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Scientific opinion on four statements questioning the EU control strategy against *Xylella fastidiosa*

EFSA Panel on Plant Health (PLH)

Abstract

This opinion addresses questions concerning the EU control strategy against *Xylella fastidiosa*: i) factors affecting symptom expression and spread of *X. fastidiosa*; ii) the aetiology of the CoDiRO (Complesso del Disseccamento Rapido dell'Olivo) disease; iii) host plant removal as an option for containment or eradication; and iv) secondary effects of pesticides. *Xylella fastidiosa* subsp. *pauca* was shown to be the causal agent of the CoDiRO disease of olives by recent biological assays fulfilling Koch's postulates. Symptoms in *X. fastidiosa* infected plants develop because of wilting from water stress induced by bacteria clogging the xylem vessels. Therefore, interventions supporting vigorous growth and development of the plant may improve its health status, its resilience, prolong its productive phase and extend the symptomless phase of the disease. The Panel considers removal of infected plants, in a system-based approach, as the only option to prevent further spread of the pathogen to new areas. In the outer strip of the containment area bordering the buffer zone, removal of infected plants and stringent monitoring can be effective in preventing pathogen spread into the buffer zone. In areas of recent introduction, such as new outbreaks in the buffer zone, the stringent removal of both infected plants and all host plants irrespective of their health status within a radius, as described in current EU legislation, can be effective in reducing pathogen spread, when rigorously administered and new infections are detected in time. Finally, the reduction of vector populations by application of chemical or biological means, mechanical treatments, or other sustainable methods, can have effects in slowing down the pathogen spread. Concerning the use of pesticides, there is currently no evidence of negative effects of such treatments on the interaction of *X. fastidiosa* with infected olive trees, the severity of symptoms and the outcome of the infection.

Keywords: CoDiRO strain, containment, eradication, Koch's postulate, pesticides, soil, symptoms expression

Requestor: European Commission

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor[1]

The purpose of this mandate is to request, pursuant to Article 29 of Regulation (EC) No 178/2002[2], scientific advice in the field of plant health as regards the regulated harmful organism *Xylella fastidiosa* (Wells et al.).

Specifically, the Commission has recently been confronted with a number of statements which are questioning the overall EU control strategy against *Xylella fastidiosa* and some relevant legal provisions laid down under Decision (EU) 2015/789[3]. Such statements are the grounds for several appeals to the European Court of Justice which are pending for final ruling. Those statements and the related questions on which the Commission requests EFSA scientific advice are presented below:

1. It is considered that *the population of* Xylella fastidiosa *subsp.* pauca, *in Apulia (Italy) is heterogeneous as several different strains are present in the infected area, on top of the unique strain (referred to Co.Di.RO) reported so far*.

   - Is there any scientific conclusive evidence for such a statement?

2. The expression of the so called 'quick declining symptoms in olive plants' (CoDiRO) seems *to be correlated, not only to the presence of* Xylella fastidiosa *or other fungi present within the xylematic vessels within the plant, but also to a number of other factors* which have not been fully taken into account in the EU Decision. Such factors are*: the degree of compactness of the soil, quantity of organic matter in the soil, presence of biodiversity between the micro-fauna of the soil, degree of salinization of the soil, concentration of glyphosate (or other chemical toxic agents), nutrient concentration, as well as any pruning activities carried out, including plowing of the soil and other agricultural practices*.

   - Is this statement in agreement with current scientific knowledge? Please advise whether this would affect the risk of *Xylella fastidiosa* for the rest of Union.

3. *The causing link between* Xylella fastidiosa *and the quick declining symptoms of olive trees is still not established and Koch's postulates have not yet been fulfilled. Therefore, it is not sure that* Xylella fastidiosa *is the only and confirmed causing agent of the plant death*.

   - Can EFSA provide an update on the current scientific knowledge about this topic? In case Koch's postulates have not yet been fulfilled for the 'quick declining symptoms' in olives, please advise whether this would affect the risk of *Xylella fastidiosa* for the rest of Union compared to what reported in the Pest Risk Assessment of January 2015 (EFSA Journal 2015, 13(1):3989)?

4. *Removal of infected trees is not considered to be a feasible option to contain or eradicate the bacterium, nor to prevent the further spread of the quick declining symptoms of olive plants, as also experienced in USA, Brazil and Taiwan.* Even more*, the removal of host plants, regardless of their health status, within a radius of 100 m around the infected plants as requested by Decision (EU) 2015/789* for any outbreak identified outside the province of Lecce, where the bacterium is not yet established, *is considered to be not scientifically validated*.

   - Can EFSA review such a statement on the basis of current scientific knowledge with regards to the level of prevention of further spread of *Xylella fastidiosa* in areas not yet infected? In particular:

     i. In a system based approach, as proposed in Article 7 of Decision (EU), 2015/789, can EFSA advise about the efficacy of removing infected plants located within an area where the bacterium is considered to be established, (so

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[2] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety, OJ L 31, 1.2.2002, p. 1–24, as last amended.

[3] Commission Implementing Decision (EU) 2015/789 of 18 May 2015 as regards measures to prevent the introduction into and the spread within the Union of *Xylella fastidiosa* (Wells et al.). OJ L 125, 21.5.2015, p. 36–53

called 'containment area'), and particularly located in proximity of the buffer zone, where the bacterium is not yet present with the aim to prevent further spread?

ii. In a system based approach, as proposed in Article 6 of Decision EU 2015/789, can EFSA advise about the efficacy of removing host plants, regardless of their health status, located in proximity of recently detected infected plants, located in areas where the bacterium was not known to occur before that detection (e.g. buffer zone or outside the 'containment area') with the aim to prevent further spread?

5. It is alleged solutions to treat diseased plants in open field would be currently available. In this respect, it is often referred to experiments carried out by Prof. Marco Scortichini of CREA (Caserta, Italy) and the ones carried out by Prof. Francesco Lops and Dr. Antonia Carlucci from the University of Foggia (Italy).

   - Can EFSA contact these researchers and assess the outcome, if provided, of these on-field experiments aiming at curing diseased plants?
   - Can EFSA also provide an update about recent treatment solutions, scientifically validated, if any, to cure diseased plants?

6. From the Pest Risk Assessment of EFSA (EFSA Journal 2015, 13(1):3989), reference is made to Section 3.5.2 '*The intensive use of insecticide treatment to limit the disease transmission and control the insect vector may have direct and indirect consequences for the environment by modifying whole food webs with cascading consequences, and hence affecting various trophic levels. For example, the indirect impact of pesticides on pollination is currently a matter of serious concern (EFSA, 2013b). In addition, large-scale insecticide treatments also represent risks for human and animal health*'; Section 4.3.2.2. '*large-scale application of insecticides may lead to the development of insecticide resistance as well as to environmental and human health issues*'; Section 4.3.3.4. '*Similarly, insecticide treatments could have a negative result by modifying insect population dynamics and favouring insect vectors, e.g. by placing proportionally higher pressure on the insects' natural enemies*'.

   - Can EFSA provide clarification on this matter in relation to the phytosanitary treatments required by Decision (EU) 2015/789 to be carried out prior to the removal of plants referred to in paragraph 2 of Article 6 and Article 7 against the vectors of *Xylella fastidiosa* and plants that may host those vectors? It is to be noted that those treatments, as appropriate, may include as well the removal of herbs where insect vectors lay down their eggs.

