STATEMENT

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# Susceptibility of *Citrus* spp., *Quercus ilex* and *Vitis* spp. to *Xylella fastidiosa* strain CoDiRO

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## Abstract

Following a request from the European Commission, the EFSA Plant Health Panel analysed a dossier submitted by the Italian Authorities to reach a conclusion on the status of *Vitis* spp., *Citrus* spp. and *Quercus ilex* as hosts for *Xylella fastidiosa* strain CoDiRO. The Panel acknowledges the difficulty to provide compelling evidence for non-susceptibility of a particular plant species. In the case of *Vitis* spp., the Panel considers that convergent lines of evidence provide sufficient demonstration that at least the tested varieties (Cabernet Sauvignon, Negroamaro and Primitivo) do not support a systemic infection by the CoDiRO strain. The extension of this conclusion to other grapevine varieties and to *Vitis* species other than *Vitis vinifera* is associated with significant uncertainties. The Panel therefore considers it premature to conclude that all *Vitis* species are unable to support a CoDiRO systemic infection. In addition, although the local accumulation detected in the mechanical inoculation experiments may represent an artefact, the Panel considers it premature to conclude that the tested grapevine varieties are not able to support local multiplication of the CoDiRO strain. Further extension of this conclusion to other grapevine varieties and to non-*vinifera* species is also premature. For *Citrus* spp., the data available provide coherent and converging lines of evidence suggesting that sweet orange may be a non-systemic host of strain CoDiRO. However, given the limited scope of the data available on other species, the Panel considers it premature to reach a general conclusion for all *Citrus* species. The potential epidemiological consequences of non-systemic infections remain to be fully evaluated. In the case of *Quercus ilex*, the Panel concludes that the limited data available provides some evidence suggesting that it may not be a systemic host of the CoDiRO strain, but that it would be premature to consider this tentative conclusion as firmly established.



**Keywords:** citrus, grapevine, holly oak, host plant, inoculation

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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 Articles 29 and 31 of Regulation (EC) No 178/2002[2], scientific advice and technical assistance in the field of plant health as regards the regulated harmful organism *Xylella fastidiosa*.

Pursuant to Article 31 of Regulation (EC) No 178/2002, the European Food Safety Authority (EFSA) is requested to further specify and update the host plants database of *X. fastidiosa* currently available,[3] taking into account the different *X. fastidiosa* subspecies and strains (with particular reference to the European isolates), with inclusion of information on non-susceptible host plants and varieties and negative results of diagnostic tests where available. EFSA is requested to maintain and update this database periodically and to make new releases available on EFSA website, together with a report. Such report should specify the list of plants confirmed to be infected by at least two detection methods in field conditions or via vector transmission under experimental conditions and be published at least annually, or according to needs following agreements between our Services. Such request is for the period 2016–2020 and the needs for its continuation will be re-assessed by the end of this period.

Additionally, following the recent 'EFSA pilot project on *Xylella fastidiosa* to reduce risk assessment uncertainties' published on 29 March 2016, the Italian Authorities have requested delisting of *Vitis*, *Citrus* and *Quercus ilex* from Annex I of Commission Implementing Decision (EU) 789/2015[4] as considered to be not suitable hosts for the colonisation and multiplication of *X. fastidiosa* subsp. *pauca*, strain CoDiRO, present in the Apulia region. Consequently, in order for the Commission and the Member States to further analyse such request, and make a decision in the relevant Standing Committee, EFSA is invited to provide scientific advice pursuant to Article 29 of Regulation (EC) No 178/2002 on current scientific knowledge to support a decision on possible delisting of the indicated plant species for *X. fastidiosa* subsp. *pauca* strain CoDiRO. When preparing this scientific advice, EFSA is invited to take into account, where needed, the EFSA Scientific Opinion of 20 November 2015 on *Vitis* sp. response to *X. fastidiosa* strain CoDiRO,[5] and be in direct contact with the Italian Authorities in case further scientific or technical information are needed. This advice should not only focus on *Vitis vinifera* but also on other relevant *Vitis* species.

Furthermore, the Costa Rica National Plant Protection Organisation has recently requested delisting of *Phoenix roebelenii* from Annex I of Commission Implementing Decision (EU) 789/2015 as not found to be infected by *X. fastidiosa* in their territory. Consequently, in order for the Commission to further analyse such a request, EFSA is invited to review the technical and scientific information submitted by Costa Rica (annexed to the mandate) and provided a scientific advice pursuant to Articles 29 of Regulation (EC) No 178/2002 on susceptibility of *P. roebelenii* to *X. fastidiosa* based on current knowledge.

EFSA is therefore requested to prepare the first scientific report on the updated *X. fastidiosa* host plants database at latest by April 2017 with regular updates as soon as available, while delivering the above-mentioned scientific advice on *Vitis* spp., *Citrus* spp. and *Q. ilex* by 15 September 2016 and the scientific advice on *P. roebelenii* by 30 October 2016 at the latest.

## 1.2. Interpretation of the Terms of Reference

In this opinion, the EFSA Panel on Plant Health (PLH; hereafter 'the Panel') replies to the Commission request concerning the susceptibility of *Citrus* spp., *Q. ilex* and *Vitis* spp. to *X. fastidiosa* subsp. *pauca* strain CoDiRO.

[1] Submitted by European Commission, ref. SANTE/G1/PDR/svi (2016) 3575400.
[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] EFSA (European Food Safety Authority), 2016. Scientific report on the update of a database of host plants of *Xylella fastidiosa*: 20 November 2015. EFSA Journal 2016;14(2):4378, 40 pp. doi:10.2903/j.efsa.2016.4378
[4] 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.
[5] EFSA PLH Panel (EFSA Panel on Plant Health), 2015. Scientific opinion on *Vitis* sp. response to *Xylella fastidiosa* strain CoDiRO. EFSA Journal 2015;13(11):4314, 20 pp. doi:10.2903/j.efsa.2015.4314

The *X. fastidiosa* bacterial populations that are considered in the present opinion belong to subsp. *pauca* and correspond to isolates classified by multilocus sequence typing (MLST) as a sequence type ST53. They are present in Apulia and found infecting olive and other plants. All bacterial isolates causing the CoDiRO disease in olives in Apulia belong to ST53 (EFSA PLH Panel, 2016) and, as clarified previously by the Panel, although the denomination 'strain CoDiRO' is not formally correct, it is now in common use in the scientific literature on the Italian *X. fastidiosa* outbreak (and in the Terms of Reference (ToR) of the present opinion). Therefore, for consistency and simplicity reasons, the term 'CoDiRO strain' will be used throughout the present opinion when referring to Apulian ST53 *X. fastidiosa* subsp. *pauca* isolates.

In the present opinion, the Panel distinguishes between systemic infection and non-systemic infection of host plants. In the first case, *X. fastidiosa* is able to multiply and systemically spread within the inoculated host. This compatible interaction is referred to in the present opinion as 'systemic infection' and the corresponding host as 'systemic host'. In the second case, *X. fastidiosa* is able to multiply at or near the inoculation point but is unable to systemically invade more distant parts of the inoculated plant. Although the underlying mechanisms are not understood, this situation is well documented for some hosts/bacteria combinations and will be referred to as 'non-systemic infection' or 'localised infection' and the corresponding host as 'non-systemic host'. This second situation is equivalent to the term 'local infection' used in the previous opinion on *Vitis* spp. response to strain CoDiRO (EFSA PLH Panel, 2015).

