanitary procedures to be implemented for containment of the spread of *Xylella fastidiosa* subsp. *pauca* CoDiRO strain. Year 2016. `http://www.regione.puglia.it/index.php?page=delibere\&id=16993\&opz=downfile\&fs\_id=21749`

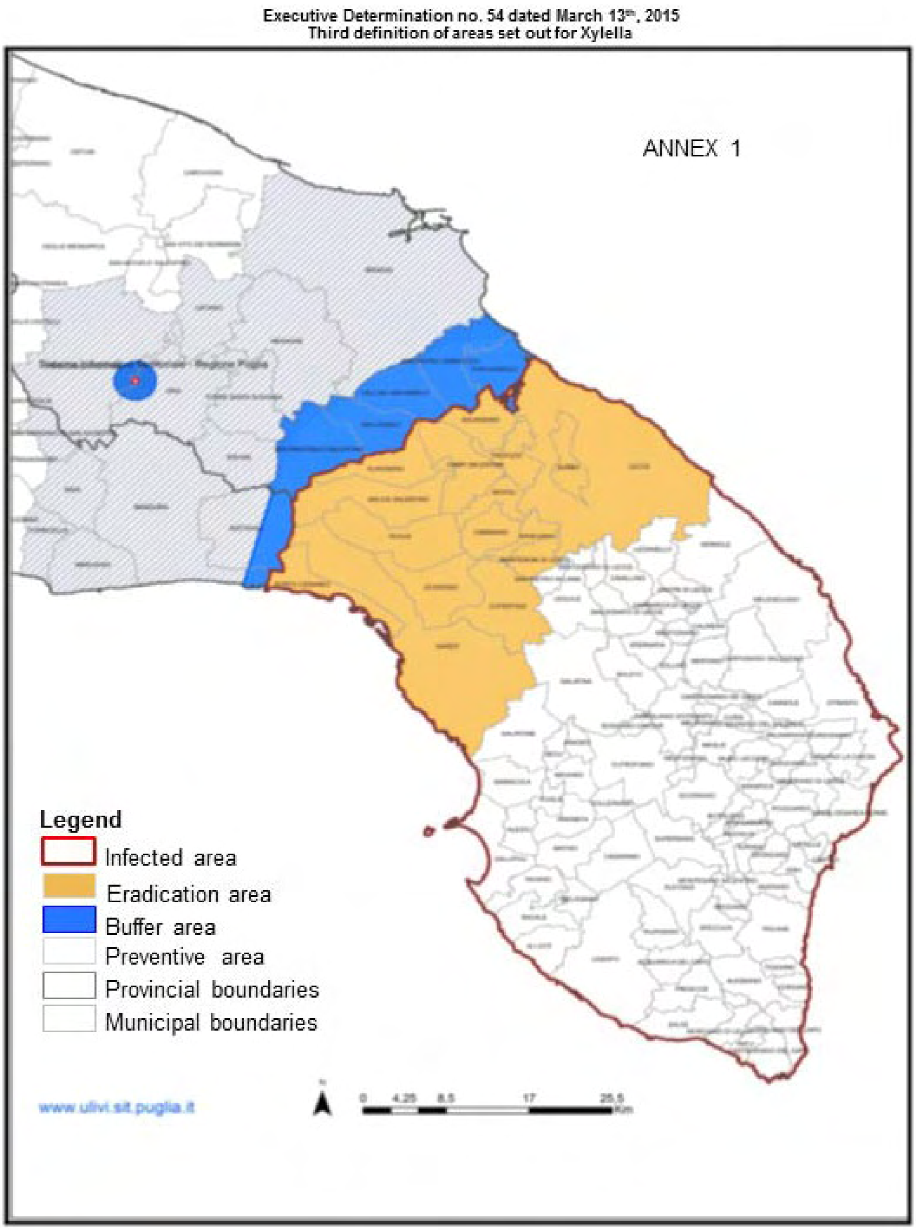
Executive Determination no. 54 dated March 13th, 2015
Third definition of areas set out for Xylella
ANNEX 1
Legend
Infected area
Eradication area
Buffer area
Preventive area
Provincial boundaries
Municipal boundaries
www.ulivi.sit.puglia.it
0
4,25
8,5
17
25,5
Km

Fig. 3

Annotation 12. In Brazil the disease known as *Citrus Variegated Chlorosis*, CVC, is also tackled though the elimination of infected plants. Despite this, the disease has increased. However, it should be considered that the interventions were implemented when the disease was already well established, supporting the conclusion that bacteria eradication is feasible only in the very early stages of the epidemic. The interventions adopted include the mandatory supervision of all nurseries phases to be carried out in *screenhouses* in the absence of vectors, the selection of mother plants for reproduction, the reduction of vectors and inoculi, the genetic improvement even with cisgenic procedures. Orange varieties resistant to infection are already available (Della Colletta-Filho, 2014; De Souza *et al.*, 2014). Pruning proved to be effective (do Amaral *et al.*, 1994), but it was rigorously adopted at the beginning of the infection and supplemented with other containment measures. An essential measure was the intensification of monitoring which reveals the premature phases of the infection's development.

Annotation 13. In California 104 million dollars are invested each year to control *Pearce's disease*. Within the State, 40% of the grapevines in the Temecula area were infected. Temecula's response was to replace most of the diseased grapevines and to remove the source of inoculum by controlling the vector even in citrus plantations, which are the vectors' preferred hosts. The disease symptoms become more severe under drought *stress*, radical pruning of the roots, other diseases, super-production, senescence state. It follows that good crop practices are effective in reducing symptoms. The interventions adopted include containment, surveillance, rapid responses, advertising, research, biological control. During the vector's peak season, 38,000 traps placed in nurseries and in urban areas are activated (Almeida, 2016). The solution to the problem was the cultivation of resistant grapevines (Hopkins and Purcell, 2002).

Annotation 14. Monteduro M. - De Bellis L. - Brocca M. - Buongiorno P. - Di Benedetto S. - Isoni A. - Luchena S.S. - Pierri M. - Tommasi S. - Troisi M. - Denuzzo A. - Buia G. - Greco R.F. - Gusmai A. - Quarta D. - Stradiotti S. - Tascagni V., *Xylella fastidiosa* emergency: why the obligation to eradicate all infected olive trees (those lacking symptoms indicating possible infections and those not believed to be infected) within a radius of 100 meters from the infected ones is a legally and scientifically contestable measure, Research document (version specifically for public communication) November 12th, 2015.

The academic document is composed of 2 parts, the first dealing with juridical aspects and the second (elaborated by De Bellis) dealing with scientific aspects. The latter are developed in two chapters: 1. Inconsistencies in the translation of the Decision of execution of the Commission 2015/789/EU into the different languages of the Union; 2. Illogical and contestable elements present in the new Silletti Plan, decision of Execution 2015/789/EU and DM June 19th, 2015. `https://www.scienzegiuridiche.unisalento.it/c/document\_library/get\_file?uuid=e6554e67-f46f-46ef-b98c-7ea53ec76999\&groupId=10122`

Annotation 15. Funds for research activities on *Xylella*. The EU financed the Project called POnTE, coordinated by the IPSP-CNR of Bari with the participation of 25 *partners* including Research Institutes as well as small and medium-sized companies, involving 13 different countries, both European and Extra-European. The research activity, funded with a total sum of EU 6.8M, is focused on important pathogens, among which *Xylella* (a35). The EU, through an announcement expired on 2/17/2016, made available other EU 7M for further research on *Xylella*. `http://ec.europa.eu/research/participants/portal/desktop/en/opportunities/h2020/topics/6071-sfs-09-2016.html.`

MiPAAF allocated EU 4M for research activities coordinated by CREA `http://www.crea.gov.it/wp-content/uploads/2015/08/emergenza\_xylella\_ministro\_Martina.pdf`

In October 2015, Apulia published 3 announcements for projects of prevention and containment of the olive quick decline syndrome, financed for a total sum of EU 2M. `http://www.regione.puglia.it/index.php?at\_id=1\&te\_id=82\&page=curp\&opz=display\&id=9810.`

Other EU 5M should be made available in 2016.

Annotation 16. Resolution of commitment by the General Government to CRSA, DIBCA, IAMB for: (i) local monitoring activities for the quarantine of harmful organisms and subject of mandatory fight; (ii) the study of the main crops' diseases in the region, and development and divulgation of control methods; (iii) the control of plants, of plant-based products, and especially of plant propagating material.

This activity will be extended in December 2013 to the SELGE network coordinated by the University of Bari, which also includes the laboratories of the University of Foggia and Lecce. `http://www.selge.uniba.it/content/accreditamento-dei-laboratori-di-diagnosi-fitosanitaria-della-rete-selge-da-parte-del`

In particular, the Lecce unit within SELGE takes part to varietal identification activities. `https://www.disteba.unisalento.it/c/document\_library/get\_file?uuid=72561cdb-1c18-4843-8387-707b1eb0b914\&groupId=6046092.`

ANNEXES

Annex A. Chronology of the events in the development of *Xylella*, including a list of regional, national and international legislation which regulates similar situations. Active actions by MiPAAF, Apulia, and the EU

From 1992.

(a1). *Xylella fastidiosa* is regulated at the EU level as an organism subject to quarantine (Directive 2000/29/EC).
http://ec.europa.eu/food/fs/sfp/ph_ps/harm/legal/dir00_29_it.pdf

2013

(a2). October 21st, 2013.The Italian Authorities notify the first confirmed epidemic of *Xylella fastidiosa* subsp. *pauca* (strain CoDiRO) on olive trees in Apulia.

(a3). November 22nd, 2013. Emergency procedures for prevention, control, and eradication of the *Xylella fastidiosa* quarantine bacterium associated to *CoDiRO* are published. They provide for both the eradication of infected plants in the outbreak areas, and insecticide treatments carried out on host plants, and practices to limit the spreading of the infection (Decision of the Regional Council of October 29th, 2013, n. 2023).
http://www.sit.puglia.it/portal/portale_gestione_agricoltura/Documenti/normRegionale/PortalXylellaNormativaRegionaleIstanceWindowIDNEWS=58\&action=e\&windowstate=normal\&mode=view\&ACTION_NEWS=DETAIL

(a4). November 26th, 2013. EFSA publishes an academic document on host plants, means of entrance and diffusion, and risk reduction options for *Xylella fastidiosa* (Wells *et al.*).

2014

(a5). February 13th, 2014. The European Commission implements emergency actions to prevent the spread of *Xylella* within the EU (Decision 2014/87/EU).
http://eur-lex.europa.eu/legal-content/IT/TXT/HTML/?uri=CELEX:32014D0087\&from=IT

(a6). From February 10th to 14th, 2014, the Food and Veterinary Office (FVO) undertakes an *audit* to evaluate the situation and on site official controls for *Xylella*, confirming the rapid spread of the bacterium in the province of Lecce.

(a7). July 23rd, 2014. The Commission adopts more accurate emer-

gency measures to prevent the diffusion of *Xylella* within the EU (Decision 2014/497/EU);
http://eur-lex.europa.eu/legal-content/IT/TXT/HTML/?uri=CELEX:32014D0497\&from=IT

(a8). September 12th, 2014. MiPAAF sets up a scientific Committee to support the phytosanitary Committee in an in-depth examination of technical-scientific problems related to the *Xylella* emergency. Boscia, D'Onghia, Nigro, Porcelli, Saponari, Savino, Surico take part in the Committee which also benefits of the expert advice of Almeida and Purcell.
https://www.politicheagricole.it/flex/cm/pages/ServeBLOB.php/L/IT/IDPagina/9262

(a9). From November 18th to 25th, 2014, the FVO performs a second *audit* which confirms a dramatic deterioration of the situation compared to February 2014.

2015

(a10). January 6th, 2015. EFSA publishes a complete evaluation on the phytosanitary risk for the health of plants infected by *Xylella fastidiosa* in the EU, with an identification and an evaluation of the options to reduce the risk.
https://www.efsa.europa.eu/it/efsajournal/pub/3989

(a11). February 10th, 2015. The Commission organizes a meeting with representatives from member States, with the aim to constitute a group of technical and law experts for the revision of the EU's emergency measures.

(a12). February 10th, 2015. The Italian Ministry Council declares the state of emergency after the spread of the quarantine pathogenic bacterium *Xylella fastidiosa* in Apulia (GU no. 42 dated 2/20/2015).
http://www.gazzettaufficiale.it/eli/id/2015/02/20/15A01161/sg;jsessionid=DSgBQrf5H4tL+d3rGPdU5Q__.ntc-as1-guri2a

(a13). February 11th, 2015. Giuseppe Silletti, Regional Commander of the State Forestry Corps for Apulia, is appointed Delegate Commissioner for the *Xylella* emergency, by order No. 225 dated February 11th, 2015 of the Civil Protection Department - Presidency.
http://www.protezionecivile.gov.it/jcms/it/view_prov.wp?contentId=LEG50783

(a14). February 25th, 2015. The Commission organizes a high-level meeting with the Head of the Phytosanitary Service of the Italian Authorities to discuss the recent developments of the case *Xylella fastidiosa* in Apulia and of the actions envisaged.

(a15). March 9th, 2015. The Italian Authorities notify the Commission of new breeding grounds in the north of the province of Lecce, and a new breeding ground in the municipality of Oria, in the neighboring province of Brindisi, outside the circumscribed area of Lecce.

(a16). March 11th, 2015. The Commission organizes a high level meeting with the Permanent Representative of Italy to the European Union to discuss recent developments in Apulia and the actions envisaged.

(a17). March 16th, 2015. The Commission presents the progression of *Xylella fastidiosa* within the EU to the AGRIFISH Council.

(a18). March 16th/17th, 2015. Delegate Commissioner's Interventions Plan. Phytosanitary measures for the control of *Xylella* in the infected area in the province of Lecce.
`http://www.gazzettaufficiale.it/eli/id/2015/04/03/15A02500/sg;jsessionid=ugdgDE6QRqy1VFQXyGsKA\_\_.ntc-as2-guri2a`

(a19). March 20th, 2015. EFSA publishes a categorization of plants intended for cultivation, excluding seeds, according to the introduction risk of *Xylella fastidiosa*.
`http://www.efsa.europa.eu/it/efsajournal/pub/4061`

(a20). March 27th, 2015. The Commission submits to the Standing Committee the results from all Member States' annual assessment on the presence of *Xylella fastidiosa* in the EU.

(a21). April 17th, 2015. EFSA publishes a statement in response to the scientific and technical information provided which maintain that *Xylella* is not the cause of the decline of olive trees in the province of Lecce in southern Italy, but only an endogenous element present in trees which is neither active nor aggressive, unless a series of fungi infect the plants and create the conditions for the development of *Xylella fastidiosa*.
`https://www.efsa.europa.eu/it/efsajournal/pub/4082`

(a22). April 23rd, 2015. The Commission organizes a high-level meeting with the Delegate Commissioner appointed by the Italian Authorities for the *Xylella* case, with the purpose to discuss the developments of the outbreak and the measures envisaged.

(a23). April 30th, 2015. In a European Parliament plenary meeting, the Commission replies to an oral question on *Xylella fastidiosa* requested by

COMAGRI (Committee on Agriculture and Rural Development).

(a24). May 18th, 2015. The Commission adopts the measures to prevent further introduction and spreading of *Xylella fastidiosa* within the EU (Decision 789/2015/EU); a different definition of the Buffer Zone and of the Infected Area is set out, and a Surveillance zone is created, and new containment measures as well as the possibility to adopt specific containment measures in the Infected Area in the province of Lecce, where it is no longer possible to eradicate *Xylella*, are established.
`http://eur-lex.europa.eu/legal-content/IT/TXT/HTML/?uri=CELEX:32015D0789\&from=IT`

(a25). May 20th, 2015. The European Parliament implements a resolution on the *Xylella fastidiosa* epidemic affecting olive trees.

(a26). From June 8th to 19th, 2015 a third audit is carried out by FVO in Italy, particularly in Liguria, Tuscany and Apulia.

(a27). June 1st, 2015. Redefinition and update of *Xylella* circumscribed areas (Determination of the Director of Agricultural Service of June 1st, 2015, n. 195).
`www.regione.puglia.it/web/files/agricoltura/xilelladelimitazioneaeree.pdf`

(a28). June 29th, 2015. A Law Decree is published, containing the phytosanitary measures to prevent and contain the spreading of the *Xylella fastidiosa* harmful organism in Italy (Decree June 19th, 2015, GU no. 148 dated 06/29/2015).
`http://www.gazzettaufficiale.it/eli/id/2015/06/29/15A05031/sg`

(a29). July 6th, 2015. A report on the actions for contrast of *Xylella fastidiosa* in Italy is published. On the basis of the results obtained following 33,600 inspections, it is declared that the whole of Italy is unharmed by *Xylella* with the exception of the circumscribed areas of the provinces of Brindisi and Lecce.
`https://www.politicheagricole.it/flex/cm/pages/ServeBLOB.php/L/IT/IDPagina/8869`

(a30). July 19th/20th, 2015. Vytenis Andriukaitis, European Commissioner for health and food safety, visits the area affected by the epidemic in Apulia.