In view of a quick reaction expected by the Commission as part of the on-going appeals to the European Court of Justice, EFSA is requested to prepare an urgent opinion by 18 March 2016. As regards specifically point 1 and point 5 above, an extended deadline could be set for 30 June and 31 March 2016 respectively.

1.2. Interpretation of the Terms of Reference

The Terms of Reference (ToR) are organised into six points, each of which refers to a different aspect of risks connected to *X. fastidiosa* presence in the EU. Under each point, the European Commission addresses one or more questions.

In the current opinion, the PLH Panel replies to points 2, 3, 4, 6 and related questions as required by the indicated deadline. The replies to points 1 and 5, with different deadlines, are not dealt in this scientific opinion.

The current opinion was prepared in light of the Italian outbreaks of CoDiRO (Complesso del Disseccamento Rapido dell'Olivo, whose English equivalent is OQDS, from Olive Quick Decline Syndrome). Therefore, unless specified otherwise, the focus of the replies is on *X. fastidiosa* subspecies *pauca* strain CoDiRO.

The following specific aspects, parts of which were directly clarified by European Commission representatives (see paragraph 2.2) were considered in the interpretation of the mandate and the preparation of the opinion:

- Statement 2: The Panel noted that diseases resulting in wilting of leaves and branches resembling drought stress can be caused by a number of organisms and abiotic stressors. However, this question focuses on the contribution of *X. fastidiosa* subspecies *pauca* isolated in Apulia and other factors to the expression of the symptoms of the CoDiRO disease. The Panel carried out a scientific literature search and reviewed the available evidence for each of the listed factors, in order to evaluate their contribution to disease development and the severity of the symptoms.
- Statement 3: the Panel provided the requested update on current knowledge regarding the link between *X. fastidiosa* subsp. *pauca* and the CoDiRO disease. For the reply to this question, it is noted that the EU control strategy is targeted at controlling the presence and spread of the plant pathogenic bacterium *X. fastidiosa* in the EU and not at controlling the CoDiRO disease.
- Statement 4: the Panel evaluated the phytosanitary measure of removal of infected plants or host plants for its effectiveness in containing or preventing the further spread of *X. fastidiosa* outside of its area of establishment. The concept of 'system-based approach' is provided in ISPM 14 (FAO, 2016).
- Statement 6: the Panel considered in its reply those 'pesticides' which are 'plant protection products' i.e. aimed at protecting crops or desirable or useful plants.

1.3. Additional information

The current opinion is the first output that the Panel was requested to produce in order to reply to the mandate. Points 1 and 5 from the mandate will be provided with separate opinions at a later stage.

2. Data and Methodologies

2.1. Data

To revise each statement and reply to connected questions, targeted extensive literature searches were conducted. Searches were carried out on the research platform ISI Web of Science. The references retrieved were reviewed together with those cited in the EFSA risk assessment on *X. fastidiosa* produced earlier (EFSA PLH Panel, 2015). Further references and information were obtained from citations within the reviewed references, from experts and from information provided in the External Scientific Report: 'Pilot project on *Xylella fastidiosa* to reduce risk assessment uncertainties' published on the EFSA website (Saponari et al., 2016).

2.2. Methodologies

The assessment was conducted in line with the principles described in the EFSA Guidance on transparency in the scientific aspects of risk assessment (EFSA Scientific Committee, 2009). The present document is structured according to the Guidance on the structure and content of EFSA's scientific opinions and statements (EFSA Scientific Committee, 2014). The main assessment is composed by four sections reflecting the mandate: i) factors affecting symptom expression and spread of *X. fastidiosa* (corresponding to point 2 in the mandate), ii) confirming the aetiology of CoDiRO (corresponding to point 3 in the mandate), iii) host plants removal as an option for containment or eradication (corresponding to point 4 in the mandate), and iv) secondary effects of pesticides (corresponding to point 6 in the mandate).

European Commission representatives participated as observers and contributed to the clarification of the mandate and terms of reference.

3. Assessment

3.1. Point 2 – factors affecting symptom expression and spread of *X. fastidiosa*

This question concerns factors influencing the expression of CoDiRO symptoms in olives. In particular, the contribution of the soil and its physical, chemical and biological characteristics and the contribution of crop management interventions to disease expression shall be taken into consideration in the context of the evaluation of the risk concerning *X. fastidiosa*.

Plant development and growth are influenced by environmental conditions which have considerable effects on the health status of the plant, its vigorous growth and the resilience to temporary stress situations inflicted by abiotic and biotic factors. The incidence and in particular the severity of symptoms and the final outcome of plant diseases can be influenced by the health status of the plant, as plants growing under suboptimal conditions or exposed to stress situations (e.g. drought periods) may react more strongly and with more severe symptoms to pathogen infections than otherwise healthy plants (Hearon et al., 1980). In particular, drought conditions can compromise the plant response making it more vulnerable to pathogen attacks (Ramegowdaa and Senthil-Kumar, 2015), increasing symptom severity and disease progression (McElrone et al., 2001, 2003). If, in addition, the disease is caused by a pathogen invading the plants vascular system, like *X. fastidiosa*, additive effects from waters stress may aggravate wilting symptoms.

Plants cultivated in intense agricultural systems require comprehensive crop management for sustainable production while plants grown for many years in habitats like old olive groves are often less intensively managed and more in synchrony with their particular environment. Space taken by an individual plant, extent of its root system, nutrient balance etc. are adjusted to the location after long adaptation processes, and this shapes the plant phenotype and its response to the environment. It is conceivable that a tree existing in a habitat for many hundreds of years is less affected by temporary stress presented by the environment e.g. water stress or herbivore attacks, however plants grown in natural environments are still sensitive to plant diseases. As shown in the case of *X. fastidiosa* infecting olives in Apulia, newly emerging diseases can have dramatic consequences for plant populations in natural stands and in particular old olive trees are showing severe symptoms (Cariddi et al., 2014; EFSA PLH Panel, 2015).

Xylella fastidiosa, as many other plant pathogens of vegetatively propagated crops, can be disseminated by infected planting material which contributes to its spread over long distances. In nature, however, *X. fastidiosa* spread relies mainly on the transmission by xylem sap sucking vectors, mostly sharpshooter and spittlebugs (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 and Almeida, 2014). In Apulia, the spittlebug *Philaenus spumarius* is widely and abundantly present, and is a proven vector of *X. fastidiosa* (Elbeaino et al., 2014, Saponari et al., 2014). Critical to the dispersal of the disease by vector insects are the acquisition of the bacteria from source plants, the retention of the bacteria in the insect anterior foregut (precibarium and cibarium), the inoculation or release of bacteria to a new plant host, and the subsequent plant infection. Thus, i) the number of infected plants – olives and/or other cultivated or wild species – serving as hosts for the bacterium and as sources of inoculum for insects to acquire the pathogen and ii) the density of insect vector populations, are the key parameters determining the efficiency of pathogen transmission and pathogen spread and the speed by which the epidemic advances in the environment. Transmission by vectors from a tree to adjacent trees appears to be very significant for pathogen dissemination. As indicated in Plantegenest et al. (2007) and Baumgartner et al. (2006), the density and pattern of host plants in the landscape have significant effects on spread of the pathogen and the disease. The role of infected wild host plants in the olive epidemics is not conclusively studied, however considering the broad host range of *X. fastidiosa*, landscapes characterised by a *continuum* of host plants are considered more conducive to spread of the disease.