### 1.3. Additional information

In order to review the host status of *Vitis* spp., *Citrus* spp. and *Q. ilex* for the CoDiRO strain, EFSA contacted the Italian Authorities to obtain scientific and technical information. The requested information concerned all available data not included in the report of the EFSA-funded pilot project on host plants of the CoDiRO strain (Saponari et al., 2016). Upon reviewing this new data, further clarifications were requested by EFSA and obtained from Dr Maria Saponari. All the new information received was considered in the light of the previous EFSA opinion on the CoDiRO host status of grapevine (EFSA PLH Panel, 2015).

## 2. Data and methodologies

### 2.1. Data

Survey data and the technical dossier prepared by the Italian Authorities were provided to the Panel after request, as follows:

1) Email of the 11th August 2016 with the following attachments
    a) '*Xylella fastidiosa* strain CoDiRO: additional information on non-susceptible hosts (*Vitis*, *Citrus* spp. and *Quercus ilex*)' – August, 2016; prepared by Istituto per la Protezione Sostenibile delle Piante, CNR Bari, Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti dell'Università di Bari, Centro di Ricerca, Sperimentazione e Formazione in Agricoltura 'Basile Caramia'.
    b) Annex I: manuscript in press 'Transmission of *Xylella fastidiosa* to grapevine by the meadow spittlebug' by Cornara D, Sicard A, Zeilinger A.R., Porcelli F, Purcell AH and Almeida RPP (hereafter Cornara et al., publication A).
    c) Annex II: accepted manuscript 'Transmission of *Xylella fastidiosa* by naturally infected *Philaenus spumarius* (Hemiptera, Aphrophoridae) to different host plants' by Cornara D, Cavalieri V, Dongiovanni C, Altamura G, Palmisano F, Bosco D, Porcelli F, Almeida RPP and Saponari M (hereafter Cornara et al., publication B).
    d) Annex III: table with the sampled *Vitis* spp. plants in winter 2015–2016.
    e) Annexes IV and V: descriptive fiches of the surveyed vineyards.

Targeted extensive literature searches were carried out on the research platform ISI Web of Science and on the EFSA *X. fastidiosa* host plant database (EFSA, 2016). 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).

For a thorough evaluation of *Vitis* spp., *Citrus* spp. and *Q. ilex* as possible hosts of the strain CoDiRO, the Panel considered all the data and information provided by the Italian Authorities and relevant literature to assess these novel host–pathogen interactions. The assessment is divided into three main sections, specific for each genus (*Vitis* spp. and *Citrus* spp.) and species (*Q. ilex*). Under each section the Panel provided (i) all relevant background information used in support to its assessment, (ii) the analysis of the data provided by the Italian Authorities and (iii) the specific conclusions.

# 3. Assessment

## 3.1. Assessment of *Vitis* spp. as a host of *Xylella fastidiosa* strain CoDiRO

### 3.1.1. Background on CoDiRO–*Vitis* spp. interactions as previously analysed by EFSA Plant Health Panel (EFSA PLH Panel, 2015)

The genus *Vitis* has been included in Annex I of the Commission Implementing Decision (EU) 2015/789 as a 'specified plant' known to be susceptible to the European and non-European isolates of *X. fastidiosa*. However, *Vitis* species have never been shown to be infected under natural conditions by the CoDiRO strain responsible for the Apulian outbreak.

To investigate the host range, incidence and spread of this newly emerging pathogen, studies on the susceptibility of some important perennial Mediterranean species to CoDiRO isolates were conducted in the framework of a pilot project initiated in 2013 by three Italian research institutes (CNR of Bari in collaboration with University Aldo Moro of Bari and research centre of Locorotondo) and funded by EFSA. Grapes, citrus, peach, plum as well as *Q. ilex* were included in the trials and the final report of this project was published in March 2016 (Saponari et al., 2016). The trials included inoculation experiments with selected grapevine cultivars performed either with isolated bacteria or with field-collected *P. spumarius* insects.

In September 2015, EFSA received a dossier from the Italian Authorities with preliminary results from the pilot project and additional data from field surveys of vineyards located in the epidemic zone and with presumably high inoculum pressure from surrounding CoDiRO infected olive trees. In the light of a request by the Italian Authorities for the delisting of *Vitis* from Annex I of the Commission Implementing Decision (EU) 2015/789, EFSA was requested to assess the data and to provide an opinion on the host status of *Vitis* spp. for the CoDiRO strain. The EFSA PLH Panel (2015) identified concerns with the available data and uncertainties that did not allow a definitive statement excluding *Vitis* spp. as a susceptible host for the CoDiRO strain.

The uncertainties expressed were related to technical aspects of the artificial inoculation method used, in particular the efficiency of the method, the limited number of test plants and the single cultivar used in the experiments (EFSA PLH Panel, 2015). An artificial infection of grapevine by needle inoculation of bacterial cultures resulted in detection by quantitative polymerase chain reaction (qPCR) of bacterial DNA at the inoculation site even 12 months after inoculation. This raised concerns that localised infection foci might establish from which bacterial cells could potentially be transmitted (EFSA PLH Panel, 2015).

Uncertainties were also associated with insect transmission experiments to infect grapevine using *P. spumarius* vectors. The available data did not demonstrate whether *P. spumarius* would visit grapevine for sufficiently long periods and would actively probe and feed on the plants to guarantee bacterial acquisition and transmission. Thus, the Panel questioned whether *P. spumarius* would be a competent vector to transmit the CoDiRO strain to grapevine (EFSA PLH Panel, 2015). Compounded with the observed low efficiency of transmission to olive plants in control experiments, the uncertainties on *P. spumarius* interactions with grapevine made it very difficult to conclude whether the negative results obtained truly reflected a non-host status of grapevine.

The results of surveys for the CoDiRO strain in *Vitis* spp. plants in vineyards under natural infection pressure from the surrounding CoDiRO-infected olive trees were also associated with uncertainties. While symptoms on grapevine were never observed and bacteria detection by enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) was negative, concerns were raised on the sensitivity of the detection method used with the possibility that low levels of infections would not be detected. The major uncertainty affecting the survey results, however, came from the fact that these results were not supported by information on the presence of infective *P. spumarius* in the vineyards (EFSA PLH Panel, 2015). In the absence of such information, it was not possible to conclude whether failure to detect *X. fastidiosa* resulted from a non-host status of grapevine or from limited contacts with insects vectoring the CoDiRO strain.

Because of the uncertainties identified, and in consideration of the difficulty to provide compelling evidence that a compatible host–pathogen interaction does not exist, the Panel considered premature, in its previous opinion, to conclude that grapevine was not a host of the CoDiRO strain (EFSA PLH Panel, 2015).

Investigations on the host range of the CoDiRO strain have been pursued. The novel data not included in the already published report of the pilot project (Saponari et al., 2016) has been integrated in a new dossier prepared by the Italian Authorities and titled '*Xylella fastidiosa* strain CoDiRO: additional information on non-susceptible hosts (*Vitis*, *Citrus* spp. and *Quercus ilex*)' (Section 2.1). This dossier provides an update on the current knowledge on the susceptibility of grapevine to the CoDiRO strain and is analysed by the Panel in the following sections.

