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Treatment solutions to cure *Xylella fastidiosa* diseased plants

EFSA Panel on Plant Health (PLH)

Abstract

This opinion addresses the question of the efficacy of current treatment solutions to cure *Xylella fastidiosa* diseased plants, and discusses the experimental treatments under evaluation by two research groups in Apulian olive orchards infected by strain CoDiRO (Complesso del Disseccamento Rapido dell'Olivo). The increasing problems from newly emerging vascular bacterial diseases and the limited success to cure plants from such infections have stimulated numerous studies on treatments with chemical and biological compounds. Under field conditions, various formulations of copper and zinc as spray or root drench are currently used while further options, for example the application of bioactive substances, are at an experimental stage. In Apulia, preliminary results from intensive treatments with such formulations, in combination with the use of good crop management practices, reported more vigorous new growth of diseased trees. However, results provided so far confirmed the continued presence of *X. fastidiosa* after the treatments under evaluation. This is in agreement with current knowledge that there are no means to cure plants from this bacterial disease, in the sense of eliminating the pathogen from plant tissues. The reported positive response of the treated olive trees is most probably due to the effect of micronutrients and other bioactive compounds that, together with soil cultivation and agronomical practices, improve the vigour of the plants and their resilience to stress caused by bacterial infections. Notwithstanding the preliminary status of these findings, the Panel acknowledged the potentially positive effects of such treatments in prolonging the productive phase of olive trees and their putative relevance for the management of olive orchards, particularly in the containment area where eradication of the pathogen is considered no longer possible. The Panel also concluded that long-term studies are needed to confirm that the reported positive effects on crop performance can be sustained over many years.

Keywords: CoDiRO strain, copper, olive disease management, symptom expression, zinc

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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 European Union (EU) control strategy against *X. 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 the European Food Safety Authority (EFSA) scientific advice are presented below:

1) It is considered that *the population of* X. 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 as CoDiRO) 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 X. 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 microfauna 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 ploughing 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 *X. fastidiosa* for the rest of Union.

3) *The causing link between* X. 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* X. 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 *X. 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 *X. 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-called 'containment area'), and

[1] Submitted by European Commission, ref. SANTE/G1/PDR/mm(2016)1031036.

[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.

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 (Fruit Tree Research Unit, 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 *X. 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 ongoing 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 in six points, each of which refers to a different aspect of risks connected to the presence of *X. fastidiosa* in the EU. Under each point, the European Commission addresses one or more questions to EFSA.

In this opinion, the EFSA Panel on Plant Health (PLH Panel) replies to point 5 and related questions as required by the indicated deadline.

This opinion was prepared in the 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* subsp. *pauca* strain CoDiRO.

The question whether scientifically validated treatments are available to cure plant diseases caused by *X. fastidiosa* in open field conditions is addressed in this opinion by distinguishing between the improvement in plant appearance (in terms of recovery from disease symptoms and vigorous growth despite remaining infected) and the cure from infection by elimination of the bacterial pathogen from the plant.

According to the International Standards for Phytosanitary Measures (ISPM) 5, a 'treatment' is an official procedure for the killing, inactivation or removal of pests, or for rendering pests infertile or for devitalisation (FAO, 2016). In previous opinions on *X. fastidiosa*, the Panel assessed physical treatments such as pruning (EFSA PLH Panel 2015a, 2016) and temperature (EFSA PLH Panel 2015a,b). In this opinion, the term 'treatment' exclusively refers to treatments with chemical mode of action, alone or in combination with other measures.

1.3. Additional information

This opinion is the second output that the Panel was requested to produce in order to address the mandate. Points 2, 3, 4 and 6 from the mandate were already addressed in a previous Panel opinion (EFSA PLH Panel, 2016) and point 1 will be addressed in a separate opinion 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, 2015a). Further references and information were obtained from citations within the reviewed references and from experts.

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).

