

### **STATEMENT**

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## Susceptibility of Phoenix roebelenii to Xylella fastidiosa

EFSA Panel on Plant Health (PLH),

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### **Abstract**

Following a request from the European Commission, the EFSA Plant Health Panel analysed a dossier submitted by Costa Rica Authorities to reach a conclusion on the host status of Phoenix roebelenii for Xylella fastidiosa. The Panel wishes first to stress the difficulties faced in providing compelling evidence for the non-susceptibility status of any particular plant species. The Panel acknowledges that the listing of P. roebelenii as a host of X. fastidiosa rests on a single report from California. Because isolation of X. fastidiosa from some hosts can be difficult, the Panel considers that the failure to isolate X. fastidiosa from P. roebelenii cannot be used to totally discard the detection of X. fastidiosa by ELISA and PCR. The Panel concludes that the detection of X. fastidiosa by two independent techniques provides sufficient evidence, although not totally conclusive, for the listing of P. roebelenii as a X. fastidiosa host plant. Concerning the survey data provided in the Costa Rican dossier, the Panel wishes to stress that such surveys cannot demonstrate the non-host status but can only provide a probability bound, upper estimate of the proportion of infected plants in the field. In the present case, and assuming all survey parameters to be optimal, the 95% confidence incidence threshold obtained is 0.2%, leaving the possibility that close to 25,000 P. roebelenii plants could be infected but undetected in the country. Accepting a scenario of local, non-systemic infection of P. roebelenii by X. fastidiosa would further increase uncertainties. In addition, the absence of data on the vector infection pressure further affects the ability to derive meaningful information on the P. roebelenii host status from the survey data. Appropriately conducted mechanical and/or vector-mediated inoculation experiments are critical to reach a more solid conclusion on the X. fastidiosa host status of P. roebelenii.

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**Requestor:** European Commission

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### 1. Introduction

## 1.1. Background and Terms of Reference as provided by the requestor<sup>1</sup>

The purpose of this mandate is to request, pursuant to Articles 29 and 31 of Regulation (EC) No 178/2002<sup>2</sup>, 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, EFSA is requested to further specify and update the host plants database of *X. fastidiosa* currently available, <sup>3</sup> 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<sup>4</sup> 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, the European Food Safety Authority (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, <sup>5</sup> 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 (NPPO) 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 *Quercus 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 the present opinion, the EFSA Panel on Plant Health (hereafter the Panel) replies to the request concerning the susceptibility of *P. roebelenii* to *X. fastidiosa*.

Because the request is based on a dossier submitted by the Costa Rica NPPO, the Panel's reply focuses first on the information and data provided in this dossier. However, as the request is more

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<sup>&</sup>lt;sup>1</sup> Submitted by European Commission, ref. SANTE/G1/PDR/svi (2016) 3575400.

<sup>&</sup>lt;sup>2</sup> 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.

<sup>&</sup>lt;sup>3</sup> 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

<sup>&</sup>lt;sup>4</sup> 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.

<sup>&</sup>lt;sup>5</sup> 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



general, the Panel considers in its analysis all the available evidence retrieved both from scientific literature and from technical reports and data.

The sections in the dossier provided by the Costa Rica NPPO which do not directly concern the request formulated in the Terms of Reference (ToR) are not considered in the present opinion. This regards:

- Section 2.3 'Overview of *Xylella fastidiosa* and *Phoenix roebelenii'* (p. 3–5)
- Section 5 'Actions for phytosanitary management of *Phoenix roebelenii* to be exported to the EU' (p. 15–16)

For the same reason, *P. reclinata*, also mentioned in the dossier, is not included in the analysis of the Panel presented here.

### 1.3. Additional information

The inclusion of *P. roebelenii* among *Xylella* host plants is based on a single disclosure/outreach document (Wong, 2005). This reference provides very few details on the methodologies used and on the results obtained. In order to obtain additional information, in July 2016, EFSA tried to contact the authors of this work via email but no answers were received.

## 2. Data and methodologies

### 2.1. Data

Survey data and the technical dossier (hereafter the Dossier) prepared by the Costa Rica NPPO were provided to the Panel together with the mandate letter.

In a second phase, the Costa Rica NPPO provided, upon request from EFSA, clarifications on aspects concerning the survey methodology.