### 3.1.2. Assessment of new data provided by the Italian Authorities

#### 3.1.2.1. *Philaenus spumarius* as an efficient vector of *Xylella fastidiosa* on *Vitis* spp.

Besides an initial demonstration proving transmission of *X. fastidiosa* by *P. spumarius* (Severin, 1950) and reports of *P. spumarius* as a vector for *X. fastidiosa* on almond (Purcell, 1980) and pecan (Sanderlin and Melanson, 2010), there is only limited data available on transmission of *X. fastidiosa* by this vector species. More recently, Saponari et al. (2014) and Cornara et al. (2016), using field-collected insects, confirmed that *P. spumarius* acts as a vector of the CoDiRO strain.

The paper by Cornara et al. (publication A), provides additional information on the transmission of *X. fastidiosa* subsp. *fastidiosa* STL strain (Almeida and Purcell, 2003) by *P. spumarius*.

The authors confirm that the transmission parameters determined for *P. spumarius* are generally similar to those for leafhopper vectors. A positive correlation between the bacterial estimates by qPCR in the insect heads and the rate of plant infection was found, but transmission was already possible with only few bacterial cells or negative PCR detection, confirming the results of Purcell and Finlay (1979) with other vectors. The authors thus underline that, up to now, *there is still no reliable protocol for monitoring for the presence of* X. fastidiosa *in vectors and connecting those results to pathogen transmission risk*.

Similar to previous work (Rashed et al., 2011) qPCR was used according to Francis et al. (2006) to estimate *X. fastidiosa* populations in insects. The measured bacterial populations in *P. spumarius* heads ranged from 10 to $10^3$ cells per insect head, a concentration considerably lower than the ca. $10^5$ cells reported for *Graphocephala atropunctata*, a well-studied vector species (Killiny and Almeida, 2009). Nevertheless, efficient transmission to grapevine was obtained with *P. spumarius* (Cornara et al., 2016).

Taken together, the results of Cornara et al. (publication A) provide convincing evidence that *P. spumarius* is able to transmit *X. fastidiosa* subsp. *fastidiosa* to grapevine, confirming experiments by Severin (1950) who determined a transmission rate of 0–66% between grapevines.

It was already known that *P. spumarius* is a vector of the CoDiRO strain and efficiently transmits it to olives (Saponari et al., 2016). The demonstration that *P. spumarius* is an efficient vector for *X. fastidiosa* subsp. *fastidiosa* to *Vitis vinifera* cv. Cabernet Sauvignon provides a good indication that the insect also interacts efficiently with grapevine. The Panel therefore concludes that it is very likely that *P. spumarius* would be able to similarly inoculate the CoDiRO strain to other grapevine cultivars.

#### 3.1.2.2. New CoDiRO insect transmission experiments to grapevine

In Cornara et al. (publication B), transmission experiments using field-collected *P. spumarius* were performed with olive, oleander, sweet orange, grapevine cv. Cabernet Sauvignon, and the *Prunus persica* × *Prunus amygdalus* stone fruit rootstock GF677. Depending on the experiment, the proportion of *P. spumarius* carrying detectable levels of the CoDiRO strain ranged from 25% to 71%. The number of *X. fastidiosa* cells (colony-forming unit (CFU) equivalents) detected in the vector heads,

using the qPCR protocol from Francis et al. (2006) was estimated between 35 and 400 CFU which was well within the range reported for *X. fastidiosa* subsp. *fastidiosa* by Cornara et al. (publication A).

*X. fastidiosa* was detected in all plant species included in the transmission experiments with the exception of grapevine. None of the 75 Cabernet Sauvignon plantlets, which were subjected to a total of 375 field-collected *P. spumarius* over a period of 2 years, became infected. The number of insects used in these experiments is in line with those commonly used in other transmission studies (Redak et al., 2004), although in the 2015 experiments, only 40% of the insects (102 of 250 specimen) tested positive for *X. fastidiosa*.

The results obtained show that the experimental conditions allowed an efficient transmission of the CoDiRO strain to olive (41.3%, 150 test plants) and oleander (74%, 50 test plants) while none of the 75 Cabernet Sauvignon grapevine plants tested positive at the time intervals 3, 6 and 14 months post-inoculation in 2014 (total of 25 plants inoculated in 2014). The corresponding testing time intervals were 3 and 6 months post-inoculation for the 50 plants inoculated in 2015. The transmission of CoDiRO to sweet orange resulted in non-systemic infections observed in 13.3% of the 75 test plants (see Section 3.2.2) while in the stone fruit rootstock non-systemic infection was found in one of the 65 test plants.

The conclusion reached by Cornara et al. (publication A) that *P. spumarius* is able to efficiently interact with grapevine has essentially no associated uncertainties. Extending this conclusion to other *P. spumarius* populations (e.g. European populations), to other *X. fastidiosa* subspecies, to other experimental or natural conditions, or to other grapevine genotypes or other *Vitis* species is perceived by the Panel as introducing some level of uncertainty:

- Concerning the first two points (other *P. spumarius* populations and *X. fastidiosa* subspecies), given the known ability of the European *P. spumarius* populations to transmit the CoDiRO strain to olive and oleander, the introduced uncertainties appear rather limited.
- Concerning the experimental conditions, available data shows that transmission efficiency is affected by a range of parameters including, for example, the nature of the acquisition host, the length of the acquisition and inoculation periods or the bacterial concentration in the acquisition host. However, the inclusion of suitable positive controls in transmission experiments would limit the uncertainties associated with changes in any of these parameters.
- Lastly, concerning the extension of the results to *Vitis* species and grapevine cultivars, this is perceived as the aspect which introduces the most important uncertainties. This is because the *Vitis* genus encompasses a range of species and cultivars which are known to show significant variability in their sensitivity to *X. fastidiosa* (Fry and Milholland, 1990; Krivanek and Walker, 2005; Krivanek et al., 2005; Fritschi et al., 2008; Rashed et al., 2013). As a consequence, it is very difficult to evaluate the uncertainty associated with the generalisation to the whole *Vitis* genus of a result obtained with a single genotype. These uncertainties likely cannot be resolved in the absence of additional experimental data.

Cornara et al. (publication B) strongly suggest that Cabernet Sauvignon is not a systemic host for the CoDiRO strain. The uncertainties associated with this conclusion are limited because the experiments involved a large number of plants and showed high infection rates in susceptible control species. As explained above, the generalisation of this conclusion to other grapevine cultivars or *Vitis* species does, however, carry significant uncertainty.

These experimental results also suggest that grapevine might not even support local accumulation of bacterial populations of the CoDiRO strain. However, such a conclusion would appear to contradict the results of the mechanical inoculation experiments which showed prolonged PCR detectability of *X. fastidiosa* at or near the inoculation point (see below). The uncertainties associated with the results obtained by Cornara et al. (publication B) are mostly linked to the experimental set up. In particular, one could wonder whether higher inoculation pressure (use of a larger number of insects or of insects having acquired *X. fastidiosa* in the glasshouse on experimental hosts and therefore likely to carry a higher bacterial titre) would have provided the same results.

#### 3.1.2.3. Mechanical inoculation experiments

Experiments to infect plants by mechanical inoculation of isolated bacteria mimic introduction and translocation of bacteria by insect vectors competent to deliver the pathogen to the plant (Wistrom and Purcell, 2005; Backus et al., 2015). Experimental conditions are artificial and, particularly when unknown interactions are to be studied, each variable of the procedure (e.g. cell density, pricking sites and intensity) have to be optimised to maximise infection efficiency. The experimental conditions used

to infect plants are described in great detail in the pilot project on *X. fastidiosa* host plants (Saponari et al., 2016), which provides information on all specific requirements and conditions for mechanical inoculation of various host plants with high efficiency. The methods described are based on knowledge and experiences shared by the most proficient experts in the field.

