SCIENTIFIC OPINION

Effectiveness of *in planta* control measures for *Xylella fastidiosa*

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Abstract

This opinion updates the information included in the previous EFSA Scientific Opinion concerning the *in planta* control measures for *Xylella fastidiosa*, with a systematic review and critical analysis of the potential treatment solutions that have been published against this pest so far. The output of this opinion focuses on the application of chemical or biological treatments on living plants. *In vitro* studies, hot water treatments, use of resistant varieties and vector control are excluded from the review. The use of antibiotics is not considered due to the risk of antimicrobial resistance development. The use of weakly virulent or avirulent strains of *X. fastidiosa* is covered in this review, although this organism is an EU quarantine plant pest and its introduction in the EU territory is banned. Experiments were recently conducted to assess the effect of application of zinc, copper, and citric acid biocomplex, of *N*-acetylcysteine, and of 'diffusible signal factor' (and of its homologs). Their results showed that these control measures were sometimes able to reduce symptoms caused by *X. fastidiosa*. Recent experiments also showed that several species of endophytic microorganisms, some bacteriophages and inoculation of weakly virulent/avirulent strains of *X. fastidiosa* could offer some protection against the Pierce's disease. However, based on the reviewed results, the Panel concludes that, although several published experiments show some effects in reducing symptoms development, the tested control measures are not able to completely eliminate *X. fastidiosa* from diseased plants. The Panel confirms as previously stated that there is currently no control measure available to eliminate the bacteria from a diseased plant in open field conditions.

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1. Introduction

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

This Scientific Opinion for *Xylella fastidiosa* was requested to EFSA by the European Commission DG SANTE, pursuant to Article 29(1) of Regulation (EC) No 178/2002, as per letter to EFSA's Director, dated 22 December 2017 reference ARES(2017)6346828. The opinion has as deadline end of March 2019.

EFSA was requested to 'update the Scientific Opinion on the risks to plant health posed by *Xylella fastidiosa* in the EU territory, published on 6 January 2015. That update should take into account the subspecies and Sequence Types (STs) of *X. fastidiosa* and the susceptible plant species detected so far in the Union territory since the first outbreak notified by Italy in October 2013. The probability of short and long distance spreading and establishment in the rest of the Union territory should be assessed, together with their consequences on the plant species concerned. In addition, based on recent scientific developments, EFSA should identify and evaluate relevant risk reduction options to prevent further spread of those subspecies and STs into the rest of the Union in order to allow, if needed, the update of the EU control measures as laid down under Decision (RU) 2015/789. EFSA should also assess the latency period of those isolates, taking into account the different climatic conditions of the Union territory, with the aim to provide an indication about the minimum number of years needed before lifting the demarcated area after the implementation of the eradication measures.'

1.2. Interpretation of the Terms of Reference

The Terms of Reference (ToR) specify that the requested opinion should update the previous EFSA Scientific Opinion, published on 6 January 2015, addressing establishment, spread and risk reduction options (EFSA PLH Panel, 2015). In the present scientific opinion, the Panel, with regard to the identification and evaluation of risk reduction options, reviews the available measures in scientific literature for the control in the plant (*in planta*) of *X. fastidiosa*. In particular, the Panel updates here the information included in the previous EFSA Scientific Opinion concerning the *in planta* control measures for *X. fastidiosa*, with a review and critical analysis of the potential treatment solutions that have been published against this pest so far.

This output focuses on the application of treatments to control *X. fastidiosa* on living plants (*in planta*) such as the application of chemical or biological treatments. *In vitro* studies are not included because the review is only about the effectiveness on living plants. Physical treatments (such as hot water treatments), agricultural practices (such as pruning), plant breeding (e.g. use of resistant varieties and GM plants) and vectors control are excluded from this review as they are included in the updated pest risk assessment for *X. fastidiosa* (EFSA PLH Panel, 2019). The use of antibiotics is not considered in this review due to the risk of antimicrobial resistance development. The use of weakly virulent or avirulent strains of *X. fastidiosa* is covered in this review, although this organism is an EU quarantine plant pest and its introduction in the EU territory is banned.[1]

2. Data and methodology

The systematic literature review was divided into the three following steps, according to EFSA guidelines (EFSA, 2010):

- Extensive literature search to identify relevant references.
- Study selection of the collected references based on title, abstract and full-text.
- Data extraction of relevant information from the selected references for the assessment of the effectiveness of control measures *in planta* for *X. fastidiosa*.

The review question 'What is the effectiveness of *in planta* control measures against *Xylella fastidiosa*?' was broken down into key elements using the PICO conceptual model (EFSA, 2010):

- Population of interest (P)

The population of interest is that of host plants of *X. fastidiosa*.

- Intervention (I)

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[1] Directive 2000/29/EC Annex I Part A section I.

The intervention is that of *in planta* control measures defined in Section 1.2.

- Comparator (C)

The comparator is that of untreated plants.

- Outcome (O)

The outcome is the level of effectiveness of *in planta* control measures.

2.1. Step 1: Extensive literature search

Two main elements were considered for the extensive literature search: the sources of information to be consulted (Table 1) and the development of the search strategy (Table 2).

2.1.1. Information sources

The defined search strategy was run in all databases listed in Table 1 via the Web of Science (Clarivate Analytics) and Scopus platforms. No limits of time or language were applied in order to retrieve as many references as possible.

Table 1: Sources of information

Database	Time span	Platform
Scopus	inception–present	Scopus
BIOSIS Citation Index	1926–present	Web of Science
CABI: CAB Abstracts®	1973–present	
Chinese Science Citation DatabaseSM	1989–present	
Current Contents Connect	1998–present	
Data Citation Index	1990–present	
FSTA® – the food science resource	1969–present	
KCI-Korean Journal Database	1980–present	
MEDLINE®	1950–present	
Russian Science Citation Index	2005–present	
SciELO Citation Index	1997–present	

Web of Science Core Collection
  • Science Citation Index Expanded
  • Social Sciences Citation Index
  • Arts & Humanities Citation Index
  • Conference Proceedings Citation Index- Science
  • Conference Proceedings Citation Index- Social Science & Humanities
  • Book Citation Index – Science
  • Book Citation Index – Social Sciences & Humanities
  • Emerging Sources Citation Index
  • Current Chemical Reactions
  • Index Chemicus
	1975–present	

Zoological Record	1864–present	

2.1.2. Search terms

The specific search strategies were developed combining controlled vocabulary, when available (CAB Thesaurus terms) and natural vocabulary to represent the concepts in the search strings. The search syntax was adapted to each platform. The search strings are detailed in Table 2 and were run in each platform databases, listed in Table 1, on 27 November 2018 and 7 March 2019.