Targeted extensive literature searches were carried out on the research platform ISI Web of Science (last update on 31 August 2016) and on the EFSA *Xylella* host plant database (EFSA, 2016). Keywords used were '*Xylella fastidiosa'*, '*Phoenix roebelenii'*, 'Arecaceae', 'Palmaceae', 'palm' 'infection', 'vector' and variants as search terms. Further references and information were obtained from citations within the reviewed references and from Panel members and external 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 *P. roebelenii* as a possible host of *X. fastidiosa*, the Panel considered all literature relevant to support an assessment. The assessment is divided into two main sections: (i) assessment of the uncertainties affecting the initial Californian report of infection of *X. fastidiosa* on *P. roebelenii*, (ii) assessment of data provided by Costa Rica throughout field surveys to determine the absence of *X. fastidiosa* from *P. roebelenii*. Uncertainties are identified and discussed under each section.

### 3. Assessment

## 3.1. Background on *Phoenix roebelenii* as a host plant for *Xylella fastidiosa*

The bacterium *X. fastidiosa* is found in many plant species, colonising the xylem vessels of its hosts. In some plants, it exists as an endophyte, the plants appearing unaffected and without any symptoms of its presence (e.g. Hopkins, 1989; Purcell and Saunders, 1999; Hopkins and Purcell, 2002), while in other plants, it can be a serious pathogen causing a wilting or scorching disease (EFSA PLH Panel, 2015a). *X. fastidiosa* is reported from many countries and from a great number of host plants including important tree crops like coffee (Rodriguez et al., 2001), citrus (Hartung et al., 1994; Aguilar et al., 2005), avocado (Montero-Astua et al., 2008a) and grapevine (Hill and Purcell, 1995; Aguilar et al., 2008). It can also infect many ornamental plants, like *Nerium oleander* (Oleander, Purcell



et al., 1999; Montero-Astua et al., 2008b) and *Polygala myrtifolia* (Saponari et al., 2014; Martelli, 2016). The current EFSA database on *X. fastidiosa* host plant species lists 359 plant species (including hybrids) from 204 genera and 75 different botanical families (EFSA, 2016).

*Xylella fastidiosa* shows a large biological and genetic diversity and has been divided into several subspecies (Nunney et al., 2014) and strains that may present different strain-specific host ranges.

*Xylella fastidiosa* is found in many plant species in Costa Rica including coffee (Rodriguez et al., 2001), citrus (Aguilar et al., 2005), as well as in grapevine with symptoms of Pierce's disease, avocado and oleander. A considerable number of potential leafhopper vector species of *X. fastidiosa* associated with coffee, citrus and avocado has been identified in Costa Rica (Garita-Cambronero et al., 2005a,b). The ornamental dwarf palm *P. roebelenii* is mostly grown by small-scale producers located in the north of the country (Figure 4 of the Dossier), an area that overlaps with the citrus production zone (Odio, 2009; Gonzalez, 2014), but with limited overlap with the coffee production zone (the Dossier).

Phoenix roebelenii is listed in the EFSA database as a host of *X. fastidiosa*. Its inclusion is based on the report of a survey conducted in 2003/2004 in Southern California (US), to document the incidence of *X. fastidiosa* in landscape ornamental hosts (Wong, 2005). In this survey, *X. fastidiosa* was detected by enzyme-linked immunosorbent assay (ELISA) and by polymerase chain reaction (PCR) in symptomatic landscape species (mostly trees) and typed by sequence analysis to identify the respective subspecies involved. Bacteria were isolated from some infected plants and for some of the isolates Koch's postulates were also fulfilled but not for *P. roebelenii* (see below). *X. fastidiosa* was detected in *P. roebelenii* by ELISA and by PCR (Wong, 2005; Table 2). This document is not a refereed publication and many details are lacking. In particular, there is no information on the number of plants of *P. roebelenii* tested and on the percentage of tested plants for which a positive detection was obtained. Attempts to isolate and culture bacteria from *P. roebelenii* plants giving positive ELISA and PCR results were, however, not successful. This report is the only reference for *X. fastidiosa* detection in *P. roebelenii*. A report by the same authors refers to detection of *X. fastidiosa* by ELISA and PCR in *P. reclinata* (Wong et al., 2004; positive detection in two plants). These two reports are the only available data on infection of *X. fastidiosa* in members of the *Phoenix* genus that the Panel could identify.

