STATEMENT

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Susceptibility of *Olea europaea* L. varieties to *Xylella fastidiosa* subsp. *pauca* ST53: systematic literature search up to 24 March 2017

European Food Safety Authority (EFSA)
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Abstract

EFSA was requested by the European Commission to produce a report on the susceptibility of olive varieties to the Apulian strain of *Xylella fastidiosa* (subsp. *pauca* strain CoDiRO, ST53). A systematic literature search identified 21 references providing results of primary research studies on olive plants infected (naturally or artificially) by ST53. From experimental infectivity studies and from surveys in olive orchards, converging lines of evidence indicate tolerance of the Leccino variety to ST53 infections, although no long term observations on yield are available yet. While the variety Leccino can become infected with the pathogen, it develops milder symptoms compared to those observed on susceptible varieties (e.g. Cellina di Nardò, Ogliarola salentina). Also the size of the *X. fastidiosa* bacterial populations measured in Leccino infected plants is lower compared to susceptible olive varieties. Preliminary results show that tolerance or resistance traits can also be found in other olive varieties. New research is now in place in the EU to study the level of susceptibility of many olive varieties to ST53 infections, therefore more relevant results will become available in the coming years.

Keywords: Apulian strain, CoDiRO, cultivar, DistillerSR, olive, resistance, tolerance

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1. Introduction

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

Pursuant to Articles 29 and 31 of Regulation (EC) No 178/2002[2], European Commission DG SANTE requested EFSA for scientific advice and technical assistance in the field of plant health as regards the regulated harmful organism *Xylella fastidiosa* (letter dated 30 June 2016 (ref SANTE/G1/PDR/svi (2016) 3575400). In particular, pursuant to Article 31 of Regulation (EC) No 178/2002, EFSA was requested to further specify and update the host plants database of *X. fastidiosa* currently available[3], taking into account the different *X. fastidiosa* subspecies and strains (with particular reference to the European isolates), with inclusion of information on non-susceptible host plants and varieties and negative results of diagnostic tests where available. EFSA was requested to maintain and update this database periodically and to make new releases available on EFSA website, together with a report. The 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. The request is for the period 2016-2020 and the needs for its continuation will be re-assessed by the end of this period.

With regard to the request to update and maintain the EFSA host plants database of *X. fastidiosa*, EFSA was requested to deliver by the end of March 2017 a preliminary report on the hosts of the Apulian strain of *X. fastidiosa* subspecies *pauca* (letter dated 2 March 2017 (Ref. Ares(2017)1157971 – 02/03/2017)). The report should include information on non-susceptible host plants and varieties and negative results of diagnostic tests when available, as specified in the above mentioned mandate, and with a particular focus on available scientific information on research results regarding tolerant or resistant olive varieties. EFSA was also requested that the complete new release of the EFSA host plants database of *X. fastidiosa*, taking into account scientific and technical literature and the up-to-date validated information provided by MS on the other European outbreaks, is delivered by end September 2017.

1.2. Interpretation of the Terms of Reference

Given the time constraints, this report only focuses on the susceptibility of *Olea europaea* L. varieties[4] to the Apulian strain of the pathogen (*X. fastidiosa* subsp. *pauca* strain CoDiRO, ST53, hereafter ST53). The review of the other plant species will be included in the new report linked to the update of the EFSA host plant database of *Xylella* that will be delivered in September 2017.

1.3. Additional information

In order to obtain the most updated overview on the state of the art of current research activities on the topic in the EU, requests for information were sent via email to:

- XF-ACTORS[5] project coordinator (6 March 2017)
- POnTE[6] project coordinator (6 March 2017)
- Department of agriculture, rural development and environment of Apulian Region (8 March 2017)
- EUPHRESCO[7] network Coordinator (14 March 2017)

[1] Submitted by European Commission, ref. SANTE/G1/DA/as Ares (2017) 1157971

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

[3] EFSA (European Food Safety Authority), 2016. Scientific report on the update of a database of host plants of *Xylella fastidiosa*: 20 November 2015. EFSA Journal 2016;14(2):4378, 40 pp. doi:10.2903/j.efsa.2016.4378

[4] In support to the reader, in this report only the term "variety" has been used, consistently with the terms of reference, however "cultivar" would have been equally acceptable.