(a31). July 27th, 2015. The French Authorities notify the Commission of the first outbreak of *Xylella fastidiosa* subsp. pauca on Polygala

myrtifolia plants in Corsica.
https://www.eppo.int/QUARANTINE/special_topics/Xylella_fastidiosa/Xylella_fastidiosa.htm

(a32). September 2nd, 2015. EFSA publishes a scientific opinion on the treatment of grapevine seedlings with hot water against *Xylella fastidiosa*.
http://www.efsa.europa.eu/it/efsajournal/pub/4225

(a33). September 18th, 2015. The French Authorities notify the Commission of the first outbreak of *Xylella fastidiosa* subsp. *multiplex* in France, in the Provence-Alpes-CÃť te d'Azur area.
https://www.eppo.int/QUARANTINE/special_topics/Xylella_fastidiosa/Xylella_fastidiosa.htm

(a34). October 14th, 2015. A research announcement specifically focusing on *Xylella fastidiosa* is issued during the 2016/2017 work program of Horizon 2020. The announcement aims at promoting a comprehensive package of activities to increase the knowledge on the bacterium and to develop options for its prevention and control, and includes risk assessment tools and policies for plant health.

(a35). November 1st, 2015. The Commission finances a European project titled *Pest Organisms Threatening Europe* (POnTE) which addresses, among others, the study of *Xylella fastidiosa*.
http://www.ponteproject.eu

(a36). From November 9th to 20th, 2015 the fourth *audit* of FVO is carried out in Sicily and Apulia. The official report is not yet available.

(a37). November 12th/13th, 2015. EFSA, in cooperation with the Commission, organized a seminar in Brussels with scientists from the EU and from other countries in order to fill the blanks and define research priorities on *Xylella fastidiosa* for the EU.

(a38). November 19th, 2015. EFSA publishes a scientific opinion concluding that, at the present, it can be excluded that grapevine is a potential host plant for the Apulian strain (CoDiRO) of *Xylella fastidiosa*. Experts observed that, although investigations on the field are negative, there is no available information on infective vectors present in grapevines. On inoculum experiments, scientists questioned the low number of grapevines used, the rigor of the inoculation procedure, and the use of a single grapevine variety.
http://www.efsa.europa.eu/it/efsajournal/pub/4314

(a39). November 23rd, 2015. Member States approve additional actions, proposed by the Commission, against *Xylella fastidiosa*. The Commission's hearing takes place on December 17th, 2015.

(a40). December 16th/17th, 2015. Member States approve the CEE directives proposed by the Commission, which are aimed at strengthening coordination and harmonization of investigation programs on *Xylella fastidiosa* to be carried out in Member States in 2016.

2016

(a41). February 9th, 2015. EFSA publishes an updated database of *Xylella* host plants which includes 44 new species. Most of the new species (70%) is located in southern Italy (Apulia), in Corsica and southern France (Provence-Alpes-Côte d'Azur area).
`http://www.efsa.europa.eu/it/efsajournal/pub/4378`

(a42). February 26th, 2016. Review of the June 19th, 2015, Decree on emergency actions for the prevention, control and eradication of *Xylella fastidiosa* in Italy (Decree of February 18th, 2016, GU no. 47, 02/26/2016).
`http://www.gazzettaufficiale.it/eli/id/2016/02/26/16A01688/sg;jsessionid=dF3yNiMW92I3P-RUENg6gg\_\_.ntc-as5-guri2b`

(a43). March 29th, 2016. EFSA publishes the results of a work commissioned by the IPSP-CNR confirming that *Xylella fastidiosa* is responsible for the disease which is destroying olive trees in southern Italy. Even oleander, rosemary and myrtle-leaf milkwort are very sensitive to the Apulian strain of the bacterium, while citrus fruits, grapevines and holly oak seem to be immune.
`https://www.efsa.europa.eu/it/press/news/160329`

(a44). March 31st, 2016. EFSA publishes the PLH Panel's scientific opinion on four assertions which questions on the EU's control strategy against *Xylella*. `http://www.efsa.europa.eu/it/efsajournal/pub/4450`

(a45). April 20th, 2016. EFSA publishes the PLH Panel's scientific opinion on the effectiveness of some treatments against *Xylella fastidiosa*.
`https://www.efsa.europa.eu/it/press/news/160420`

(a46). April 8th, 2016. Apulia publishes a new Plan including phytosanitary measures to be implemented for the containment of the diffusion of *Xylella fastidiosa* subspecies pauca CoDiRO strain.
`http://cartografia.sit.puglia.it/doc/xylella/DGR\%20n.459\%20del\%2008-04-2016.pdf`

(a47). June 9th, 2016. The sentence of the European Court of Justice is published. The Court rejected the appeals brought before the TAR of Lazio as opposition to the decrees of removal of all host plants within a radius of 100 meters around the plants infected by the quarantine bacterium.

The sentence concludes that:

The examination of the questions raised has not disclosed any factor such as to affect the validity of article no. 6, paragraph no. 2, letter a) of the decision of execution (EU) 2015/789 of the Commission, dated May 18th, 2015, concerning measures to prevent the introduction and the diffusion within the European Union of Xylella fastidiosa (Wells et al.), as regards to Directive 2000/29/EC dated May 8th, 2000, concerning protective measures against the introduction into the European Community of organisms harmful to plants or plant products and against their diffusion within the European Community, as amended by Directive 2002/89/EC of the Council, dated November 28th, 2002, read in light of the principles of precaution and proportionality, as well as in relation to the obligation to state reasons in accordance to Article 296 TFEU and to Article 41 of the Charter of Fundamental Rights of the European Union.

http://curia.europa.eu/juris/celex.jsf?celex=62016CJ0078\&lang1=en\&lang2=FR\&type=TXT\&ancre=

Annex B. Schedule of visits and interviews. Annotations from visits and interviews with specialists and researchers

Schedule

3/22/2016 morning; Bari research area

Giovanni Martelli. Linceo. Full Professor, Unibari, Department of Soil, Plants and Food Sciences (Di.S.S.P.A.). Via Amendola, 165/A. 70126 Bari. Associate to Institute for Sustainable Plant Protection (IPSP).

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3/22/2016 afternoon; Mediterranean Agronomic Inst. of Bari (IAMB)

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Giandomenico Consalvo. Vice President of Confagricoltura and President of CIVI ITALIA (national Consortium among nursery groups and growers' union associations). Corso Vittorio Emanuele II, 101. 00186 Rome.

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3/23/2016 morning; Bari Research Area

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3/23/2016 afternoon; University of Matera

Cristos Xiloyannis. Full Professor. University of Basilicata, Department of European and Mediterranean Cultures: Architecture, Environment, Cultural Heritage (DiCEM). Via S. Rocco, 3. 75100 Matera.
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3/24/2016 morning; Lecce and University of Salento

Giovanni Seclì. Lecce Coordinator of the Environment and Health Forum (Nonparty network coordinating movements, committee and associations for the safeguard of the environment and of people's health), Via Vico dei Fieschi - Corte Ventura 2. 73100 Lecce.
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Giorgio Doveri. Member of the LAIR (Law and Agro-ecology - Ius et Rus) research Group, active since 2012 as project financed by the 0.5% (five per mil tax) funds at the University of Salento.
Marilù Mastrogiovanni. Journalist; blog curator and author of the *Xylella* Report.
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Annotations on the content of the meetings

b1. *Boscia on the discovery of the disease.* In August 2013 in Taviano (LE) an olive grower warned him about the disease. The symptoms were visible on different plants and became the object of investigations by DiSSPA and IPSP. It should be noted that a few months earlier, during a Conference, the symptoms had been associated to the presence of the insect *Zeuzera pyrina*. Saponari from CNR ruled out this possibility and advised a search for other etiological agents. In September, during an inspection, Martelli, basing his opinion on his knowledge of similar desiccation observed on grapevines in California, was the first to suggest the hypothesis of Xylella. Martelli asked J.K. Uyemoto (*Department of Plant Pathology, University of California*, Davis) if there were known cases of *Xylella* infections on olive trees (9/11/2013). Late September: Saponari, through the PCR and sequencing, confirmed the suggestion made by Martelli on 10 olive trees, as well as on almond and oleander trees. On October 13th, 2013, Saponari, Martelli, Boscia and Nigro reported the case to the regional sanitary Service, which reported it to the national sanitary service and to Bruxelles.

b2. *Boscia on suspects.* On October 28th, 2013, in Racale (LE), during a conference with farmers, suspicions arose on IAMB activities which, in October 2010, had organized a theoretical-practical workshop on *Xylella* within COST's periodic activities related to quarantine pathogens. Strains of *Xylella* received from Holland and infected grapevines were used, and a superficial conclusion was that the strains spread out in the environment. Bari, however, demonstrated that the Salento strain (classified as *Xylella pauca*, strain CODIRO ST53) was completely different from the one of the exercises (*Xylella fastidiosa*). Molecularly, a very similar strain was found in Costa Rica on coffee and oleander trees. The COST workshop on phytopathogenic organisms was organized by some of the leading international experts, including P.H. Janssen and R. Almeida. The practical exercises were carried out with the use of grapevine seedlings affected by *Pierce's disease* which were destroyed at the end of the course. It is a common practice to organize such conferences in an international context of large movement of goods, including plant material. Other similar conferences - in Caserta, for example - were organized for the purpose of prevention, in quite similar ways even as pertains to exercises.

b3. ***Boscia on strains.*** ST53 in Salento was identified on 22 different plant species, 5/6 of them perhaps asymptomatic. Asymptomatic olive trees also host the bacterium. A second strain found in Italy in Bolzano on an asymptomatic coffee plant (ST73) differs from *pauca*, and is completely different from *fastidiosa*. It is estimated that, in the last few years, 3000-5000 coffee plants have been imported in Europe. Boscia hypothesized that the infection may have spread from Alezio (6 Km from Gallipoli, 10 Km from Taviano). The climatic conditions of the Gallipoli Bay are similar to the ones of San Francisco Bay where the bacterium is present (Flint, 2016). He also separated the parasitic scorch (*Brusca*) symptoms on olive trees (present in the Adriatic region) from those produced by *Xylella* (Ionian). He concluded by making two important notations: first, only one strain is present in Salento; secondly, the strain was not there before, and this is deduced from evidence related to the 22 species which were not sick before the arrival of the strain. Additionally, new outbreaks on olive trees are all due to ST53, including its presence on asymptomatic olive trees. The ST53 strain was only found in Salento on olive trees and on other 22 plant species, as well as in Nicaragua on oleander trees. This cannot be confused with the strain that causes *Pierce's disease*, which is caused by the subspecies *fastidiosa*. In Loconsole *et al.* (2016), it is proven that a single strain is reported in Apulia.

b4. ***Boscia on regional monitoring of the diffusion of the epidemic.*** Conduced through Elisa, and if positive confirmed with PCR, it considered about 60,000 plants, almost all asymptomatic in the 2013-2015 period. In 2013 the bacterium was found in Gallipoli, after a few months in Lecce, then many outbreaks occurred, and in 2015 it was found in Brindisi and Taranto (December). The 2% of monitored plants is positive.

b5. ***Boscia on the removal-destruction plan.*** In April 2014, in Trepuzzi, infected plants were cut down. Nevertheless, in the summer 2015 the symptom spread out: 2000 plants were found positive to ST53. In February 2014, the EU had determined the block of nursery plants' exports from Salento's infected areas. In July 2014, the EU also had considered that only newly identified outbreaks should be removed and destroyed, including contiguous healthy plants and the diseased ones, as it was done for the Plum pox's *Sharka virus*, for the citrus's tristeza *virus* and for the fire blight.

b6. ***Boscia on the local non-acceptance of the plan.*** In addition to a criminal investigation, which led to the requisition of plants throughout Apulia, a litigation was brought by landowners in front of the TAR of Lecce, which blocked the destruction of trees in the appellants' plantations. The TAR of Lazio was also involved.

With reference to the provisions of the internal Judicial Authority, please visit the link `http://www.informatoreagrario.it/ita/files/Decreto\_Procura\_Lecce.pdf`

b7. ***Martelli on the peculiarity of the strain harmful to olive trees.*** In Brazil a strain devastating for citrus trees differs from ST53. In Puglia citrus trees tested exclude the presence of *Xylella*.

b8. ***Martelli and the origin of the epidemic recent invasion.*** His conclusion is in favor of a recent invasion on the basis of the following observations: i) in Europe, the presence only of *Xylella fastidiosa* subsp. *fastidiosa* (Kossovo) and of *Xylella* on ornamental plants in Corsica (strain *multiplex*) was known; ii) nevertheless, the Salento strain is *pauca*; iii) a strain of *pauca* similar to ST53 was identified in Costa Rica; iv) the strain ST53 transmits the disease to healthy plants; v) all samples from olive trees with symptoms reveal the presence of ST53.

b9. ***Martelli on measures.*** The Leccino variety seems to tolerate the *Xylella* infection better than others. A measure should concern the transplantation of this variety in the infected areas.

b10. ***Savino on possible concauses*** which contribute, together with *Xylella*, to the symptomatology, or which alone determine the symptoms appearance. He is determined to rule out concauses such as wood moth, fungi, and poor agronomy. His opinion is based on the existence of young sick plants which are not exposed to concauses other than *Xylella*.

b11. ***Savino on the epidemic.*** There is a progression front of the disease but also a spreading of point outbreaks explained through the migration of infected ornamental plants (Polygala, and at least other 15 ornamentals including myrtle and acacia). He suggests that even in Corsica the presence of several outbreaks of *Xylella* (*multiplex*) can be attributed to ornamental plants. He points out the lack of a European quarantine center for plants imported from other continents.

b12. ***Savino on measures.*** He is in favor of control interventions based mainly on eradication and vector control. He predicts future problems for almond and cherry plantations. At the current stage of development of the epidemic, he deems an expressed political will to solve the problem necessary.

b13. ***Porcelli on the disease.*** Quick desiccation is often an elusive and difficult symptom to be interpreted. Now we can assert that we are in the presence of a real disease.

b14. ***Porcelli on the strain.*** The almost absolute identity of ST53 with the Costa Rica strains and the low appearance of micro mutations within the bacterium (a nucleotide mutated every 15 years) assigns to Costa Rica the origin of the strain which has recently reached Salento through plant migration.

b15. ***Porcelli on vector and measures.*** Salento is quite fortunate because fewer vectors have been reported in the area compared to North America. The vector is the spittlebug (*Philaenus spumarius*) which transmits the bacterium canonically for this type of insects. The insect does not transmit when it is young, as it is not very mobile, and in the absence of reservoir plants. Herbaceous plants are possible vectors. The symptoms are evident in olive and oleander trees. An important measure is attention to ground cleanliness by good soil management practices. In serious epidemic conditions, between 250,000 and 500,000 vectors are located in each hectare, a density correlated to the level of symptoms. To control the vector, he proposes the use of crushing for younger insects. Neonicotinoid insecticides, as imidacloprid, may also be used especially against adult insects. Bees do not frequent olive trees, so the treatment has no consequences on beehives. A weeding delayed treatment would be of great success and have low impact.

b16. ***Porcelli on the local situation.*** The local social world holds varied opinions on the case, which are frequently opposite to the ones held by those assaying the etiology of CoDiRO. There are also political and ideological positions which oppose the possible technology transfer of scientific knowledge now available on the subject. Scientific institutions in Bari are criticized for their management of the funds designated to the study of the disease.

b17. ***Bari IAM on the 2010 Xylella course.*** The Coordinator of the COST 873 course was Prof. Daffi, Agroscoop, Zurich; the program examined crop plants' diseases, and the training with *Xylella fastidiosa* was crucial because it was feared that it could attack grapevines as it was doing in California. The use of the bacterium was authorized, and the infected vines and strains, which were required and received by an international bank for germplasm, did not belong to the subspecies *Xylella fastidiosa* subsp. *pauca*.

b18. ***Bari IAM on the bacterium's presence in Europe.*** After the detection of *Xylella* pathogenic for olive trees, and from 2010 onwards, the bacterium's interceptions became numerous, as if the problem had been underestimated before. It is now clear that ornamental plants nurseries, especially from Costa Rica and Honduras, are an opportunity for new arrivals. It is also true that, until 2013, the pathogenicity of the bacterium concerned mainly vines and citrus plants.

b19. ***Bari IAM (Cosimo Lacerignola Director, Maurizio Raeli Deputy Director. Vincenzo Verrastro, Annamaria d'Onghia, Michele di Giaro) and Xylella epidemic.*** The data published shows that the sample, curated by the regional Service, of symptomatic plants ascertains the presence of the bacterium in 99% of cases, while the value found for asymptomatic ones is 2-4% (analyzed by Elisa). This low incidence of asymptomatic hosts was mentioned by opponents of the *Xylella* causal agent to deny its role in the epidemic. The case of the Oria outbreak, where in the first instance only some of the symptomatic plants resulted infected by *Xylella*, was clarified by a second sample with 100% symptomatic plants carriers of the bacterium. In Torchiarolo, a town 20 km north of Lecce where the epidemic was signaled and where positive symptomatic plants were absent two years prior, now the disease is fairly widespread, probably transported by the vector.

b20. ***IAMB on strain ST53.*** Even local isolates in species different from the olive tree have brought back only to the ST53 strain in Apulia.