## 3.2. Assessment of the Dossier by Costa Rica

The Dossier presented by Costa Rica to justify the removal of *P. roebelenii* from 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* addresses the question from two directions. It first highlights and discusses the uncertainties affecting the Californian report of infection. It then provides survey results comprising more than 1,500 samples taken in the region were ornamental dwarf palms are produced. The survey results are further substantiated by the testing of samples from a consignment of *P. roebelenii* conducted by the NPPO of the Netherlands.

# 3.2.1. Assessment in the light of the Dossier by Costa Rica of the uncertainties affecting the initial Californian report of infection of *Xylella fastidiosa* on *Phoenix roebelenii*

The finding of positive results in two independent detection assays (i.e. relying on different methodologies) is generally considered as providing sufficient evidence of infection by a pathogen and constitutes the standard requested by many scientific journals for reports of new hosts (ISPM 27 by FAO, 2006). Such concordant results obtained by two independent assays indeed reduce the risk of erroneous conclusions that may result from false-positive detections.

The detection of *X. fastidiosa* in *P. roebelenii* samples by the Californian team by ELISA and by PCR appears to meet this requirement of detection by two independent assays. The conclusions reached from this initial report are, however, associated with some uncertainties:

- This initial detection has not been further pursued or published by the team, and *P. roebelenii* has not been included in the Californian list of *X. fastidiosa* hosts
- Attempts at isolation of the bacteria from ELISA and PCR positive plants have not been successful, so that it is uncertain whether viable/live bacterial cells were detected by the assays
- While the team generally typed the detected isolates by sequencing the obtained PCR product, such a typing has not been performed in the case of the *X. fastidiosa* isolate(s) from *P. roebelenii*



The positive detection of *X. fastidiosa* in the material tested by two independent techniques is considered by the Panel as carrying significant weight despite the uncertainties listed above. In addition, it should be noted that Wong (2005) report the presence of symptoms in the positive *P. roebelenii*.

The Panel conclusions are in agreement with a statement by the reporting Californian team obtained by Costa Rica and cited at paragraph 6.7 of the Dossier: that there is great uncertainty to determine with certainty that some plants genus are hosts of Xylella fastidiosa, due to the fact that the bacteria could not be recovered from the samples taken.

Nevertheless, the Panel wishes to emphasise that isolation of *X. fastidiosa* from particular plant species is sometimes difficult (Purcell and Saunders, 1999) and that a failure to isolate and cultivate bacteria cannot be considered as a proof of the absence of bacterial multiplication in a particular host. The positive detection of *X. fastidiosa* by ELISA and by PCR in *P. roebelenii* cannot therefore be totally discarded on the basis of the negative result of isolation attempts.

Based on these considerations and on the technical details of the experiments provided in the California report, the Panel concludes that the detection of *X. fastidiosa* by ELISA and by PCR provides sufficient, although not totally conclusive, evidence to list *P. roebelenii* as a host plant for *X. fastidiosa*.

In the assessment of *P. roebelenii* as a host plant for *X. fastidiosa*, the Panel refers to the scientific opinion prepared by EFSA on *Vitis* sp. response to *X. fastidiosa* strain CoDiRO (EFSA PLH Panel, 2015b). With reference to susceptibility and suitability of *P. roebelenii* as a host, *Biological assays provide the most conclusive evidence that pathogen and plant undergo compatible interactions leading to successful colonisation of the host which consists of both multiplication of the pathogen and its systemic movement through the plant (EFSA PLH Panel, 2015b). These experiments albeit very difficult and time consuming also can provide proof of whether the bacteria, once introduced, systemically invades its host or whether it can locally multiply without systemic movement. In the latter case, insect vectors are known to be sometimes able to acquire bacteria from such non-systemic hosts (Hill and Purcell, 1995, 1997; EFSA PLH Panel, 2015b), so that bacterial cells could still potentially be transmitted by vectors feeding on <i>P. roebelenii*.

The uncertainties highlighted above on whether *P. roebelenii* is a host of *X. fastidiosa* would therefore be best addressed by properly conducted mechanical and/or vector-mediated inoculation experiments. Appropriately conducted surveys, documenting in particular the vector infection pressure in the surveyed plant would also help reducing the uncertainties. However, it should be stressed that the demonstration of the total absence of the pathogen in a species necessitates the testing of all plants with recommended assays (see below).