Plant infections establish in compatible pathogen/host/vector interactions, and in general the pathogen persists in its host for the entire life span of the plant. This is independent of symptoms being produced. Invasion of plants by *X. fastidiosa* during which the pathogen moves freely through the xylem vessels can remain symptomless for a long time in certain individuals or simply be asymptomatic on certain species (Purcell and Saunders, 1999, Hopkins and Purcell, 2002, Harris et al., 2014). Only when bacterial cells develop into biofilms, xylem sap flow is eventually impaired and water stress symptoms become apparent (Fry and Milholland, 1990; Newman et al., 2003). The increasing occlusion of the xylem leads to the typical wilting symptoms shown in the CoDiRO infected olives, because the water transport is disrupted. All interventions supporting the plant's vigour may reduce the severity of symptoms caused by *X. fastidiosa* (Hopkins and Purcell, 2002), and prolong the asymptomatic phase, however without any proven effect on curing the plant infection. Actively growing plants may maintain all vital functions, however they are not cured to eventually be freed from bacteria nor are they protected from the expression of severe disease symptoms eventually developing. It is therefore conceivable that crop management practices such as irrigation, fertilization, application of plant growth activator substances, pruning, pests and diseases control can affect the severity of CoDiRO symptoms. However, despite maintaining their appearance of good health, the infected plants will still act as reservoirs for *X. fastidiosa* and as sources of inoculum for insect vectors to acquire the bacteria. Because insects preferentially feed on succulent plant and plant parts with good vigour (Daugherty et al., 2011), they might even acquire higher numbers of bacteria than by feeding on wilting and severely declined trees.

Pruning is a good horticultural/silvicultural practice that is used to maintain the productivity of the plant and to keep its health status by removing deadwood, diseased, damaged, dead or non-productive branches and tissues. Based on current knowledge however, it is not known whether the removal of branches and other plant parts of olives infected by *X. fastidiosa* has any positive effects on the elimination of bacteria. Pruning was shown to be effective in eliminating CVC (citrus variegated chlorosis) from sweet orange in Brazil (do Amaral et al., 1994), but it was rigorously done at the onset of the infection, area wide, supported by removal of infected hosts, effective vector control and strictly followed by monitoring of the plantations. Pruning has been shown to be effective in sweet orange however only if applied early in infection. In general, it is not considered as an effective means to eliminate *X. fastidiosa* (EFSA PLH Panel, 2015). This is because of the difficulties in identifying early *X. fastidiosa* plant infections and of the rapid translocation of the bacteria to the roots. While symptomatic plant parts are removed by pruning, bacterial concentrations are not reduced significantly (Holland et al., 2014). On the contrary, new growth from flushing buds is likely to be readily invaded by *X. fastidiosa* and attract vector insects for bacteria acquisition (Marucci et al., 2004). Furthermore excessive pruning activity for phytosanitation could exert a high level of stress to the plant, then responding with forced budding and production of water shoots. Hence pruning, while in general contributing to plant health, could also have detrimental effects on diseases caused by *X. fastidiosa*.

Infections in olives occur because of transmission of the bacteria by insects. Thus the population of infected insect vectors has significant influence on the extent and speed by which new plant hosts are infected and the disease is spread. Insect vectors of *X. fastidiosa* and in particular their immature life stages are associated with herbaceous hosts and weeds (see Table 2 in PLH Panel, 2015) and this is also true for *P. spumarius* in the olive groves in Apulia (Cornara and Porcelli, 2014). Weed control, consisting in the removal of vegetation by mechanical or chemical means, can reduce vector populations, and the dissemination of the disease when done at the proper time (Purcell, 1979, Purcell et al., 1999). Thus *X. fastidiosa* spread and disease dissemination is significantly influenced by measures that have direct effects on vector populations and indirect on pathogen transmission (Almeida et al., 2005). A late elimination of weeds may however result in a massive migration of adult insects from weeds to the olive crop, resulting in an increased transmission. Early elimination of the weeds, before emergence of adults, can prevent the emergence of spittlebug populations that would transmit *X. fastidiosa* within the olive orchard. Keeping olive groves free of weeds can therefore be considered as an effective measure against *X. fastidiosa* spread as it removes host plants and insect habitats. Vectors control and vegetation/weeds management can significantly reduce *X. fastidiosa* inoculum in the environment and thus the risk of pathogen spread and disease.

Additive effects of other stress factors contributing to symptom severity and disease progress under field conditions are difficult to assess because of limited data. This also comes true for statements on side effects from herbicides, e.g. the influence of glyphosate on the composition of the microorganism flora of both the soil and of endophytic communities in the plant (Kuklinsky-Sobrel et al., 2005; Imparato et al., 2016; Newman et al., 2016). The current body of evidence does not allow one to draw clear conclusions on the direction of the effects or their impact. From all evidence currently available, it can be stated that the major component of the CoDiRO disease is *X. fastidiosa* (see also Section 3.2 below) with possible additive effects from other stressors that may contribute to the speed by which this decline progresses. Furthermore, as shown for Pierce's disease in grapevine (Black and Kamas, 2007), soil conditions may affect the composition of plant communities suitable for *X. fastidiosa* and its vectors.

In conclusion, the Panel states that it is not possible to quantify the effects of the environment, the physical structure and biological composition of the soil and to evaluate the positive or negative effects of particular treatments (herbicide/insecticide applications) on *X. fastidiosa* subsp. *pauca* bacteria and on the CoDiRO disease in olives. This is because data from long term studies under field conditions are lacking. However, agronomic practices to increase the availability of nutrients and the use of water to support development and growth of plant organs above and below the soil surface can contribute to the plant health status and the resilience to diseases and may prolong the productive time of the crop before the disease eventually enters a symptomatic stage and plant decline cannot be further prevented. The Panel therefore concludes that incidence, severity and progression of the disease are influenced by abiotic/biotic factors. Improving the health status of a plant infected by *X. fastidiosa* can prolong its productive life but cannot cure it from the bacterial infection. All factors indicated in the statement may have an effect on the expression of the disease. However, despite the validity of these factors for the disease expression, the risk posed by the presence of the bacterium *X. fastidiosa* for the rest of the EU, remains (EFSA PLH Panel, 2015). It has also been demonstrated that *X. fastidiosa* is the causal agent of the CoDiRO disease in olives (see Section 3.2).

3.2. Point 3 – confirming the aetiology of CoDiRO

This question addresses the direct link between the presence of *X. fastidiosa* bacteria in olives and the expression of wilting symptoms and the 'quick declining symptoms of olive trees' or CoDiRO. The Panel is requested to provide further evidence that *X. fastidiosa* is the cause of the disease leading to death of olive trees and whether pending confirmation would have consequences for the risk assessment.

3.2.1. Koch's postulates and their limitations

Koch's postulates, also known as Henle-Koch's postulates (Carter, 1985), were published in the nineteenth century, as a methodology for the demonstration that a bacterium was the cause of a specific disease. Specifically, these postulates state the particular criteria that have to be fulfilled to demonstrate that an organism is the causative agent of a disease: 1 – the microorganism (agent) must be present in all cases of the disease; 2 – the microorganism (agent) must be grown in pure culture outside the diseased organism; 3 – when inoculated with the microorganism (agent), healthy test organisms must develop the same symptoms as in the original host; and 4 – the microorganism (agent) must be present in the experimentally infected plants and constantly associated with the disease. Koch's postulates remain widely used in the field of plant pathology since biological assays provide the most conclusive evidence for compatible interactions between a pathogen and its host plant.