b21. ***IAMB and current situation.*** There is the possibility that the European Court of Justice will require the destruction of olive trees. If so, what will the institutions do?

b22. ***Consalvo, Confagricoltura, on the case.*** He declares himself to be astounded by the denial of the evidence of *Xylella* as causal agent. He claims that the Italian agricultural system did not react properly and that many damages have been created, especially in regards to the block of the plant nursery sector.

b23. ***Perrino and demolition.*** He is against the destruction of olive trees and questions the disease's progression. The reasons he advances are the following: olive trees monoculture is the epidemic's predisposing cause, as is the case for Salento; the available data is insufficient to conclude in favor of destruction; fungi presence is at least a concause; poor agronomical practices, such as errors in pruning and in soil management, errors with treatments and herbicides, and in particular the use of glyphosate - an herbicide - predisposes the plant to the disease, if it does not actually harm it. He warns that the use of the above mentioned herbicide in Salento is higher than in other provinces, and that he is aware of data showing its negative effect on beneficial microorganisms and its positive effect on harmful microorganisms. He acknowledges that experimental data on the role of these causes and concauses is not available or not accessible. He maintains that those adopting good agronomical practices and, even better, engaged in organic agriculture have no quick desiccation problems, a position which needs to be verified.

b24. ***Saldarelli on causes and concauses.*** In response to the first question, he says that experimental data on the epidemic possible concauses does not exist. He mentions a visit to the Gallipoli fields conducted during a conference on DOP Bitonto production in October 2013, where olive trees showed bacterial symptoms. The symptoms were present in well managed plots, irrigated, and with appropriate pruning. The same applies to organic olive growing (of low incidence in the Salento productions) which is not significant on diverting from the conventional one. He concludes that the etiological agent is *Xylella* and that the epidemic cannot be explained otherwise.

b25. ***Saldarelli and remedies.*** There is a low perception of the severity of the disease. However, if we compare the year 2015 with the year 2013, we notice progression of the disease and its seriousness. The Ugento area (south of Taviano) is greatly affected by the epidemic. The Leccino may be part of the solution: its bacterial concentration is 100 times lower than the one of other varieties, and the comparative transcriptomics of the genotype indicates some tolerant and resistant genes. On a visit to the fields around Gallipoli for territorial monitoring activities, he observed bacterial symptoms also in well-managed olive groves, irrigated, and with appropriate pruning. As the interviewee is from Terlizzi, a town near Bitonto where a

DOP is present, he affirms to have extensive experience in fields where herbicides are used without observing any desiccation symptom assimilable to the ones determined by *Xylella* on the Salento olive trees.

b26. ***Xiloyannis on good agronomic practices.*** Since 2001, he has conducted an experiment in management of 1 ha of sustainable olive grove in comparison with 1 ha of conventional olive grove. The sustainable one accumulated organic matter (from organic waste treatments from recycling) with evident increase in bacterial and fungal species in the soil. He reports being able to control plant infections with antagonists and not with fungicides. His experience should be useful for the case of *Xylella*, which he deems to be the etiological agent. He asserts it is possible to live with the disease without proceeding with destruction, but rather adopting good agronomic practices such as the reduction of tall foliage, the use of *compost* and water, the control of fungi which worsen the soil's state of health, tolerant variety, and superficial and minimal soil tillage.

b27. ***Xiloyannis and perspectives.*** Conducting research on possible concauses also using questionnaires. He solicits the definition of guidelines, lamenting the absence of agronomists in the regional technical- scientific Committee. He considers the deadlock to be detrimental.

b28. ***Xiloyannis and Xylella transmission.*** To a precise question, he answers that *Xylella* can be transmitted through pruning, mentioning a scientific publication (5% of transmission through pruning vs. 20% through vector).

b29. ***Seclì and desiccation symptoms.*** He asserts that groups of farmers noticed desiccation already in 2008, reporting them in 2009. He excludes that the IAMB workshop had a causal role in the epidemic. He has no certainties on the primary cause (for instance, he mentions the incorrect management of a phytopharmaceutical product in the area of Gallipoli where it was used against olive tree scab). Even so, he accepts the important role of *Xylella*, which might have existed locally since the end of the 18th century (described by A. Ciccarone, Foggia, who, in one of his publications, refers to a desiccation epidemic occurred in the mid-1700s). Mutation of the parasite?

b30. ***Seclì, concauses and measures.*** Concauses may exist if in the Gallipoli area both healthy and destroyed olive groves are identified, an evident fact only partially explained by the current knowledge on the development of bacterial epidemic. The factors involved may include: agronomical negligence, fungi, *Zeuzera pyrina*, pruning if it spreads fungi, excessive pruning, lack of definite rules on management of infected and healthy olive groves. He reports that, in 2014, SANCO inspectors signaled the presence of fungi which may cause the desiccation of olive trees. He states that senseless pruning may facilitate fungal infections, whereas it was observed that intensive pruning accelerates desiccation. He cites Dr. Scortichini (CREA, Caserta) who claims to know a useful antibacterial product, to be possibly evaluated as tool for the containment of *Xylella*. He reports on the use of a mixture of bacteriophages, an approach proposed in the USA for *Xylella* in grapevines (Carlos Gonzales, `http://www.oliveoiltimes.com/olive-oil-making-and-milling/phages-xylella-fastidiosa/48797`). He complains about delays in territorial monitoring and speaks out against felling of trees which were revealed to be ineffective in Brazil.

b31. ***De Bellis and multiplicity of causes.*** The discussion was not very productive. It emerged from it that the existence of various causes has no targeted experimental investigations. From specific inquiries on the speed of the spreading of the disease within the area, on hypothesis on the causes of the disease and on pruning infectious or non-infectious outcomes stemmed only uncertain and useless answers. He does not deem the bacterium to be the cause of *CoDiRO*: the symptoms are not the same.

Leccino is tolerant? That is not true! Leccino too reaches very high titers. There are no known causes for strain heterogeneity. It is necessary to ascertain with a questionnaire the overlap between agronomic practice and infection. It may be the scorch (*Brusca*), which was present here in the past.

b32. ***Doveri and Mastrogiovanni on the genesis from environmental pollution.*** The cause of the epidemic is not the bacterium. At most, it is a concause within the primary role of environmental pollution (contaminations from ILVA powders, cement, refuse-derived fuel waste, ashes-obtained fertilizer). The excessive use of water from groundwater through the exploitation of artesian wells generates dangerous infiltrations of polluted and marine water which makes the effects of irrigation less evident if not harmful. At the request for data relating to this role of pollution, they do not know or do not have reliable data available, if not that related to the slaughtering of livestock due to the presence of dioxin.

Annex C. *Xylella* epidemic in Apulia: genomic analysis and source of infection

The recently published data established that the responsible agent of the *Olive Quick Decline Syndrome (OQDS, CoDiRO)* is the bacterium *Xylella fastidiosa* subspecies *pauca*. The genomic analysis allowed scientists to determine that the bacterium responsible for *OQDS* is found with an identical sequence in all the symptomatic plants and in the ones infected during the incubation period, which may protract over time (Cariddi *et al.* 2014, Bleve *et al.* 2016). The bacterium isolated from infected plants, and cultured in pure form in laboratory, once re-injected in healthy olive and polygala plants, is able to reproduce the symptoms of the disease, fulfilling Koch's postulates (EFSA Report, 2016).

The subspecies of Xylella fastidiosa

It is necessary to mention concepts and procedures used to establish phylogenetic relationships among organisms, both if they are closely related (for example belonging to the same species but to different subspecies) and if they are taxonomically distant. One must consider that the polymerase synthesizing the DNA starting from its template and the repair system of the newly duplicated molecule cause errors in each replication (Shapiro, 2008). The error corresponds to the mutation rate which for bacteria varies from 10^{-8} to 10^{-9}, that is from 1 to 10 substitutions of a single nucleotide with a different one every 0,1 - 1 billion of individuals deriving from the reproduction of the same progenitor (Drake *et al.*, 1998; Rosche e Foster, 2000). It follows that every bacterial strain obtained by the same isolate has a certain probability to be somehow different from its parent and brothers (see Annotation annex C). This, although being an instance of levels of intermediation of theories and scientific processes, does not prevent taxonomists from producing certain interpretations of the phylogenetic relationships among and within species. In the case of *Xylella fastidiosa*, the bacterial pathogen was divided into the subspecies *Xylella fastidiosa* (*X.f.*) *multiplex*, *X.f. pauca*, *X.f. sandyi* (Almeida *et al.*, 2008; Kung and Almeida, 2011). The molecular approach to this categorization uses DNA sequences of microorganisms' gene fragments. The fragments are amplified (operation which, on its own, may add some mutations) and sequenced. The strength of the attribution of a strain to a specific *Xylella* subspecies is enhanced when the genes and their haplotypes considered in an experiment are at least ten (13 genes, of which 7 constitutive, in Elbeaino *et al*., 2014, for a total of 4161 base pairs, figure 1).

This procedure based on a set of genes - usually not involved in evolutionary processes leading to particular forms of evolution as for the genes resistant to diseases - is known by taxonomists as MLST method, *Multilocus sequence typing* (Maiden *et al*., 1998).

Figure 2 summarizes the concept visually: the isolates of the subspecies *X.f. pauca*, for example, have in common the same haplotypes of the genes *leuA*, *petC*, *lacF* e *holC*. The existence of so topographically close "mutations", that rules out a role of genetic recombination in separating them, attests that the strains *pauca* are molecularly similar "for ascendance", and that, while new mutations may appear randomly on the genomic DNA of new isolates of the bacterium, the isolates with the same haplotypes are to be included in the subspecies *pauca*. The analysis highlights also the differences between the *pauca* strain isolated in citrus plants in South America and the strain *pauca*/CoDiRO isolated in olive trees in Apulia (Elbeaino *et al*., 2014).

A very recent contribution to *Xylella* taxonomy (Marcelletti and Scortichini, 2016) uses a genomic approach applied to 21 bacterial strains, and reduces the subspecies to three: *fastidiosa*, *multiplex* and *pauca*. The *sandji* subspecies is included in the *fastidiosa* subspecies, and the strain present on pear trees in Taiwan would be an isolate of a new *Xylella* species. The methods adopted in the above mentioned contribution are based on the sequence of 956 genes corresponding to about 820 thousand nucleotides, sequences which were then primarily analyzed with a *Neighbor-joining* procedure generating a phylogenetic tree, and with a *Neighbor-net network* resulting in a phylogenetic network comparable to a more articulated phylogenetic tree. In the two trees the ST53 strain is, topologically, a typical member of the *pauca* subspecies, different from other strains and closer to the CFBP8072 strain, from which it differs, however, in 3% of nucleotides, confirming the uniqueness of the CoDiRO strain.

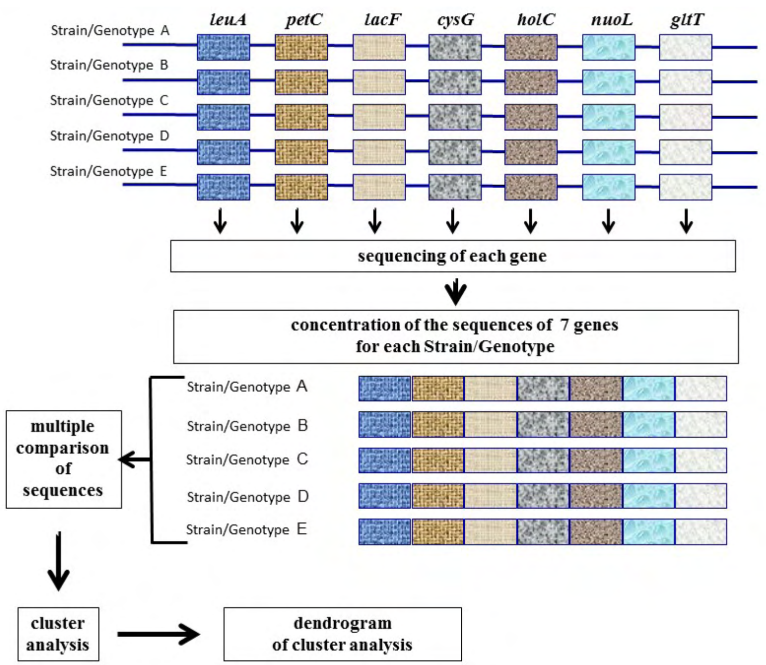
leuA
petC
lacF
cysG
holC
nuoL
gltT
Strain/Genotype A
Strain/Genotype B
Strain/Genotype C
Strain/Genotype D
Strain/Genotype E
sequencing of each gene
concentration of the sequences of 7 genes
for each Strain/Genotype
Strain/Genotype A
Strain/Genotype B
Strain/Genotype C
Strain/Genotype D
Strain/Genotype E
multiple comparison of sequences
cluster analysis
dendrogram of cluster analysis

Fig. 4

Family / genotype	Host	Loci MLST								
Xf6	Olive tree	*	*	*	*	*	*	*	pauca/ CoDiRO	53
Xf9	Olive tree	*	*	*	*	*	*	*	pauca/ CoDiRO	53
OLG2	Olive tree	*	*	*	*	*	*	*	pauca/ CoDiRO	53
KM13	Olive tree	*	*	*	*	*	*	*	pauca/ CoDiRO	53
OLDR-1	Oleander	*	*	*	*	*	*	*	pauca/ CoDiRO	53
PW1	Periwinkle	*	*	*	*	*	*	*	pauca/ CoDiRO	53
ALM1	Almond	*	*	*	*	*	*	*	pauca/ CoDiRO	53
9a5c	Orange	*	*	*	&	*	&	&	pauca	13
CVC0018	Orange	*	*	*	&	*	&	&	pauca	13
Dixon	Almond	o	o	o	o	o	o	o	multiplex	6
M12	Almond	o	o	o	o	o	o	o	multiplex	7
GB514	Grapevine	^	^	^	^	^	^	^	fastidiosa	1
M23	Orange	^	^	^	^	^	^	^	fastidiosa	1
Temecula1	Grapevine	^	^	^	^	^	^	^	fastidiosa	1
Ann1	Grapevine	§	§	§	§	§	§	§	sandyi	5

Table 2

The plants affected by Xylella

For the implications on understanding the genesis and the subsequent Apulian epidemic, the analysis of recent cases of *Xylella* infection offers the possibility to adopt a control of these bacterial strains. *Xylella fastidiosa* (Wells *et al*., 1987), especially in the Americas in a warm temperate climate, can attack many plant species (Janse and Obradovic, 2010; Purcell, 2013). In the last decade of the past century, the diseases caused by *Xylella* became a serious risk for the American continent, "*emerging diseases*" not noticed before. Vine, peach, plum, almond, maple, coffee, oak, pear and alfalfa plants are species which can be severely damaged by the disease, but the pathogen can be hosted by hundreds of asymptomatic plants (Chatterjee *et al*., 2008). For some of these diseases, the pathogenicity in areas newly colonized by exotic strains of *Xylella* is the cause of host-pathogen association not reported previously. This is the case of *Citrus variegated chlorosis*, *CVC* (Hopkins and Purcell, 2002). The *CVC* chlorosis by *Xylella* is a destructive disease in citrus trees, first detected in Brazil in 1987, which attacks all commercial varieties of sweet orange. In 1993, its causative agent was identified in a strain of *X.f.* subsp. *pauca* which, until 2013, seemed to have its diffusion confined to South America, particularly to Brazil but with recent findings in Argentina (Haelterman *et al*., 2015) and Paraguay. In Brazil *X.f. pauca* severely attacks coffee and orange plants but also olive trees; however, it should be noted that this strain of *pauca* isolated from infected olive trees has a sequence indicated with ST16, which differs from the one of the strain isolated in Apulia (Della Coletta Filho *et al*., 2016). Moreover, although the strains attacking orange and coffee are genetically similar, so much so to be both classified as *X.f. pauca*, each of them is specific to a single plant species. In citrus trees, the graft can transmit the *CVC*, and the bacterium is found in symptomatic plants' xylematic vessels. After the identification of the etiology in 1996, the bacterium's vectors were subsequently recognized to be 13 species of insect of the Cicadellidae family (leafhopper). Importantly, the symptoms of the infection may occur over a period ranging from 6 months to a few years (Colletta-Filho, 2014). The severity of the disease led to the adoption of stringent measures in 1993, such as the mandatory supervision of all nursery phases to be carried out in the absence of vectors in nursery or *tunnel*. Currently, CVC is endemic in all orange production areas in Brazil, and threatens to spread to North America (Redak *et al*., 2004). Therefore, orange varieties resistant to the *Xylella* infection have been developed, which is obviously the most effective way to fight the disease (Colletta-Filho, 2014).