Following a pilot experiment with three grapevine plants performed in July 2014, the two initial inoculation experiments in December 2014 involved 10 cuttings per experiment of the grapevine cv. Cabernet Sauvignon, which is a systemic host of *X. fastidiosa* subsp. *fastidiosa* (Almeida and Purcell, 2003). A third experiment was initiated in June 2015 involving the cvs. Negroamaro and Primitivo (10 plants for each cultivar). Therefore, the last observation time for Cabernet Sauvignon was 19 months after inoculation (from December 2014 to July 2016) and for the two other cultivars 13 months. Inoculated plants were maintained under glasshouse conditions at 24–28°C for constant growth and to avoid the dormancy period during fall/winter.

In its previous scientific opinion on *Vitis* spp. response to *X. fastidiosa* strain CoDiRO (EFSA PLH Panel, 2015), the Panel raised concerns on the applied methodology regarding: (i) the efficiency of artificial inoculation tests with plants that might either be less susceptible to a particular subspecies/strain of *X. fastidiosa* or only show non-systemic infections; (ii) the low number of plants used might not have allowed detection of a low frequency of infection; (iii) the positive qPCR detection obtained for several plants near the inoculation sites even 12 months after inoculation but not in the stems and petioles above the inoculation point. In addition, despite the failed attempts to isolate and culture bacterial cells from those inoculation foci, the concern was expressed by the Panel that the DNA detected could correspond to viable but non-culturable cells and that grapevine might serve as an asymptomatic reservoir for the CoDiRO strain from which transmission by xylem-feeding insects could occur (EFSA PLH Panel, 2015).

The updated information provided in the dossier submitted by the Italian Authorities for this mandate (Section 2.1) follows up on the *V. vinifera* cv. Cabernet Sauvignon plants inoculated in December 2014 and novel information on the results of the experiments with cvs. Negroamaro and Primitivo. The follow up of the seven remaining Cabernet Sauvignon plants (out of the 20 originally inoculated plants) failed to detect bacterial DNA in old stem segments above the inoculation points and in new sprouts recovered after the main stem had been trimmed in April 2016. In the experiments with cvs. Negroamaro and Primitivo, in 7 out of 10 inoculated plants from cv. Negroamaro and 2 out of 10 from cv. Primitivo, bacterial DNA was detected by qPCR at the inoculation point (table 2 of the technical dossier). However, similar to the experiments with Cabernet Sauvignon, there was no qPCR detection of *X. fastidiosa* DNA in dissected stems or petioles above the inoculation points. All test plants developed healthy appearing sprouts and leaves and attempts to isolate bacteria from the inoculated Negroamaro and Primitivo plants have so far yielded negative results.

The Panel acknowledges that experiments providing negative evidence inherently present many difficulties. In the particular case of grapevine, which is a known host of *X. fastidiosa* subsp. *fastidiosa*, a hitherto unknown host/pathogen interaction with a different subspecies (subsp. *pauca* strain CoDiRO) might warrant more precautionary considerations than with plants for which similar pathogens have never been recorded.

Taken together the results of the two consecutive experiments with Cabernet Sauvignon and of the experiment with Negroamaro and Primitivo showed a positive qPCR detection of *X. fastidiosa* DNA near the inoculation points for extended periods of time in some of the inoculated plants but there was no detection of bacteria in distant/non-inoculated tissues nor successful isolation of bacteria by culturing when attempted. These reproducible results support the conclusion that the tested varieties are not systemic hosts of the CoDiRO strain, with limited uncertainty.

The qPCR positive detection for extended time periods at the inoculation points is at odds with the results obtained following insect-mediated inoculation in which qPCR tests of inoculated plants were systematically negative (Cornara et al., publication B). It could suggest local accumulation of bacterial cells and possibly a non-systemic host status for the inoculated cultivars. The Panel had reflected on this situation in its previous opinion (EFSA PLH Panel, 2015), also discussing the possibility that a non-systemic host with only localised infection could still contribute to the spread of the disease to other plants, as was demonstrated in other cases (Hill and Purcell, 1995, 1997).

The Panel recognises that very different bacterial loads are delivered in the two types of inoculation and that the positive qPCR detection after mechanical inoculation might be an artefactual amplification of residual DNA from dead bacterial cells. Such an interpretation would be consistent with the failure of the attempts at bacterial isolation from the inoculated plants. However, bacterial isolation may sometimes be difficult (Purcell and Saunders, 1999) and there are uncertainties about whether higher inoculation pressure in the insect-mediated transmission experiments could have resulted in a positive

qPCR signal (see above). In its previous opinion, the Panel had already reflected on the need for additional experiments to try to resolve the uncertainties associated with these positive qPCR results. Despite limited efforts at culturing, no isolation results are provided in the new dossier since the additional data concern mostly the testing of new varieties or a qPCR follow up of the inoculated plants to exclude systemic infection. The Panel still sees a need for such experiments, involving, for example, PCR assays allowing a distinction between live and dead cells (Hu et al., 2013; RT-PCR detection of bacterial RNA; Navarrete and De La Fuente, 2014), more extensive isolation efforts or *in situ* cytology to try to observe bacterial cells.

As for the insect-mediated inoculation experiments, a significant element of uncertainty is added when trying to generalise the conclusions obtained in these mechanical inoculation experiments to all grapevine cultivars and *Vitis* species.

#### 3.1.2.4. Follow up on the survey

A survey is 'an official procedure conducted over a defined period of time to determine the characteristics of a pest population or to determine which species occur in an area' (ISPM 5 by FAO, 2016). Surveys for the presence of the CoDiRO strain in grapevine were conducted in vineyards in the epidemic zone in Apulia during November 2013, late summer 2014, January 2015 and September 2015, and the findings were reviewed in EFSA PLH Panel (2015). The surveys were conducted in vineyards close to infected olive groves. No visual signs of disease or positive samples were discovered during these surveys. However, the Panel noted that several uncertainties affected the results of these surveys including (i) the sensitivity of the sampling and diagnostic procedure, (ii) the timing of the surveys in relation to lack of symptom expression and seasonal pathogen population variation, and (iii) a lack of information on the density and behaviour of vectors for transmission (see EFSA PLH Panel, 2015 for further information). Despite high *P. spumarius* summer populations in Apulia and anecdotal reports of high *P. spumarius* populations in European vineyards (Carle and Moutous, 1966; Nicoli Aldini et al., 1998; Braccini and Pavan, 2000; Pavan, 2006; Filippin et al., 2009; Kunz et al., 2010; Cvrković et al., 2011; Trivellone et al., 2015), the lack of information on vector populations in the surveyed plots is particularly critical, because in its absence it is not possible to precisely evaluate the infection pressure. As a consequence, failure to detect the pathogen does not necessarily demonstrate a non-host status of the surveyed crop but might rather reflect an absence of confrontation between pathogen and host.