Table 2: Search strings

Platform	Query	Results
Scopus	(TITLE-ABS-KEY (zinc OR copper OR (citric W/3 acid*) OR acetylcysteine OR nacetylcysteine OR "N Acetyl L cysteine" OR (pathogen* W/3 confusion) OR *phage* OR bacteriophage* OR avirulent OR "weakly virulent" OR benign OR nonpathogenic OR "non pathogenic" OR (biologic* W/3 control*) OR biocontrol*)) AND (TITLE-ABS-KEY (xylella OR "X fastidiosa" OR ((pierce* OR crespera OR phony OR "leaf scorch") W/2 disease*) OR (phony W/2 peach*) OR ((almond OR bacterial OR coffee OR mulberry OR oleander OR pecan OR pear) W/2 "leaf scorch") OR (plum AND "leaf scald") OR (citrus AND "variegated chlorosis") OR "Periwinkle wilt" OR "Ragweed stunt" OR "olive quick decline syndrome"))	135
Web of Science	TS=(Xylella OR "X fastidiosa" OR ((pierce* OR crespera OR phony OR "leaf scorch") NEAR/2 disease*) OR (phony NEAR/2 peach*) OR ((almond OR bacterial OR coffee OR mulberry OR oleander OR pecan OR pear) NEAR/2 "leaf scorch") OR (plum AND "leaf scald") OR (citrus AND "variegated chlorosis") OR "Periwinkle wilt" OR "Ragweed stunt" OR "olive quick decline syndrome") AND TS=(Zinc OR Copper OR (citric NEAR/3 acid*) OR Acetylcysteine OR Nacetylcysteine OR "N Acetyl L cysteine" OR (pathogen* NEAR/3 confusion) OR *phage* OR (biologic* NEXT/3 control*) OR biocontrol* OR avirulent OR "weakly virulent" OR benign OR nonpathogenic OR "non pathogenic")	321

The outputs of the searches, 135 records retrieved in Scopus and 321 in the databases of the Web of Science platform, have been exported into an EndNote X8 library (Clarivate Analytics). Duplicates were removed using automatic and manual detection of duplicates in EndNote X8. One hundred four records were considered as duplicates and removed from the EndNote library, and 352 were considered as unique records. Pierce's disease website (www.piercedisease.org) was consulted to retrieve additional relevant references on *in planta* control measures against *X. fastidiosa*. Three recent project reports (Lindow et al., 2017, 2018b; Rolshausen et al., 2018), thus containing experimental results unlikely published in scientific journals, were considered for the study selection. The Proceedings of the Pierce's Disease Research Symposium were also consulted and two reports were taken into account (Hopkins et al., 2015; Lindow et al., 2018a).

2.2. Step 2: Study selection

The collected references were screened for relevance in two steps:

- Title/abstract screening of all references collected through the extensive literature search.
- Full-text screening of the references that passed the previous step.

Inclusion/exclusion criteria, listed in Tables 3 and 4, were applied to each step and two reviewers screened in parallel all the references.

The first step required the reviewers to reply to two questions, listed in Table 3, considering only title and abstract of the references. The aim of this first step was to select references reporting results of experiments assessing *in planta* control measures for *X. fastidiosa*. A positive answer to both questions was needed to select the reference. If the information provided by title and abstract was not sufficient to answer to both questions, the reference was passed to the following step for further consideration.

Table 3: Inclusion criteria for the title/abstract screening

Question text	Type of answer	Answer text	Inclusion/ exclusion criteria
Is the paper dealing with *in planta* control measures for *X. fastidiosa?*	Only one of the possible alternative answers can be selected	Yes	Included
		No	Excluded
Does the paper report results of experiments with plants in fields or controlled conditions?	Only one of the possible alternative answers can be selected	Yes	Included
		No	Excluded

All the selected publications were submitted to the full-text screening (second step). The reviewers were required to reply to one question, listed in Table 4. Only the references reporting data of effectiveness of control measures were included and considered for the data extraction step.

Table 4: Inclusion criteria for full-text screening

Question text	Type of answer	Answer text	Inclusion/ exclusion criteria
Does the paper report data of effectiveness of *in planta* control measures for *X. fastidiosa*?	Only one of the possible alternative answers can be selected.	Yes	Included
		No	Excluded

2.3. Step 3: Data extraction

The last step of the process was the extraction of informative data reported in the selected references. The extracted information is listed in Table 5. The extracted data are available in the Excel file reported in Annex A.

The selected publications were divided between the two reviewers that worked in sequence: the first reviewer performed the data extraction of the assigned references while the second reviewer conducted the quality check of the extracted information.

Table 5: Data extraction structure

Extracted data	Description
Reference	Full reference
Publication year	Year of the publication
Starting/Ending Year	Starting and ending year of the study, if reported
Location	Place where the study was conducted
Host plant	Plant species
X. fastidiosa subspecies/strain	*X. fastidiosa* subspecies and/or strain used in the study
Control measure	Control measure applied in the study
Plant location	Place where the plants were located: field or controlled conditions
Presence of control	Information on the presence/absence of untreated plants
Initial level of bacteria	Initial amount of bacterial population in the plants
Replicates	Information on the presence and number of replicates in the study
Measures of effectiveness	Definition of measure of effectiveness
Final level of bacteria after treatment	Final amount of bacterial population in the plants
Statistical analysis	Statistical analysis applied
Comment	Additional relevant information

3. Assessment

3.1. Results of the literature review

The extensive literature search was conducted in November 2018 and March 2019 in Scopus and Web of Science platforms and 456 references were obtained. Duplicate entries were removed and 352 references went through the screening for relevance, together with five additional references retrieved in Pierce's disease research symposium proceedings and website.

In the first step, title and abstract screening, 330 references were excluded either because they do not deal with chemical or biological control strategies for *X. fastidiosa* or they do not report results of *in planta* experiments.

The accepted 28 references were subjected to the full-text screening, and 12 references were excluded at this step as they do not report data on the effectiveness of the control measures for *X. fastidiosa*.

Therefore, 16 references (11 retrieved in Scopus and Web of Science platforms and 5 in Pierce's disease website – Appendix A) were accepted for the data extraction step and afterwards considered for the assessment of the effectiveness of *in planta* control measures for *X. fastidiosa*.

3.2. Effectiveness of control measures based on published results

We reviewed the experimental results found in the literature and listed in Table 6 and including chemical and biological measures.