Xylella epidemic in Apulia

In 2013, Saponari and coauthors reported for the first time the presence of *Xylella* on olive trees with desiccation symptoms (Saponari *et al*., 2013). *Xylella fastidiosa* had not been identified in Europe, other than in two unconfirmed cases: one in Kosovo on grapevines and the other in Turkey on almond trees (Martelli, 2015). The Xf9 strain isolated in Apulia from olive trees (Elbeaino *et al*., 2014a) is assigned to *Xylella* subsp. *pauca* (Simpson *et al*., 2000), and has a sequence known as ST53. In a phylogenetic tree, ST53 is part of the cluster *pauca* with the 9a5C and CVC0018 strains (Gianpetruzzi *et al*., 2016). In the area where the epidemic is present, new foci and plant species which the bacterium can infect indicate that the bacterial strain is just the same known as ST53 or CoDiRO (Loconsole *et al*., 2016; Bleve *et al*., 2016). The genome of the CoDiRO strain consists of 2,507,614 bp. In comparison with other strains, variations in genes encoding virulence factors have been found. The CoDiRO strain has the highest similarity to bacterial strains isolated in Central America, but not to strains found in California on olive trees (Gianpetruzzi *et al*., 2016). The vector of the bacterium is *Philaenus spumarius* a very common insect that, when collected in olive groves in areas affected by *Xylella*, has a percentage of infection up to 50% (Saponari *et al*., 2014). Also *Neophilaenus campestris* and *Euscelis lineolatus* insect species may host the bacterium. Plants showing symptoms of the presence of *Xylella* are cherry trees, *Polygala myrtifolia*, and rosemary plants. All symptomatic plants examined were found to be infected by the bacterium (Saponari, 2015). The sequences of 5 genes amplified by these plants are all 100% identical to homologous amplified by Apulian infected olive tree samples (Cariddi *et al*., 2014). *P. myrtifolia* and *W. fruticosa* were not known to be hosts of the bacterium. Many of the species infected with *Xylella* are asymptomatic, or the symptoms are identified many months after the infection, or it is hard to use them as diagnosis indexes as they are similar in shape and color to effects of drought. A synergistic role on the severity of the desiccation symptoms played by *Phaeoacremonium-, Pleurostomophora-, Phaeomoniella-* and *Neofusicoccum*-type fungi was often reported (Digiaro and Valentini, 2015; Carlucci *et al.*, 2013a; 2013b; 2015). Some of this data has been often mentioned to oppose the final association of *X.f. pauca* to the causal agent of olive tree desiccation. Conversely, the authors of this document approve both the conclusion of the European Agency EFSA confirming the identification of *Xylella* as causal agent of the disease, and EFSA's documentation giving a negative response to the proposition of primary infective causes, both abiotic and biotic, different from bacterial infection (EFSA PLH Panel, 2015; EFSA PLH Panel, 2016a; EFSA, 2016b (*Xylella* is causing the disease of olive trees in Italy); EFSA, 2016c; EFSA PLH Panel 2016d).

The origin of the Apulian strain of Xylella

The Apulian strain *X.f.* 9 of *X.f. pauca* has an allelic profile (ST53) very similar to one of *X.f. pauca* strains isolated in coffee and oleander plants in Costa Rica (Nunney *et al.*, 2014b; Digiaro e Valentini, 2015). *X.f. pauca* in Costa Rica has been associated to a coffee disease known as "crespera" (irregular leaf growth, leaf margins curling, and chlorotic mosaics). In June 2015, *X.f. multiplex* was found in Corsica on polygala plants. The European Union, facing the threat posed by the bacterium, strengthened the measures for the movement of plants. As a result, infected plants were isolated from coffee plants imported from Central America. It is a well-known fact that strains of *X.f.* subsp. *pauca* and subsp. *fastidiosa* can infect this host. It must be concluded that South and Central America are a reservoir of the bacterium's strains (Gianpetruzzi *et al.*, 2015a). However, as early as 2014 *X.f. pauca* and *X.f. fastidiosa* had been identified on plants imported from Ecuador, and diagnosed in coffee ornamental plants probably imported in Holland from Costa Rica and Honduras (Bergsma-Vlami *et al.*, 2015). After the detection of *Xylella* in Apulia and Corsica, plants infected by the bacterium were reported also in the South of Paris, and other 11 plants were detected in European ports (Loconsole *et al.*, 2016). Among intercepted plants, sequences similar at a 97-98% were obtained

(Bergsma-Vlami *et al*., 2015); in Northern Italy, three coffee bacterial isolates have originated from different subspecies of the bacterium (Loconsole *et al*., 2016). One of these isolates, CO33, has a molecular profile similar to ST72 (the two are probably recombined strains as they are similar to the possible new *X.f. sandyi* and *morus* subspecies). The Dutch bacterial isolates from coffee plants imported from Costa Rica are assigned to the same CO33 group (Gianpetruzzi *et al*., 2015b). In France, 2 of the strains isolated from 3 coffee plants were assigned to *pauca* and the third to *fastidiosa* (Legendre *et al*., 2014; Legendre, 2016). In Costa Rica six strains isolated from coffee and oleander plants and six strains isolated in Apulia from olive, almond, periwinkle, and oleander plants are molecularly identical (Bleve *et al*., 2016). The data provided lead to the conclusion that: globalization has undermined the international phytosanitary system (de Mesa, 2015) which, now more than ever before, the system is unable to identify and stop plants infected by pathogenic microorganisms. De Mesa (2015) summarizes the various passages which the bacterium *Xylella* has probably made from Costa Rica via Holland up to Apulia. EFSA concludes its statement on the *Xylella* risk by assessing that the vectors of the disease were and are plants which were spread via the transport from nurseries to olive orchards; the entrance of the bacterium into new areas is followed by the infection spreading via insect vectors (Stancarelli *et al*., 2015).

Delays in intervention to contain the disease

Already in 2000, at the time of the Brazilian researchers' publication on the decoding of the genome of *Xylella*, what the authors reported should have warned the Institutions interested in crop plants' health about the danger the bacterium posed. In fact, in the publication of Simpson and collaborators (2000), the authors commented that *Xylella*'s genes are similar to the *rpf* gene *cluster* 21 of *Xanthomonas* (*Rpf*, regulation of pathogenicity factors), which encodes regulatory proteins of the enzymes synthesis involved in the virulence activity. The fact that *Xanthomonas* is a fearsome pathogen with a broad host range (it affects nearly 400 species including solanaceae, brassicaceae and plums) is a clear indication that, as the other bacterium, *Xylella* can express pathogenic factors and regulate their synthesis (Chatterjee *et al*., 2008). It is ascertained that two crop plants typical of southern Italian, such as vine and orange, are extremely sensitive to some isolates of the bacterium. Hence, in Bari in 2010 the organization of a course on *Xylella* was logical and appropriate. Similar concerns motivated Brazil to sequence the bacterium's genome, first in the world to do that.

Regarding this, several local researchers and specialists are skeptical about the causal agent of the olive trees' disease. Nonetheless, the observation of symptoms - probably occurred in 2012 - of the disease was followed, already in 2013, by the first certain data on the bacterium's presence, on the molecular confirmation of the involvement of the *pauca* species (2014) and of its insect vector (2015), on the genome of the CoDiRO strain (2016), and on the disease's transmissibility via inoculum with CoDiRO (very quickly retracing the series for the same events in Brazil: 1987, 1993, 1996, 2000). The current deadlock status concerning the control of the disease's diffusion is partly due to the intense debates on its etiology.

Yet, the objection made that until the transmission via inoculum is not proved the cause of the disease is undefined, is unjustifiable: the Brazilian and Californian experiences made a clear prediction of the long wait for the symptoms to develop after artificial inoculation. Similarly, it is pointless to maintain that *Xylella* has been present in Apulia for decades, or that the strains attacking olive trees are different, which suggest that the bacterium is a usual host of this plant, including the CoDiRO strain viewed as secondary agent in the insurgence of the disease. In California, *Olea europaea* trees showing *leaf scorch* symptoms were analyzed for the presence of *Xylella fastidiosa*. 17% of them were positive, but the symptoms were not transmissible. Six strains isolated from these olive trees belong to *multiplex* subspecies. Pathogenicity tests on grape and almond plants confirm that the strains isolated from olive trees determine the outbreak of the disease in both the plants with symptoms typical to *multiplex* strain. In the case of *multiplex*, olive trees' infection has low efficiency and does not spread. One may conclude that olive trees can be a refuge for *multiplex* vectors which are radically fought with chemical means in the Californian vines and citrus cultivations (Krugner, 2014). Moreover, in light of the Brazilian experience, it appears that different isolates of the same subspecies *pauca* are specific for different plant species, a verification that renders plausible the idea that the CoDiRO strain should be deemed specific for olive trees.

The slow endeavor of containment measures precluded a targeted intervention of eradication. In fact, the experience related to the bacterium in the Americas shows that the latter can be eradicated only immediately after its appearance: once entered into a favorable area for climate and flora, the bacterium is so firmly established that its eradication is impossible and what remains to be done is to contain its spreading. The discussion on regional plans of intervention continues to this day. In the meantime, the disease proceeds: in October 2013 in Salento, after an accurate examination, it was estimated that the attacked olive trees covered approximately 8000 ha (Saponari *et al.*, 2013; Cariddi *et al.*, 2014; Lo Console *et al.*, 2014); one year later it was hypothesized that the affected area covered around 10,000 ha, comprising about a million olive trees; in June 2015 the whole province of Lecce of around 2,300 km^2 (230,000 ha) resulted infected (Martelli, 2015b, Fig. 5 and Fig. 6). Fortunately, the sensitivity of vine and citrus plants to *X.f.* CoDiRO can be excluded because naturally *pauca* has never been isolated from the two species, as confirmed by the results of artificial infections (Saponari and Boscia, 2015).

Scientific dispute

The discussion between two parts, which can be considered counterposed, has lasted for at least two years and at the present time it continues to focus on the molecular nature of the pathogenic variation of the bacterium for olive trees. The isolation and sequencing of bacterial strains present in olive trees affected by quick decline syndrome, the subject of this research, has determined, through the examination of DNA sequences, the taxonomic relationships between these isolates and other host strains of *Xylella fastidiosa* in other regions of the world, from several plant species and whose presence causes serious diseases in crop plants such as vines and citrus (and, today, olive plants). In regards to mass media, a higher attention towards the phenomenon was observed after the publication of a second work which reports the fragments' sequences of some genes by the Lecce CNR (Bleve *et al.*, 2016), given that these new sequences are 100% identical to those produced, and already available for some time, by

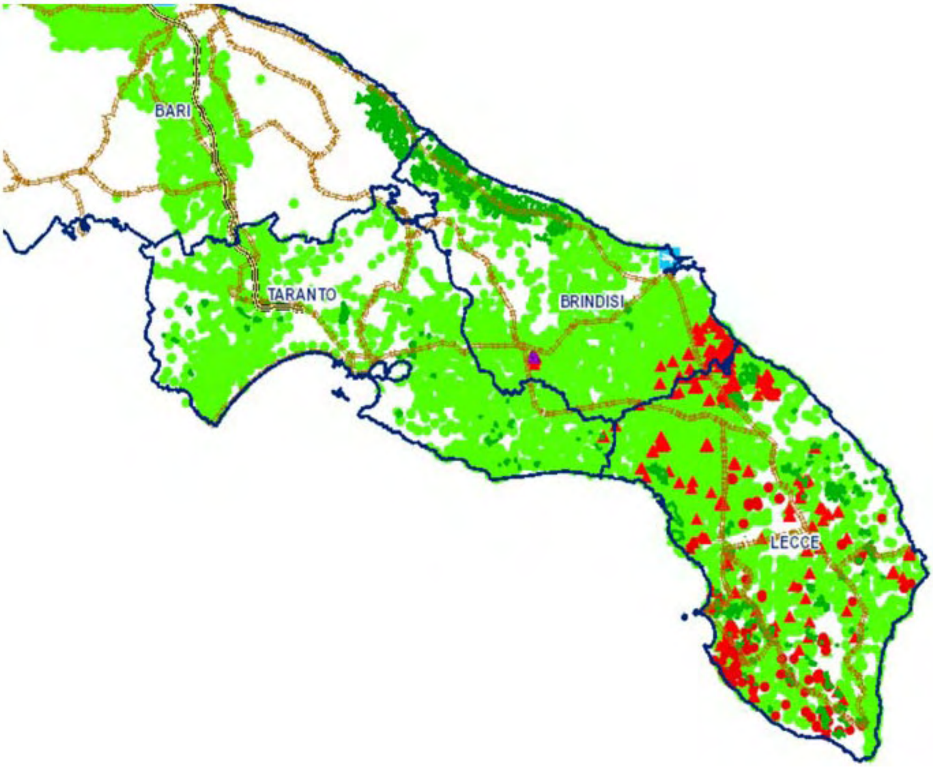

Fig. 5. – Epidemic of Xylella in Salento. The symbols in red represent areas where Xylella has been confirmed through molecular genetic testing. In light green olive trees, in dark green monumental olive trees.

Fig. 6. – Progression of the infection in an area near Gallipoli. The numbers indicate the date of aerial images. Trees analyzed where the presence of Xylella was confirmed are indicated in red, in light green are indicated the ones uninfected.

the CNR laboratory of Bari (Elbeaino *et al.*, 2014; Gianpetruzzi A. *et al.*, 2015; 2016). Nevertheless, it should be borne in mind that: i) the scientific Institutions of Lecce and Bari have expressed totally opposite views about the disease, notably in relation to its causes; ii) that identifying with certainty the *Xylella* strain responsible for quick decline syndrome has significant consequences. First of all, the hypothesis that multiple strains exist in Salento loses its value. Another hypothesis formulated by environmental groups at the first press conference where the identification of *Xylella* as cause of *OQDS* was announced (October 4^{th}, 2014) held the view that the responsible bacterial strain had leaked from laboratories where it was used in a European COST refresher course held in the Mediterranean Agronomic Institute of Bari (IAMB) in 2010. However, the Institute has proved that none of the strains used in the course matched the one associated to the olive trees' disease, a datum which is crucial to determine the possible route followed by the bacterium reaching Salento from Central and South America, the sites where it is endemic and evolves its variants (Chatterjee *et al*., 2008; Hopkins and Purcell, 2002; Nunney, 2014).

Conclusions

In this review of events, data and reports on the *Xylella* case in Apulia, we considered in interpretative terms what could be inferable from studies with a solid scientific basis. It is certain that the available molecular data represented an essential contribution to the comprehension of the nature of the disease's causal agent, its spreading, and the specificity of the *Xylella* strains for plants of relevant economic interest. The scientific information collected during the past few years in Apulia throughout the whole period of the *Xylella* alarm provided also unambiguous information, whereas the alternative hypotheses on the origin of *OQDS* were never further substantiated with objective data. Unfortunately, experimentally unverified hypothesis have delayed the implementation of measures for the containment of the disease.

Annotation to annex C

If the mutation frequency of a single specific nucleotide varies around 10^{-8} in bacteria (Drake *et al*., 1998; Rosche and Foster, 2000) (in a hundred million bacterial cells derived from the same progenitor, one cell has a nucleotide substitution to a precise DNA position), for a genome of approximately 2.5 x 10^6 nucleotides as the one of *Xylella* (Simpson *et al.*, 2000), a new mutation in any point in the sequence of the bacterial DNA has a probability of 1 x 10^{-8} multiplied by 2.5 x 10^6, that is 2.5 x 10^{-2} (a cell out of 250 has at least one of his two and a half million nucleotides different from the one of the progenitor; also cells with 2 or more mutations will be found, but with much lower frequencies). It should be noted that where Bleve *et al.* (2016) report that the sequence of their Salento-1 isolate is 100% identical to the one of the CoDiRO isolate sequenced by the Bari CNR (reported in Giampetruzzi *et al.*, 2015) and Xf9 (in Elbeaino *et al.*, 2014), both assigned to *Xylella fastidiosa* subsp. *pauca*, they refer to the 7 sequences of gene fragments. Therefore, as underlined, we cannot exclude that new mutations are present in other genome positions of the isolate. However, when two independent isolations of *Xylella, sampled from the same host plants in places where the bacterium has just established itself and where the epidemic is born at the center of an area which enlarges concentrically*, are assigned to the same subspecies, it is hard not to infer that they are ascribable to the same moment of appearance of the bacterium in the area considered. Indeed, there are differential characteristics in *Xylella*'s subspecies as concerns the *range* of host plants (Hernande-Martinez, 2007). This is important because molecular identification and assignment to subspecies of the different strains offer significant guidelines for controlling the spread of the epidemic (Saponari and Boscia, 2015). If we considered the probability of obtaining through mutation a new *Xylella* strain with a gene haplotype of another subspecies (for example, a new version with 4 mutations in the same positions and with nucleotides identical to the ones of the other subspecies), the frequency of this event would correspond to the compound probability of 1 x 10^{-8} to the fourth, equal to 1 x 10^{-32}, a value which is so low as to make the origin through mutation of a subspecies from another impossible. By contrast, the available data indicates the recombination with strains as a cause of new subspecies' formation. The MLST method is able to differentiate all subspecies (Almeida *et al.*, 2008) and, as the bacterium is able to use homologous recombination with subspecies, it is only using MLST that one can correctly determine the subspecies (Janse, *et al.*, 2012). The role of the intersubspecific homologous recombination was evident when the appearance of strains of *Xylella fastidiosa* able to attack mulberry trees was noticed. This disease referred to as *leaf scorch* was identified 25 years ago in California and is due to a *Xylella* strain which could be considered a new subspecies of the bacterium: *Xylella fastidiosa* subsp. *morus*. The gene sequence of the new isolate has among its progenitors *Xylella* subsp. *fastidiosa* (introduced from Central America) and *Xylella* subsp. *multiplex* (native of the USA) and its chimeric state is attributable to intersubspecific homologous recombination. The strain has reduced molecular variability, and, since *X.f.* subsp. *fastidiosa* is not able to attack mulberry trees, the shift to pathogenicity must have been favored by a strong selection on recombination products (Nunney, 2014). The *gene flow* via homologous recombination is currently considered an important evolutionary factor as a cause of emergence of new diseases caused by the bacterium (Kung and Almeida, 2011; Almeida and Nunney, 2015). However, the possible mutational origin of the ability to colonize a new plant which becomes host of the bacterium should be also introduced. In fact, single mutations in genes responsible for the bacterium's virulence (Simpson *et al*., 2000) may generate the compatibility between the bacterium and a new host plant. Nevertheless, even using advanced genomic platforms, it is difficult and complex to obtain certain proof that a single mutation transforms the bacterium into the causal agent of the disease.