An additional survey was conducted between November 2015 and January 2016, sampling the same vineyards as previously. Thirty-six vineyards were thus selected, and each inspected for visual symptoms and samples taken for laboratory analysis. The number of samples collected from each vineyard varied but was typically between 10 and 20. Each sample was tested with ELISA, and samples with inconclusive results were retested with qPCR. Similar to the previous survey, no samples resulted in positive qPCR tests and no visual symptoms were identified.

The continued lack of detection of symptomatic plants and the negative results of diagnostic tests conducted with *Vitis* spp. tissue from vineyards in this study and in previous surveys adds confidence, but does not unambiguously demonstrate that *X. fastidiosa* is not present in grapevines in the region surveyed.

Indeed, the uncertainties outlined in EFSA PLH Panel (2015) remain and it cannot be ruled out that grapevine infection exists in that area at some low level, i.e. when no disease is detected in a sample, this does not mean that none is present (as it could have been missed by chance). Given a survey where a proportion of the population is sampled, the binomial distribution can be used to infer the probability that disease is present in host populations where none is discovered. The binomial distribution is the appropriate model for cases when sampling is done with replacement (or when the population is sufficiently large that this can be assumed) and where all samples in a population have an equal probability of selection. In this case, using the binomial distribution, a confidence interval for disease incidence can be calculated. The lower threshold of this interval is zero and the 95% one-sided upper threshold, $P_U$, is given by

$$P_U = 1 - 0.95^{1/N},$$

where $N$ is the total sample size. Hence given a sample of size $N$, there is 95% confidence that the disease incidence is with the interval zero to $P_U$ when no disease is detected in a sample. A useful approximation to this equation is the 'rule of three' which gives the upper threshold as,

$$P_U = 3/N.$$

The rule of three works for cases where diagnostic test sensitivity is high and gives the 95% confidence interval that, if no disease is found, the true incidence of disease is less than the upper threshold $P_U$ (p. 285 of Madden et al., 2007).

In the current survey, the typical sample size was $N = 20$ per vineyard. Based on this, there is a 95% confidence that the true incidence of disease was between 0 and 0.15 as a proportion of the population (i.e. between 0% and 15%) in each individually tested vineyard. However, the lack of positive tests from multiple vineyards simultaneously and over time should also be considered. The total number of samples during the most recent survey is $N = 614$. Assuming samples were independent and could be considered to be drawn from a homogeneous population, there is a 95% confidence that the true incidence in the region is between 0% and 0.48% (equivalent to an upper bound of 10–40 infected plants/hectare assuming a planting density of 2,000–8,000 plants/hectare). Although this is considerably lower than the 15% threshold incidence when only individual vineyards are considered, it is unlikely that the assumption of independence of sampling is met at this higher scale. For example, the 614 samples were clustered within 36 vineyards in the region. *X. fastidiosa* is known to have a clustered distribution at short distances due to plant-to-plant movement of vectors (Tubajika et al., 2004) and average movement distances of around 100 m, and so samples close to each other are likely to have the same disease status and cannot thus be considered independent (Blackmer et al., 2004).

This 0.48% value is conditional to a number of assumptions which increase the uncertainties affecting it. In particular, this value supposes a perfect diagnostic of infection, a condition that is rarely met in practice. In this respect, the Panel notices that the sampling for the new survey was performed during the November–January period, when bacterial titres are likely to be reduced as a consequence of cold weather conditions (Lieth et al., 2011). In view of the conjunction of the late season sampling with the use of the less-sensitive ELISA assay, the risk of false negative results in the survey cannot be totally discounted.

Another point to consider is that the strength of such surveys lies in the number of *Vitis* genotypes analysed. However, the number of individual plants tested per genotype is limited so that the 95% confidence threshold incidence is quite high for individual genotypes. In particular, the Panel wishes to stress that a total of only 30 plants were tested for all *Vitis* spp. rootstocks and non-*vinifera Vitis* spp. cultivars, with the rule of three giving a 95% confidence upper value for infection of 10% for these species in the survey.

It should also be noted that, in any case, evidence lack of infection in the surveyed host population is not direct evidence that grapevine is not a host. For example, as highlighted in EFSA PLH Panel (2015), uncertainties exist around the availability of infectious *P. spumarius* in the sampled vineyards and the feeding preferences for grapevine in the presence of other hosts.

Lastly, it should be stressed that although such surveys can provide, within uncertainty limits, some indications on whether particular genotypes can be systemic hosts, they are ill-suited to draw conclusions on a non-systemic host status because localised infections are very unlikely to be captured with the sampling strategy used.

### 3.1.3. Conclusions on *Vitis* spp. as a host for *Xylella fastidiosa* strain CoDiRO

The Panel acknowledges the difficulty to provide compelling evidence for non-susceptibility of a particular plant to infection with a pathogen. This is because the components/nature of the infection process are barely understood and artificial inoculation experiments thus can only mimic natural processes and are therefore much less efficient and in many cases not successful at all. Nevertheless, even a very low number of infected plants resulting from those biological experiments in which bacteria are detected at sites distant from the inoculation points at several time intervals after inoculation are proof for a successful invasion of the host, independently of the symptoms expressed. However, to provide evidence that a particular plant is not susceptible to a given pathogen is much more difficult and is also invariably associated with uncertainties concerning the correctness of the experimental approaches taken for a hitherto unknown host/pathogen interaction.

The Italian Authorities substantiated their request for deregulation with survey data and vector-mediated or mechanical inoculation experiments. The Panel recognises that these different lines of evidence, although not fully demonstrative on their own, frequently complement and reinforce each other. However, in at least one instance, the confrontation of the vector-mediated and mechanical

inoculation experiments, which provide diverging results concerning *X. fastidiosa* accumulation at the inoculation point, makes it more difficult to reach a firm conclusion (see Section 3.1.2.). In reaching its conclusions, the Panel systematically tried to consider all information available as well as the associated uncertainties and the possible synergies between the different lines of evidence.

Despite the limitations and uncertainties affecting each line of evidence, the Panel considers that, taken together, the absence of systemic infections upon mechanical and insect-mediated inoculations and the failure to detect infected grapevines in the various surveys provide sufficient demonstration that at least the inoculated cvs. Cabernet Sauvignon, Negroamaro and Primitivo do not support a systemic infection by the CoDiRO strain. The extension of this conclusion to other grapevine cultivars that have not been subjected to artificial inoculation experiments and that represent at best low numbers of plants surveyed is associated with significant uncertainty. The further extension to *Vitis* species other than *V. vinifera* would be highly speculative and associated with even larger uncertainties because of the increased genetic distance between the plants compared and because very few plants for the various non-*vinifera* species are included in the surveys. Overall, the Panel considers that, contrary to the situation described above for tested grapevine cultivars, it is premature to conclude whether a systemic host status for the CoDiRO strain can be extended to all *Vitis* species. Additional data involving experimental inoculation trials of non-*vinifera* species would greatly contribute to clarify this question.

Given the possibility that a non-systemic host could contribute to the spread of *X. fastidiosa* (Hill and Purcell, 1995, 1997), the Panel also considered the possibility that *Vitis* spp. could be non-systemic hosts of the CoDiRO strain. In this respect, the Panel wishes first to stress that the available survey results are not suitable to address this question.

The two remaining lines of evidence provide different results even if efforts at bacterial isolation were negative in both cases. Upon vector-mediated inoculation, no positive qPCR detection was obtained while positive PCR detection for prolonged periods of time was observed, only at or near the inoculation point, in some of the mechanically inoculated plants. As noted in its previous opinion by the Panel (EFSA PLH Panel, 2015), it is difficult to decide on the significance of these positive PCR signals.