Table 6: List of control measures and references selected by the systematic literature review

Chemical control measure	Plant species	Reference
N-acetylcysteine (NAC)	Sweet orange	Muranaka et al. (2013)
Zinc	Tobacco	Navarrete and De La Fuente (2015)
Dentamet® (zinc, copper, citric acid biocomplex)	Olive trees	Scortichini et al. (2018)
Biological control measure	**Plant species**	**Reference**
DSF, palmitoleic acid, C16-*cis*, macadamia oil, and related DSF homologues	Grapevine	Lindow et al. (2014, 2017, 2018a,b)
Paraburkholderia phytofirmans	Grapevine	Lindow et al. (2017); Lindow et al. (2018a,b); Baccari et al. (2019)
Curtobacterium flaccumfaciens	*Catharanthus roseus*	Lacava et al. (2007)
Endophytic microorganisms (*Pseudomonas fluorescens*, *Achromobacter xyloxosidans* and *Cochliobolus* sp.)	Grapevine	Rolshausen et al. (2018)
Bacteriophages	Grapevine	Das et al. (2015)
Transfer of bacteriophages by insects	Cowpea	Bhowmick et al. (2016)
Biological control using an avirulent strain of *X. fastidiosa*	Grapevine	Hao et al. (2017)
Biological control using weakly virulent strains of *X. fastidiosa*	Grapevine	Hopkins et al. (2005; 2012; 2015)

DSF: diffusible signal factor.

3.2.1. Chemical control measures

N-Acetylcysteine (NAC) is an analogue of cysteine that disrupts disulfide bonds in mucus. This molecule is able to decrease biofilm formation and disrupt mature biofilm of a variety of bacteria. Muranaka et al. (2013) reports the results of several experiments conducted in controlled conditions on sweet orange (*Citrus sinensis*). The objective was to investigate the inhibitory effect of NAC on *X. fastidiosa* strain 9a5c population in sweet orange. NAC was supplied to *X. fastidiosa*-infected plants in hydroponics, fertigation and adsorbed fertiliser (NAC-fertiliser). Experiments using fertigation and NAC-fertiliser were done to simulate a condition closer to that normally used in the field. Statistically significant decreases of both symptoms (disease severity) and bacterial growth rate were observed in plants grown with NAC compared to control, but *X. fastidiosa* bacteria were still present in plants at the end of the experiment. Symptoms returned after treatment stopped in some of the treated plants. Although the reported results showed that NAC had an antibacterial effect against *X. fastidiosa*, it did not demonstrated that this measure provided a full control of the disease. Further studies have been conducted on NAC field application in Brazil on citrus and in Italy on olive by the H2020 projects POnTE and XF-ACTORS (De Souza et al., 2017, 2018; Dongiovanni et al., 2017a,b) but final results are not yet published at the time of writing this Scientific Opinion, and are therefore not included.

Navarrete and De La Fuente (2015) studied the role of zinc ions in the growth and biofilm formation of *X. fastidiosa* subsp. *fastidiosa* in tobacco plants. High levels of certain metals, including zinc, can be deleterious to the growth of some microorganisms. Previous studies (Cobine et al., 2013; De La Fuente et al., 2014) had shown that the higher levels of some ions promoted virulence, whereas others, such as copper and zinc, impede the growth of biofilms. The effect of zinc had been demonstrated in batch culture of the bacterium. In this study, the authors made knock-out mutants of

two genes that regulate zinc metabolism (uptake regulation and efflux) in *X. fastidiosa*. Effects of increasing zinc concentration in the growing media had varying effects on bacterial growth *in vitro*. Tobacco plants (5 plants for each of the mutants, the wild-type and a buffer-only control) were needle-inoculated, and foliar symptoms were rated at the onset of symptoms. Reduced symptom development (% symptomatic leaves per plant) in terms of either leaf-scorching or leaf chlorosis was seen in both mutants compared with the wild-type (*X. fastidiosa* subsp. *fastidiosa*, strain Temecula). Bacterial populations were also monitored, and all plants were positive for the presence of bacteria, though the wild-type populations were generally higher than those of the mutants. These results indicate that zinc concentration levels in the plant may have a role in the establishment and growth of *X. fastidiosa*, but no practical treatment was proposed in this study to increase zinc concentration at a level sufficiently high to affect bacterial growth in the xylem.

Scortichini et al. (2018) reports the results of several experiments on *X. fastidiosa* control in olive trees in southern Italy. Below, we summarise the results of three field experiments conducted by the authors to assess the effectiveness of Dentamet® (zinc, copper and citric acid biocomplex) to control *X. fastidiosa* subsp. *pauca* in olive trees.

1) Field effectiveness of Dentamet®. In this experiment, the disease was monitored during 3 years on 20 treated trees (trees sprayed with Dentamet®) and 20 untreated trees located in two facing blocks (Veglie). The 20% of the trees showed symptoms at the beginning of the trial. The disease severity was assessed for each tree through spring, summer and autumn by counting the total number of wilted twigs and branches through the whole tree canopy. In addition, *X. fastidiosa* DNA concentration was measured in two treated trees (of two different cultivars) and two untreated trees (of the same two cultivars) using quantitative real-time polymerase chain reaction (PCR). During the 3 years of the experiment, a statistically significant decrease of the disease severity was observed in the treated trees compared to the control trees, but *X. fastidiosa* was still present at the end of the experiment in both treated and untreated olive trees. Moreover, in treated trees, the mean number of wilted twigs was higher during the last year of the experiment (between 30 and 60) than during the first year (5–10). In most cases, a statistically significant decrease of the *X. fastidiosa* DNA concentration was observed in treated trees compared to untreated trees. However, the *X. fastidiosa* DNA concentration was measured in a small number of trees (two treated trees in 2016, one treated tree in 2017, and two untreated trees in 2016 and 2017) and the initial *X. fastidiosa* DNA concentration (before starting the treatment) was not clearly reported.

2) Trunk injection of severely diseased olive trees. Ten severely infected olive trees were treated through trunk injection of Dentamet® in spring 2017. Disease severity and *X. fastidiosa* DNA concentrations were measured the same year, and the measurements were compared to those obtained in control trees receiving distilled water injection. Shoot resprouting was observed in treated trees whereas control trees did not show any new vegetation. However, quantitative real-time PCR analyses showed that *X. fastidiosa* was present in several treated trees in July and September 2017.

3) Implementation of an integrated control programme. An on-farm experiment was conducted to evaluate an integrated control programme including sprays of Dentamet® in addition to agronomic techniques (pruning, removal of weeds). This programme was implemented in two farms. In each farm, 10 trees received a Dentamet® spray treatment and control trees (5 per farm) did not receive any treatment. Dentamet®-treated trees had 45% fewer wilted twigs than control trees.

These experiments showed that Dentamet® sprays may lead to a reduction in disease severity compared to untreated trees, but the results did not demonstrate that Dentamet® provided a full control of the disease over the 3 years of the experiment. Some of the results of this study are based on a limited sample size and additional data are thus needed to verify the effectiveness of this disease control measure.