In order to respond to the question requesting contacting the researchers Dr Antonia Carlucci and Prof. Francesco Lops of the University of Foggia (Italy) and Prof. Marco Scortichini of CREA (Caserta, Italy) to assess the outcome of their field experiments, EFSA proceeded as follows: a letter was sent to the three scientists, inviting them to share the applied methodologies, collected data and any relevant supporting material (such as pictures, presentations, etc.) and to participate in a technical hearing of the Working Group.

As the three researchers contacted were unable to share with EFSA the original data of their experiments due to patent or copyright issues stated, and also considering the preliminary phase of these experiments, it was decided to conduct a technical hearing with a more descriptive approach in order to obtain feedback on the ongoing activity and future expectations.

Carlucci and Lops accepted the invitation to participate and the hearing was conducted during the 6th Working Group meeting, on 22 March. The information obtained during the hearing is included in the minutes of the Working Group and available online at http://www.efsa.europa.eu/sites/default/files/plhxylellaurgentreview.pdf.

Scortichini did not accept the invitation to participate in the technical hearing but provided a written response via email on 19 March 2016, which in this document is referred to as 'Scortichini, personal communication'.

Because the data collected during the field experiments by the two above mentioned research groups were not provided to the Panel and the same researchers considered the results obtained until now preliminary pending repetition for at least another season, the Panel could neither provide a scientific peer-review nor validate their results in this scientific opinion.

3. Assessment

This request is connected to the results collected in Apulian olive orchards from experimental treatments on olive trees infected by *X. fastidiosa* strain CoDiRO by Prof. Marco Scortichini of CREA (Caserta, Italy) and by Dr Antonia Carlucci and Prof. Francesco Lops from the University of Foggia (Italy). The Panel was then asked to evaluate the outcomes of the work conducted by these two research groups that have reported promising preliminary results from their treatment approaches. In addition, the Panel was requested to provide an update on the availability of scientifically validated treatments to cure plants infected with *X. fastidiosa* in open field conditions.

With regard to this second point, the Panel has reviewed the scientific literature on this topic and observed the following: the limited success in controlling bacterial infections in plants and the increasing problems from newly emerging bacterial diseases of crops, have motivated fundamental and applied research, as well as industry efforts to explore further avenues for disease control. This is the case, for example, of fire blight of apple and pear (*Erwinia amylovora*), citrus canker (*Xanthomonas citri* subsp. *citri*), citrus greening ('*Candidatus* Liberibacter asiaticus', also known as huanglongbing (HLB)), Pierce's disease in grapevine (*X. fastidiosa* subsp. *fastidiosa*) and CoDiRO disease in olives (*X. fastidiosa* subsp. *pauca* strain CoDiRO).

A review of the available literature on treatments against vascular bacterial diseases showed that currently proposed treatments include: (i) antibiotics, used experimentally; (ii) formulations of bioactive compounds as activators to enhance plant resistance response; (iii) formulations of growth promoting micronutrients or biological compounds; (iv) antagonistic bacterial or fungal strains; (v) copper (Cu)-based formulations with fertilising, antimicrobial and/or fungicidal action; and (vi) formulations in commercial products applied in combinations and together with other crop management practices.