Although there is a distinct possibility that the tested grapevine cultivars are not host for the CoDiRO strain, and that the local accumulation detected in the mechanical inoculation experiments might represent an artefact, the Panel considers that it is currently difficult to unambiguously conclude on this point and that additional experiments, as suggested in Section 3.1.2., would greatly help in reaching a firm conclusion.

In addition, the Panel concludes that at this stage there is no experimental data that could be used to support an extension of the above conclusion, reached with a few cultivars, to other grapevine cultivars or even to non-*vinifera* species. In this respect, artificial inoculation experiments performed on non-*vinifera* species seem critical to reach any broad conclusion on a non-systemic host status of all members of the genus *Vitis.*

## 3.2. Assessment of *Citrus* spp. as hosts of *Xylella fastidiosa* strain CoDiRO

### 3.2.1. Background information

There is ample information that citrus isolates of *X. fastidiosa* subsp. *pauca* are able to systemically infect some *Citrus* species, specially *Citrus sinensis* (sweet orange), in which they cause a severe disease that has been named Citrus variegated chlorosis (CVC) (Laranjeira et al., 1998). In the susceptible sweet orange cv. Caipira, for example, a CVC isolate was shown to multiply and move systemically over time, reaching concentrations that increased from log 4–5 CFU/g of tissue at 1 week to log 5–7 CFU/g at 2–4 months after mechanical inoculation. All sweet orange cultivars tested so far are susceptible, except Navelina ISA 315, which shows very low bacterial titre (Fadel et al., 2014). However, acid lime (*Citrus aurantifolia*), lemon (*Citrus limon*), grapefruit (*Citrus paradisi*), pummelo (*Citrus grandis*), kumquats, *Poncirus trifoliata* and most mandarins (*Citrus reticulata*) and tangors (*C. sinensis* × *C. reticulata*) are highly tolerant or resistant to this pathogen (Coletta-Filho et al., 2007; Garcia et al., 2012). In some genotypes, considered to be resistant, such as hybrids of *C. sinensis* and tangor cv. Murcot, the bacterium cannot move and only local infection has been reported. It can locally multiply around the inoculation point but is unable to systemically invade the plant and to colonise tissues distant from the inoculation point (Niza et al., 2015). Although local infection by *X. fastidiosa* subsp. *pauca* can be detected in resistant genotypes 2–3 months after inoculation, bacterial populations usually decline after several months (Coletta-Filho et al., 2007; Niza et al., 2015).

The ability to multiply and establish systemic and persistent infections depends not only on the citrus host species, but also on the *X. fastidiosa* strain. In reciprocal mechanical inoculation experiments, Brazilian isolates of *X. fastidiosa* subsp. *pauca* from coffee did not infect *C. sinensis* (sweet orange), while CVC isolates were able to infect and multiply in *Coffea arabica*, but at lower rates and titres than in sweet orange (Almeida et al., 2008; Prado et al., 2008). In addition, infections by CVC isolates in coffee plants remained local (detected only a few cm around the inoculation point) and did not persist after several months post-inoculation (Almeida et al., 2008). Although closely related, coffee and citrus strains of *X. fastidiosa* subsp. *pauca* are thus genetically and biologically distinct, with differential abilities to colonise coffee and citrus plants.

*Xylella fastidiosa* accumulation is an important requirement for a host plant to serve as an inoculum source. For *X. fastidiosa* subsp. *fastidiosa*, a minimum population level of log 4 CFU/g of plant tissue is required for bacterial acquisition and subsequent transmission by an efficient sharpshooter vector (Hill and Purcell, 1997). Higher bacterial populations result in higher transmission efficiency; in grapevines, for example, transmission efficiency by the blue-green sharpshooter ranged from 4.5% to 55% for *X. fastidiosa* populations of log 4 CFU/g and log 8 CFU/g of plant tissue, respectively. Systemic movement of *X. fastidiosa* and persistence of infections increases the capacity of host plants to serve as an inoculum source (Hill and Purcell, 1995, 1997; Purcell and Saunders, 1999).

Concerning the specific case of the CoDiRO strain, the following information was available prior to the dossier provided by the Italian Authorities:

- Results of artificial mechanical inoculation experiments conducted on greenhouse-grown plants in the frame of the Pilot project on *X. fastidiosa* host plants (Saponari et al., 2016). These experiments, conducted in parallel to the grapevine cv. Cabernet Sauvignon mechanical inoculation experiment involved 10 plants each of sweet orange (cv. Madame Vinous), grapefruit (cv. Duncan) and mandarin (cv. Comune) and 10 plants each of three Citrange hybrids (Carrizo, Troyer and C35).
- Results of one experiment of exposure to naturally infective *P. spumarius* in the field, involving 10 plants of each of sweet orange (cv. Navelina), clementine (cv. Hernandina) and Citrange Troyer (Saponari et al., 2016). In the report of this project, only data on the sweet orange plants was provided. Concerning this experiment, it is worth mentioning that it is unclear to the Panel whether the cv. Navelina used is the same as Navelina ISA 315 reported to be the only variety of sweet orange which shows resistance to *X. fastidiosa* subsp. *pauca* CVC causing strains in Brazil (Fadel et al., 2014).
- Results of field surveys carried out in the Apulia region and involving various numbers of *Citrus* spp. plants, including sweet orange, clementine, mandarin and lemon, which failed to detect *X. fastidiosa* in a total of over 350 plants (Martelli, 2016).

Overall, this data has been developed by the same teams and methodologies as the data on *Vitis* spp. susceptibility analysed by EFSA in its previous opinion (EFSA PLH Panel, 2015). As a consequence, all major uncertainties or limitations of such data previously pointed out by the Panel also apply in the case of the *Citrus* spp. data.

Contrary to the Cabernet Sauvignon grapevines inoculated in parallel, *X. fastidiosa* was detected by qPCR at the node above the inoculation point of *Citrus* spp. plants at 3-month post-inoculation (in 1–5 of the 10 inoculated plants of each tested species; Saponari et al., 2016). According to additional data obtained by EFSA from the Italian Authorities upon request (Section 2.1), positive culture isolation of the bacteria from Madam Vinous sweet orange 3 months post-inoculation was also obtained (two colonies from a 1:100 sap dilution of a composite leaf sample). Twelve months post-inoculation, the bacteria were still detected by qPCR in a few plants in stem segments 10 cm from the inoculation point (3 plants out of a total of 36 analysed plants, with another 3 giving positive PCR only in petioles 10 cm away but not in the stem). Fourteen months post-inoculation, qPCR indicated titres in the inoculated section of a few plants of between 9.9E + 04 and 1.9E + 06 CFU/mL. The bacterium could not be re-isolated from the stem or leaf petiole 12 months post-inoculation, when six plants were entirely sectioned and tested by culturing.

### 3.2.2. Assessment of new data provided by the Italian Authorities

The new data provided in the dossier prepared by the Italian Authorities concerns two different aspects: (i) experimental inoculations of sweet orange (cv. Madam Vinous) using infectious

*P. spumarius* (Cornara et al., publication B) and (ii) a follow up at a later time on the plants remaining from the mechanical inoculation experiment described above.