The identification and consistent detection of a pathogen in a particular plant makes it possible to associate a pathogen with a disease but, because of the complex origin of diseases, only the fulfilment of Koch's postulates provides an ultimate proof of the identity of the causal agent. However, Koch's postulates are in many cases difficult or impossible to fulfil. Evans (1993) acknowledged such limitations, for example (i) when the pathogen cannot be obtained in pure culture such as in the case of viruses or phytoplasmas, (ii) in the case of asymptomatic plant infections that may eventually develop symptoms or remain symptomless under particular conditions or, (iii) when multiple agents are to constitute disease symptoms (syndrome). For diseases not caused by pathogens, Hill's considerations for causal inference (1965) are mostly used instead of Koch's postulates. More recently, Fredericks and Relman (1996) have also proposed to reconsider Koch's postulates based on advances in knowledge and technologies, to propose molecular guidelines for establishing microbial disease causation. The debate about causality in epidemiology remains strong and complex (Broadbent, 2011; Vandenbroucke et al., 2016).

The Panel acknowledges that experiments to prove the host status of a plant for a specific pathogen, considering both symptomatic (disease) and asymptomatic infections, are among the most challenging in plant pathology. As observed for *X. fastidiosa* and for other pathogens, the introduction of a bacterium into a plant and the observation of its systemic movement within the host do not always result in symptomatic infections (Purcell and Saunders, 1999; Feil et al., 2003; de Souza Prado et al., 2008). In some cases, bacterial populations even after an extended period of movement and invasion do not establish in the plant (de Souza Prado et al., 2008). These transient infection situations complicate the assessment of the susceptibility of a host, because even hosts that support only small and transient bacterial populations might still function as sources of inoculum for insect vectors (Hill and Purcell, 1997; Purcell and Saunders, 1999; Wistrom and Purcell, 2005; Marucci et al., 2005; Krugner et al., 2014).

3.2.2. *Xylella fastidiosa* and Koch's postulates

Since the first description of Pierce's disease in grapevine in 1892, almost a century elapsed before Koch's postulates were duly fulfilled. This was facilitated by the isolation and establishment of a pure culture of the bacterium *X. fastidiosa* in 1978 (Davis et al., 1978). The tedious isolation process of *X. fastidiosa* and the complex back transmission experiments may explain why the demonstration that *X. fastidiosa* is the causal agent of a particular disease was often not brought to an end or performed adequately (EFSA PLH Panel, 2015). Koch's postulates have been performed for the four different subspecies of *X. fastidiosa* (Chang et al., 1990; Lee et al., 1993; Hartung et al., 1994; Sanderlin and Heyderich-Alger, 2000; Hernandez-Martinez et al., 2006; Bove and Ayres, 2007; Chang et al., 2009; Su et al., 2013) but only for a limited set of host – *X. fastidiosa* subspecies combinations.

For a large proportion of plants, including those that carry asymptomatic infections, neither back transmission experiments to verify host plant status, nor Koch's postulates to determine the causation of the disease have been performed. Consequently for many cases, host reports for *X. fastidiosa* are based on pathogen detection in suspicious field samples only (EFSA PLH Panel, 2015).

Nevertheless, despite pending experimental confirmation and proof of disease, a confirmation of the continuous association of a pathogen and a particular plant species demonstrates infection and confirms the host status for the pathogen. Thus, the finding of *X. fastidiosa* subsp. *pauca* in wilting diseased olive trees in Argentina in 2013 (Haelterman et al., 2015) and the confirmation of the pathogen in olive plants showing leaf scorching in Brazilian olive orchards (Colhetta-Filho et al., 2016) and the detection and identification of *X. fastidiosa* subsp. *pauca* in CoDiRO affected olives in Apulia (Saponari et al., 2013) confirm that olives are host plants for this pathogen. Despite missing evidence from Koch's postulates, these observations then also provide circumstantial evidence for the tight association of the pathogen with the disease.

In a recent publication by Krugner et al. (2014), *X. fastidiosa* subsp. *multiplex* was isolated from symptomatic olives in California and mechanically inoculated to almond, grapevine and olive. In inoculated almonds, symptoms were observed while in olives *X. fastidiosa* was detected in the plants for a few months after inoculation only, without observation of symptoms. Consequently, the authors were unable to confirm *X. fastidiosa* subsp. *multiplex* as the causal agent of the olive leaf scorch disease that affected the Californian olive plants from which the studied isolate had been originally obtained (Krugner et al., 2010, 2014).

Transmission of *X. fastidiosa* from olives to other hosts by the vector *P. spumarius* was experimentally demonstrated, thus confirming its vector competence (Saponari et al., 2014). Recent experimental results from mechanical inoculations to infect olives with pure culture of *X. fastidiosa* proved bacterial transmission and infection (Saponari et al., 2016) and demonstrated wilting and branch dieback in olives. These inoculation experiments done in December 2014 with the *X. fastidiosa* subsp. *pauca* isolate 'De Donno' provided evidence that Koch's postulates have been fulfilled. *X. fastidiosa* isolate 'De Donno' was obtained in pure culture from naturally infected olive trees in the field that were showing typical CoDiRO symptoms. This isolate was used to inoculate olive seedlings of four cultivars grown under controlled conditions. Only after more than 12 months of observation, these olive plants started to develop leaf rolling symptoms followed by severe wilting and branch dieback. Evidence provided in the above-mentioned report, and previous evidence provided by the same group of scientists include: Postulate 1: *X. fastidiosa* has been consistently found associated with symptomatic olive trees in the outbreak region; Postulate 2: *X. fastidiosa* isolate 'De Donno' was isolated from a symptomatic olive tree and cultivated as pure culture; Postulate 3: Inoculation of four different olive cultivars (10 plants each) was successful and proven by qPCR to verify bacterial establishment in the host and systemic movement (acropetally and in some cases basipetally to the roots). Most importantly, symptoms (chlorosis, wilting, and desiccation) similar to those observed for CoDiRO were found with varying degrees in all cultivars tested; and Postulate 4: re-isolation of the bacteria from symptomatic plants and growth in vitro of pure bacterial cultures from plants artificially infected were conducted.

The Panel concludes that the evidence provided by recent experiments (Saponari et al., 2016) demonstrates that *X. fastidiosa* isolate 'De Donno' causes CoDiRO symptoms in olives and thus is the causal agent of this disease. However the Panel also wishes to state that the fulfilment of Koch's postulates provides evidence for the direct link between the pathogen and the disease in olives, but that the occurrence of *X. fastidiosa* infections in olives was sufficient for its risk rating as stated in the 2015 by the Panel (EFSA PLH Panel, 2015).

3.3. Point 4 – removal of host plants (Articles 6 and 7 of Decision (EU) 2015/789)

This question addresses the efficacy of removal of *X. fastidiosa* host plants regardless of their health status within a radius of 100 m around a plant found infected in case of eradication efforts (Art. 6 of Decision (EU) 2015/789) and of at least all *X. fastidiosa* infected plants in case of containment efforts (Art. 7 of Decision (EU) 2015/789). The question is focused on measures set in place in some parts of the demarcated area in Apulia (Italy) to prevent further spread of *X. fastidiosa* to areas not yet infected. An analysis of scientific evidence was requested in particular concerning the efficacy of the measures in the buffer zone outside the infected demarcated area and in the 20 Km outer strip, in proximity of the buffer zone, of the 'containment area' in Lecce province where *X. fastidiosa* is considered established.