Based on the request, the Panel considered in its evaluation the approaches to treat bacterial diseases of field-grown plants and, in particular, compounds that are used for the management of *X. fastidiosa* diseases in olive or other crops. From the review of the available literature, the Panel can confirm that numerous studies exist on the efficacy of chemical and biological compounds on the growth of bacteria. Many of these experiments are however *in vitro* studies and thus of limited informative value as it is impossible to draw clear conclusions on potential *in vivo* activity. Moreover, for many experimental products, notwithstanding their potential efficacy, it remains to be shown whether environmental or economic considerations would allow or prevent their use under field conditions. For example, antibiotics (oxytetracycline,[4] streptomycin,[5] tetracycline) have been used experimentally to treat fire blight and, in the field, to treat citrus greening in individual trees (Stockwell and Duffy, 2012; Divya et al., 2013; Lokesh et al., 2014; Zhang et al., 2014) and on grapevine plants affected by Pierce's disease or on other *X. fastidiosa*-infected hosts (Hopkins and Mortensen, 1971; Hopkins, 1979; Kostka et al., 1985; Chang, 1987; Leu and Su, 1993; Kirkpatrick et al., 2003; Hartman et al., 2010). Reduction or temporal suppression in Pierce's disease symptoms were reported on treated grapevines as they remained symptomless for a number of years, but none of the treatments did eliminate *X. fastidiosa* infections. Notwithstanding, antibiotics are not used outside experimental set-ups to treat *X. fastidiosa* and they are not approved for use as bactericides in plant and crop protection in the EU. This is due to the technical limitations connected to their application but, more so, because of the risk of antimicrobial resistance development and thus the high concerns at the EU level regarding the use of antibiotics in agriculture per se (ECDC/EFSA/EMA, 2015).

Other types of treatments, for which the most valid information comes from experience gained from long-term experiments to fight the Pierce's disease of grapevine in the USA, are the application of micronutrients, Cu, zinc (Zn), manganese (Mn) at various concentrations and chemical forms, with additives to enhance their availability and uptake by the plant. A broad variety of commercial products based on the toxic activity of specific mineral elements is available and in use for disease control as well as plant fertilisers. Many of those compounds enhance plant resistance (Fones et al., 2010) and have fungicidal and bactericidal effects (Kirkpatrick et al., 2003; Sayler and Kirkpatrick, 2003). Copper ions (Cu^{++}) and zinc ions (Zn^{++}), for instance, are beneficial to living organisms because they serve as stabilisers and catalysts in many enzymes; however, at high concentrations, they can become toxic. In agriculture, Cu has been used for disease control for many decades. In Cu formulations, it is the toxic effect of Cu^{++} ions which block enzyme reactions leading to inactivation and denaturation of fungal spores and bacteria when applied above the homeostatic range for microbes (Borkow and Gabbay, 2005; Mackie et al., 2012). In particular, for the control of fungal pathogens, Cu preparations, copper sulfate, etc. are widely used, especially for pathogen control in fruit orchards, vineyards and hop gardens. For field control of plant pathogenic bacteria, Cu^{++} is commonly sprayed to aerial parts of the plant to control diseases like citrus canker caused by *Xanthomonas citri* subsp. *citri* (Behlau et al., 2010, 2011; Scapin et al., 2015), bacterial canker of stone fruits caused by *Pseudomonas syringae* pv. *syringae* (Sayler and Kirkpatrick, 2003) and others. In contrast to the xylem-invading *X. fastidiosa*, all of these bacteria controlled by Cu sprays live on leaf surfaces and aerial parts of the plant, hence foliar

[4] Prohibited in EU as plant protection product by Commission Regulation (EC) No 2076/2002 of 20 November 2002 extending the time period referred to in Article 8(2) of Council Directive 91/414/EEC and concerning the non-inclusion of certain active substances in Annex I to that Directive and the withdrawal of authorisations for plant protection products containing these substances. OJ L 319, 23.11.2002, p. 3–11.

[5] Prohibited in EU as plant protection product by Commission Decision of 30 January 2004 concerning the non-inclusion of certain active substances in Annex I to Council Directive 91/414/EEC and the withdrawal of authorisations for plant protection products containing these substances. 2004/129/EC. OJ L 37, 10.2.2004, p. 27–31.

applications of Cu in concentrations toxic to bacteria can reach the pathogens before they enter the plant. *X. fastidiosa* is distributed along the xylem vessels, where it forms biofilms that protect bacteria from environmental stresses. Therefore, to be effective against *X. fastidiosa,* a compound should make direct contact with the bacterium and this is more likely to occur with injected formulations than with spraying or soil drenching.