[5] http://www.xfactorsproject.eu/

[6] http://www.ponteproject.eu

[7] http://www.euphresco.net/

Feedback was received in form of a joint report from the two Horizon 2020 research projects (Technical Report by POnTE and XF-ACTORS, 2017; 14 March 2017) and email replies (from EUPHRESCO on 20 March, from INIAV, Portugal, on 21 March, and from INRA, France, on 23 March).

2. Data and Methodologies

A systematic literature search was performed for the extraction of data on susceptibility to ST53 in *O. europaea* varieties, in line with the systematic literature review approach proposed in the EFSA guidelines (EFSA, 2010). The methodology was adapted to the scope of the mandate and to its implementation with the DistillerSR software (Evidence Partners, Ottawa, Canada). Furthermore, the forms created in DistillerSR for pre-screening and data extraction were kept generic (i.e. not only suitable for the data extraction on olive varieties and ST53, but more general) in order to permit the application of the same methodology and tool to the complete *Xylella* host plants database, whose update is planned for September 2017.

The process included the following steps (detailed in Appendix A):

1. *Literature search* to collect references on the topic (see section A.1 of Appendix A)
2. *Screening for relevance* to exclude references not suitable to the scope (see section A.2 of Appendix A)
3. *Data extraction* from the selected references (see section A.3 of Appendix A)

All the steps were performed by two reviewers who acted in parallel or in sequence depending on the tasks.

3. Assessment of the susceptibility of *Olea europaea* L. varieties to *Xylella fastidiosa* subsp. *pauca* ST53

3.1. Scope of the assessment

The first outbreak of the quarantine plant pathogen *X. fastidiosa* in the EU was discovered on olive trees (*O. europaea*) showing a massive scorching of leaves followed by wilting of entire branches. From its first discovery in a restricted area near the city of Gallipoli in Lecce province (Apulia region, Italy) the disease spread fast, reaching north up to the provinces of Brindisi and Taranto. In October 2013, the first scientific report on the association between the disease, termed olive quick decline syndrome, and the presence of *X. fastidiosa* was published (Saponari et al., 2013). Since then, the number of plant species found infected (either with or without symptoms) in the Apulian region has been constantly increasing (EFSA, 2015 and 2016; EFSA PLH Panel, 2015) together with the expansion of the infected area. While more host plants of *X. fastidiosa* are continuously reported, the most striking impact of this pathogen is the olive quick decline syndrome. One of the first studies on the biology of *X. fastidiosa* strain CoDiRO[8] and its causal association to the olive quick decline syndrome was funded by EFSA. This pilot project included major Mediterranean crops and some olive varieties and was finalised in March 2016 (Saponari et al., 2016). The experiments and observations initiated during the project are continuing and are now merged in the recent EU-funded projects (see section 3.4).

As *O. europaea* is the most affected crop of the *X. fastidiosa* outbreak in Apulia, a large part of all research activities over the last years focused on epidemiological studies of *X. fastidiosa* in olives and on the vectoring capacity of xylem sap-feeding insects commonly present in the area where infected olive plants are found.

Given the urgency to provide an updated scientific overview on the level of susceptibility of olive varieties to ST53, EFSA conducted a review of literature on this topic.

[8] *Xylella fastidiosa* subsp. *pauca* strain CoDiRO belongs to the single sequence type ST53 and to date is the only sequence type present in the Apulian region (EFSA PLH Panel, 2016).