In ISPM 5 (FAO, 2016), containment is defined as 'the application of phytosanitary measures in and around an infested area to prevent spread of a pest' whereas eradication is 'the application of phytosanitary measures to eliminate a pest from an area'. Commission implementing decision (EU) 2015/789 Articles 6 and 7 outline host removal as part of the overall phytosanitary measures aimed at, respectively, eradication and containment of *X. fastidiosa* within specified areas of the EU. Host plant removal is a widely adopted control measure and element of phytosanitary interventions to contain or eradicate a pest from a given area (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 effectiveness of containment and eradication depends on a range of epidemiological factors (Pluess et al., 2012) and, in particular, on the degree of pathogen invasion which is reflected by the number and density of infection foci at the onset of a control programme. Broadly, in areas where the bacterium is established, only containment may be possible whereas in areas of recent introduction, removal of infected plants and host plants, regardless of their health status, may lead to the eradication of the pathogen and the disease.

3.3.1. Removal of infected plants as a containment measure

The containment area as specified in article 7 is where *X. fastidiosa* is known to be established (see the updated map by the Apulian Region[4]). The article specifies the requirement to remove infected plants in certain locations, particularly within a distance of 20 km from the border of the containment area with the rest of the EU territory (buffer zone). Removal of infected host plants (synonymous with 'roguing') is a practice which removes inoculum sources that contribute to pest spread. In many cases, removals are combined with other phytosanitary measures which also contribute to reducing incidence and spread of the pathogen. For example, the citrus greening disease caused by '*Candidatus Liberibacter* spp.', another plant bacterium, has been controlled in large farms (e.g. > several thousand hectares) in Brazil by applying tree removal, to exclude inoculum sources, in

[4] Determinazione del dirigente sezione agricoltura 12 febbraio 216, n. 23. Bollettino Ufficiale della Regione Puglia - n. 16 del 18-2-2016, 7554–7560. Available online http://www.regione.puglia.it/web/files/agricoltura/aggiornamento_aree_xilella.pdf

combination with psyllid control by insecticide application programmes, to reduce inoculum dispersal (Belasque et al., 2010; Bassanezi, 2013a and b). On the other hand, in spite of these measures *X. fastidiosa* epidemics on citrus and grape have continued to increase in parts of the US and Brazil (Lopes et al., 2000; Purcell, 2013). However, it has to be stated that in these interventions, treatment of already established diseases was tried. In fact, to make removal of infected trees effective, studies have shown that roguing must occur at an early stage of epidemics to minimise the opportunity for inoculum production, and subsequent spread (de Boer and Boucher, 2011; Gordillo et al., 2012; Behlau et al., 2014) and involve high levels of compliance (Sisterson and Stenger, 2013). This necessitates timely intervention (Ward, 2016) which requires the ability to properly identify infected plants and their timely removal to interrupt inoculum build-up and to guarantee the overall effectiveness of the intervention. Moreover, for citrus greening disease, studies have shown that for removal of positive-only trees (roguing) to be effective, disease must be at low incidence (Belasque et al., 2010). A key prerequisite is therefore sufficient intensity of disease monitoring to allow the identification of early stage infections at low intensity foci and, to this effect, appropriate sampling and testing protocols (Pluess et al., 2012).

Given that infected olive trees are sources of inoculum for *X. fastidiosa* vector transmission and pathogen spread, a stringent monitoring for early detection of newly infected trees and their timely removal can reduce *X. fastidiosa* inoculum. In combination with other phytosanitary practices specified in Art. 7, the rates of epidemic spread within and from the containment area into neighbouring areas not yet infected will consequently be reduced. This will be particularly effective in areas where foci have recently been introduced, like those in proximity of the buffer zone, where localised removal of foci is possible provided that identification of diseased plants is done at a sufficiently early stage of infection (Genovesi and Shine, 2004). For *X. fastidiosa* detection in olives, this can present a challenge because of latent infections requiring sensitive diagnostic tools to detect the pathogen. To this effect, Article 7(3) prescribes sampling and testing of host plants located within the 100 m radius around an infected host plant removed, to be done at regular intervals, at least twice a year.

Similarly, removal of all infected plants can have significant effects to reduce inoculum sources. However, while uncertainty still exists that wild plants infected with *X. fastidiosa* have a significant direct role as inoculum sources, it would be very difficult to identify all infected plants as the broad list of host plants of *X. fastidiosa*[5] is not complete and monitoring for pathogen presence in all plants would exceed diagnostic capacity. Nevertheless, it should be stated that more intensive surveillance in the buffer zone as specified by Art. 6 is a prerequisite to prevent spread and reduce the expansion range of the bacterium. Surveys should be conducted in accordance with the EU guidelines on *X. fastidiosa* surveys[6] which aim to 'ensure the highest possible level of early detection of outbreaks of *X. fastidiosa* in the Union territory'.

Considering the danger of high inoculum levels in the containment area bordering the buffer zone and the danger of inadvertent dispersal of infected sources (either hosts or vectors) from the containment zone to the buffer zone, the measures stated in Art. 7 comprising infected olives and all other infected host plants have a risk based justification, as the removal of infected plants may limit further spread by the elimination of infection sources. However, because of the wide host range *of X. fastidiosa*, uncertainty exists on the number of plant species other than olives and the host plants to be included in monitoring and on the effective identification of asymptomatic plants. The width of the zone of application of these measures is a risk management decision and is not part of the Panel assessment.

Options for containment of *X. fastidiosa* have been discussed in Section 4.3.3 'Containment strategies' of the risk assessment by the EFSA PLH Panel (2015).

3.3.2. Removal of host plants, regardless of their health status, in areas of recent introduction

Article 6 provides details of actions to be taken relating to the eradication of outbreaks of *X. fastidiosa*, in areas where it was previously not known to occur, outside the containment area. Due

[5] See Annex II of the Commission Implementing Decision (EU) 2015/789 of 18 May 2015 as regards measures to prevent the introduction into and the spread within the Union of *Xylella fastidiosa* (Wells et al.)

[6] European Commission, Directorate General for Health and Food Safety, Brussels, 16 December 2015. Guidelines for the survey of *Xylella fastidiosa* (Wells et al.) in the Union territory. 8 pp. Available online http://ec.europa.eu/food/plant/docs/ph_biosec_legis_guidelines_xylella-survey.pdf

to its recent introduction and the absence of previously known disease in this area, local eradication of emerging foci is possible only if all new foci are identified at an early infection stage and infected hosts are efficiently removed. A number of studies have reinforced that prerequisite to successful eradication is the early detection of infected sites (Genovesi and Shine, 2004; Pluess et al., 2012). Appropriate survey and sampling practices (see available EU guidelines on *X. fastidiosa* survey) can significantly reduce the uncertainty to correctly detect *X. fastidiosa* also in asymptomatic infections.

Article 6 requires a range of measures including the removal, within a radius of 100 m around an infected host, of all host plants known to be susceptible to an European isolate of *X. fastidiosa* (regardless whether infected or not), other plants known to be infected by *X. fastidiosa*, and other plants showing symptoms indicating possible infection by *X. fastidiosa* or suspected to be infected.