3.2.2. Biological control measures

Xylella fastidiosa was found to be affected by a compound called the 'diffusible signal factor' (DSF), produced by the pathogen itself. Mutants which overproduce DSF adhere more readily, produce more abundant biofilms and, more importantly, seem to lack virulence (Lindow et al., 2014). This gives rise

to the possibility that an external production of DSF could reduce the pathogen movement and symptom development due to *X. fastidiosa*. Lindow et al. (2014) studied the DSF hypothesis by transforming grapevine with genes from either *X. fastidiosa* or *Xanthomonas campestris* pv. *campestris*. These GM grapevines allow to verify the role of DSF and were inoculated with *X. fastidiosa* subsp. *fastidiosa* strain Temecula. A statistically significant reduction in disease susceptibility was observed, as indicated by a reduced number of symptomatic leaves (less than 2) compared to the untransformed controls which had typically 8 symptomatic leaves. This reduced susceptibility was correlated to a detection of DSF in the transformed lines using fluorescent biomarker specific for DSF. Movement along and between xylem vessels of *X. fastidiosa* was shown using a fluorescent-marked strain of the bacterium. A statistically significant increase in the adhesiveness of the bacterium was observed in the DSF-producing plants, and DSF movement from transgenic rootstocks to wild type scions was also documented. Field trials with the transformed lines were conducted with both natural insect-mediated inoculation as well as artificial needle inoculation, and the reduction in disease severity (as measured by the number of symptomatic leaves) was checked here as well. A statistically significant reduction in disease severity was observed in the transformed grapevines compared to the untransformed plants. No disease reduction was observed in scions grafted on the DSF-producing rootstocks in these field trials, indicating that there was not sufficient migration of DSF from a transformed rootstock for disease control. These studies indicate that DSF could reduce symptom development, even under field conditions.

The project report by Lindow et al. (2017) presents the results of three studies addressing the practical issues about how DSF molecules might be applied to plants for disease control. As a project report, the information presented is more sparse, and it has not been peer reviewed. First, the authors identify several DSF-producing transgenic grape varieties. A number of these plants were produced for further testing in field conditions, but these results are not directly relevant since this control measure is specifically excluded. Their results do confirm, however, the ability of DSF to affect disease development. Second, the authors evaluate the efficacy of direct spray applications of palmitoleic acid, C16-*cis* and related DSF homologues to grape in various ways to achieve disease controls. A statistically significant reduction in disease severity was observed after palmitoleic acid and macadamia oil applications (with various adjuvants) in grape (about by half compared to the control). While intact plants were used in this portion of the study, it was not clear if these studies were performed in a greenhouse or in the field. The most promising treatment consisted of spraying palmitoleic acid or macadamia oil soap 2 weeks before inoculation with *X. fastidiosa* (Pierce's disease strain), and monthly applications afterwards. These results are based on a project report and further experiments are needed. In a third study, Lindow et al. (2017) present the results of an experiment assessing the ability of the endophytic bacteria *Paraburkholderia phytofirmans* (PsJN) to control Pierce's disease (see below).

Baccari et al. (2019) reported on studies which used an endophytic bacterium, PsJN, to colonise grapevines. A statistically significant reduction in the severity of Pierce's disease was observed in these studies. These studies were conducted primarily in greenhouse, but in a separate publication Lindow et al. (2018a,b) reported on limited field studies with the grape cultivar Cabernet sauvignon. PsJN was successful in spreading within the grapevine, and was also successful in reducing symptoms of Pierce's disease when co-inoculated with the pathogen. After simultaneous puncture inoculation with PsJN and *X. fastidiosa* (subsp. *fastidiosa* strain Temecula), almost no disease symptoms were observed (up to 16 weeks after inoculation), nor were viable *X. fastidiosa* bacteria recovered at 4 and 8 weeks after inoculation. Treatment with PsJN does not need to take place at the same physical site as inoculation with *X. fastidiosa*, and while pretreatment with PsJN 4 weeks before inoculation with *X. fastidiosa* gave limited protection, treatment with the PsJN 30 days after inoculation with *X. fastidiosa* gave good disease control. Inoculation of grapevines was also conducted by spraying the plants with a suspension of bacteria together with an organosilicon surfactant, which generally gave results similar to puncture inoculating the plants, though some disease symptoms were observed and viable *X. fastidiosa* could be recovered. A field trial (Lindow et al., 2018a,b) with PsJN was conducted in early 2018 and preliminary results of this experiment indicated that good disease control could be obtained with treatment of PsJN by either spray or needle inoculation the month after inoculation with *X. fastidiosa*.

Lacava et al. (2007) tested the efficacy of the endophytic bacterial species *Curtobacterium flaccumfaciens* to reduce symptoms caused by *X. fastidiosa* subsp. *pauca* in the model plant *Catharanthus roseus* (Madagascar periwinkle). *Curtobacterium flaccumfaciens* is the principal endophyte isolated from asymptomatic *Citrus sinensis* plants affected by *Citrus variegated chlorosis* (CVC), the disease caused by *X. fastidiosa* subsp. *pauca,* in citrus plants in South America.

Catharanthus roseus plants were artificially inoculated with bacterial cultures of *X. fastidiosa* and *Curtobacterium flaccumfaciens,* either separately and simultaneously, and maintained in controlled conditions. Plants inoculated with *X. fastidiosa* exhibited a reduced number of flowers and height, and severe symptoms such as stunting, leaf malformation and wilting. Plants inoculated with both *X. fastidiosa* and *Curtobacterium flaccumfaciens* did not show symptoms and the height of the plants did not statistically differ from that of non-inoculated control plants. Those results show that the endophyte *Curtobacterium flaccumfaciens* reduces the symptom severity in the model plant *Catharanthus roseus* plants inoculated with *X. fastidiosa* subsp. *pauca*. Additional studies are required to further explore the potential use of *Curtobacterium flaccumfaciens* against *X. fastidiosa*.

Rolshausen et al. (2018) explored the use of grape endophytic microorganisms as a practical management tool for Pierce's disease. The authors have assembled a collection of microbes and antimicrobial products that possess anti-*X. fastidiosa* properties. They evaluated the ability of three grape endophytic microorganisms to control Pierce's disease on grapevine; two bacteria (*Pseudomonas fluorescens*, *Achromobacter xyloxosidans*) and one fungus (*Cochliobolus* sp.) were considered as potential biocontrol agents. Both disease severity and *X. fastidiosa* titre (bacteria per 2 ng of total DNA) were monitored on untreated and treated plants. Results showed that the disease severity and the quantities of bacteria were lower in treated plants than in the control if the endophytic microorganisms were introduced *in planta* either through vacuum infiltration or needle inoculation, but *X. fastidiosa* was still present at the end of the experiment in the treated plants. This is a project report and the statistical significance of these differences should be further confirmed. No effect was observed in the treated plants if the endophytic microorganisms were applied through foliar spray or drench application. This study did not demonstrate that the introduction of microorganisms provides a full control of the disease.