### 3.2.2. Assessment of field survey data provided in the Dossier of Costa Rica

The Costa Rica NPPO conducted a survey in 2015 to detect *X. fastidiosa* in open air, commercially produced *P. roebelenii*. This section describes the sampling principles necessary to ascertain the absence of disease using an analysis of the Costa Rica survey data to this end. Surveys can be conducted to provide evidence that a plant population is uninfected. The probability that some infection was missed during a survey cannot, however, be ruled out (Cannon, 2002; EFSA, 2012), except in the unrealistic case that a survey encompasses a complete census of all plant tissue in a population and is conducted with a perfect sampling and diagnostic process. Rather, information from a survey can be analysed to say with 95% probability (or any other prescribed confidence level) that, if no infection is found, the true incidence of infection is less than some threshold level (Cannon, 2002; EFSA, 2012).

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,  $P_{\text{U}}$ , 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  $P_{\rm U}$  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  $P_{II}$  (p. 285 of Madden et al., 2007).

The Panel wishes to stress that failure to detect a pathogen in a survey does not provide proof that a plant species is not a host. This is because the absence of infection in a population can be the consequence of absence of inoculum suitable vectors (EFSA PLH Panel, 2015b). Therefore, the absence of information on vector presence and infectivity in a survey compromises the ability to reach a conclusion on host status.

Furthermore, the type of survey performed by Costa Rica is particularly ill-suited to detect situations of non-systemic infection that are known to occur with *X. fastidiosa* in some hosts (Coletta-Filho et al., 2007; Garcia et al., 2012; Niza et al., 2015) to have potential epidemiological consequences (Hill and Purcell, 1995, 1997).

The Dossier indicates that Costa Rica contains around 500 ha of *P. roebelenii* plants for planting across the country. The size of production sites is highly variable ranging from < 1 to > 100 ha. Between September and December 2015, official surveys were conducted on 40 *P. roebelenii* producers which represent, according to the clarifications provided to EFSA in a second phase (Section 2.1), all producers in Costa Rica. A map and a list of all sampled producers were made available to EFSA. At each producer, three plants were sampled per hectare. For each sampled plant, two leaves were collected and combined, one from the top of the plant and one from the bottom. Samples were stored in sealed, clean plastic bags with unique identification numbers. In total, 1,558 plants were sampled over three survey rounds. The first round of survey involved collection of 527 samples in August distributed across all 40 producers. The second round involved collection of 919 samples in October, again distributed across all 40 producers. The third round of survey involved 72 samples collected in November and December to confirm previous doubtful results from the laboratory analysis of earlier samples. All samples were sent to and analysed by the Agdia laboratory, the US Company that has validated a method for PCR analysis for *X. fastidiosa*. Agdia performed the analyses in accordance with ISO/IEC 17025.

Assuming a perfect test, the 95% confidence interval that the true level of disease is at or below a certain incidence threshold can be determined (Cannon, 2002, Madden et al., 2007; EFSA 2012). Given that 1,558 plants were sampled, and assuming perfect test sensitivity, this would imply that the true incidence of *X. fastidiosa* in *P. roebelenii* populations in Costa Rica has a 95% confidence upper bound of 0.2%. The sensitivity of the test is, however, not completely clear from the Dossier and complementary information received from Costa Rica Authorities. However, given the very large number of samples analysed, the upper confidence limit for the proportion of infected plants in the population is not affected in a major way by the test sensitivity (e.g. a test with a low sensitivity of only 0.5 would result in a confidence limit of 0.4% (EFSA 2012)). However, if considering the possibility of existence of only localised, non-systemic infections, the test sensitivity is likely to be degraded in a major way because sampling only leaves might not allow to capture a localised infection. In such a scenario, the overall upper confidence limit for the proportion of infected plants in the population determined by the survey could be heavily increased.

Assuming that the production values provided in the Excel file included in the Dossier are correct, the Panel calculated a total production of almost 13 million plants for Costa Rica. Applying the confidence limit for the proportion of infected plants in the population of 0.2%, determined from the survey results under the most optimistic scenario, could still leave close to 25,000 undetected infected palms for the whole country or close to 49 infected plants/ha of cultivation.

## 3.2.3. Assessment of the information on testing of imported *Phoenix roebelenii* samples by the Netherlands provided in the Dossier by Costa Rica

The Dossier contains in Annex IV information showing that the Netherlands performed PCR assays for detection of *X. fastidiosa* in imported *P. roebelenii* samples. Although it is difficult to precisely understand how many plants were tested in total, at least 700 leaf samples were tested, all of them with negative results.

For the same reason as outlined above for the Costa Rican survey results, such analyses cannot be used to evaluate the host status of *P. roebelenii*.