Concerning the results presented in the Cornara et al. (publication B), *X. fastidiosa* was consistently detected by qPCR over a 14 months period in the leaf and stem tissues exposed to infective insects of 10 of the 75 test plants (13.3%). Under similar inoculation conditions, systemic accumulation of *X. fastidiosa* was detected in 41.3% of inoculated olive plants and in 74% of inoculated oleander plants. Taking into account the previous results obtained with experimental mechanical inoculation described above, the authors discuss the possibility that *Citrus* spp. 'may support early and localised multiplication of *X. fastidiosa*, but this plant is not a systemic host of ST53'.

The follow up on the plants remaining from the experimental inoculation experiment of 2014 provides results of qPCR testing 19 months post-inoculation of the four remaining plants of each of the six tested genotypes (the other six plants for each genotype having been tested 14 months post-inoculation). *X. fastidiosa* could not be detected in any of the retested *Citrus* plants, either in old stem parts that included the inoculation points or in new sprouts. Given the very low number of plants and the absence of information on the reproducibility of this novel observation, this second piece of new data contributes little to reducing the uncertainties.

The data presented by Cornara et al. (publication B) provide novel information from robust experimental conditions as judged by (i) the high rates of infection achieved in parallel in susceptible hosts like olive and oleander, (ii) the appropriate control of *P. spumarius* bacterial loads and (iii) the survival of *P. spumarius* on the sweet orange test plants. Uncertainties associated with these experiments mostly concern the absence of confirmatory assays, in particular isolation/culturing experiments, which would potentially have allowed the hypothesis for a local multiplication of *X. fastidiosa* in the inoculated portion of the sweet orange test plants to be confirmed.

Lastly, these new results (Cornara et al., publication B), which appear to be the most robust ones to date, deal only with a single sweet orange cultivar. Given the known variability that may exist between *Citrus* species and *X. fastidiosa* strains (see Section 3.2.1), there is a very high uncertainty as to whether these results, obtained in sweet orange, can be generalised to all *Citrus* species. A possible way to reduce this uncertainty would be to take into account the results of the surveys performed in the Apulia region (Martelli, 2016), which involve plants of four *Citrus* species (sweet orange, lemon, mandarin and clementine). However, the Panel wishes to point out that this kind of survey is particularly ill-suited to detect situations of non-systemic infection. In addition, the absence of data on the presence and infectivity of *P. spumarius* vectors in the surveyed *Citrus* spp. groves further complicates the interpretation of the survey results, as previously discussed for the grapevine surveys (Section 3.1.2.).

### 3.2.3. Conclusions on *Citrus* spp. as hosts of *Xylella fastidiosa* strain CoDiRO

The data available to date provides coherent and converging lines of evidence suggesting that sweet orange may be a non-systemic host of the CoDiRO strain. However, from the experimental evidence and the known variability in sweet orange-*X. fastidiosa* interactions, it is premature to exclude that systemic infections could not occur in cultivars other than the tested Madam Vinous. Such a scenario would be in line with the reported infection pattern observed in a range of *Citrus* spp. with *X. fastidiosa* subsp. *pauca* (Coletta-Filho et al., 2007; Garcia et al., 2012; Niza et al., 2015).

Given the very limited data available on species other than sweet orange, the Panel considers that it is similarly premature to conclude that the situation in sweet orange, if solidly demonstrated, could be extended to all other *Citrus* species.

Lastly, the Panel wishes to stress that epidemiological consequences of non-systemic infections of *Citrus* spp. plants by the CoDiRO strain are currently completely unknown. In particular, it remains to be investigated whether such non-systemic infections could serve as sources for transmission of *X. fastidiosa* by insect vectors to other susceptible species.

As exposed in Section 3.2.1., transmission studies with *X. fastidiosa* subsp. *fastidiosa* showed that the probability of pathogen acquisition by a competent vector depends on bacterial multiplication in the host reaching a minimum level of log 4 CFU/g of plant tissue (Hill and Purcell, 1997). The results presented in Saponari et al. (2016) show that the CoDiRO strain is able to non-systemically infect sweet orange cv. Madame Vinous. Fourteen months post-inoculation, population levels of log 4–6 CFU/mL around the inoculation point were found in a significant proportion of mechanically inoculated plants. Although it is not straightforward to compare an experimental concentration expressed in CFU/mL with the threshold for transmission expressed in CFU/g of plant tissue, it still appears likely that if a similar transmission

threshold applies to the CoDiRO strain it could be reached in at least some of the plants concerned. This adds a considerable level of uncertainty concerning the risks of *Citrus* spp. acting as an inoculum source of the pathogen. This uncertainty might be reduced by investigating if a competent vector (e.g. *P. spumarius*) can indeed acquire the bacterium when exposed to infected portions of citrus plants at different times after inoculation and evaluations of viable cell concentrations by culturing and qPCR.

## 3.3. Assessment of *Quercus ilex* as a host of *Xylella fastidiosa* strain CoDiRO

### 3.3.1. Background information

There is ample information that *X. fastidiosa* is able to systemically infect a range of oak (*Quercus* spp.) species (Barnard et al., 1998; EFSA, 2016), including some European species like *Quercus robur* and *Quercus suber* (Hartman, 2007; ONPV, 2015). In at least some of these species, infection by *X. fastidiosa* causes a severe leaf scorch disease. For *Q. ilex*, however, the Panel was unable to identify a specific reference in the literature showing that it can be a host for at least some *X. fastidiosa* subspecies or strains.

Early PCR results in Apulia reported presence of the CoDiRO strain in one *Q. ilex* plant (personal communication by Pasquale Di Rubbo, DG SANTE, of 26 August 2016). These initial results apparently form the basis for the initial inclusion of the entire genus *Quercus* among potential host plants.[6] Later, *Quercus* spp. was kept in Annex I of the Commission Implementing Decision (EU) 2015/789 as a 'specified plant' known to be susceptible to the European and non-European isolates of *X. fastidiosa*. Such initial results have not been reproduced (see below).

Concerning the specific case of the CoDiRO strain, the following information was available prior to the dossier provided by the Italian Authorities:

- Results of artificial mechanical inoculation experiments conducted on greenhouse-grown *Q. ilex* plants in the frame of the Pilot project on *X. fastidiosa* host plants (Saponari et al., 2016). These experiments, conducted in parallel to the grapevine Cabernet Sauvignon mechanical inoculation experiments, involved 12 *Q. ilex* plants.
- Results of one experiment of exposure to naturally infective *P. spumarius* in the field, involving 12 *Q. ilex* plants (Saponari et al., 2016).
- Results of field surveys carried out in the Apulia region and involving 130 samples of *Quercus* sp. plants, all of which turned out negative. In the data received by EFSA, there is, however, no information as to how many of these samples belong to the species *Q. ilex*, which introduces a substantial uncertainty (Annex III of the dossier).

Overall, this data has been developed by the same teams and methodologies as the data on *Vitis* spp. susceptibility analysed by EFSA in its previous opinion (EFSA PLH Panel, 2015). As a consequence, all major uncertainties or limitations of this data previously pointed out by the Panel also apply in the case of the *Q. ilex* data. In particular, as pointed out previously by EFSA in its analysis of similar survey data concerning grapevine (EFSA PLH Panel, 2015), in the absence of data on the presence and infectivity of *P. spumarius* vectors in the surveyed oak trees, the absence of detection of *X. fastidiosa* cannot be taken as an indication that the surveyed plant are not systemic hosts.