Bacteria can be infected by specific viruses and *X. fastidiosa* is no exception. The ability of viruses (here called bacteriophages or phages) to infect and lyse the bacterial cell is specific to certain strains of the bacteria as well as the specific bacteriophage. The use of bacteriophages to control plant diseases has been explored and they represent a viable control measures for some bacterial plant pests (Buttimer et al., 2017). Das et al. (2015) used a mixture of four different bacteriophages (Sano, Salvo, Prado and Paz) that can infect *X. fastidiosa* to examine the effect of these bacteriophages on Pierce's disease of grapevine. Several different experiments were conducted to examine the distribution after inoculation of the bacteriophages into healthy grapevines, and these showed limited movement within the grapevine. Grapevine plants were also needle inoculated first with *X. fastidiosa* subsp*. fastidiosa* strain Temecula, and 3 weeks later with the bacteriophages. While the development of some symptoms had already started at week 4, further symptom development did not take place after this in the treated plants. The concentration of bacteriophages (as plaque forming units, PFU per gram tissue) increased 10- to 200-fold compared to those in the plants inoculated with phages only. Concentration (CFU per g tissue) levels of *X. fastidiosa* were 10–1 000 times lower 5 and 9 weeks after the bacteriophage treatment compared to the bacteria-only treated plants. The ability of the bacteriophage cocktail to prophylactically protect the grapevines was also tested by inoculating them first with the bacteriophage cocktail, and then inoculating them with *X. fastidiosa* after 3 weeks. No symptoms developed in the plants that had received the bacteriophage treatment, which also corresponded to a statistically significant reduction in the concentration of *X. fastidiosa* bacteria and to an increase in the concentration of bacteriophages. Isolation of *X. fastidiosa* from plants that had been treated with the bacteriophages showed no resistant mutants, but some resistant mutants could be selected *in vitro*. Twitching ability and movement of these mutants were studied *in vivo*, but they were not able to produce disease symptoms nor were they able to move within the plants. The results from these experiments indicate that bacteriophages could be used therapeutically or prophylactically for treatment of Pierce's disease of grape. The authors point out that *in vitro* experiments had shown these bacteriophages were effective against *X. fastidiosa* subsp. that cause almond (*X. fastidiosa* subsp. *multiplex* (Ahern et al., 2014)), oleander (*X. fastidiosa* subsp. *sandyi* (Ahern et al., 2014)) and coffee leaf scorch. Das et al. (2015) discussed the possibility that the receptors for bacteriophages were related to the pathogenicity of *X. fastidiosa*, which would thus reduce the possibility of development of virulent bacteriophage-resistant strains of the pathogen.

As mentioned above, the phage-based therapy system may be used for the treatment of Pierce's disease. Glassy-winged sharpshooters (GWSS, *Homalodisca vitripennis*), a sap-feeding vector of *X. fastidiosa*, could potentially be used for the transfer of phages to infected plants. Bhowmick et al. (2016) reports the results of an experiment conducted in controlled conditions on phage transfer to cowpea plants. The objective was to assess the ability of GWSS to uptake and transfer a

bacteriophage (Paz) to plants for controlling *X. fastidiosa* subsp*. fastidiosa* (strain Temecula). GWSS were allowed to feed on stems immersed in a phage Paz solution. The acquisition of phages from stems by GWSS and the transmission of phages by GWSS to plants were both monitored. Results show that, while the uptake of phage by the insects is highly efficient when the phage is present in high concentration in plants, there is an apparent dilution effect due to feeding activity resulting in low transfer of phage by the insects to other plants. GWSS fed on cowpea plants harbouring the phage were thus unable to transfer phages to plants efficiently. This study did not demonstrate that the use of phages transferred by GWSS was an efficient control measure of *X. fastidiosa* in grape.

Virulence to grape of *X. fastidiosa* strains originally obtained from grapevines with Pierce's disease varies from avirulent to highly virulent. Weakly virulent strains multiply and move systemically but more slowly in the plant, producing only minor symptoms in the host (Hopkins, 2005; Hao et al., 2017). Several experimental studies were conducted to assess whether an inoculation of avirulent/ weakly avirulent strains of *X. fastidiosa* in grapevines could provide protection against Pierce's disease. Hopkins (2005) evaluated several weakly avirulent strains of *X. fastidiosa* for biological control of Pierce's disease in both greenhouse and vineyards. In greenhouse, the avirulent strains were compared to a highly virulent strain of *X. fastidiosa* (PD92-8) but, in vineyards, natural infection occurring by natural feeding of vectors was used to evaluate biological control of Pierce's disease.

- In the greenhouse tests, each treatment included four grapevine plants inoculated by pin-pricking with weakly virulent strains, highly virulent strain or both. Inoculated plants were observed for symptoms of Pierce's disease every 2 weeks for 6 months after inoculation. Plants that first were inoculated with the weakly virulent strain EB92-1 and then inoculated 2 weeks later with the highly virulent strain PD92-8 did not develop Pierce's disease symptoms during the 6-month test. Plants inoculated with other weakly virulent strains showed symptoms but with a lower severity rate compared to the control.
- Three field trials were carried out to evaluate several weakly avirulent strains.

  - In the first trial, three grapevine plants ('Himrod' hybrid) each were treated with two weakly virulent strains (PD-1 and Syc86-1, inoculated with the pin-pricking technique) and four plants were left as nontreated controls. Grapevines were rated for symptoms and disease severity was rated every 6 months for 2 years. In plants, naturally infected by *X. fastidiosa,* a statistically significant reduction in disease severity was observed in plants that were also inoculated with Syc86-1 but not in plants that were also inoculated with PD-1.
  - The second and third field trials were conducted on cv. Flame Seedless and cv. Cabernet Sauvignon, respectively, to evaluate control provided by six weakly avirulent strains. Six plants of each cultivar were used per treatment. Flame Seedless and Cabernet Sauvignon plants were rated for symptoms every 6 months for 2 and 4 years, respectively. Only strain EB92-1 (strain Syc86-1 was ineffective) provided good control of the disease in both Flame Seedless and Cabernet Sauvignon; its inoculation resulted in lower disease severity and plant mortality (statistically significant) compared to the control even at the end of the experiment, but the disease symptoms were not totally absent in the inoculated plants.

From 2011 to 2015, another field trial was conducted in on grapevine to evaluate *X. fastidiosa* strain EB92-1 for the biocontrol of Pierce's disease. Results confirmed that disease severity and mortality were lower in grapevines (Pinot Noir and Cabernet Sauvignon) inoculated with the strain EB92-1 (Hopkins et al., 2015). Disease incidence and severity reductions were also reported in a long-term field experiment for several cultivars of grapevines inoculated with EB92-1 by Hopkins (2012).