The Panel therefore concludes that this additional information does not substantially clarify the host status of *P. roebelenii*.

### 3.2.4. Further comments on the Dossier by Costa Rica

The other elements presented or discussed in the Dossier do not call for further comments in the frame of the present opinion, which is focused on the question of whether *P. roebelenii* is a host for *X. fastidiosa*.

The Panel wishes to stress that the use of the term Pest Free Area refers to an area where the pest does not occur as demonstrated by scientific evidence (FAO, 1995), which therefore implies the absence of infection in any host plant in that area and therefore is not restricted to the presence/absence on a particular host. Therefore, the Panel does not agree with the statement provided at point 6.10 of the Dossier By not detecting the presence of Xylella fastidiosa affecting plant species in the samples taken, this measure [production of plants for planting in Pest Free Areas] would not apply to Phoenix roebelenii. These are not the only parameters to make an area qualify for the pest-free status.

These conclusive statements, and other similar statements in the Dossier, seem to imply that the absence of some contaminated hosts (coffee, point 6.3. of the Dossier) or non-detection of infection in a crop (possibly *P. roebelenii*) are the only important parameters and are sufficient to declare an area free of a pest. This is clearly a misinterpretation, as to reach a Pest Free Area status a demonstration of the absence of the pest in any host in the area has to be obtained.

It has also to be remarked that the report of the audit carried out by DG SANTE in Costa Rica from 21 September to 1 October 2015<sup>6</sup> states that there are no production areas in Costa Rica, which are declared free from Xf. The greenhouses, where the re-rooting before export takes place, were not insect proof either. In addition, the same report indicates that the production of *P. roebelenii* in Costa Rica includes an open air production phase, therefore producers cannot meet requirements of Commission Decision 789/2015/EU.

#### 4. Conclusions

The listing of *P. roebelenii* as a host of *X. fastidiosa* rests on a single report from California (Wong, 2005). Because the isolation of *X. fastidiosa* from some hosts can be difficult (Purcell and Saunders, 1999), the Panel considers that the fact that the Californian team was not able to isolate and cultivate the bacteria cannot be used as a proof of the absence of bacterial multiplication in *P. roebelenii*. The positive detection by two independent techniques reported by Wong (2005) cannot therefore be totally discounted on the basis of the negative result of their isolation attempts. The Panel concludes that the detection of *X. fastidiosa* by ELISA and by PCR provides sufficient justification to keep *P. roebelenii* in the list of host plant species for *X. fastidiosa* at present.

Concerning the survey data provided in the Costa Rican Dossier, the Panel wishes first to stress that surveys cannot demonstrate that *P. roebelenii* is not a host of *X. fastidiosa* but can only provide, under the best circumstances, a probability bound upper threshold of infection (Cannon, 2002; Madden et al., 2007; EFSA, 2012). In the present case, and assuming all survey parameters to be optimal, the 95% upper confidence limit for the proportion of infected plants in the population obtained is 0.2%, leaving the possibility that under a worst case scenario close to 25,000 *P. roebelenii* could be infected but undetected in the country. Accepting the possibility that *P. roebelenii* could be a local, non-systemic host of *X. fastidiosa* would further add to the uncertainties associated with these conclusions. In addition, the Costa Rica survey does not address some important aspects; in particular, the absence of data on the vector infection pressure strongly affects the ability to derive information on the *P. roebelenii* host status from the survey data.

The Panel considers that appropriately conducted mechanical and/or vector-mediated inoculation experiments, involving the various subspecies of *X. fastidiosa* present in Costa Rica and possibly different *P. roebelenii* genotypes are critical to reach a more solid conclusion on whether *P. roebelenii* is a host of *Xylella*.

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<sup>&</sup>lt;sup>6</sup> DG(SANTE) 2015-7644 – MR. Final report of an audit carried out in Costa Rica from 21 September 2015 to 01 October 2015 in order to evaluate the system of official controls for the export of plants for planting to the European Union. Available from file:///C:/Users/tramosa/Downloads/2015-7644%20MR%20Final.pdf



### **Documentation provided to EFSA**

Dossier provided by the Costa Rica National Plant Protection Organisation. July 2016. Submitted by the European Commission (DG SANTE) together with the mandate. The dossier includes