Zn-based formulations are also used to control some plant pathogens. Zinc sulfate, for instance, has inhibitory activity against *Xanthomonas campestris* pv. *vesicatora*, the causal agent of bacterial leaf spot on peppers and tomatoes, when applied *in vitro* (Adaskaveg and Hine, 1985). For *X. fastidiosa* the effect of Zn^{++} has been studied intensively. Studies on the effect of Zn^{++} *in vitro* showed concentration-dependent effects in varying directions, as the presence of Zn in xylem vessels has been positively (Andersen et al., 2007) or negatively (Malavolta et al., 2005; Brady et al., 2010) correlated with the growth of *X. fastidiosa*. At high concentrations, Zn^{++} ions have deleterious effects on bacteria (Cobine et al., 2013; Navarrete and De La Fuente, 2014). Application of Zn drenches to grapevines was shown to decrease *X. fastidiosa* populations, but with inconsistent effects on disease development (Brady et al., 2010). Grapevines with Pierce's disease were submitted to injections of Zn formulations, soil drenching and foliar sprays by Kirkpatrick et al. (2003) but long-term positive effects, such as reduction in bacterial populations or alleviation of disease symptoms, have not been reported. The most effective treatments to protect grapevines against *X. fastidiosa* were found to be injections of streptomycin or zinc sulfate into the stems of vines. By direct delivery into the xylem, the active compound was brought in contact with the pathogen. The treatments prevented symptom expression over 3 years but disease suppression did not eliminate the causal agent, *X. fastidiosa*, and in the following years disease symptoms reappeared (Kirkpatrick et al., 2003).

In the experimental treatments of olive trees currently conducted to mitigate the CoDiRO disease in southern Italy, commercial products, such as fertilisers, fungicides and bioactive compounds, alone or in combination, also together with agronomic practices are used. In particular, the products and formulations used by the two research groups are also used in organic farming:

- The work conducted by the group of Carlucci and Lops (University of Foggia), that has been recently described in a non-peer-reviewed journal (Carlucci et al., 2016) and was the object of a hearing (Section 2.2), involves the application of commercial products, in formulations and concentrations recommended and applied either as single treatments or in combinations.
- The experiments of the Scortichini group (Scortichini, personal communication) tested the treatment of olive trees in open fields with a commercial product containing Zn (4%), Cu (2%) and citric acid.

The experiments conducted in southern Italy by the Carlucci and Lops group from the University of Foggia reported positive effects of most treatments on the vigour of olive trees affected by the disease. Although in the experimental set-up only a limited number of plants was subjected to particular treatments, the difference between untreated and treated plants was evident in their appearance (i.e. more leaves per branch with respect to the untreated controls). After only 5 months of treatment, trees with greening branches and lush green leaves were shown in their report, although all the treated trees tested positive for *X. fastidiosa*. In addition, sizable olive harvests from trees subjected to the treatments were reported. Similar results were also reported from experimental treatments conducted by the Scortichini research group (although information could only be retrieved from grey literature and video clips from the internet).[6]

In the experiments conducted in southern Italy by the Carlucci and Lops group, the treatments were applied after rigorous pruning of diseased trees to remove dead branches. Trees treated with several bioactive compounds produced vigorous new season growth of branches with leaves that lacked disease symptoms (Carlucci et al., 2016). As shown in pruning experiments with Pierce's disease, affected grapevines subjected to severe pruning regenerated quickly from the deep impact of the intervention and remained free of symptoms for a number of years before disease symptoms reappeared (Kirkpatrick, 2008). In the experiment on Apulian olive trees, soil management was carried out in combination with other measures with the objective of improving soil structure and controlling weed/vegetation in order to reduce insect vector populations. While treatment effects