Hao et al. (2017) evaluated the protective effect of the avirulent *X. fastidiosa* strain ΔPD1311. The authors carried out an experiment on grapevines inoculated (i) with the wild-type *X. fastidiosa* Temecula 1 (TM1) alone, (ii) with ΔPD1311 alone, (iii) with both TM1 and ΔPD1311 at the same time, and (iv) with ΔPD1311 2 weeks before TM1. The Pierce's disease severity was rated weekly on 10 plants in each treatment during 24 weeks in a glasshouse, and the experiment was repeated twice. A reduction in disease severity was not statistically significant in plants inoculated with both TM1 and ΔPD1311 at the same time, but a statistically significant reduction was observed in plants inoculated with ΔPD1311 2 weeks before TM1. However, some plants inoculated with ΔPD1311 2 weeks before TM1 showed Pierce's disease symptoms and the wild-type TM1 was detected in these symptomatic plants.

Based on the results listed above, biological control by inoculation of susceptible grapevines with weakly virulent/avirulent strains of *X. fastidiosa* (especially strain EB92-1 and ΔPD1311), appears to

have the potential to reduce severity of Pierce's disease in commercial vineyards. However, this control method is not able to completely eliminate the bacteria, and its implementation in farmers' fields in Europe poses both practical and regulatory issues that still need to be considered. One drawback of this control measure is that recombination between the genotypes present in the EU and any novel genotypes could lead to new pathogen variants and possibly new diseases (EFSA PLH Panel, 2015).

4.   Conclusions

In its previous pest risk assessment published in 2015, the Panel concluded that the effectiveness of the reviewed methods for disease control in planta was negligible for phytosanitary purposes and recommended the continuation and intensification of research activities, among others, on the control of *X. fastidiosa* (EFSA PLH Panel, 2015). In November 2015, EFSA, in collaboration with the European Commission's Directorates-General for Research and Innovation, Agriculture and Rural Development, and Health and Food Safety, organised a workshop in Brussels to identify and analyse the uncertainties and knowledge gaps on *X. fastidiosa* and to discuss priorities for future research on this pathogen in the EU. The workshop conclusions regarding the control of the pathogen in the plant highlighted that some research lines were on-going, but there was not yet an effective control method of the pathogen applicable in the field (EFSA, 2016).

In this opinion, the Panel updates the information included in the previous EFSA Scientific Opinion concerning the *in planta* control measures for *X. fastidiosa*, with a systematic review and critical analysis of the potential chemical or biological treatment solutions in the plant against this pest. This review does not cover the topics of plant breeding, cropping practices, vector control and antibiotics.

The effectiveness of the application of zinc, copper, and citric acid biocomplex to control *X. fastidiosa* (subsp. *pauca*) has been assessed in recent experiments. This control measure may temporarily reduce disease severity in some situations, but some of these studies are based on a limited sample size and additional data are thus needed to verify their effectiveness in reducing the disease. There is no evidence that this treatment could eliminate *X. fastidiosa* in field conditions during a long period of time.

The effectiveness of NAC to control *X. fastidiosa* strain 9a5c was evaluated in controlled experiments; a statistically significant reduction in both symptoms and bacterial growth rate were observed in citrus plants grown with NAC compared to control, but *X. fastidiosa* bacteria were still present in plants at the end of the experiment. Symptoms returned after treatment stopped in some of the treated plants. Experiments assessing the effectiveness of NAC in field conditions are ongoing but the results are not yet published.

Novel techniques that had not been described in the literature preceding the EFSA opinion in 2015 include the effects of DSF and its role in disease development. The presence of this chemical seems to promote clumping and reduces the spread *X. fastidiosa* subsp. *fastidiosa* (strain Temecula) in grape plants. Research are underway to identify microorganisms that may produce this compound and would control the disease. Application of DSF homologues, such as palmitoleic acid and macadamia oil, also reduces the amount of disease but these results are based on a preliminary report and need to be further confirmed.

A microorganism, PsJN, has shown efficacy in reducing populations of *X. fastidiosa* subsp. *fastidiosa* (strain Temecula), and reducing Pierce's disease severity in several grape cultivars. Simultaneous inoculation of both the pathogen and the PsJN produced little or no disease along with no recoverable colonies of *X. fastidiosa*, and control could be achieved with spray inoculation of PsJN, the month after inoculation with *X. fastidiosa*, in both greenhouse and field studies. The mechanism whereby PsJN controls Pierce's disease appears to be different from the pathways that rely on DSF, leading to the possibility that both methods could be combined in disease management.

Experimental results indicate that bacteriophages could be used therapeutically or prophylactically for treatment of Pierce's disease of grape. Although GWSS (a sap-feeding vector of *X. fastidiosa*) could potentially be used for the transfer of phages to infected plants, this study did not demonstrate that the use of phages transferred by GWSS was an efficient control measure of *X. fastidiosa*.

Biological control by inoculation of susceptible grapevines with weakly virulent/avirulent strains of *X. fastidiosa* (especially strain EB92-1 and ΔPD1311), appears to have the potential to reduce severity of Pierce's disease in commercial vineyards. However, this control method is not able to completely eliminate the bacteria, and its implementation in farmers' fields in Europe poses both practical and regulatory issues that still need to be considered. One drawback of this control measure is that

recombination between the genotypes present in the EU and any novel genotypes could lead to new pathogen variants and possibly new diseases (EFSA, 2015).

Based on the reviewed results, the Panel concludes that, although several published experiments show some statistically significant effects in reducing symptom development, the tested control measures are not able to completely eliminate *X. fastidiosa* from diseased plants. The Panel confirms as previously stated that there is currently no control measure available to eliminate the bacteria from a diseased plant in open field conditions.

The Panel wishes to highlight that the scope of this opinion is to present and discuss possible *in planta* control measures retrieved from the scientific literature that may have an effect on diseases caused by *X. fastidiosa*. The control measures mentioned in this opinion have not used materials/ products that have current authorisations for plant protection in the EU. The use of plant protection products in the EU is regulated by Regulation (EC) No 1107/2009[2].

References

Ahern SJ, Das M, Bhowmick TS, Young R and Gonzalez CF, 2014. Characterization of novel virulent broad-host-range phages of *Xylella fastidiosa* and *Xanthomonas*. Journal of Bacteriology, 196, 459–471. https://doi.org/10.1128/JB.01080-13

Baccari C, Antonova E and Lindow S, 2019. Biological control of Pierce's Disease of grape by an endophytic bacterium. Phytopathology, 109, 248–256. https://doi.org/10.1094/phyto-07-18-0245-fi

Bhowmick TS, Das M, Heinz KM, Krauter PC and Gonzalez CF, 2016. Transmission of phage by glassy-winged sharpshooters, a vector of *Xylella fastidiosa*. Bacteriophage, 6, e1218411–e1218411.