- 1) The main document titled 'Technical and scientific criteria for the exclusion of *Phoenix roebelenii* as a host of *Xylella fastidiosa* in Costa Rica'
- 2) Annex 1 List of sampled producers in 2015
- 3) Annex 2 Results of laboratory tests of sampling of Phoenix roebelenii
- 4) Annex 3 COHORT Bulletin
- 5) Annex 4 Response of the Commercial Attaché of the Embassy of the Kingdom of the Netherlands for Central America to the sampling of *Phoenix roebelenii*
- Annex 5 Work Programme of good phytosanitary practices for production of Phoenix roebelenii

### References

- Aguilar E, Villalobos W, Moreira L, Rodriguez CM, Kitajima EW and Rivera C, 2005. First report of *Xylella fastidiosa* infecting citrus in Costa Rica. Plant Disease, 89, 687.
- Aguilar E, Moreira L and Rivera C, 2008. Confirmation of *Xylella fastidiosa* infecting grapes *Vitis vinifera* in Costa Rica. Tropical Plant Pathology, 33, 444–448.
- Cannon RM, 2002. Demonstrating disease freedom—combining confidence levels. Preventive Veterinary Medicine, 52, 227–249.
- Coletta-Filho HD, Pereira EO, Souza AA, Takita MA, Cristofani-Yale M and Machado MA, 2007. Analysis of resistance to *Xylella fastidiosa* within a hybrid population of Pera sweet orange × Murcott tangor. Plant Pathology, 56, 661–668.
- EFSA (European Food Safety Authority), 2012. A framework to substantiate absence of disease: the risk based estimate of system sensitivity tool (RiBESS) using data collated according to the EFSA Standard Sample Description An example on *Echinococcus multilocularis*. Supporting Publications 2012:EN-366, 44 pp. doi:10.2903/sp.efsa.2012.EN-366
- 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
- EFSA PLH Panel (EFSA Panel on Plant Health), 2015a. Scientific Opinion on the risk to plant health posed by *Xylella fastidiosa* in the EU territory, with the identification and evaluation of risk reduction options. EFSA Journal 2015;13(1):3989, 262 pp. doi:10.2903/j.efsa.2015.3989
- EFSA PLH Panel (EFSA Panel on Plant Health), 2015b. 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
- EFSA Scientific Committee, 2009. Guidance of the Scientific Committee on transparency in the scientific aspects of risk assessments carried out by EFSA. Part 2: general Principles. EFSA Journal 2009;7(5):1051, 22 pp. doi:10.2903/j.efsa.2009.1051
- EFSA Scientific Committee, 2014. Guidance on the structure and content of EFSA's scientific opinions and statements. EFSA Journal 2014;12(9):3808, 10 pp. doi:10.2903/j.efsa.2014.3808
- FAO (Food and Agriculture Organisation of the United Nations), 1995. ISPM (International Standards for Phytosanitary Measures) 4 requirements for the establishment of pest free areas. Produced by the Secretariat of the International Plant Protection Convention (IPPC). Available online: https://www.ippc.int/static/media/files/publication/en/2016/01/ISPM\_04\_1995\_En\_2015-12-22\_PostCPM10\_InkAmReformatted\_oSd1 gog.pdf
- FAO (Food and Agriculture Organisation of the United Nations), 2006. ISPM (International Standards for Phytosanitary Measures) 27 Diagnostic protocols for regulated pests. Produced by the Secretariat of the International Plant Protection Convention (IPPC). Available online: https://www.ippc.int/static/media/files/publication/en/2016/01/ISPM\_27\_2006\_WithoutApp2\_En\_2015-12-22\_PostCPM10\_InkAmReformatted.pdf
- Garcia AL, Torres SCZ, Heredia M and Lopes SA, 2012. Citrus responses to *Xylella fastidiosa* infection. Plant Disease, 96, 1245–1249.
- Garita-Cambronero J, Godoy C, Villalobos W and Rivera C, 2005a. Leafhoppers (Hemiptera: Cicadellidae) as potential vectors of *Xylella fastidiosa* in Costa Rica. Phytopathology, 96, S163.
- Garita-Cambronero J, Godoy C, Villalobos W and Rivera C, 2005b. Population dynamics and ecological relationships of ten leafhopper species (Hemiptera: Cicadelliade), potential vectors of *Xylella fastidiosa* in Costa Rica. Phytopathology, 96, S163.
- Gonzalez V, 2014. Costa Rica citrus annual orange juice production and trade. USDA Report. Available online: http://gain.fas.usda.gov/Recent%20GAIN%20Publications/Citrus%20Annual\_San%20Jose\_Costa%20Rica\_12-16-2014.pdf