Similar to the situation in grapevines cv. Cabernet Sauvignon inoculated in parallel, positive qPCR detection of *X. fastidiosa* was observed 3 months post-inoculation at the inoculation point (8/12 plants) but not at the node above the inoculation point. At 12 months post-inoculation, bacterial DNA was not detected in three plants that were extensively sampled at or above the inoculation point.

### 3.3.2. Assessment on *Quercus ilex* as a host of *Xylella fastidiosa* strain CoDiRO

The new data provided in the dossier prepared by the Italian Authorities concerns a follow up at a later time point on the plants remaining from the single mechanical inoculation experiment described above. The nine remaining plants were each extensively sampled and tested at 19 months post-inoculation. *X. fastidiosa* could not be detected in any stem section of the plants from 5 cm above the

[6] DG(SANCO) 2014-7260 - MR FINAL. Final report of an audit carried out in Italy from 10 to 14 February 2014 in order to evaluate the situation and official controls for *Xylella fastidiosa*. Available from http://ec.europa.eu/food/fvo/act_getPDF.cfm?PDF_ID=11108

inoculation points. Given the low number of plants involved and the absence of information on the reproducibility of this single mechanical inoculation experiment, this piece of new data contributes little to reducing the uncertainties.

### 3.3.3. Conclusions on *Quercus ilex* as a host of *Xylella fastidiosa* strain CoDiRO

The data available to date provides some limited evidence suggesting that *Q. ilex* may not be a systemic host of the CoDiRO strain. However, from this limited evidence, which mostly concerns a single mechanical inoculation experiment that has not been reproduced, the Panel concludes that it would be premature to exclude the possibility that systemic infections could occur in this *Quercus* species. It also wishes to stress that, similar to the situation in grapevine and in citrus, the currently available evidence could point to the possibility of an initial local accumulation of *X. fastidiosa* upon inoculation, the epidemiological consequences of which remain unknown.

A simple consideration of the volume of experimental data developed on the study of the host status of *Vitis* spp. and of the difficulties encountered to reach an unambiguous conclusion provides a road map for future efforts to develop data that would allow reaching a conclusion in the case of *Q. ilex*. Such novel data would in particular involve mechanical and vector-mediated inoculation experiments encompassing significant numbers of plants and appropriate controls and surveys including information about bacterial population levels *in planta* and infectious vectors populations.

## 4. Conclusions

In answer to a request of the European Commission, the PLH Panel performed an analysis of a dossier submitted by the Italian Authorities and of the relevant scientific literature in order to reach a conclusion on the host status of *Vitis* spp., *Citrus* spp. and *Q. ilex* to *X. fastidiosa* subsp. *pauca* strain CoDiRO involved in the current Apulian outbreak.

The Panel acknowledges the difficulty in providing compelling evidence for non-susceptibility of a particular plant to infection with a pathogen. It also wishes to stress that novel experimental data developed since its previous evaluation (EFSA PLH Panel, 2015) has significantly reduced the uncertainties associated with the evaluation of the grapevine–CoDiRO host status.

In the case of *Vitis* spp., the Panel recognises that the different available lines of evidence, although not fully demonstrative on their own, frequently complement and reinforce each other. Therefore, in reaching its conclusions, the Panel systematically tried to consider all information available as well as the associated uncertainties and the possible synergies between the different lines of evidence.

Despite the limitations and uncertainties affecting each line of evidence, the Panel considers that the absence of systemic infections upon mechanical and insect-mediated inoculations and the failure to detect infected grapevines in the various surveys provide sufficient demonstration that at least the inoculated cvs. Cabernet Sauvignon, Negroamaro and Primitivo do not support a systemic infection by the CoDiRO strain.

The extension of this conclusion to other grapevine cultivars that have not been subjected to artificial inoculation experiments and that represent at best low numbers of plants surveyed is associated with significant uncertainty. The further extension to *Vitis* species other than *V. vinifera* is associated with even larger uncertainties because of the increased genetic distance between the plants compared and because very few plants for these various non-*vinifera* species are included in the surveys. Overall, the Panel considers that, contrary to the situation described above for tested grapevine cultivars, it is premature to conclude whether a systemic host status for the CoDiRO strain can be excluded for all *Vitis* species. Additional data involving experimental inoculation trials of non-*vinifera* species would greatly contribute to clarify this question.

Given the known possibility that a non-systemic host could contribute to the spread of *X. fastidiosa*, the Panel also considered the possibility that *Vitis* spp. could be non-systemic hosts of the CoDiRO strain. Considering all available evidence, the Panel concludes that although there is a distinct possibility that the tested grapevine cultivars are not host for the CoDiRO strain, and that the local accumulation detected in the mechanical inoculation experiments might represent an artefact, it is currently difficult to unambiguously conclude on this point. Additional experiments would greatly help in reaching a firm conclusion.

In addition, the Panel concludes that at this stage there is no experimental data that could be used to support an extension of a tentative conclusion concerning the non-systemic host status of a few cultivars to other grapevine cultivars or even to non-*vinifera* species. In this respect, artificial

inoculation experiments performed on non-*vinifera* species seem critical to reach any broad conclusion on a non-systemic host status of all members of the genus *Vitis*.

In the case of *Citrus* spp., the Panel notes that many species are known to be systemic or non-systemic hosts of *X. fastidiosa* subsp. *pauca*, clearly raising the question of whether the same may apply in the case of the CoDiRO strain. After reviewing the available evidence, the Panel concludes that the data available to date provides coherent and converging lines of evidence suggesting that sweet orange may be a non-systemic host of the CoDiRO strain. However, from the experimental evidence and the known variability in sweet orange–*X. fastidiosa* interactions, it is premature to exclude that systemic infections could not occur in cultivars other than the tested Madam Vinous.

Given the very limited data available on species other than sweet orange, the Panel considers that it is similarly premature to conclude that the situation in sweet orange, if solidly demonstrated, could be extended to all other *Citrus* species.

Lastly, the Panel wishes to stress that the epidemiological consequences of non-systemic infections of *Citrus* spp. plants by the CoDiRO strain are currently completely unknown. In particular, it remains to be investigated whether such non-systemic infections could serve as sources for transmission of *X. fastidiosa* by insect vectors to other susceptible species.

In the case of *Quercus ilex*, the Panel notes that this species was included in the list of host species on the basis of an initial report that could not be confirmed later. However, given the number of oak species that have proven to be hosts to *X. fastidiosa*, the question of whether *Q. ilex* could be a host of the CoDiRO strain appears to be a valid one. The Panel concludes that the data available to date provides some limited evidence suggesting that *Q. ilex* may not be a systemic host of the CoDiRO strain. However, from this limited evidence, which mostly concerns a single mechanical inoculation experiment that has not been reproduced, the Panel concludes that it would be premature to exclude the possibility that systemic infections could occur in this *Quercus* species. It also wishes to stress that, similar to the situation in grapevine and in citrus, the currently available evidence could point to the possibility of an initial local accumulation of *X. fastidiosa* upon inoculation, the epidemiological consequences of which remain unknown.

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## Abbreviations

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| CFU | colony-forming unit |
| CVC | Citrus variegated chlorosis |
| ELISA | enzyme-linked immunosorbent assay |
| MLST | multilocus sequence typing |
| PCR | polymerase chain reaction |
| PLH | EFSA Plant Health Panel |
| qPCR | quantitative polymerase chain reaction |
| TOR | Terms of Reference |