Annex D. Annotations extracted from the bibliography

Almeida and Nunney, 2015. Why did a relatively unknown bacterium have its genome sequenced, being the first bacterium causing plant disease? The bacterium is able to establish itself in two distinct ecological habitats: vector insect's intestine and the plants' xylematic vessels. After mechanical or insect inoculation, in many plants *Xylella* is able to remain at the site of infection for long periods of time. However, asymptomatic infections diminish over time (Purcell and Saunders, 1999). An isolate of the same subspecies can be transmitted by various vectors in new colonized habitats. This promotes the bacterium's establishment into new areas. Symptomatic plant species are susceptible to specific isolates of specific subspecies; for this reasons the phylogenetic clades of the bacterium have a limited number of susceptible host plants (Nunney *et al.*, 2013). *Xylella fastidiosa* especially attacks tree species such as oak, elm, plane, *Liquidambar* and *Caria*. The bacterium is introduced into new environments mainly through the transport of potentially asymptomatic infected plants. ST53 in Costa Rica is identified in oleander plants (Nunney *et al.*, 2014). Homologous recombination within the species leads to the bacterium spread in new habitats.

Almeida *et al.*, 2008. *Cross-inoculation* experiments of 26 and 20 isolates of *X.f. pauca* - inducing, respectively, *CVC* (*citrus variegated chlorosis*) in orange trees and *CLS* (*coffee leaf scorch*) in coffee plants - indicate that the isolates from the two plants are biologically distinct: they infect only one species, i.e. the one they attack in nature.

Almeida, 2016. California has 4 problems due to *Xylella*: *Pierce's disease* in the North has been present for 100 years and now shows new epidemic forms; *Pierce's disease* in the South spread by a new vector which absorbs the greatest effort of state and federal governments; small outbreaks of desiccation of almond and oleander plants. Over the past 15 years, millions of dollars have been invested in monitoring, control, research, and technical assistance. However, there is no definite cure for vine infections. The only intervention is an integrated approach to the disease which includes multiple actions and strategies against the pathogen and its vector, as well as economic and environmental agricultural practices and social actions. *Pierce's disease* costs to California about 104 million dollars per year (Tumber *et al.*, 2014). Measures adopted (% for each action): pesticides in vineyards - 17.67; insecticides away from vineyards - 1.51; elimination of diseased plants - 38.88; pruning - 3.53; weed control - 3.53; cleaning field borders - 8.58; monitoring of both disease and vectors - 14.64. What happened in Southern California? The vector arrived in the 1990s. In the second half of the 90s, vector populations were extremely high, from 100 to 1000 insects per plant. The proximity to citrus crops accentuated the disease. A very serious infection reached 100% of the plants in a year; in the Temecula region 40% of vines were infected. Temecula's response was to replace most of the diseased grapevines, to remove the source of the inoculum by controlling the vector (monitoring; organic control; chemical control also in citrus crops), and to appropriately handle the vineyard. Today, vectors populations have been radically reduced. Chemical control of the vector: insecticide treatments on citrus crops which are vectors' preferred hosts; vineyard treatments to reduce the vector pressure. The disease diffusion is currently low: less than 1% of cultivations. California's State interventions: containment, surveillance, rapid response, advertising, research, biological control, regional area-wide projects. Quick responses: limits, rules, treatments, monitoring, 17 eradications. Monitoring: verification of non-infected areas; new vector's and bacterium's foci; applied to 49 counties (6 with infection, 36 without, 7 partly); in the vector peak season, use of 38,000 traps in nurseries and in urban areas.

Barba, 2016. The olive quick decline syndrome is a complex phenomenon associated with the management of olive groves and with infestations of insects like *Zeuzera pyrina*, with fungi of the *Phaeoacremonium* genus and with the *Xylella fastidiosa* bacterium. *Zeuzera* plays a secondary role, and fungi can aggravate the disease. According to the 2009/EC Directive, the presence of the bacterium in Europe stimulated the research necessary to clarify many unresolved issues. Vine and citrus plants are not infected by the CoDiRO strain. Maps showing the spread of the disease in 2013, 2014, 2015, and March 2016 are reported. Dramatic. In Italy only Apulia is declared infected.

Bergsma-Vlami *et al.*, 2015. *Xylella fastidiosa* subsp. *pauca* in Costa Rica has been associated to a coffee disease known as "crespera" (irregular leaf growth, leaf margins curling, and chlorotic mosaics). *Xylella fastidiosa* subsp. *pauca* and *Xylella fastidiosa* subsp. *fastidiosa* were recently found in coffee plants imported from Ecuador and in *C. canephora* plants from Mexico. *Xylella* was allegedly diagnosed in coffee ornamental plants imported to Holland from Costa Rica and Honduras in the fall of 2014. The molecular analysis confirms the presence of *Xylella* (from 96 to 100% identity with standard strains). From coffee plants, three different sequences similar at 97-98% were obtained.

Blave *et al.*, 2016. Salento-1 is not distinguishable from the CoDiRO strain which was assigned to *Xylella* subsp. *pauca*. On the basis of data and comparative analysis with other results, the subspecies *pauca, multiplex*, and *fastidiosa* can attack olive trees worldwide (California, Italy, Argentina and Brazil). *Xylella fastidiosa* (Wells *et al.*, 1987) is a gram negative bacterium that, especially in the Americas in a warm temperate climate, can attack many plant species (Janse and Obradovic, 2010; Purcell, 2013). Olive trees are infected with *Xylella* subsp. *multiplex* in California (Hernandez-Martinez *et al.*, 2007; Krugner *et al.*, 2014; partial indication). In the Salento peninsula the attacked olive trees cover about 8000 ha (Saponari *et al.*, 2013; Cariddi *et al.*, 2014; Loconsole *et al.*, 2014). Xf9, the first strain isolated from olive trees (Elbaino *et al.*, 2014), is phylogenetically assignable to *Xylella* subsp. *pauca* 9a5c (Simpson *et al.*, 2000). Xf9 has the same allelic profile (ST53) of the strains of *Xylella* subsp. *pauca* of Costa Rica isolated from coffee and oleander plants (Nunney *et al.*, 2014). Salento-1 *Xylella* has the same sequence of ST53 described for *Xylella* subsp. *pauca* Xf9 (Elbeaino *et al.*, 2014) and CoDiRO (Giampetruzzi *et al.*, 2015). Six strains of *Xylella* subsp. *pauca* from Costa Rica isolated from coffee and oleander plants and six strains isolated from olive, almond, periwinkle and oleander plants in Apulia are molecularly identical to ST53. In Brazil, olive trees are infected with *Xylella* subsp. *pauca* which has a DNA sequence serotype ST16, different from the strain isolated in Apulia (Della Coletta Filho *et al.*, 2016).

Boscia *et al.*, 2014. History of symptomatology.

Bosco, 2016. Bacteria are restricted to the insect vector's alimentary canal and not to its whole body. *Xylella* transmission is only possible for insects feeding on the xylematic sap; there is no species specificity, but all insects sucking the xylematic sap are to be considered potential vectors. The transmission efficiency varies depending on the insect, the host plant, and the *Xylella* genotype. At least 38 Cicadellidae species, Cicadellinae subfamily and 6 Aphrophoridae and Cercopidae species are *Xylella fastidiosa* vectors.

Among all the European species of insects feeding on the xylematic sap, only *Philaenus spumarius* transmits the CoDiRO strain of *Xylella fastidiosa* (Saponari *et al.*, 2014).

Almond, sweet cherry, oleander, broom, *Polygala myrtifolia*, *Westringia fruticosa* and *Acacia saligna* plants (Boscia *et al.*, 2014; Saponari *et al.*, 2013), and recently *Rosmarinus officinalis*, *Rhamnus alaternus* and *Myrtus communis* plants (Martelli *et al.*, 2016) are sources of inoculum for olive trees. Oleander plants are a terminal host of the bacterium (Boscia *et al.*, 2014) because *P. spumarius* is not able to feed itself and live on this plant. Further research on vectors would be necessary, especially on: phenology, ecology and *P. spumarius* preferences in olive groves of different Italian regions and in vineyards; even in areas where olive trees are not a monoculture; molecular characterization of different populations of *Philaenus spumarius*; chemical ecology and vector's behavioral response to plant's volatile compounds; insecticides for conventional and organic agriculture to use against the vector; characterization of the bacterium transmissibility; temporal dynamic of plant-host association. Conclusions: "*Little information at this time! A lot of research work is needed*".

Bosso *et al.*, 2016. These researchers at the University of Naples argue that the bacterium has the ability to spread throughout the Mediterranean basin, affecting olive trees and native plants in Portugal, Spain, Italy, Greece, Albania, Montenegro, Corsica and Turkey, as well as in the whole of North Africa and in the Middle Eastern countries. In Italy, the bacterium can spread over the north of Apulia into Calabria, Basilicata, Sicilia, Sardinia, Campania, Lazio and the south of Tuscany.

Bragard, 2016. The natural way of bacterium spreading is the insect vector which can fly for distances up to 100 meters, but which is transportable over longer distances by the wind. EFSA was tasked: to assess the risk associated with *Xylella* and its vector for the whole Europe; to identify options to reduce the risk and to assess their effectiveness; to evaluate phytosanitary interventions against the disease under the 2000/29/EC Directive; to consider the data emerging from investigations conducted in the infected area. Diseases caused by *Xylella*: *Pierce's disease* in vine plants; dwarfism in alfalfa plants; *Leaf scorch* in almond trees; *Phony dis-*

ease in peach trees; *Leaf scald* in plum trees; *Variegated chlorosis* in citrus trees; *Leaf scorch* in maple trees; *Leaf scorch* in oleander plants. Currently *Xylella*'s hosts (359) belong to 75 botanical families and host species vary for each bacterium strain. The bacterium is a serious risk to Europe. Conclusions on the arrival of the bacterium: ornamental plants transplanted into gardens. The probability is very high because 1) they are sources of the bacterium; 2) they may be asymptomatic; 3) they were imported from countries where *Xylella* is present; 4) bacteria survive transport; 5) especially in asymptomatic plants; 6) the transfer to a suitable host is simple. Conclusions on the arrival of the bacterium: infection from infected vectors. There is moderate probability that the bacterium moves via vector transportations as the disease is often associated with how the goods exit the exporting countries; the transport conditions can be non-permissive for the vector; it depends on existing phytosanitary practices in risk areas. Conclusions on bacterium establishment in a new area. The probabilities are high because the bacterium can infect one host out of the many in which he can live, and the vector can feed on several plant species; the bacterium adapts to different environmental conditions (there is conflicting data on its ability to survive the winter); control measures and cultural practices to prevent the establishment of bacterium are often inefficient.

Conclusions on the spreading of the disease: chances are very high due to the high number of host species, to the polyphagia and the spread of the *P. spumarius* vector, to the difficulties to control human movement from the infected area outwards, to the difficulty of containing the vector. Conclusions on the consequences. They are evaluated as relevant as: the production losses would be high; negative aspects relate to agriculture but also the activities derived from it; the impact on cultural, historical and recreational heritage is high; the impact of insecticides on trophic chains exists.

Cariddi *et al.*, 2014. The bacterium is isolated and cultured in infected olive trees where, however, other bacterial contamination makes this operation difficult. Pure cultures are easily obtained from oleander and periwinkle plants. Isolated strains allow for the sequencing of gene fragments. The sequences indicate that strains from oleander and periwinkle trees are identical to those amplified by olive trees, and comparing them to sequences available in the literature allows us to assign the bacterium to the *pauca* subspecies.

Chatterjee *et al.*, 2008. The disease caused by *Xylella fastidiosa* in its numerous subspecies is described for alfalfa, peach, plum, almond, maple, coffee, oak, pear and for several other plant species, and the bacterium can be identified in hundreds of other asymptomatic plants. Already in 2008, the *Xylella* allegation and its similarity to that of *Xanthomonas* indicated this species as extremely dangerous to plants. *Xylella fastidiosa* has been associated with many diseases in the past, new and important diseases caused by the bacterium and the dissemination of its vectors makes *Xylella* significantly interesting from an economic point of view for the damage it causes. At the genomic level the pathogen hosts virulence systems similar to those of *Xanthomonas*, but it is different for specific gene complexes of pathogenicity.

Chauvel *et al.*, 2015. In Corsica, 56 *multiplex* cases were isolated from Polygala, and one from broom. Plants which are contiguous to those positive to the bacterium were free from infection. The report supports the need for further research on the bacterium and its phylogeny, on the still unknown vector, favoring territory surveillance. Objectives. Origin: plan for possible measures (14 signaled) to know the bacterium's origin in Corsica. Vector: potential insect species; considering *P. spumarius*; crop plants potentially hosting the vector; biology of vectors and of bacterium reservoir plants. Agricultural production: control of citrus and of exported citrus plant; verification of favorable climatic conditions; information campaigns. In the Americas the bacterium is reported from Argentina to Ontario.

In Apulia more than 200,000 ha are infested with *pauca*. *X.f.* reported in Iran (Amanifar *et al.*, 2014).

France. National surveillance plan from May 13th, 2015. The number of vector insects is correlated to the transmission rate of *Xylella*. The 139-page report reflects the French concern that *Xylella* will establish itself becoming harmful in the country.

Choi *et al.*, 2013. Although under a transcriptome analysis the response of vine to the *Xylella* infection is different from the one induced by water shortage stress, the data indicates that the two situations have a partially common modification of the gene expression, often with evidences of synergistic molecular alterations.

Cornara *et al.*, 2016. Four insect species feeding on the xylematic sap have been found in olive groves and in the surrounding vegetation. *P. spumarius* is the most abundant species and the only one positive for *Xylella*. The insect's nymphs are formed within the spit-shaped foam, and adult insects migrate from olive trees from May to October. The insect transmits the bacterium from infected to uninfected olive trees.

Della Colletta-Filho *et al.*, 2014. The bacterium colonizes fruits and seeds; however, after experiments repeated for 7 years, it is not found in seedlings derived from infected seeds.

Della Colletta-Filho, 2014. Until 2013, The *X.f.* subsp. *pauca* bacterium was restricted to South America, especially to Brazil but with recent findings in Argentina and Paraguay. In Brazil, it severely attacks coffee, orange and plum trees. Although the strains attacking coffee and orange trees are so genetically similar to be both classified as *pauca*, the latter do not attack the above-mentioned species. In 1987, the orange disease known as CVC emerged. It does not lead to the plant's death, but it reduces its production very significantly. It is not transmitted by seed but is widespread by 13 insect species. The symptoms are environment-dependent. The insect can transmit CVC, and electron microscopic studies revealed that bacteria were present in symptomatic plants' xylematic vessels. In 1993, the etiological cause of CVC was identified in the *Xylella fastidiosa* bacterium. Later, also the bacterium's vectors were identified. The post-infection asymptomatic period ranges from 6 months to a few years. In 1993, the severity of the disease recommended the adoption of stringent measures such as mandatory supervision of all nursery phases to be carried out in *screenhouses* in the absence of vectors, selection of mother plants for reproduction, vectors and inoculum reduction, genetic improvement even with cisgenic procedures. All sweet orange varieties are susceptible (with exceptions); mandarin varieties are resistant; hybrids between *C. sinensis* and *C. reticulata* are resistant, as well as lemon trees. Today, CVC is endemic in all orange production areas in Brazil. Orange varieties resistant to *Xylella* infection have been developed.

Das *et al.*, 2015. *Pierce's disease* in grapevines is caused by *Xylella fastidiosa* subsp. *fastidiosa*. The effect of a mixture of 4 lytic phages on *Xylella* was evaluated for therapeutic and prophylactic purposes to cure infections on grapevine. The bacterium's level of presence resulted to be significantly reduced. In particular, a slowdown in the progress of PD symptoms has been noticed, and the appearance of *Xylella* strains resistant to phages has not been noticed. The experiment may be a basis for developing methods for the bio-control of the bacterium.

de Mesa, 2015. He reports the transfer of *Xylella* from Costa Rica via Holland up to Apulia. Globalization has undermined the international phytosanitary system.

De Souza *et al.*, 2014. He describes two strategies for CVC control; the first is based on the improvement of orange trees to the *Xylella* infection using crosses of *Citrus sinensis* with *Citrus reticulata*. The second uses the N-Acetylcysteine compound, used against human infections. Significant symptom remissions and reduced bacterial replication are observed after treatment. A form of slow-release fertilizer has been developed to apply the compound to crops.