[6] Some sources: http://www.trnews.it/2015/05/08/xylella-ecco-gli-effetti-della-cura-israeliana-sugli-ulivi/123114229/
http://www.csvsalento.it/notizie/index.php?id=5567
http://xylellareport.it/2015/10/17/lo-strano-caso-della-lebbra-dellulivo-che-divenne-co-di-ro-e-poi-xylella/
https://www.youtube.com/watch?v=gDxGDgw8bzw

were recorded during the experiments (Carlucci et al., 2016), it was stressed during the hearing that the combined effects of all interventions (i.e. crop management practices, such as pruning, soil cultivation/aeration, weeding, irrigation, fertilisation, application of micronutrients, plant growth activator substances) contribute to improving the health status of the plants. While this is a well-acknowledged fact, the positive results from this preliminary study cannot hide the fact that definite controls for each treatment are missing and thus effects of individual measures are difficult to assess.

The experimental treatment started by the group of Scortichini in 2015, with a commercial product containing Zn (4%), Cu (2%) and citric acid is considered by the researcher as promising as all the treated plants, which were all infected by the bacterium from the beginning of the experiment, survived both the last hot summer and the current winter (Scortichini, personal communication).

Both research groups stated that those treatments are tested with the scope to suppress the disease symptoms and not to eliminate the pathogen in the olive trees infected by *X. fastidiosa* in Apulia.

From the knowledge gained during the hearing, together with available information from the management of bacterial diseases (citrus, grapevine and other tree crops), the Panel can draw the following considerations on the treatment of olive trees currently tested in Apulia:

1) The mode of action – directly on the pathogen, or indirectly on the plant – of the compounds listed above and used to control plant diseases, in particular those caused by bacteria, is not known. However, based on extensive research on the management of Pierce's disease in grapevine, it can be inferred that such treatments neither can protect nor cure the plant from *X. fastidiosa* infections. Furthermore, those treatments can have impacts on other pathogens in the plant, and thus positively affect plant responses by reducing the severity of disease symptoms.
2) While for a number of compounds an *in vitro* activity on the growth of bacterial cultures is demonstrated, further proof of their efficiency under natural conditions (*in vivo*) is lacking in most cases. The most promising therapeutic treatments with direct bactericidal effects would be expected from application of antibiotics and from the use of Zn^{++} preparations. However, the necessity to bring these antibacterial treatments into direct contact with the bacteria would require repeated injections into the xylem of individual plants. There are no antibiotics approved in the EU as bactericides in crop or plant protection. Due to the increased risk of resistance development to antibacterial compounds in bacteria, the use of antibiotics, in particular in agriculture, is expected to be of high concern in the EU.[7]
3) The Panel acknowledges that the positive effects from good crop management practices, including pruning and soil cultivation, applied in combination with the treatments under evaluation, may have contributed to the reported improvement of the healthy appearance of plants infected with *X. fastidiosa*. The overall effects from such crop management practices are likely to promote vigorous growth of the plant, by increasing water and nutrient availability and utilisation, and plant metabolism.
4) Despite the reported more vigorous growth and healthy appearance, the treated olive trees are still infected by *X. fastidiosa*. While it can be considered that pruning or direct effects from treatments can result in a reduction – not in the elimination – of bacterial load, this reduction could be temporary and could also be compensated by invasion of recovering bacterial population into newly formed branches and leaves. Plants in such a state may still function as sources of infection for insects to acquire the pathogen, and insect vectors might even be more attracted to plants with fresh and lush growth (EFSA PLH Panel, 2016).
5) The Panel acknowledges the relevance of testing the treatments at short intervals (e.g. every 8 weeks) and in combination with intense crop management practices during this experimental phase. However, while the strategy could require a later adjustment also to reduce the frequency of the treatments, only experiments over several seasons can prove the effectiveness and applicability of such interventions.
6) While many Cu/Zn products are in use as fertilisers and for disease control in conventional and organic farming, there can be negative long-term effects from their use, such as the

[7] See Commission's Action Plan (2011–2016) against the rising threats from antimicrobial resistance. Available online: http://ec.europa.eu/dgs/health_food-safety/amr/action_eu/index_en.htm

accumulation of Cu in soils and problems from Cu toxicity.[8] As shown for other bacteria, the intensive use of Cu and Zn can also lead to an increased resistance of the bacteria to these elements (Behlau et al., 2010, 2012, 2013). Therefore, it has to be considered that positive effects on crop performance, also on olive trees, need to be sustained over many years and not counterbalanced by the acknowledged adverse effects to the environment.