Such an action can help to limit further spread outside of the infected area. While there is uncertainty about the complete host range of European plant species susceptible to the European isolates of *X. fastidiosa*, the extensive list of host plants of *X. fastidiosa* (more than 200 in the list of 'specified plants[7]') to be monitored within a radius of 100 m around the infected host plants would provide comprehensive coverage of possible infections. The definition of a radial distance – here 100 m – addresses the activity of the vectors transmitting the pathogen and considers an infection gradient from the focal point. Gilligan and van den Bosch, (2008) highlight the need to 'match the scale of control with the intrinsic scale of the epidemic'. In fact, knowledge of the dispersal scale of the pathogen or vector has been used to define the appropriate size of removal areas.

For disease response and eradication programmes, such radius demarcations are common practice and significant for the success of the intervention. Such measures have been successfully put into practice to respond to, for instance, Plum pox virus eradication in New York State (Rimbaud et al., 2015) and fireblight of apple and pear in Australia (Sosnowski et al., 2009). Sudden oak death spread was significantly reduced in Oregon following an eradication protocol that prescribed cutting and burning of host plants within a minimum radius of 100 m from an infected specimen (Peterson et al., 2015). This was to ensure that all inoculum sources are removed and to prevent secondary spread from individuals that are infected but not yet testing positive (Cunniffe et al., 2015). Once secondary spread events have occurred, the efficiency of eradication measures decreases.

In the case of *X. fastidiosa*, dispersal seems to be primarily limited by the short-range flight of infectious insect vectors. For the leafhopper *Homalodisca vitripennis*, it has been reported that active flight ranges about 100 m, as stated in the PLH Panel risk assessment on *X. fastidiosa* (EFSA PLH Panel, 2015). In addition, wind can contribute to insect vector dispersal; however the main factor is active flight of the insects. Short distance spread was also shown by Gottwald et al. (1993), who conducted spatial analyses of the spread of CVC in Brazil and found strong associations between citrus trees immediately adjacent to each other, suggesting a dominance of tree-to-tree spread. Similar studies are not yet available for the CoDiRO disease in Apulia.

Moreover, an optimum removal radius of host plants around an infection focus is difficult to pinpoint in practice and will depend on the density of infection foci and the timely detection following removal of host plants (Cunniffe et al., 2015). Thus, the same pathogen can be subjected to different eradication radii depending on the specific epidemic situation. The successful eradication of Plum pox virus from New York State was by adhering to a 50 m removal radius around infected trees (Rimbaud et al., 2015). In contrast, a removal radius of 500 m was used in an eradication program for the same pathogen in Canada and this was partly due to higher numbers of initial infections (Rimbaud et al., 2015). Retrospective analysis of the Canadian epidemic demonstrated that '95% of new infections occurred in the first year within 628 m, the second year within 465 m and the third year within 317 m distances' suggesting that the removal radii of 500 m, thus between 317 and 628 m was appropriate for the Canada situation (Gottwald et al., 2013). However, in some situations far larger removal areas have been demarcated. For example, the Northern Territory Government in Australia initially used a 1 km removal radius in the Banana Freckle (*Phyllosticta cavendishii*) Eradication Program, later extending this to remove all Banana plants within infestation zones (McMaster et al., 2015) to guarantee successful eradication of the pathogen.

[7] See Annex II of the Commission Implementing Decision (EU) 2015/2417 of 17 December 2015 as regards measures to prevent the introduction into and the spread within the Union of *Xylella fastidiosa* (Wells et al.)

In summary, eradication from areas where a disease is already well established is unlikely without significant numbers of host removals. This is the probable explanation for the limited success of *X. fastidiosa* control programmes conducted in Brazil and the US (Amaro et al., 1998; Lopes et al., 2000; Purcell, 2013) where *X. fastidiosa* epidemics already were far advanced and the number of infected plants too high at the onset of the control programmes. Nevertheless, at the outer border of an outbreak area, the pathogen range expansion will be from infected foci to neighbouring host plants, therefore the removal of infected host plants in a system-based approach including other measures (e.g. measures to control the vectors by chemical or biological control means and vegetation management) contributes to reduce or prevent the spread of the pathogen to other areas. Uncertainties regarding the level of the efficiency of the measure may come from the *X. fastidiosa* extensive host range, which has to be fully explored for the EU and the difficulty to identify early infections in olives and in other hosts.

The success of measures to eradicate the pathogen from areas recently invaded is due to a number of factors in particular early detection of infected trees, comprehensive monitoring to define the infection foci thus the extend of the invasion and the timely intervention that is elimination of infected hosts and removal of all host plants infected or not, in the vicinity of the infection foci. While the length of an eradication radius may be difficult to define for all circumstances, its definition takes into account epidemic parameters, the flight activity of the insect vector, the timeliness of detection and an infection gradient. The entire area of host plant removal then depends on the detection of 'new' infection foci and their density. Successful eradication thus is possible in areas of recent introduction and low incidence provided an early detection of foci and its timely removal (Genovesi and Shine, 2004).

Options for eradication of *X. fastidiosa* and its vectors have been discussed in Section 4.3.2. 'Eradication' of the risk assessment by the EFSA PLH Panel (2015).

Finally, the Panel concludes that removal of infected plants is a risk-reducing option that, by removing inoculum sources, can contribute to reduce the incidence of infections in the outer zone of the outbreak and to prevent further spread of the pathogen. Particularly in the epidemic situation of Southern Italy, which is characterised by a containment zone demarcated by surrounding sea and a buffer zone advancing the front of the epidemics into which the pathogen has not yet been introduced, the measures indicated in article 7 and in article 6 can provide means to counter pathogen spread into areas not yet infected. In the containment area, in the northern strip bordering the buffer zone, the removal of infected plants and stringent monitoring can be effective in preventing further spread of the pathogen into an area that has not yet been reached. With regard to the elimination of new introduction foci in areas of recent invasion, such as the buffer zone, the stringent removal of both infected plants and all host plants irrespective of their health status within a radius, as described in current EU legislation, can be effective in reducing pathogen spread, when rigorously administered and new infections are detected in time.

3.4. Point 6 – secondary effects of pesticides

The question addresses the negative effects of pesticide applications, direct and indirect consequences for the environment and risks for human and animal health. Insecticide treatments to control vector populations of *X. fastidiosa* as well as herbicide applications to treat host plants of pathogen and vectors shall be assessed with regard to the efficacy of the measure and the negative impact identified. Outbreaks of *X. fastidiosa* are invariably linked to presence of vector insects. Xylem sap-feeding insects are the natural vectors of *X. fastidiosa* worldwide and in Europe. So far, few spittlebug species have been found positive for *X. fastidiosa* in the Apulian infected area: *Euscelis lineolatus*, *Neophilaenus campestris* and *P. spumarius*, while only the last was proven to be able to transmit the pathogen (Elbeaino et al., 2014; Saponari et al., 2014).

Control of vector insects as discussed in the PLH Panel risk assessment on *X. fastidiosa* (2015) is a crucial element of strategies to combat the disease. In a systemic approach, the management of insect vectors is a key component requiring multiple interventions and targeted strategies taking into consideration many aspects in order to be effective. This explains why the use of pesticide treatments is mentioned so many times and from different angles in the Panel opinion (Sections 3.2.1.1., 3.2.2.2., 3.2.2.3., 3.2.3.1., 3.2.3.2., 3.2.3.3., 3.5.1.1, 3.5.1.4., 3.5.1.5., 3.5.1.6., 3.5.2., 4.1.1.2., 4.1.2.1., 4.1.3.7., 4.2.1.2., 4.2.1.3., 4.2.1.5., 4.2.2.3., 4.3.2.2., 4.3.3.3., 4.3.3.4., 4.5., 4.6.8.,), and not only in the quotations provided under point 6 of the mandate as specified later in this section.