Digiaro and Valentini, 2015. In favor of the ST53 strain as causal agent of the epidemic.

Synergistic role on the severity of the symptoms of fungi of the *Phaeoacremonium* and *Phaeomoniella* type. In June 2015, approximately 90,000 acres infected in Salento. The whole genome of *Xylella fastidiosa* subsp. *pauca* ST53 is sequenced (Gianpetruzzi *et al.*, 2015). Close resemblance to Costa Rica's *pauca* where it infects flowers often exported to Europe. Grapevine and citrus plants susceptible to CoDiRO in nature should be excluded as *pauca* has never been isolated from these species. The vector is *P. spumarius*. The *Neophilaenus campestris* and *Euscelis lineolatus* species may host the bacterium. Commentary on the DM 2777 Law dated 9.26.2014 for the bacterium's mandatory control. The need for the general public to be educated to avoid movement of infected plants and the risk of new outbreaks requires finding olive trees with genetic resistance to *Xylella*. Molecular techniques for quick monitoring. Vector control.

It is not the only recent case of invasion. Other cases are attributable to *Rhynchophorus ferrugineus, Drosophila suzukii, Dryocosmus Kuriphilus, Tuta absoluta, Anoplophora chinnensis* and *Pseudomonas syringae actinidiae.*

Dongiovanni *et al.*, 2016a. The best outcomes for the control of insect vectors have been accomplished with formulations of neonicotinoid insecticides: acetamiprid and imidacloprid.

Elbaino *et al.*, 2014a. Use of *Multilocus sequence typing* (MLST) for *Xylella* phylogeny based on fragments of 13 genes (4161bp). The strain isolated in Apulia from olive trees shows the highest molecular similarity with *pauca*, despite being different from the known *pauca*, defining the ST53 serotype. In a phylogenetic tree, ST53 is part of the cluster *pauca* with the 9a5C and CVC0018 strains. The genetic proximity of the ST53 strain to the *pauca* subspecies suggests it to be potentially pathogenic for other agricultural species, particularly for citrus trees which are heavily attacked by *pauca* (the etiological agent of the CVC disease). According to D. Boscia, ST53 is not infectious for citrus trees.

Elbaino *et al.*, 2014b. *Xylella* is found *P. spumarius*, *Neophilaenus campestris* and *Euscelis lineolatus* insects, but only in insects caught in areas infected by the bacterium.

Fadel *et al.*, 2014. After noticing that a variety of sweet orange resisted to *Xylella fastidiosa*, a consideration of bacterium transmission experiments shows that the variety has no symptoms after inoculation and has low bacterial concentrations in its tissues.

Frisullo *et al.*, 2014. In the past, several cases of olive tree desiccation referred to as “scorch” have been noticed in the province of Lecce. The coincidence of these symptoms with those of CoDiRO generated speculation on the presence of *Xylella fastidiosa* in the province of Lecce in the last 250 years. This work denies this hypothesis and blames the *Xylella* bacterium for the olive tree quick decline syndrome. 50-70-year-old trees or younger have a less severe disease syndrome than the one found in 100-year-old trees. These trees may not even die.

Gianpetruzzi *et al.*, 2015A. The CoDiRO strain’s genome consists of 2.507.614 bp (52% GC). The genome has also been annotated (6 genes rRNA, 49 tRNA, 2,053 genes encoding proteins). A 35.318 bp plasmid is present and has a 98% similarity with pXF-RIV5 conjugated plasmid, although the first differs from the latter in encoding genes of the toxin-antitoxin system. In comparison with other strains, variations in genes encoding virulence factors have been found. The CoDiRO strain is genetically assigned to *Xylella* subsp. *pauca* having the highest similarity with the bacterium’s strains isolated in Central America but not with strains found on olive trees in California.

Gianpetruzzi *et al.*, 2015b. In June 2015, *Xylella* was found in Corsica. The European Union, facing the threat posed by the bacterium, strengthened measures regarding the movement of plant material. As a result, infected plants were isolated in Holland from coffee plants imported from Central America. It is a well-known fact that strains of *Xylella* subsp. *pauca* and *fastidiosa* can infect this host. It must be concluded that South and Central America are a reservoir of genetic and biological *X. fastidiosa* diversity. CO33 isolates coming from a coffee plant intercepted in Northern Italy has a molecular profile similar to ST72. Other isolates from coffee plants imported in Holland in October 2014 from Costa Rica are assignable to the same CO33 group. CO33 is genetically close to several subspecies of X. *fastidiosa*. The prevailing part of read fragments of its sequence is associable with strains of subsp. *sandyi* or subsp. *morus*, a datum in support of the genetic complexity of this plant pathogenic bacterium and of the role of sub-interspecific homologous recombination in generating new variations.

Gianpetruzzi *et al.*, 2016. The presence of *Xylella* at gene expression level is evident both in the cv Ogliarola (susceptible to the bacterium) and in the Leccino (tolerant). The over-expression of coding genes for *kinase receptor like* (RLK) and *protein receptor like* (RLP) is typical of Leccino and absent in Ogliarola. It is plausible that Ogliarola reacts to *Xylella* with a similar response to that elicited by drought, while Leccino, which shows a lower bacterium titer, being less sensitive to *X.f.* and better suited to containing the bacterium's presence, does not have the same reaction as Ogliarola and does not over-express genes in response to drought stress.

Gualano *et al.*, 2014. Aerial images would facilitate an early detection of the disease symptoms and, thereby, the detection of new outbreaks.

Guario *et al.*, 2013. The authors write: "*The pollution of groundwater and irrigation water or the introduction of toxic substances in the soil are not enumerated among the causes of desiccation for the reason that the symptomatology affected exclusively olive trees and not other crops (citrus trees and other orchard plants including grapevine), ornamental plants, or plant species commonly present in the same area, even in the form of combined crops, which did not show any symptom of vegetative decline, although identified as sensitive to xenobiotics*". They report the key role of *Xylella fastidiosa*. They report the assignment of the bacterium strains to four major subspecies, including *pauca* which infects citrus and coffee.

Haelterman *et al.*, 2015. The *pauca* subspecies was found on olive trees with desiccation symptoms in Argentina and assigned to the subspecies through molecular methods.

Hartung, 2014. Results as in Colletta-Filho *et al.*, 2014.

Hernande-Martinez *et al.*, 2007. Phylogenesis in the *Xylella* genus.

Hill and Purcell, 1995. The bacterium's permanence in the plant depends on the infection period: in summer, the shoot recovers over the winter and frees itself from the bacterium in the following season. Bacterium hosts are a source of infection. The bacterium's multiplication has a different kinetics according to the different plant hosts.

Hopkins, 2014. Data on systemic insecticides against vectors, the removal of host species, abiotic stress reduction, genetic resistances, and inoculation with benign strains are reported.

Hopkins and Purcell, 2002. In the last decade of the past century, the diseases caused by *Xylella* have become a serious hazard for the American continent. They are "*emerging diseases*", not noticed before but which today represent a serious issue. For some of these diseases, the pathogenicity by exotic strains of *Xylella* represent a new host-pathogen association. This is the case of *Citrus variegated chlorosis* (CVC) in South America in the 1980s, and of the appearance of the *oleander leaf scorch* in California in the 1990s. *Pierce's disease* (PD). In 1880, a new disease basically destroyed vines in the area around Los Angeles. Newton Pierce described the symptoms of the disease so thoroughly that they are still rigorously recognizable in currently sick plants. However, neither him nor his successors were able to indicate the causal agent of the disease. Fifty years later, an epidemic in California created new interest in the disease, and in the period between 1939 and 1945 the xylematic sap sucking insects were discovered to be the vectors of the infections. The solution to the problem posed by *Xylella fastidiosa* was the cultivation of resistant grapevines. The PD symptoms become more severe under drought stress, radical pruning of roots, other diseases, super-production, and senescence state. It follows that good crop practices are effective in reducing the symptoms. The use of attenuated strains of the bacterium which do not provoke the disease's insurgence can limit the effect of virulent strains. A realistic possibility is to prevent the introduction and the spread of exotic strains of *Xylella*.

Krell *et al.*, 2007. Four possible transmission mechanisms of *Xylella* in *Vitis vinifera* were examined: natural graft transmission between roots, transmission via pruning, and the transmission via two Californian insects, nymphs and adults of *Homalodisca liturata* and adults of *Diceroprocta apache*. Natural grafts between roots of grapevines both immune or infected with *Xylella* were not found and, therefore, bacterium transmission via natural graft is excluded. One out of 21 transmission attempts via pruning induced the *Xylella* infection. Although the chance is relatively low, further investigations of potential mechanisms of new *Xylella* infections are still needed.

Krugner, 2014. In California, *Olea europaea* trees showing *leaf scorch* or *branch dieback* symptoms were analyzed for the presence of *Xylella fastidiosa*. 17% of them were positive, but the symptoms were not transmissible in greenhouse. Six strains were isolated and attributed to *Xylella* subsp. *multiplex*. Pathogenicity tests on grape and almond plants confirm that the strains isolated from olive trees determine the outbreak of the disease in both plants with typical symptoms caused by *multiplex*. Olive tree infection has low efficiency and does not spread. *Homalodisca vitripennis* may transmit *multiplex* and *fastidiosa* strains to olive trees but with low efficiency. One may conclude that the *fastidiosa* subspecies does not cause disease in the olive tree, a plant that can be considered an alternate suboptimal host for *Xylella*. Olive trees can be a shelter for *multiplex* vectors which are radically fought with chemical means in grapevines and citrus cultivations.

Janse *et al.*, 2012. It provides a table illustrating plant species from which *Xylella* strains were obtained. The complete list of hosts can be found at `http://www.cnr.berkeley.edu/xylella.`

It reviews diagnostic methods used in COST 873.

Important: Figure 1 shows symptoms of the presence of *Xylella* on *Citrus sinesis* and *Vitis vinifera* by experiments performed in the COST 873 course. The symptoms are observed in the two species after inoculation with an LMG 9061 strain, a not *pauca* strain. A review of PCR methods for *Xylella* was prepared by Doddapaneni *et al.*, (2007). The MLST method is able to differentiate all subspecies (Almeida *et al.*, 2008). The bacterium is capable of activating homologous recombination, and, for this reason, it is only when using MLST that *Xylella* subspecies can be identified with certainty.

Joudar, 2016. *Xylella fastidiosa*, a quarantine controlled organism (annex 1AI of the 2000/29/EC Directive). Organism for which the fight is mandatory everywhere (annex A of the French Ordinance dated June 31^{st}, 2000). First category organism (Ordinance dated December 15^{th}, 2014).

Lacirignola *et al.*, 2015. It is believed that *P. spumarius* is the vector, and other two insect species are potential vectors. Spy insects: bioindicators for the bacterium's presence in areas considered to be bacterium free. Development of rapid methods based on the improvement of ELISA and PCR tests (Djelouah *et al.*, 2014; Yaseen *et al.*, 2015).

Lapedota, 2015. 60 million olive trees in Apulia, almost 190 thousand companies of which 64% have a medium extension under 2 ha. It brings back a piece of history of what happened. In 2014 the regional Plant Protection Service identifies the areas infected by *Xylella* (decision no. 157 dated 4/18/2014), and reports the case to the Agricultural Ministry and to the EU. The Council of Ministers declares the state of emergency (February 10^{th}, 2015) and the Civil Protection manager appoints an emergency Commissioner, Gen. G. Silletti (OCPDC 225 dated February 11^{th}, 2015). The Commissioner must draw up an intervention plan. At the EU Standing Committee on Plant Health it is argued that eradication within a radius of 1 km had to be enlarged to cover 15 km. The plan is approved on March 18^{th}, 2015 and considers the measures listed in the DM 2777 dated 9.26.2014. *P. spumarius* is certainly the vector of the *pauca* CoDiRO strain. The plan includes the elimination of herbaceous plants in spring and the use of insecticides in fall.

Legendre *et al.*, 2014. In 2012, 3 coffee plants carrying *Xylella* were detected in France. Two *Xylella* strains isolated from these plants were assigned to *pauca*, the third to *fastidiosa*.

Legendre, 2016. June 2015: first finding in Corsica. October 2015: first finding in the PACA region. 2012: first detection on coffee intercepted in France. 2014: laboratory tests for the validation of the bacterium; Italy, the Netherlands, New Zealand, England, and France participate in the laboratory tests. Two different profiles of *Xylella fastidiosa* concern Corsica and the PACA region isolates: *Xylella* subsp. *multiplex*, profile A (ST7) and profile B (ST6), other than the CoDiRO *X.f.* subsp. *pauca* present in Apulia.

Loconsole *et al.*, 2016. In Apulia, in the area where the epidemic is ongoing, new foci and plants infected by the bacterium indicate that the bacterial strain is still the one known as ST53 or CoDiRO. Many of the species infected with *Xylella* are asymptomatic, or the symptoms are identified many months after the infection, or it is hard to use them as diagnosis indexes as they are similar to the ones induced by drought. After the detection of *Xylella* in Apulia and Corsica, plants infected by the bacterium were identified also in south of Paris, 11 were detected in European ports, and more recently in Corsica. The taxonomy of *Xylella fastidiosa* is complex, depending also on homologous recombination acting in cross-overs between species and subspecies (Kung and Almeida, 2011). Three coffee isolates in Northern Italy belong to different bacteria subspecies, while the strain already described as ST53 for Apulia is a *pauca* strain. The gene flow via homologous recombination is an important evolutionary factor inducing the emergence of new diseases caused by the bacterium (Almeda and Nunney, 2015).

Loconsole *et al.*, 2014. Setting up of Elisa and PCR analyses to reveal the presence of *Xylella* in plant tissue samples. Elisa is preferable to epidemiological investigations.

Martelli, 2013. Martelli communicates the presence of *Xylella* on infected olive trees, but suggests that it may be a contributory cause of the disease as it is also accompanied by pathogenic fungi.

Martelli, 2015a. Detailed summary of the case. It reports how we came to regional measures. In the buffer zone (2 km alongside the infected area) and in the sanitary cordon zone (2 km distant 10 km from recent outbreaks) the following interventions are scheduled: i) insecticide treatments against vectors; ii) agronomical interventions against vectors' juvenile stages and against herbaceous weeds; iii) the elimination of host plants present along roads, streams, ditches, green areas; iv) monitoring of the presence of *Xylella* on host plants.

Martelli, 2015b. In fall 2014 the infected area was estimated to cover 10.000 ha and diseased olive trees 1.000.000 ha. In fall 2015, the diseased olives covered an area of 2300 km^2 approximately 230.000 hectares. The bacterium's detection is reported for the first time by Saponari *et al.* (2013). *Xylella fastidiosa* had not been identified in Europe other than in two unconfirmed cases, in Kosovo on vines and in Turkey on almond trees.

The experience related to the bacterium in the Americas shows that once the bacterium enters into a favourable territory for its appropriate climate and flora, it is so firmly established that its eradication is impossible. In spring 2014, the situation was such that eradication became impossible and the only plausible approach was the containment of the diffusion area. There followed the "saga" of regional intervention plans which continues to this day, even with the judiciary intervention. The Cellina di Nardò and Ogliarola olive tree varieties, almost exclusively grown in Apulia, are susceptible to the disease.

Mastrogiovanni, 2015. The one in Salento is not an olive forest but an olive monoculture. The distinction is important because it is precisely monocultures which predispose agricultural crops to epidemics. However, in this booklet the author does not mention this concept. In general it should be noted that the author would subject the hypothesis of researchers who were interested in the case referring to *X.f. pauca* to a strict and proper scientific criticism, while, in formulating its own hypothesis or anti-*Xylella* circles hypothesis, the author does not provide supporting data.

A second observation: a marked skepticism was noticed regarding how much and what "scientists" (at least some) do. The author presents Rodrigo Almeida as a global expert on *Xylella*. The author is instead very appreciative toward Dr. Massimiliano Virgilio who was asked to evaluate the hypothesis which saw *Xylella* as the cause of the disease, and to whom are attributed the words "weak scientific evidence" against Almeida. Almeida published 70% of his works on the *Xylella* bacterium, which are widely cited (index HI of 22), while Virgilio is a fly expert (insects included in Dipteran; *Xylella* vectors are Hemipterans). The farmers treat successfully with traditional methods plants showing desiccation symptoms. Still today (April 2015), there is no certainty about *Xylella* as a causal agent and on its vector. At a IAMB course, Almeida proposes the cause of *Xylella* and role of the spittlebug (but the author's note considers him scarcely credible). The author goes into a detailed description of the Regional Council's opinions and resolutions contrasting with those of the Agriculture Committee of the House of Representatives which is silent on the disease's cause. The Commission justifies this position mentioning Krugner who in California injects *Xylella* into olive trees and does not register symptoms. The Commission makes a serious error: in the cited work (*a report to the California*

Olive Committee) a *multiplex* strain was used. The Commission writes that several olive trees in the heavily infected area produce suckers and new recovered buds. The booklet elaborates on complaints submitted to the Public Prosecutor's office, one of which suggests that the *Xylella* strain may have leaked out of laboratories during the 2010 course. It reports in 4 points on page 28 the quarantine procedure. The desiccation of olive trees in Apulia is a phenomenon which farmers have recorded since 2008.