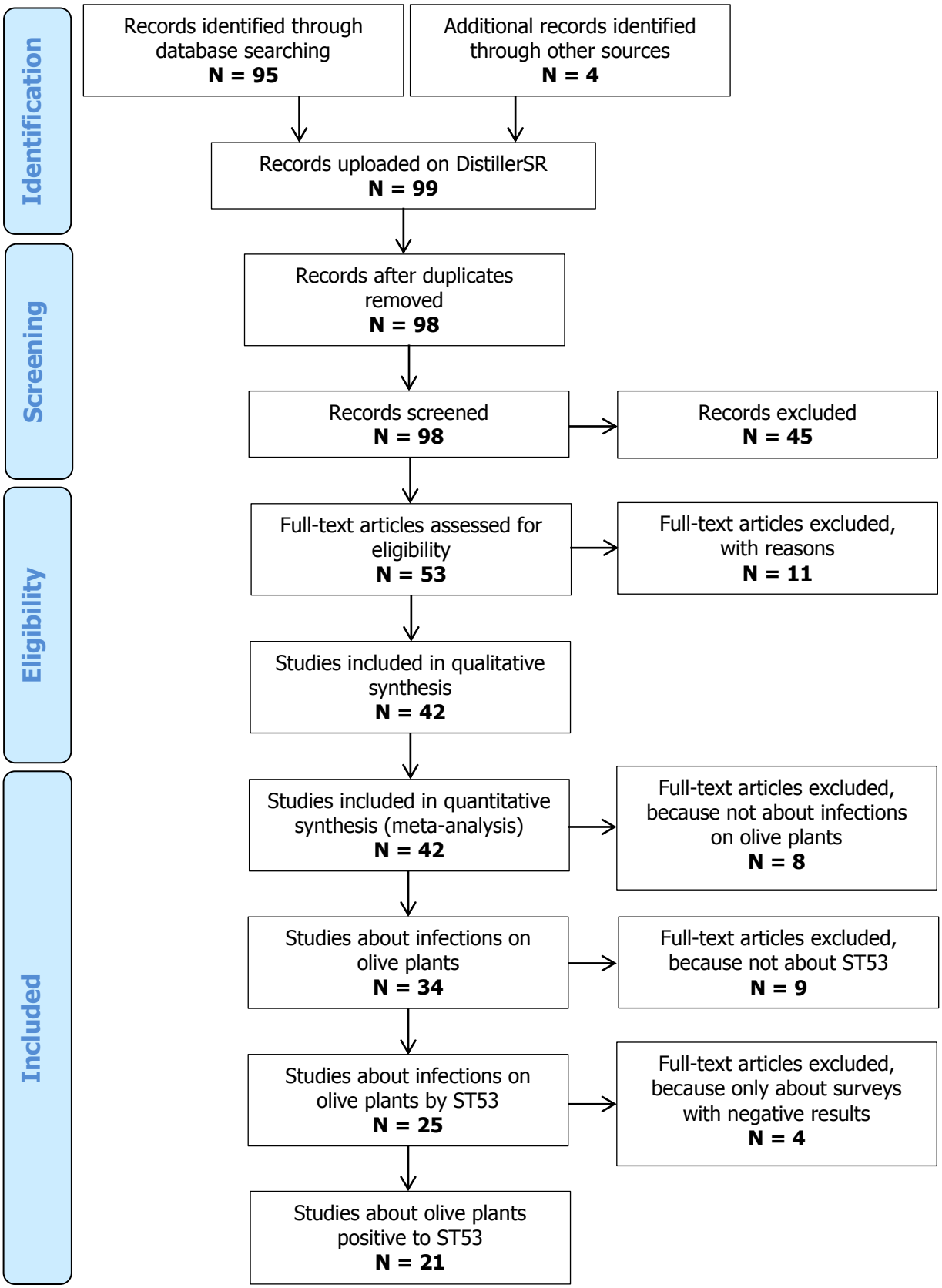

Figure 1: Flow diagram on the data extraction conducted on the DistillerSR tool

3.2. Collected literature

The literature search conducted at the end of January 2017 produced 95 references complemented later by four more scientific articles and reports (section A.1).

During the screening for relevance (section A.2), 46 articles not focusing on *Xylella* and/or not describing primary studies were excluded by title/abstract screening (section A.2.1) while a further 11 reports were removed after full text screening because they were not describing *in vivo* studies (section A.2.2).