Buttimer C, McAuliffe O, Ross RP, Hill C, O'Mahony J and Coffey A, 2017. Bacteriophages and bacterial plant diseases. Frontiers in Microbiology, 8, 34. https://doi.org/10.3389/fmicb.2017.00034

Cobine PA, Cruz LF, Navarrete F, Duncan D, Tygart M and De La Fuente L, 2013. *Xylella fastidiosa* differentially accumulates mineral elements in biofilm and planktonic cells. PLoS ONE, 8, e54936. https://doi.org/10.1371/journal.pone.0054936

Das M, Bhowmick TS, Ahern SJ, Young R and Gonzalez CF, 2015. Control of Pierce's disease by phage. PLoS ONE, 10, e0128902. https://doi.org/10.1371/journal.pone.0128902

De La Fuente L, Navarrete F, Oliver JE, Cruz L and Cobine PA, 2014. CHAPTER 13: The influence of metal elements on virulence in plant-pathogenic bacteria. In: Jonathan EO, Fernando N, Luisa C and De La Leonardo F (eds). Paul AC. Virulence mechanisms of plant-pathogenic bacteria, The American Phytopathological Society. pp. 231–262.

De Souza AA, Takita MA, Picchi SC, Machado MA, Cristofani-Yali M and Coletta-Filho H, 2017. Advances in controlling *Xylella fastidiosa* in the host plants. Book of Abstracts of the European Conference on *Xylella* 2017: findings answers to a global problem. Palma de Mallorca (Spain), 13-15 November 2017, 15 pp.

De Souza AA, Takita M, Machado M, Caserta R, Mitre L, Gòmez-Krapp L, Nascimento C, Rebelatto D, Safady N, Picchi S and Colhetta-Filho H, 2018. Advances and innovative concepts to control *Xylella fastidiosa* colonization in citrus plants. Abstracts of Concurrent Session Presentations at ICPP2018. Phytopathology, 108:S10, S1.242. https://doi.org/10.1094/phyto-108-10-s1.240

Dongiovanni C, Di Carolo M, Fumarola G, Ciniero A, Tauro D, Palmisano F, Silletti MR, Pollastro P, Altamura G, Morelli M, Coletta-Filho H, De Souza AA, Saldarelli P, Boscia D, Saponari M and Faretra F, 2017a. Evaluation of field treatments to reduce the impact of *Xylella fastidiosa* infections in olive trees. Book of Abstracts of the European Conference on *Xylella* 2017: findings answers to a global problem. Palma de Mallorca (Spain), 13–15 November 2017, 16 pp.

Dongiovanni C, Di Carolo M, Fumarola G, Ciniero A, Tauro D, Palmisano F, Silletti MR, Pollastro P, Altamura G, Cavalieri V, Morelli M, Saldarelli P, Boscia D, Saponari M and Faretra F, 2017b. Recenti sperimentazioni per il controllo di *Xylella*. Olivo e olio, 4/2017, 25–29.

EFSA (European Food Safety Authority), 2010. Application of systematic review methodology to food and feed safety assessments to support decision making. EFSA Journal 2010;8(6):1637, 90 pp. https://doi.org/10.2903/j.efsa.2010.1637

EFSA (European Food Safety Authority), 2016. Workshop on *Xylella fastidiosa*: knowledge gaps and research priorities for the EU. EFSA supporting publication 2016:EN-1039, 74 pp.

EFSA PLH Panel (EFSA Panel on Plant Health), 2015. Scientific opinion on the risks 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. https://doi.org/10.2903/j.efsa.2015.3989

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[2] Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. OJ L 309, 24.11.2019, p. 1–50.

EFSA PLH Panel (EFSA Panel on Plant Health), Bragard C, Dehnen-Schmutz K, Di Serio F, Gonthier P, Jacques M-A, Jaques Miret JA, Justesen AF, MacLeod A, Magnusson CS, Milonas P, Navas-Cortes JA, Potting R, Reignault PL, Thulke H-H, Van der Werf W, Vicent Civera A, Yuen J, Zappalà L, Boscia D, Chapman D, Gilioli G, Krugner R, Mastin A, Simonetto A, Spotti Lopes JR, White S, Abrahantes JC, Delbianco A, Maiorano A, Mosbach-Schulz O, Stancanelli G, Guzzo M and Parnell S, 2019. Update of the Scientific Opinion on the risks to plant health posed by *Xylella fastidiosa* in the EU territory. EFSA Journal 2019;17(4):5665. https://doi.org/10.2903/j.efsa.2019.5665

Hao L, Johnson K, Cursino L, Mowery P and Burr TJ, 2017. Characterization of the *Xylella fastidiosa* PD1311 gene mutant and its suppression of Pierce's disease on grapevines. Molecular Plant Pathology, 18, 684–694. https://doi.org/10.1111/mpp.12428

Hopkins DL, 2005. Biological control of Pierce's disease in the vineyard with strains of *Xylella fastidiosa* benign to grapevine. Plant Disease, 89, 1348–1352. https://doi.org/10.1094/pd-89-1348

Hopkins DL, 2012. Long-term control of Pierce's disease in various grape genotypes with a benign strain of *Xylella fastidiosa*. Phytopathology, 102, 55–55.

Hopkins DL, Kirkpatrick B, Rolshausen P and Daugherty M, 2015. Biological control of Pierce's disease of grapevine with a benign strain of *Xylella fastidiosa*. Proceedings of Pierce's Disease Research Symposium, 72–76.

Lacava PT, Li W, Araujo WL, Azevedo JL and Hartung JS, 2007. The endophyte *Curtobacterium flaccumfaciens* reduces symptoms caused by *Xylella fastidiosa* in *Catharanthus roseus*. Journal of Microbiology, 45, 388–393.

Lindow S, Newman K, Chatterjee S, Baccari C, Lavarone AT and Ionescu M, 2014. Production of *Xylella fastidiosa* diffusible signal factor in transgenic grape causes pathogen confusion and reduction in severity of Pierce's disease. Molecular Plant-Microbe Interactions, 27, 244–254. https://doi.org/10.1094/mpmi-07-13-0197-fi

Lindow S, Antonova E and Baccari C, 2017. Comparison and optimization of different methods of different methods to alter DSF-mediated signalling in *Xylella fastidiosa* in plants to achieve Pierce's disease control. Final progress report for CDFA Agreement Number 14-0143-SA. Available online: www.piercedisease.org

Lindow S, Antonova E, Koutsoukis R and Baccari C, 2018a. Biological control of Pierce's disease of grape with an endophytic bacterium. Proceedings of the Pierce's disease Research Symposium, December 17-19, pag. 43-57.