The author offers an interpretation of part of the EFSA's report dated January 2015, arguing that this agency has stated that eradication is senseless.

Meng *et al.*, 2005. *Xylella fastidiosa* develops a biofilm which blocks the xylematic sap flow. It was still not clear how the bacterium moves in xylematic vessels against the sap movement. The bacterium migrates via type IV pili with 5 microm min^{-1}motility against the sap flowing at 20.000 microm min^{-1}.

Two mutants for pilB and pilQ loci are defective for pili of type IV and do not colonize the plant. Mutants with type I pili have increased biofilm production, while mutants with type IV pili alone produce less biofilm.

Muranaka *et al.*, 2013. *Xylella fastidiosa* causes the disease known as *Citrus Variegated Chlorosis* (CVC) in citrus trees. The inhibitory effect on the N-Acetylcysteine disease (NAC), a cysteine analogue used in human medicine, was investigated. Above 1 mg/mL concentration, the compound reduces the bacteria's adhesion to glassy surfaces, the formation of biofilm and EPS. Sweet orange plants treated with NAC (0.48 and 2.4 mg/mL) show a remission of the symptoms, which is associated with a reduction of the bacteria. The use of NAC in agriculture may fight bacterial infections.

Nigro *et al.*, 2013. The discoloration of olive wood's vascular elements was reported in 2008 by Carlucci and collaborators, and associated to the presence of fungi (Carlucci *et al.*, 2008). The presence of fungi of the *Phaeoacremonium* and *Phaeomoniella* varieties was identified in olive trees showing desiccation symptoms. The presence of *Phaeoacremonium parasiticum* and *Phaeoacremonium alvesii* is detected for the first time.

Nunney, 2014. The role of homologous inter-subspecific recombination in the evolution of numerous bacteria was evaluated. The case described concerns the emergence of *Xylella fastidiosa* strains able to attack mulberry trees. The *leaf scorch* disease was identified 25 years ago in

California and is caused by a *Xylella* strain which can be included among the bacterium subspecies as *Xylella fastidiosa* subsp. *morus*. The gene sequence from the new isolate has, among its progenitors, *Xylella* subsp. *fastidiosa* (introduced from Central America) and *Xylella* subsp. *multiplex* (originated in the USA) and its chimeric genome is to be ascribed to an inter-subspecific homologous recombination. The strain has a reduced molecular variability, and, since *fastidiosa* is not able to attack mulberry trees, the transition to pathogenicity has been favoured by a strong selection on recombination products.

Perrino, 2011. The author mentions several works which should be verified. After 2013, Perrino extends his absolutely negative opinion about glyphosate on the *Xylella* case and attributes the same to herbicide use. However, for the time being, in Apulia there is no available experimental data on the use of glyphosate as herbicide around olive trees and on its association with olive tree desiccation. The text reports the results of several works by Kremer R.J. treating this subject, which were all published in the *European Journal of Agronomy.* The majority of the publications cited is in two journals only: "Science in Society" and "The Organic & Non-GMO Report".

Perrino, 2015. As all complex diseases, *CoDiRO* is caused by several biotic factors (bacteria, fungi, insects and probably other yet unknown pathogens) and abiotic factors (abuse of chemical fertilizers, anticryptogamics or pesticides, insecticides, phytopharmaceuticals, herbicides, weed-killers, desiccant, adverse climatic factors such as humidity, temperature, thermal excursion, wind, water imbalance and other stress factors). Some researchers from the University of Foggia and the University of California researchers emphasize that *Xylella fastidiosa* is not always present in the samples obtained from trees showing CoDiRO symptoms, or, if is it present, it is systematically associated with a variety of species of fungi existing solely in Apulia such as *Phaeoacremonium aleophilum, Phaeoacremonium alvesii* and *Phaeoacremonium parasiticum*, and with other three species, which appeared in Italy for the first time: *Phaeoacremonium italicum, Phaeoacremonium sicilianum* and *Phaeoacremonium scolyti*, which besides being the most common worldwide are also the most pathogenic. The same conclusion was reached by other pathologists at the University of Florence and by the Directors of the Tuscany Phytosanitary Service who, on the Salento olive materials showing CoDiRO symptoms, disclosed the presence of attacks by *Zeuzera pyrina* and the presence of some tracheomycotic fungi such as *Phaeoacremonium parasiticum, P. rubrigenum, P. aleophilum, P. alvesii, Phaemoniella* sp., in addition to the occasional presence of *Xylella fastidiosa*'s DNA. The author reports data on *Xylella* in olive trees in California not mentioning that it is a *X.f. multiplex* strain. He interprets EFSA as favouring the non-intervention thesis on eradication. Several Salento olive growers remain unheard; they showed that diseased plants, if treated appropriately with traditional farming techniques opportunely reinterpreted from a modern point of view, show signs of recovery and healing within a short period of time. According to the author, those results are also supported by an extensive literature. The author insists that the causes of *CoDiRO* are not reliable and at the same time emphasizes that plants become vulnerable because of the use and abuse of chemicals, which have depleted the soil's micro-flora, reduced the organic matter almost to zero, and impoverished biodiversity. The solution to the Salento olive trees' disease, called CoDiRO, is not the eradication of diseased plants but their care and healing through good agricultural practices. He adds that a document on the containment of *CoDiRO* signed by 16 agronomist and forest doctors in the province of Brindisi identifies useful practices such as foliage aeration, regular pruning, suckering, good management of soil fertility, adopting improving and conservative techniques for the organic matter, which exclude the use of chemical herbicides.

Redak *et al.*, 2004. Over the last few decades, two diseases caused by *Xylella*, *citrus variegated chlorosis* (*CVC*) and *Pierce's disease* (*PD*) in vines, have emerged as really important for the economic losses they cause. For some of these diseases, the pathogenicity within areas newly colonized by exotic strains of *Xylella* results in the host-pathogen combinations not reported previously. This is the case of *CVC* (Hopkins and Purcell, 2002). The *CVC* chlorosis by *Xylella* is a citrus' destructive disease, first detected in Brazil in 1987, which attacks all commercial varieties of sweet orange. The recent arrival and establishment in California of the *Homalodisca coagulata* insect threatens most of U.S. vine and almond crops. *H. coagulata* colonization of Southern California and *CVC* diffusion from Argentina to Brazil show the biological risk posed by exotic species of bacteria. The close genetic resemblance between *Xylella* strains in coffee plants and *CVC* in orange trees suggests the possibility that the orange strain has recently evolved from the coffee strains.

Saponari *et al.*, 2013. For the first time, the presence of *Xylella* is identified in olive trees with desiccation symptoms, as well as in almond trees and oleander plants.

Saponari *et al.*, 2014. In a recent *Xylella* review in Europe, *Eurymela fenestrata* and *Philaenus spumarius* are identified as potential vectors of *Xylella* (Janse and Obradovic, 2010).

70% of *P. spumarius* insects collected in the first week of November are positive for *X. fastidiosa*. The sequence of 19S rDNA and gyB genes amplified via PRC from periwinkle plants is 100% identical to the bacterium's sequence isolated from olive trees in Apulia. The results prove that *P. spumarius*, which is very common in olive groves, has percentage of Xylella infection up to 50%.

Saponari, 2015. The following plants revealed symptoms of *Xylella*: (i) Cherry trees (*Prunus avium*); (ii) *Polygala myrtifolia*; (iii) rosemary (*Westringia fruticosa*) plants. All the symptomatic plants examined were infected by the bacterium, as proved by PCR and ELISA methods following Loconsole *et al.*'s protocol (2014). The products amplified from 5 genes, using two specific-strain primers designed for *Xylella*, were sequenced. They all have a sequence 100% identical to their counterparts amplified in Apulia from infected olive trees (Cariddi *et al.*, 2014). With the exception of cherry trees for which in California a *Xylella* subsp. *fastidiosa* infection was reported (Hernandez-Martinez *et al*, 2007), *P. myrtifolia* and *W. fruticosa* were not known as the bacterium's hosts.

Simpson *et al.*, 2000. The genome consists of 2.679.305 bps where the circular chromosome has 52.7% of GC-rich sequences and is accompanied by two plasmids of 51.158 and 1.285 bp. A putative function was assigned to 47% of the 2.904 units encoded. The mechanisms associable with the bacterium's pathogenicity for plants consist of toxins, antibiotics and of ion sequestration systems, as well as of proteins mediating plant-bacterium and bacterium-bacterium relationships. Orthologous genes coding for some of these proteins was identified in human and animal pathogens, and their presence in *Xylella* indicates that the molecular basis of the bacterium's pathogenicity is driven by its genome and is independent from the host plant. At least 83 *Xylella* genes derive from bacteriophages, the case of genes for virulence deriving from other bacteria, a direct evidence of the horizontal transfer of genes mediated by phages. The *CVC* is a citrus destructive disease, first detected in Brazil in 1987, which attacks all commercial varieties of sweet orange. In 1993, a strain of *Xylella fastidiosa* was identified as causal agent, and in 1996 the bacterium was associated to diffusion via *sharpshooter leafhoppers*. Other *Xylella* strains cause economically-relevant diseases such as vine's *Pierce's disease*, alfalfa's dwarfism, peach trees' *phony disease*, periwinkle plants' withering, plum trees' *leaf-scorch*, and *Xylella* is at least associated with other diseases in pear, mulberry, almond, maple, oak, pecan and coffee plants. In 1992 in Bordeaux (France), a 9a5c-sequenced pathogenic strain was obtained from a sweet orange shoot collected in Macaubal (Sao Paulo, Brazil) in 1992. The 9a5c strain inoculated in orange produces *CVC* symptoms.

In planta, the bacterium is contained within a translucent extracellular matrix. Bacterial clots are formed blocking xylematic vessels that causes stress symptoms from lack of water. The matrix is, at least on a sequencing basis, composed of extracellular polysaccharides (EPSs) synthesized by enzymes similar to those of *Xanthomonas campestris* pv *campestris* (Xcc) which produce a gum (*xanthan gum*). *Xylella* gum might be less viscous than the *Xanthomonas* one. The cluster 21 of *rpf* genes of *Xanthomonas* (*regulation of pathogenicity factors*) codifies for regulatory proteins of extracellular enzymes synthesis and of EPS. *Xylella* has genes codified for RpfA, RpfB, RpfC and RpfF, indicating that both the bacteria can regulate the synthesis of pathogenic factors EPS.

Stancanelli *et al.*, 2015. *Xylella* infects plants belonging to at least 68 families (mono-and dicotyledonous plants, 3 of Gymnosperms), 187 genera and 300 species. *Xylella* diseases are transmitted by insects that feed on the xylematic sap. The super-families are Cercopoidea (*spittlebugs* or *froghoppers*), Cicadoidea (cicada) and Membracoidea (includes one species feeding on the xylem, a leafhopper). All the American species are absent in Europe (*P. spumarius* originated in Europe and was introduced in America). In the USA there are 68 species of *Sharpshooter* (7 in Europe), 6 of *Spittlebug* (34 in Europe) and 2 of Cicadae (54 in Europe).

Conclusions on EFSA's risk evaluation. ESFA's *Plant health* Panel concluded that the disease's vectors are insects and plants which spread via transport from the nurseries to olive-growing areas. Uncertainties arise from the little knowledge about distribution and bacterium symptoms in plants growing where the bacterium originated. After the bacterium has entered a new geographical area, it establishes itself on account of its ability to infect several plants species. After its arrival, the bacterium's spreading probability is very high, again because of its numerous hosts and vector populations. The consequences of the bacterium's presence in Apulia are a serious threat to the whole of Europe.

Yaseen *et al.*, 2015. It illustrates a method (real-time LAMP) which uses mobile components (Smart-Dart *device*) and can be directly used on the fields. A Smartphone connection displays real time results. It analyses plant and insect samples for the presence of *X. fastidiosa*.

Yang *et al.*, 2014. In the vine's *Pierce's Disease* (*PD*) caused by *Xylella fastidiosa*, apparently healthy branches contiguous to others heavily infected can be observed. The work assessed whether the difference could depend on causes other than genetic, more specifically on microbiome differences. Thus, an attempt was made to identify endophytic organisms living in grapevine. In a 3-year experiment, DNA sequencing contiguous to ribosomal genes amplified by the endophytic community reveals differences between diseased plants and plants without symptoms. 180 species of fungi were isolated and cultivated, 8 of which have an antagonistic effect towards *X.f.*, 4 of which reduce the bacterium titer and symptoms' severity in experiments on plants grown in nurseries. Natural substances with antagonistic action against *Xylella* were isolated from antagonist fungi.

Annex E. Annotations from documents by the *European Food Safety Authority*, (EFSA)

EFSA, 2015a. A list of *Xylella* host plants is produced, focusing on market plants and on the cultivation of the same in infected areas. The definition of host plant is based on the determination of natural infection status confirmed by at least two determination methods for the presence of the bacterium.

EFSA PLH Panel, 2015b. *Xylella* can colonize several plant species, both cultivated and spontaneous. *Philaenus spumarius*, a polyphagous hemipteran which is very common in Apulia's infected area, is the main infection vector. The risk that the bacterium will enter into new areas through plant movement and the import, especially of ornamental plants is very high; it is lower if the bacterium is transmitted by vectors present on traded plants. The probability of the bacterium's establishment in Europe is very high, with serious consequences of loss of agricultural production and regarding the infection containment cost. In addition, the pesticides use for vector control will have a strong impact on the environment. Prevention strategies should be based on monitoring plants used in new implantations and on vector containment, combining the procedures in an integrated approach: i) transplant plants produced in areas where the bacteria is absent, supervision, certification, monitoring, and possible therapy or antibacterial treatments for small plants; ii) inspections and insecticide treatments. The Panel recommends the intensification of research on host plants, epidemiology, control and local confinement.

EFSA PLH Panel, 2010. Grapevine is not an important host of *X.f. pauca* CoDiRO strain.

EFSA PLH Panel, 2016a. Introduces the theme of the care of diseased plants on the basis of two experiment series conducted in Apulia, and more precisely of an assessment by Prof. Scortichini and Drs Carlucci and Lops. It produces an *assessment* based on involved scientists' interviews or their preliminary documents. Both groups consider their data requiring another year for confirmation. Various copper and zinc concentrations are used in field conditions, while the use of bioactive substances is at experimental stage. Treatments in combination with appropriate agronomic care translates into infected olive trees' greater vegetative vigour, but the bacterium persists in the treated tissue after the treatment. Currently, there is no cure able to eliminate the bacterium from tissues where *Xylella* is present. The Panel recognizes that this approach may prolong infected plants' productive life. Long-term specific studies on the treatment of diseased plants are required. A bibliographic review of interventions against bacterial infections consider i) antibiotics; ii) endogenous opposition bio-activators; iii) compounds stimulating plant growth; iv) bacterial and fungal antagonists; v) copper-based treatments; vi) treatments combined with good agronomic practice. Antibiotics treatments against *Xylella* (a long series of publications is cited, for example, on grapevine) do not eliminate the bacterium. The same can be concluded for copper and zinc. One of the two interviewed groups used commercial products, the use of which is allowed, and the other a product containing Zn (4%) and Cu (2%) in citric acid solutions. For what concerns the experiments carried out by Carlucci and Lops, the Panel reasoned that in *Pierce's disease* affecting grapevine a severe pruning may lead to new branches showing no disease symptoms for years. Nevertheless, the reported combination of good treatments and agronomic practices does not allow a clarification of the role of individual measures. Both groups maintain that their experiments are aimed at the elimination of symptoms and not of the bacteria.

The Panel concludes: i) the grapevine experience shows that treatments similar to those adopted in Apulia do not eliminate the disease. However, they may protect the plant from other fungi. ii) some of the above mentioned compounds are effective *in vitro*, but evidence on the treatment of entire plants in open fields are lacking. Zinc and antibiotics can be effective but need continuous injections to put them into contact with the bacterium. Additionally, currently antibacterial antibiotics are not allowed for the treatment of plants in the fields. iii) the Panel recognizes the positive effect of good farming practices on the development of the disease. iv) plants remain infected after treatments anyway. v) The Panel observed that the number of exogenous treatments used should be reduced for many reasons. vi) the Panel recommends the consideration of the long-term effect of repeated copper and zinc treatments.

EFSA, 2016b. *Xylella fastidiosa* is responsible for the disease which is destroying olive trees in southern Italy. Even oleander and myrtle-leaf milkwort plants succumb to the Apulian bacterium strain, while citrus, grapevine and holly oak trees seem to resist it. Findings are derived from investigations conducted in the past two years in Apulia on plants hosting Xylella CoDiRO strain. Scientists at the National Research Council, through artificial inoculation and in field exposure to infected insect vectors, put the bacterium into contact with important varieties of perennial crops such as olive trees, grapevine, citrus trees, almond trees, peach trees, cherry trees and plum trees, but also holly oak trees, oleander and myrtle-leaf milkwort plants. G. Stancanelli, Director of EFSA's "*Animal and plant health*" unit, states: these results confirm that the *Xylella* CoDiRO strain causes olive trees' decline. "*We can now accurately assess the risk that the epidemic will spread from Puglia*". Inoculated olive plants have severe desiccation and decline symptoms similar to those of plants attacked by the bacterium in the fields. It also seems that the bacterium requires more time to infect the Coratina, Leccino and Frantoio varieties than the Cellina di Nardò variety. *Philaenus spumarius* transmits the bacterium to olive, oleander and polygala plants. The infection is detected six months after the plant's exposure to insects, when they are still asymptomatic. Citrus, vines and holly oak plants do not reveal the bacterium's presence after inoculation. Stancanelli added: "*The project's outcomes significantly reduce the uncertainties surrounding the risks for the EU territory associated to the CoDiRO strain of Xylella and will contribute to the planning of future research*".