Section 3.5.2 mentioned in the terms of reference is a standard element of risk assessments conducted by the EFSA PLH Panel and should be viewed in the context of the entire Section 3.5. on the assessment of consequences. The articles 'Control of the pest in the risk assessment area in the absence of phytosanitary measures' (Section 3.5.1.4.) and 'Control measures currently applied in the risk assessment area' (Section 3.5.1.5.) explain that current practices (either integrated pest management or insecticides currently used) can have an effect on the pest. Similarly, Section 3.5.1.6. on control measures currently applied in the infected area of Lecce province, refers to the Italian Ministry of Agriculture (Italian Ministerial Decree No 2777 issued on 26 September) and implemented in the area under the surveillance of the Phytosanitary Service of the Apulian Region (Resolution 1842 (Apulia Region), 5 September 2014). The measures are based on an integrated pest management strategy that includes insecticide applications against the vectors, agronomic measures to suppress nymphal stages of the vector on the weeds and removal of infected plants. Section 3.5.2. is in accordance with the ISPM 11 (FAO, 2016) and with the EFSA PLH Panel Guidance on a harmonised framework for pest risk assessment and evaluation of risk reduction options (EFSA PLH Panel 2010), the assessment of the consequences of the introduction of a plant pest should include both direct and indirect pest effects. Environmental consequences of control measures fall among those indirect effects: this is why, besides considerations on the efficacy of such measures, the potential effects of insecticide and herbicide applications on human health, non-target organisms, ecosystem functions and biodiversity are to be stated.

A similar consideration applies to Sections 4.3.2.2. and 4.3.3.4. of the EFSA PLH Panel risk assessment (2015), also mentioned in the terms of reference. Those quotations were extracted from the sections on identification and evaluation of options to reduce the probability of establishment of *X. fastidiosa* subject to eradication and containment measures respectively are discussed. Here again, these sub-sections should be viewed in the general context of the Sections 4.3.2. (Eradication) and 4.3.3. (Containment). In these sections, advantages and contraindications of each measure or combination of measures are provided, in support of decision makers. Consideration of potential indirect effects under the scenario of the overall risk for the whole EU territory (e.g. impacts on the environment, insecticide resistance, impacts on non-target organisms, human and animal health) are included in the 'Analysis of the applicability of the risk reduction option' following the PLH Panel guidance on methodology for evaluation of the effectiveness of options for reducing the risk of introduction and spread of organisms harmful to plant health in the EU territory (EFSA, 2012). Concerning more specifically the Commission Implementing Decision[8] currently in place, both article 6 and 7 dedicate paragraph 4 to phytosanitary treatments, with the following statement: 'The Member State (MS) concerned shall carry out appropriate phytosanitary treatments prior to the removal of plants referred to in paragraph 2 against the vectors of the specified organism and plants that may host those vectors. Those treatments may include, as appropriate, removal of plants'. In this context, appropriate phytosanitary treatments should include, among others, chemical or non-chemical control measures (including biological control measures) as well as vegetation management, as concluded in the PLH Panel risk assessment (2015), particularly when applied in open field conditions.

In general, the process regulating the use of plant protection products in the EU ensures a high level of protection for human health, animal health and the environment. Pesticide active substances are approved at European level and then Plant Protection Products are authorised at National level according to the provisions under Regulation (EC) No 1107/2009[9]; additional information on the legal framework is provided in the DG SANTE website under the specific page on Legislation on Plant Protection Products (PPPs) (http://ec.europa.eu/food/plant/pesticides/legislation/index_en.htm). Only Plant Protection Products authorised at the national level can be used, and always adhering to the conditions authorised and applicable restrictions and risk mitigation measures. The conditions

[8] Commission Implementing Decision (EU) 2015/789 of 18 May 2015 as regards measures to prevent the introduction into and the spread within the Union of *Xylella fastidiosa* (Wells et al.). Official Journal of the European Union L 125/36-53, 21.5.2015.

[9] Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. L 309/1-50. Available from http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32009R1107&from=EN

prescribed are based on a risk assessment conducted at the European level for the active substance and by the MS National Authorities for each authorised product, in accordance with the legislation and applicable guidelines. The EFSA Conclusions on pesticides (published in the EFSA Journal) present the outcome of the European evaluation of each active substance. The updated approval conditions for active substances are available in the European Pesticides Database (European Commission, online). The EFSA Pesticides Unit, in close collaboration with the risk assessors in the MSs, is in charge of the EU evaluation, which includes an initial risk assessment of the active substance used in plant protection products by a Rapporteur MS and a scientific peer review by EFSA. The assessment includes a comprehensive evaluation of the potential hazards of the pesticide active substance, covering human and animal health as well as the environment, and the risk assessment for a set of representative uses. The output is summarised in the EFSA Conclusion, which includes descriptions of the physical/chemical properties, toxicology and risk for operators, workers, bystanders and residents; expected residues in crops and the risk for consumers; environmental fate and behaviour, and ecotoxicology and environmental risk assessment. The main active substance properties are summarised in the List of Endpoints, which describes the parameters and information validated during the EFSA peer-review and to be used in the National assessments of Plant Protection Products containing the active substance. Any assessment, from Pesticides Unit and PLH Panel is freely available, accessible from the EFSA website and published on the EFSA Journal; there are also a number of guidance documents and scientific opinions of the PPR Panel describing the methodology to be applied in the risk assessment

Each MS can find in this regulation support in defining the most adequate phytosanitary strategy, an evaluation of pesticides and respective databases available at the EU level and specifically implemented at national level. Each MS should consider if the Plant Protection Products already authorised in the MS, taking into account the conditions for use and possible restrictions, provide sufficient coverage for the phytosanitary treatments required for setting a proper strategy. The use of Plant Protection Products for controlling *X. fastidiosa* vectors may require application patterns different from those covered by previous risk assessments and current authorisation conditions. According to Regulation (EC) No 1107/2009, in special circumstances a MS may authorise, for emergency situations, a product containing an active compound not approved at the EU level for limited and controlled use. This is to respond to urgent situations when pests can otherwise not be contained by other means. The use of Plant Protection Products in protected and vulnerable areas may require additional authorisation to adhere to National legislations. For more details concerning the specific Italian situation, information on the products authorised can be found on the website of the Ministero della Salute[10].

However, as also indicated in the PLH Panel opinion, the appropriate use of plant protection products following prescribed procedures both in outbreak areas and buffer zones is considered a practice with considerable effects on insect vector populations, directly, by reducing the number of actively transmitting insects and nymphal stages and indirectly by limiting the sources of inoculum and reservoirs for both insects and bacterium (see paragraphs 4.3.2.2. and 4.3.3.4. of PLH Panel, 2015). The EFSA conclusions on pesticides, including the list of validated endpoints for risk assessment, and the specific guidance for the risk assessment of pesticides, complemented by the scientific opinions of the EFSA PPR Panel, can be used for assessing the risks of the phytosanitary treatments for human health, animal health and the environment. These assessments are not only relevant for supporting the national authorisations required prior to the use of the plant protection product; the need for specific *ad hoc* assessments of the overall risk of the phytosanitary treatment strategy may be considered in some cases, e.g. for phytosanitary treatments requiring the use of several Plant Protection Products.