Lindow S, Lowe-Power T and Baccari C, 2018b. Biological control of Pierce's disease of grape with an endophytic bacterium. Interim progress report for CDFA Agreement Number 16-0514-SA. Available online: www.piercedisease.org

Muranaka LS, Giorgiano TE, Takita MA, Forim MR, Silva LFC, Coletta-Filho HD, Machado MA and de Souza AA, 2013. N-Acetylcysteine in agriculture, a novel use for an old molecule: focus on controlling the plant-pathogen *Xylella fastidiosa*. PLoS ONE, 8, e72937. https://doi.org/10.1371/journal.pone.0072937

Navarrete F and De La Fuente L, 2015. Zinc detoxification is required for full virulence and modification of the host leaf lonome by *Xylella fastidiosa*. Molecular Plant-Microbe Interactions, 28, 497–507. https://doi.org/10.1094/mpmi-07-14-0221-r

Rolshausen P, Roper C and Maloney K, 2018. Greenhouse evaluation of grapevine microbial endophytes and fungal natural products for control of Pierce's disease. Final report of CDFA Agreement Number 16-0512-SA. Available online: www.piercedisease.org

Scortichini M, Chen J, De Caroli M, Dalessandro G, Pucci N, Modesti V, L'Aurora A, Petriccione M, Zampella L, Mastrobuoni F, Migoni D, Del Coco L, Girelli CR, Piacente F, Cristella N, Marangi P, Laddomada F, Di Cesare M, Cesari G, Fanizzi FP and Loreti S, 2018. A zinc, copper and citric acid biocomplex shows promise for control of *Xylella fastidiosa* subsp. *pauca* in olive trees in Apulia region (southern Italy). Phytopathologia Mediterranea, 57, 48–72. https://doi.org/10.14601/Phytopathol_Mediterr-21985

Abbreviations

CFU	colony forming unit
CVC	*Citrus variegated chlorosis*
DSF	diffusible signal factor
GWSS	glassy-winged sharpshooters (*Homalodisca vitripennis*)
NAC	*N*-acetylcysteine
PCR	polymerase chain reaction
PsJN	*Paraburkholderia phytofirmans*
ST	Sequence type
ToR	Terms of Reference

Appendix A − List of references selected by the systematic literature review

1. Baccari C, Antonova E and Lindow S, 2019. Biological control of Pierce's Disease of grape by an endophytic bacterium. Phytopathology, 109, 248–256. https://doi.org/10.1094/phyto-07-18-0245-fi

2. Bhowmick TS, Das M, Heinz KM, Krauter PC and Gonzalez CF, 2016. Transmission of phage by glassy-winged sharpshooters, a vector of *Xylella fastidiosa*. Bacteriophage, 6, e1218411–e1218411.

3. Das M, Bhowmick TS, Ahern SJ, Young R and Gonzalez CF, 2015. Control of Pierce's disease by phage. PLos One, 10, e0128902. https://doi.org/10.1371/journal.pone.0128902

4. Hao L, Johnson K, Cursino L, Mowery P and Burr TJ, 2017. Characterization of the *Xylella fastidiosa* PD1311 gene mutant and its suppression of Pierce's disease on grapevines. Molecular Plant Pathology, 18, 684–694. https://doi.org/10.1111/mpp.12428

5. Hopkins DL, 2005. Biological control of Pierce's disease in the vineyard with strains of *Xylella fastidiosa* benign to grapevine. Plant Disease, 89, 1348–1352. https://doi.org/10.1094/pd-89-1348

6. Hopkins DL, 2012. Long-term control of Pierce's disease in various grape genotypes with a benign strain of *Xylella fastidiosa*. Phytopathology, 102, 55–55.

7. Hopkins DL, Kirkpatrick B, Rolshausen P and Daugherty M, 2015. Biological control of Pierce's disease of grapevine with a benign strain of *Xylella fastidiosa*. Proceedings of Pierce's Disease Research Symposium, 72–76.

8. Lacava PT, Li W, Araujo WL, Azevedo JL and Hartung JS, 2007. The endophyte *Curtobacterium flaccumfaciens* reduces symptoms caused by *Xylella fastidiosa* in *Catharanthus roseus*. Journal of Microbiology, 45, 388-393.

9. Lindow S, Newman K, Chatterjee S, Baccari C, Lavarone AT and Ionescu M, 2014. Production of *Xylella fastidiosa* diffusible signal factor in transgenic grape causes pathogen confusion and reduction in severity of Pierce's disease. Molecular Plant-Microbe Interactions, 27, 244–254. https://doi.org/10.1094/mpmi-07-13-0197-fi

10. Lindow S, Antonova E and Baccari C, 2017. Comparison and optimization of different methods of different methods to alter DSF-mediated signalling in *Xylella fastidiosa* in plants to achieve Pierce's disease control. Final progress report for CDFA Agreement Number 14-0143-SA. Available online: www.piercedisease.org

11. Lindow S, Antonova E, Koutsoukis R and Baccari C, 2018a. Biological control of Pierce's disease of grape with an endophytic bacterium. Proceedings of Pierce's Disease Research Symposium, 43–57.

12. Lindow S, Lowe-Power T and Baccari C, 2018b. Biological control of Pierce's disease of grape with an endophytic bacterium. Interim progress report for CDFA Agreement Number 16-0514-SA. Available online: www.piercedisease.org

13. Muranaka LS, Giorgiano TE, Takita MA, Forim MR, Silva LFC, Coletta-Filho HD, Machado MA and de Souza AA, 2013. N-Acetylcysteine in agriculture, a novel use for an old molecule: focus on controlling the plant-pathogen *Xylella fastidiosa*. PLos One, 8, e72937. https://doi.org/10.1371/journal.pone.0072937

14. Navarrete F and De La Fuente L, 2015. Zinc detoxification is required for full virulence and modification of the host leaf lonome by *Xylella fastidiosa*. Molecular Plant-Microbe Interactions, 28, 497–507. https://doi.org/10.1094/mpmi-07-14-0221-r

15. Rolshausen P, Roper C and Maloney K, 2018. Greenhouse evaluation of grapevine microbial endophytes and fungal natural products for control of Pierce's disease. Final report of CDFA Agreement Number 16-0512-SA. Available online: www.piercedisease.org

16. Scortichini M, Chen J, De Caroli M, Dalessandro G, Pucci N, Modesti V, L'Aurora A, Petriccione M, Zampella L, Mastrobuoni F, Migoni D, Del Coco L, Girelli CR, Piacente F, Cristella N, Marangi P, Laddomada F, Di Cesare M, Cesari G, Fanizzi FP and Loreti S, 2018. A zinc, copper and citric acid biocomplex shows promise for control of *Xylella fastidiosa* subsp. *pauca* in olive trees in Apulia region (southern Italy). Phytopathologia Mediterranea, 57, 48–72. https://doi.org/10.14601/phytopathol_mediterr-21985

Annex A – Excel file reporting informative extracted data

Annex A can be found in the online version of this output ('Supporting information' section): https://doi.org/10.2903/j.efsa.2019.5666