EFSA, 2016c. In 2015, EFSA published a list of *X. fastidiosa* host plants. In the same year, EFSA (EFSA, 2015b) produced an update, adding 36 new species. The European Commission then asked EFSA to review the matter with particular reference to the host plants of the CoDiRO Apulian strain. The work integrates the list of host plants published by EFSA on the 20th of March 2016 (EFSA-Q-2015-00168). It cites recent bacterium appearances in Europe: in Apulia, Corsica, southern France. It lists in total 369 *Xylella* host species belonging to 204 varieties and 75 botanical families. The majority of the new species comes from Apulia, Corsica and southern France. 5 hosts sensitive to *pauca* after artificial infection: *Olea europea, Catharanthus roseus, Nerium oleander, Polygala myrtifolia, Coffea arabica*. 26 after natural infection: *Olea europea, Catharanthus roseus, Nerium oleander, Polygala myrtifolia, Coffea arabica, Coffea* sp., *Vinca minor, Asparagus acutifolius, Cistus creticus, Euphor-*

bia terracina, Acacia saligna, Spartium junceum, Lavandula angustifolia, Rosmarinus officinalis, Westringia fructicosa, Westringia glabra, Eugenia myrtifolia, Polygala myrtifolia, Grevillea juniperina, Rhamnus elaternus, Prunus avium, Prunus dulcis, Citrus sinensis, Citrus sp.*, Dodonea viscosa purpurea, Myoporum insulare*). *Multiplex* can infect olive trees naturally.

EFSA PLH Panel, 2016d. This document, more than others, has an informational value detailing EFSA's position on the *Xylella* case (and thus the European Commission's position). For this reason, a long summary is presented here.

The original publication and its related bibliography, part of which is mentioned in the summary, is available at: `http://www.efsa.europa.eu/it/efsajournal/pub/4450`

Members of the Panel. They are 21 scientists of internationally recognized value and level: Claude Bragard, David Caffier, Thierry Candresse, Elisavet Chatzivassiliou, Katharina Dehnen-Schmutz, Gianni Gilioli, Jean-Claude Grégoire, Josep Anton Jaques Miret, Michael Jeger, Alan MacLeod, Maria Navajas Navarro, Bjoern Niere, Stephen Parnell, Roel Potting, Trond Rafoss, Vittorio Rossi, Gregor Urek, Ariena Van Bruggen, Wopke Van Der Werf, Jonathan West e Stephan Winter.

Opposition. It is expressed by the Lecce local groups against the European Community's control strategy (EU 2015/7893 Decision). It was manifested in the form of an appeal to the European Court of Justice. The opponents' positions are listed below together with the *terms of reference* assigned by the EFSA Panel. 1. In Apulia *Xylella fastidiosa* subsp. *pauca* populations are heterogeneous because of various existing strains. *Is there any conclusive scientific evidence for such a statement?* 2. *OQDS* depends not only on the presence of *Xylella fastidiosa* or of other fungi colonizing the xylematic vessels, but also on other factors such as soil compaction degree, soil organic matter, micro-fauna biodiversity, soil salinization, concentration of glyphosate (or of other toxic chemicals), nutrients' concentration. Causes also include pruning, ploughing and other agronomic practices. *Is this statement in agreement with current scientific knowledge?* 3. it is not certain that *Xylella fastidiosa* is the only causative agent of plant death. *Can EFSA provide an update? Please advise whether this would affect the risk of Xylella fastidiosa for the rest of the Union?* 4. The removal of infected plants is not a solution, as demonstrated in the USA, Brazil and

Taiwan. The removal of host plants within a 100 m radius around infected plants (EU 2015/789) for outbreaks outside the province of Lecce is not based on scientific evidence. *Can EFSA assess the level of prevention of further spreading of Xylella fastidiosa in areas not yet infected? Can EFSA advise on the efficacy of removing infected plants located in the 'containment area' to prevent further spreading? Can EFSA advise about the efficacy of removing host plants in proximity of recently detected infected plants in areas where the bacterium was not known to have occurred?* 5. Field treatment methods would be available for diseased plants, as by experiments of Prof. Francesco Lops and Dr. Antonia Carlucci (University of Foggia). *Can EFSA contact these researchers and assess the outcome of these on-field experiments? Can EFSA provide an update on treatment solutions, scientifically validated, to cure diseased plants?* 6. The EFSA Journal (2015, 13:3989) refers to *'the intensive use of insecticides to control the insect vector may modify the trophic networks with cascading effects. In addition, large-scale insecticide treatments pose risk to human and animal health; the use of insecticides may stimulate the emergence of resistance and the treatments can lead to the reduction of vector's natural enemies'. Can EFSA clarify this matter in relation to the phytosanitary treatments to be carried out prior to the removal of plants against the vectors of Xylella fastidiosa and plants that may host those vectors?*

In the document the Panel considers only points 2, 3, 4 and 6. The questions answered are i) which factors control *X. fastidiosa*'s observable symptoms and spread; ii) the etiological cause of the CoDiRO disease; iii) host plants elimination as an option for containment or eradication; iv) secondary effects of pesticides, including herbicides.

Positions of the Panel. Xylella fastidiosa subsp. *pauca* is the causative agent of *OQDS*. The symptoms are due to water drought stress due to the occlusion of xylematic vessels. Interventions improving the plant's vegetative state prolong its production phase and extend the disease's asymptomatic stage. The removal of infected plants is the only option preventing the parasite's appearance into new areas. In the containment area bordering the *buffer* zone, the removal of infected plants and their monitoring could prevent the pathogen from spreading. In new outbreaks, the radical removal of *Xylella* host plants, both infected and uninfected, within the range defined by the EU regulations can be effective. The reduction of vector population using chemical, biological and mechanical methods or other sustainable procedures may contribute to the reduction of the bacterium's spreading. There is no evidence of the pesticides' negative effects in the interaction of *Xylella* with olive trees, neither with the increase in symptoms severity or with the insurgence of infection.

Specific comments.

(i) Quantifying the effects of *OQDS* on the environment in the general sense, of soil composition, and of herbicide-insecticides treatments is not possible. Despite the possible relevance of secondary factors also contributing to the alleviation of the disease, the risk caused by the presence of *Xylella* for the rest of Europe is serious. In fact, it has been shown that the bacterium is the causative agent of the disease. *Xylella* is spread by vectors sucking xylematic fluids (Almeida *et al*., 2005; Almeida and Purcell, 2006; Chatterjee *et al*., 2008; Daugherty and Almeida, 2009; Daugherty *et al*., 2009; Backus and Morgan 2011; Killiny e Almeida, 2014). In Apulia, the spittlebug *Philaenus spumarius* is abundantly found and is ascertained to be a *Xylella* vector (Elbeaino *et al*., 2014; Saponari *et al*., 2014). Data on the role, in the olive tree epidemic, of other host plant species is not sufficient but, once the broad spectrum of *Xylella* infectivity will have been established, it is correct to consider that a host species *continuum* accentuates the spreading of the disease. The presence of *Xylella* in xylematic vessels is, for a long time, asymptomatic in some species and in other always asymptomatic (Purcell and Saunders, 1999; Hopkins and Purcell, 2002, Harris *et al*., 2014). Asymptomatic plants are a reservoir of bacteria and of vectors' infectious inoculum. The vector prefers plants in good vegetative condition, and symptom-free plants (Daugherty *et al*., 2011). It is not known whether the removal of infected branches from olive trees has a positive impact on the elimination of the bacterium. Pruning was found to be effective in *CVC* cases in Brazil (do Amaral *et al*., 1994), but it was strictly adopted at the beginning of the infection and integrated with other containment measures. The pruning of symptomatic plants does not reduce the concentration of bacteria (Holland *et al*., 2014). It might instead encourage the development of more vigorous and infected shoots which attract vectors, causing them to become infected (Marucci *et al*., 2004). *Xylella* insect vectors, especially in immature young stages, frequent herbaceous plants, a behaviour also verified for *P. spumarius* in the Apulian olive growing areas (Cornara and Porcelli, 2014). As demonstrated for *Pierce's disease* in grapevines (Black and Kamas, 2007), soil management can affect plant associations favourable to *Xylella* and its vectors.

(ii) The Panel concludes that recent experiments (Saponari *et al.*, 2016) demonstrate that 'De Donno' isolate causes *OQDS* symptoms in olive trees and that it is the disease's causal agent. *Xylella* detection on olive trees is sufficient to assign it the risk category indicated in the 2015 Panel. The presence of *Xylella* subsp. *pauca* in dried olive trees in Argentina in 2013 (Haelterman *et al.*, 2015), the confirmation of the pathogen's presence in olive trees with *leaf scorch* in Brazil (Colhetta-Filho *et al.*, 2016), and the finding and identification of *Xylella* subsp. *pauca* in the Apulian olive trees affected by *OQDS* (Saponari *et al.*, 2013) confirm that olive trees are host plants of this pathogen. In California, Krugner *et al.* (2014) isolated *Xylella* subsp. *multiplex* from symptomatic olive trees and inoculated mechanically the strain isolated in vines and almond trees. Disease symptoms were observed in almond trees, while *Xylella* was found in olive trees only after a few months from the inoculum and without symptoms. Therefore, the above mentioned authors have not been able to confirm *Xylella* subsp. *multiplex* as a causal agent of olive trees' *leaf scorch* in California (Krugner *et al.*, 2010, 2014). The transmission of *Xylella* from olive trees to other species via *P. spumarius* is demonstrated (Saponari *et al.*, 2014). Recent inoculation mechanical experiments on olive trees with *Xylella* pure cultures produced evidence of the transmission and infection of the bacterium (Saponari *et al.*, 2016), which resulted in the withering and death of olive tree branches (December 2014 experiments related to De Donno isolate of *Xylella* subsp. *pauca*, which fulfill Koch's postulate for this disease). Only after 12 months did inoculated plants show leaf curling symptoms, followed by severe desiccation and dead branches. The bacterium was re-isolated from symptomatic first-inoculated plants and grown *in vitro* in pure cultures. Koch's postulate is fulfilled, but the Panel believes that the presence of *Xylella* in olive trees is sufficient to assign the risk inherent in the bacterium to the level defined in 2015 by the EFSA Panel.

(iii) In the epidemic situation in southern Italy, characterized by a containment area demarcated by two seas flanking it and a buffer zone where the bacterium spreads - the epidemic's front - measures from art. no. 6 and no. 7 may slow down the bacterium's progression toward non-infected areas. The containment area under article no. 7 corresponds to one where *Xylella* has been found. The provision requires the removal of infected plants within a distance of 20 km (*buffer zone*) from the containment area's edge. The elimination of infected plants is accompanied by other measures in many cases. Despite this, the *Xylella* epidemic on citrus trees and grapevines has increased in the USA and in Brazil (Lopes *et al*., 2000; Purcell, 2013). However, the interventions mentioned were adopted when the disease was already well-established in the area. Thus, plant removal must be enacted at the very early stages of the epidemic (de Boer and Boucher, 2011; Gordillo *et al*., 2012; Behlau *et al*., 2014). An important measure relates to the monitoring of the disease's intensity to identify the very early stages of its development. In order to monitor *Xylella* in olive trees, infection in asymptomatic plants are a problem because they require sensitive analytical techniques. In fact, article no. 7 prescribes to sample and analyze the host plants within a radius of 100 m around the already removed infected plants, and interventions to be carried out at regular intervals. The radius definition is a common practice in eradication cases. The elimination of causative agent host plants is a widely accepted measure to eradicate a pathogenic agent (Mumford, 2006; Thomson, 2006; Sosnowski *et al*, 2009; Belasque *et al*., 2010; de Boer and Boucher, 2011; Filipe *et al*., 2012; Gordillo *et al*., 2012; Palacio-Bielsa *et al*., 2012; Sosnowski *et al*., 2012; Bennett *et al*., 2013; Su *et al*., 2013; Behlau *et al*., 2014; Cunniffe *et al*., 2014 and 2015; Gottwald and Graham, 2014; MacMaster *et al*., 2015; NTG, 2015; Rimbaud *et al*., 2015). There are several epidemiological factors to be considered (Pluess *et al*., 2012), and especially the degree of pathogen invasion depends on the number and density of infected sites, including the timing of adoptable interventions. When the agent is well established, the only strategy is containment while in cases of recent introduction the elimination of infected plants and host species, both healthy and infected, may result in the eradication of both the pathogen and the disease.

(iv) Regarding the use of products applied to plants, and despite their adverse side effects, the Panel concludes that a comprehensive approach should be adopted and adapted to specific local situations. New *Xylella* foci are always connected to the presence of insect vectors. In Apulia, a few spittlebug species were reported to be positive to the presence of

Xylella in infected areas: *Euscelis lineolatus, Neophilaenus campestris* and *P. spumarius*. Only the latter species has been proved to be able to transmit the pathogen (Elbaino *et al*., 2014; Saponari *et al*., 2014). The control measures that should be in place in the infected area in Lecce refer to the Italian Ministry of Agriculture (No 2777 of DM dated September 26th, 2014), as they were implemented in the area under the surveillance of the Apulia Phytosanitary Service (Resolution 1842, Apulia, September 5th, 2014). The measures include the use of insecticides against vectors and agro-techniques to suppress the vectors' nymph stages on weeds and the elimination of infected plants. As regard to the European Commission and its current Implementing Decision (8), both art. no. 6 and no. 7 dedicate subparagraph no. 4 to phytosanitary treatments, reciting: '*The Member State (MS) concerned shall carry out appropriate phytosanitary treatments prior to the removal of plants referred to in paragraph no. 2 against the vectors of the specified organism and plants that may host those vectors. Those treatments may include, as appropriate, the removal of plants'*. In this context, appropriate phytosanitary treatments should include chemical and non-chemical (including biological) control measures as well as vegetation management, as concluded by the PLH Panel risk assessment (2015), related particularly to field operations.

Each Member State can find necessary support in the European regulation to define the most appropriate phytosanitary strategy and an evaluation of the pesticides to be used. The Panel considers that the products' appropriate use in accordance to what the procedures prescribed both in the new foci of the disease and in the buffer zone is a practice with significant effects on vector population.

Saponari and Boscia, 2015. Grapevine is not infected with *Xylella* subsp. *pauca*, strain CoDiRO. Artificial inoculations work well on *pauca* hosts but not on grapevines, on which the bacterium's DNA was amplified after 9 months only at the inoculation site. Under the same conditions the bacterium spreads in olive trees. There are characteristic differences between *Xylella* subspecies regarding the host plants' range (Hernande-Martinez, 2007). This is relevant as the identification and assignment of strains offers a meaningful guideline to fight the spreading of the bacteria.

Saponari *et al.,* 2016. The CoDiRO strain is similar to the ST53 from Costa Rica. Symptoms like the Italian ones were observed in Argentina and Brazil, caused by the presence on olive trees of *Xylella* sup. *pauca* strains, but different from the Salento isolates. *Pauca* is the causal agent of citrus CVC and of coffee withering. A list of the bacterium hosts is reported. The experimental inoculation procedure was defined in collaboration with Almeida and Purcell. Experiments are conducted in nurseries and *screenhouses*, the latter in Gallipoli. Olive trees were also exposed to natural infection.

Nurseries experiments. Olive plants, oleander plants, polygala, almond trees, cherry trees (known hosts) and, in addition, oak trees, vines, citrus trees, and other plum pox plants were inoculated. *Olive tree.* Varietal differences in the bacterium titer were noted after inoculation. The bacterium is absent in uninoculated control. The bacterium is isolated from inoculated plants. The first symptoms are noticed after 12 months on the Cellina di Nardò variety and after 14 months on other varieties. Symptom evolution is similar to the one of on field plants. *Oleander* and *polygala plants.* The bacterium is present and disease symptoms are noticed after 10 months from inoculation. *Citrus trees*: they do not show symptoms after CoDiRO strain inoculation. The same for grapevine.

Experiments in screenhouses. Temperature alternation in various seasons seems to have drastically reduced the presence of the inoculated bacterium. The same for oleander and polygala plants.

Natural infections with the bacterium carried by P. spumarius. The bacterium reproduces itself in olive, oleander, and polygala plants. It does not reproduce itself in *Quercus ilex*, grapevine and in citrus trees.

On field inspections. In the first under-control site, the bacterium was initially present on 3 plants (2,7% of the total area); after 2 years it was present on 59 trees (52,7%). In the second site, plants increased from 1 to 66 to 220 when monitored in March 2015, July 2015 and December 2015.

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