Based on the results collected till now, the Panel considers it premature to draw conclusions on long-term benefits of such treatments. Notwithstanding the preliminary nature of the experimental results and the risk of limited statistical robustness when small numbers of plants are treated, the Panel acknowledges the potentially positive effects of such treatments in prolonging the productive phase of olive trees. While an experimental confirmation is still pending on data from long-term experiments over several seasons, such treatments have potential relevance for the management of olive orchards in the area currently identified as the containment zone, where eradication is no longer possible.

4. Conclusions

The PLH Panel was requested to contact the researchers Marco Scortichini of CREA (Caserta, Italy), and Francesco Lops and Antonia Carlucci from the University of Foggia (Italy), in order to assess the outcomes of their experiments aiming at treating CoDiRO diseased plants. The Panel was also requested to provide an update about any recent scientifically validated treatment to cure *X. fastidiosa* diseased plants.

From the collected feedbacks and the evidence reviewed, the Panel could draw the following conclusions on the treatment of olive trees currently tested in Apulia:

1) The treatments under consideration may reduce the symptoms of *X. fastidiosa* in olive trees, but do not eliminate the pathogen *X. fastidiosa* from the treated infected plants.
2) There is currently no treatment known to cure plants, by eliminating the pathogen, from xylem-limited bacterial infections, under field conditions.
3) The positive effects from good crop management practices applied in combination with the treatments under evaluation may have contributed to the reported vigorous growth and improved appearance of the diseased plants.
4) Despite the reported more vigorous growth and healthy appearance, the treated olive trees are still systemically infected by *X. fastidiosa*, and could be acting as sources of inoculum for insect vectors which might be even more attracted to plants showing good vegetative conditions.
5) Treatments tested at short intervals (e.g. every 8 weeks) and in combination with intense crop management practices during this experimental phase, may require further adjustments in the frequency of treatments. In addition, only treatments conducted over several seasons can provide information on the long-term effectiveness of these approaches.
6) While many Cu/Zn products are in use for fertilisation and disease control in conventional and organic farming, negative long-term effects from their use are the accumulation of Cu/Zn in the soil, problems from Cu/Zn toxicity and the possible decrease in bacterial susceptibility. Therefore, long-term studies are required to confirm that positive effects on crop performance can be sustained for many years and not counterbalanced by adverse effects to the environment.

Based on the results collected till now, the Panel considers it premature to draw conclusions on long-term usefulness of such treatments. Notwithstanding the preliminary nature of the experimental results and their limited statistical robustness, due to the small number of plants treated, the Panel acknowledges the potentially positive effects of such treatments in prolonging the productive phase of olive trees. While an experimental validation is still pending data from long-term experiments over several seasons, such treatments have a potential relevance for the management of olive orchards in the area currently identified as the containment zone and supports the need for further studies in this regard.

[8] See DG SANTE, 2015. Review report for the active substance Copper compounds. Copper compounds SANCO/150/08 final 10 October 2014. 9 pp. Available online: http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=activesubstance.detail&language=FR&selectedID=1158

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Abbreviations

CoDiRO	Complesso del Disseccamento Rapido dell'Olivo
HLB	huanglongbing
ISPM	International Standards for Phytosanitary Measures
OQDS	olive quick decline syndrome
PLH Panel or the Panel	EFSA Panel on Plant Health