After data extraction (section A.3), more references were excluded in order to achieve high precision in the final dataset for this report. In this phase, 8 references were excluded because they did not contain any entry on *O. europaea*, 9 references were excluded because they were not on ST53, and 4 references were excluded because they only described surveys with negative results.

At the end of the selection process (22 March 2017), 21 papers and reports were retained (full list in Appendix B) for a total number of 128 data extraction forms. The first scientific paper on *X. fastidiosa* in Italy was published in 2013 (Saponari et al., 2013) and the most recent article was published in an Italian technical magazine on 23 March 2017 (Boscia et al., 2017). A flow diagram on data extraction is shown in Figure 1 (above).

3.3. Results

Most references (15) provide results on detection of ST53 in open fields and orchards (study category "field"). Three references are exclusively dedicated to experimental trials under controlled conditions (study category "experimental") and three cover both categories.

Six papers describe pathogenicity tests and vector transmission studies (natural inoculation) and two reports focus on mechanical inoculation experiments, both conducted by the CNR (Consiglio Nazionale delle Ricerche) of Bari (Saponari et al., 2016; Technical Report by POnTE and XF-ACTORS, 2017).

Currently, no results are available from long term studies over an extended geographical range comprising multiple seasons, due to the fairly recent occurrence and the complex nature of the problem: such studies on tree pathogens are in fact difficult to execute and require long observation times for meaningful conclusions in particular on the host status.

The selected 21 references provided a list of 28 olive varieties in which ST53 was either detected during surveys or developed infections under experimental conditions. Most studies only focus on a few varieties (mainly on Leccino and Ogliarola salentina, followed by Cellina di Nardò, Coratina, Frantoio, Cima di Melfi, Nocellara and Picholine) representing some of the main olive varieties grown in the infected area (Table 1). While the number of reported susceptible varieties is expected to increase with intensified research (section 3.4), the few studies available also indicate a differential susceptibility of the olive varieties to ST53 (Tables 2 and 3).

Most evidence has been collected by research groups located in Apulia[9]. From their work, the variety Leccino was identified as tolerant to *X. fastidiosa* infections (Saponari et al., 2016). From experimental infectivity studies and from surveys in olive orchards, converging lines of evidence indicate tolerance of the Leccino variety to ST53 infections, although long term production data are not available yet. While the variety Leccino can become infected with the pathogen, it develops milder symptoms compared to those observed on susceptible varieties (e.g. Cellina di Nardò, Ogliarola salentina) (Boscia et al., 2017; Giampetruzzi et al., 2016). Also the size of the ST53 bacterial populations measured in infected plants is shown to be two orders of magnitude lower in Leccino than in the susceptible varieties Cellina di Nardò (Saponari et al., 2016; Technical Report by POnTE and XF-ACTORS, 2017) and Ogliarola salentina (Giampetruzzi et al., 2016; Boscia et al., 2017) (Tables 2 and 3).

Giampetruzzi et al. (2016) also showed that the transcriptome profiles of the two varieties Leccino and Ogliarola salentina upon infection by ST53 were distinctively different, whereas those of the healthy

[9] Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti (DiSSPA), Università degli Studi di Bari (DiSSPA-UNIBA), Aldo Moro
Centro di Ricerca, Sperimentazione e Formazione in Agricoltura – Basile Caramia, Locorotondo, Bari
Istituto per la Protezione Sostenibile delle Piante (IPSP), Consiglio Nazionale delle Ricerche (CNR), Unità Organizzativa di Bari

plants were similar. Such findings are consistent with previous studies performed on *Vitis vinifera* (Choi et al., 2013) or on *Citrus* sp. (Rodrigues et al., 2013). Further investigations are needed to understand the molecular mechanism underlying such varietal differential response of olive to *X. fastidiosa* spp. *pauca* ST53.

Recent observations on olive trees under field conditions identified the olive rootstock FS-17® as an olive genotype with possible resistance traits: in a heavily-infected multivarietal olive orchard, the average size of ST53 bacterial populations in infected FS-17® was only about half the size of ST53 bacterial populations observed in infected Leccino plants grown in the same plot (Boscia et al., 2017) (Table 3).