- Hartung JS, Beretta J, Brlansky RH, Spisso J and Lee RF, 1994. Citrus variegated chlorosis bacterium: Axenix culture, pathogenicity, and serological relationships with other strains of *Xylella fastidiosa*. Phytopathology, 84, 591–597.
- Hill BL and Purcell AH, 1995. Multiplication and movement of *Xylella fastidiosa* within grapevine and four other plants. Phytopathology, 85, 1368–1372.
- Hill BL and Purcell AH, 1997. Populations of *Xylella fastidosa* in plants required for transmission by an efficient vector. Phytopathology, 87, 1197–1201.
- Hopkins DL, 1989. *Xylella fastidiosa*: xylem-limited bacterial pathogens of plants. Annual Review of Phytopahotlogy, 1989, 271–290.
- Hopkins DL and Purcell AH, 2002. *Xylella fastidiosa*: cause of Pierce's disease of grapevine and other emergent diseases. Plant Disease, 86, 1056–1066.
- Madden LV, Hughes G and van den Bosch F, 2007. *The study of plant disease epidemics*. The American Phytopathological Society, APS Press, St. Paul, MN.
- Martelli GP, 2016. The current status of the quick decline syndrome of olive in southern Italy. Phytoparasitica, 44,
- Montero-Astua M, Saborio-R G, Chacon-Diaz C, Garita L, Villalobos W, Moreira L, Hartung JS and Rivera C, 2008a. First report of *Xylella fastidiosa* in avocado in Costa Rica. Plant Disease, 92, 175.
- Montero-Astua M, Saborio G, Chacon-Diaz C, Villalobos W, Rodriguez CM, Moreira L and Rivera C, 2008b. First report of *Xylella fastidiosa* in oleander in Costa Rica. Plant Disease, 92, 1249.
- Niza B, Coletta-Filho HD, Merfa MV, Takita MA and de Souza AA, 2015. Differential colonization patterns of *Xylella fastidiosa* infecting citrus genotypes. Plant Pathology, 64, 1259–1269.
- Nunney L, Ortiz B, Russell SA, Ruiz Sanchez R and Stouthamer R, 2014. The complex biogeography of the plant pathogen *Xylella fastidiosa*: genetic evidence of introductions and subspecific introgression in Central America. PlosOne, 9, e112463.
- Odio CE, 2009. Citrus in Costa Rica. Presentation at the International Citrus and Beverage Conference (ICBC), 15–18 September 2009, Florida, USA. Available online: https://conference.ifas.ufl.edu/citrus09/Presentations/Wednesday/1040%20Odio.pdf
- Purcell AH and Saunders SR, 1999. Fate of Pierce's disease strains of *Xylella fastidiosa* in common riparian plants in California. Plant Disease, 83, 825–830.
- Purcell AH, Saunders SR, Hendson M, Grebus ME and Henry MJ, 1999. Causal role of *Xylella fastidiosa* in oleander leaf scorch disease. Phytopathology, 89, 53–58.
- Rodriguez CM, Obando JJ, Villalobos W, Moreira L and Rivera C, 2001. First report of *Xylella fastidiosa* infecting coffee in Costa Rica. Plant Disease, 85, 1027.
- Saponari M, Boscia D, Loconsole G, Palmisano F, Savino V, Potere O and Martelli GP, 2014. New hosts of *Xylella fastidiosa* strain CoDiRO in Apulia. Journal of Plant Pathology, 96, 611.
- Wong F, 2005. Update on *Xylella fastidiosa* in landscape plant hosts. CoHort (Outreach and Cooperation, University of California) 7.2 (Winter 2005): 1–3.
- Wong F, Cooksey DA, Costa HS, Downer J, Henry M, Kabashima J, Karlik J, LeStrange M and Shaw D, 2004. Documentation and characterization of *Xylella fastidiosa* strains in landscape hosts. Proceedings of the Pierce's Disease Research Symposium, 7–10 December 2004, Coronado, California. Available online: https://www.cdfa.ca.gov/pdcp/Documents/Proceedings/2004 Proc.pdf

### **Abbreviations**

ELISA enzyme-linked immunosorbent assay NPPO National Plant Protection Organisation

PCR polymerase chain reaction PLH EFSA Plant Health Panel ToR Terms of Reference