In addition to the information already provided in the PLH risk assessment on *X. fastidiosa* (PLH Panel, 2015) the Panel considers that one single general strategy for the whole EU on pesticide application conceived for a system-based approach is not expected to cover the different situations that could be envisaged in the European territory. The current legislation provides to the MS the possibility to define

[10] Ministero della Salute. Home > Temi e professioni > Alimenti > Prodotti fitosanitari. Available online: http://www.salute.gov.it/portale/temi/p2_4.jsp?lingua=italiano&tema=Alimenti&area=fitosanitari [Accessed: 17 March 2016]

the most effective control strategy (targeting insects, cultivated or wild host plants and ruderal vegetation) to the specific pest/host/vector combination observed in a given containment area, outbreak area or buffer zone. Furthermore, the Panel highlights the fact that, in spite of undesired effects of plant protection products, there is currently no evidence of any negative effect of such treatments on the interaction of *X. fastidiosa* with infected olive trees and in particular on the severity of CoDiRO symptom expression and the outcome of the infection (see also Section 3.1. of current opinion. The Panel therefore concludes that the application of plant protection products against *X. fastidiosa* should be seen in a system-based approach and targeted to the specific local situations. EU MSs can count on a very structured system of evaluation of plant protection products (in which EFSA plays a key role) ensuring a high level of protection for humans health, animals health and the environment. The PLH Panel, in its assessment, considered the use of pesticides in their complexity, evaluating different aspects concerning their efficacy and applicability with a holistic approach. The application of appropriate phytosanitary treatments against *X. fastidiosa* vectors should include, among others, chemical or non-chemical control measures (including biological control) as well as vegetation management.

4. Conclusions

The PLH Panel was requested to provide a scientific evaluation of several statements questioning the overall EU control strategy against *X. fastidiosa* and some relevant legal provisions laid down under Decision (EU) 2015/789. The specific conclusions on four of those statements are:

- on factors affecting expression of symptoms and spread of *X. fastidiosa*: the Panel states that it is not possible to quantify the effects of the environment, the physical structure and biological composition of the soil and to evaluate the positive or negative effects of particular treatments (herbicide/insecticide applications) on *X. fastidiosa* subsp. *pauca* bacteria and on the CoDiRO disease in olives. This is because data from long term studies under field conditions are lacking. However, agronomic practices to increase the availability of nutrients and the use of water to support development and growth of plant organs above and below the soil surface can contribute to the plant health status and the resilience to diseases and may prolong the productive time of the crop before the disease eventually enters a symptomatic stage and plant decline cannot be further prevented. The Panel therefore concludes that incidence, severity and progression of the disease are influenced by abiotic/biotic factors. Improving the health status of a plant infected by *X. fastidiosa* can prolong its productive life but cannot cure it from the bacterial infection. All factors indicated in the statement may have an effect on the expression of the disease. However, despite the validity of these factors for the disease expression, the risk posed by the presence of the bacterium *X. fastidiosa* for the rest of the EU remains (EFSA PLH Panel, 2015). It has also been demonstrated that *X. fastidiosa* is the causal agent of the CoDiRO disease in olives.
- on the aetiology of CoDiRO: the Panel concludes that the evidence provided by recent experiments (Saponari et al., 2016) demonstrates that *X. fastidiosa* isolate 'De Donno' causes CoDiRO symptoms in olives and thus is the causal agent of this disease. However the Panel also wishes to state that the fulfilment of Koch's postulates provides evidence for the direct link between the pathogen and the disease in olives, but that the occurrence of *X. fastidiosa* infections in olives was sufficient for its risk rating as stated in the 2015 by the Panel (EFSA PLH Panel, 2015).
- on host plants removal: the Panel concludes that removal of infected plants is a risk-reducing option that, by removing inoculum sources, can contribute to reduce the incidence of infections in the outer zone of the outbreak and to prevent further spread of the pathogen. Particularly in the epidemic situation of Southern Italy, which is characterised by a containment zone demarcated by surrounding sea and a buffer zone advancing the front of the epidemics into which the pathogen has not yet been introduced, the measures indicated in article 7 and in article 6 can provide means to counter pathogen spread into areas not yet infected. In the containment area, in the northern strip bordering the buffer zone, the removal of infected plants and stringent monitoring can be effective in preventing further spread of the pathogen into an area that has not yet been reached. With regard to the elimination of new introduction foci in areas of recent invasion, such as the buffer zone, the

stringent removal of both infected plants and all host plants irrespective of their health status within a radius, as described in current EU legislation, can be effective in reducing pathogen spread, when rigorously administered and new infections are detected in time.

- on secondary effects of pesticides: with reference to phytosanitary treatments required by Decision (EU) 2015/789 (Articles 6 and 7), the Panel highlights the fact that, in spite of undesired effects of plant protection products, there is currently no evidence of any negative effect of such treatments on the interaction of *X. fastidiosa* with infected olive trees and in particular on the severity of CoDiRO symptom expression and the outcome of the infection. The Panel therefore concludes that the application of plant protection products against *X. fastidiosa* vectors should be seen in a system-based approach and targeted to the specific local situations. EU MSs can count on a very structured system of evaluation of plant protection products (in which EFSA plays a key role) ensuring a high level of protection for human health, animal health and the environment. The PLH Panel, in its assessment, considered the use of pesticides in their complexity, evaluating different aspects concerning their efficacy and applicability with a holistic approach. The application of appropriate phytosanitary treatments against *X. fastidiosa* vectors should include, among others, chemical or non-chemical control measures (including biological control) as well as vegetation management.

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Glossary and Abbreviations

In Commission Implementing Decision 2015/789 amended by Commission Implementing Decision 2015/2417[11], host plants of *X. fastidiosa* are defined as

Specified plants: host plants and all plants for planting, other than seeds, belonging to the genera or species listed in Annex I (Annex I of Commission Implementing Decision 2015/789). This list is currently composed of 160 plant species and 28 genera.

Host plants: plants for planting, other than seeds, belonging to the genera and species listed in the Commission database of host plants susceptible to *Xylella fastidiosa* in the Union territory, as having been found to be susceptible in the Union territory to the specified organism or, where a Member State has demarcated an area with regard to only one or more subspecies of the specified organism pursuant to the second subparagraph of Article 4(1), as having been found to be susceptible to that or those subspecies. The original list, composed by 11 species and 2 genera, was updated on 3 February 2016 and now it comprises 39 species and 4 genera (Commission database of host plants found to be susceptible to *Xylella fastidiosa* in the Union territory – update 2).

In this opinion the two terms are used as follows

Specified plants: in accordance with above mentioned regulation.

Host plants: unless otherwise stated and when directly referring to the above mentioned regulation, it is a general term used for plant species known to be susceptible to *X. fastidiosa*. As listed in the EFSA *X. fastidiosa* host plants database (update 9 February 2016) currently consisting of 359 plant species – both naturally and experimentally infected hosts – from 204 genera and 75 botanical families.

CoDiRO	Complesso del Disseccamento Rapido dell'Olivo
PLH Panel or the Panel	EFSA Scientific Panel on Plant Health
MS	Member State
OQDS	Olive Quick Decline Syndrome

[11] Commission Implementing Decision (EU) 2015/2417 of 17 December 2015 amending Implementing Decision (EU) 2015/789 as regards measures to prevent the introduction into and the spread within the Union of *Xylella fastidiosa* (Wells et al.). OJ L 333, 19.12.2015, p. 143–147.