X. fastidiosa acquisition efficiency by its vectors is thought to be correlated with the bacterial population size within the plants (Hill and Purcell, 1997). Although the lower ST53 bacterial population size observed in tolerant olive varieties could possibly reduce the vector acquisition efficiency, the occurrence of the disease and the detection of the pathogen in infected plants of these varieties show that vector transmission still occurs. Therefore it is important that the growth of tolerant olive varieties is accompanied by the implementation of good agriculture practices and integrated pest management for vectors control.

Table 1: Susceptibility of olive varieties to ST53. Host susceptibility information is based on experimental and survey data

Variety	Study category	Number of data extraction forms including the variety	Reference number(s) (Appendix B)
Arbequina	Experimental	3	[19], [20]
Arbosana	Experimental	3	[19], [20]
Ascolana tenera	Field	1	[20]
Carolea	Field	3	[2], [20]
Cellina di Nardò	Experimental and field	7	[19], [20]
Cima di Melfi	Experimental	4	[19], [20]
Cipressino	Field	1	[20]
Coratina	Experimental	7	[4], [5], [19], [20]
Dolce di Cassano	Field	1	[20]
Frantoio	Experimental	5	[19], [20]
FS17	Field	2	[2], [20]
Gioconda	Field	3	[2], [20]
Kalamata	Field	2	[2], [20]
Koroneiki	Experimental	3	[19], [20]
Leccino	Experimental and field	15	[2], [10], [15], [19], [20]
Maiatica	Field	1	[20]
Nocellara	Field	4	[2], [19], [20]
Nocellara etnea	Field	1	[20]
Nocellara messinese	Field	1	[20]
Nociara	Field	1	[20]
Ogliarola barese	Experimental	1	[20]
Ogliarola salentina	Experimental and field	10	[1], [2], [4], [10], [15], [19], [20]
Oliastro	Field	1	[20]
Pendolino	Field	1	[20]
Peranzana	Field	1	[20]
Picholine	Field	4	[2], [19], [20]
Termite di Bitetto	Field	1	[20]
Toscanina	Field	1	[20]
Seedlings (generic)	Experimental	2	[19]
Not specified	Experimental and field	44	
Total		**134***	

* The total number of forms (see glossary for definition) provided in this table (134) is higher than the one mentioned in section 3.2 (128) because in two cases a single form included four olive varieties and figures were represented in the reference as aggregated.

Table 2: Summary table of ELISA tests (average absorbance OD_{405nm} of positive samples) presented by Boscia et al. (2017) comparing olive varieties' response to *Xylella fastidiosa* ST53 infections observed in different orchards in the infected area.

Location of the survey	FS17	Leccino	Ogliarola salentina
Racale (Lecce province)		0.875 OD_{405nm}	1.677 OD_{405nm}
Sannicola (Lecce province)	0.46 OD_{405nm}	0.67 OD_{405nm}	1.69 OD_{405nm}
Ugento (Lecce province)		0.24 OD_{405nm}	1.40 OD_{405nm}

Table 3: Summary table of qPCR average values provided by different studies comparing olive varieties' response to *Xylella fastidiosa* ST53 infections.

Trial and reference	Cellina di Nardò	Coratina	Frantoio	FS17	Leccino	Ogliarola salentina
Survey in Sannicola (Lecce province), Boscia et al., 2017				5.04×10^4CFU/ml	9.93×10^4CFU/ml	4.51×10^6CFU/ml
Quantification of ST53 population size, Giampetruzzi et al., 2016					3.89×10^4CFU/ml	2.33×10^6CFU/ml
Artificial inoculation in greenhouse, results after 12 months, Saponari et al., 2016(*)	stem: 6.77×10^6CFU/ml leaf petioles: 3.34×10^6CFU/ml	stem: 5.65×10^5CFU/ml leaf petioles: 7.59×10^4CFU/ml	stem: $3,01\times10^5$CFU/ml leaf petioles: 5.18×10^5CFU/ml		stem: 6.38×10^4CFU/ml leaf petioles: 3.52×10^5CFU/ml	
Experimental plot, Technical Report by POnTE and XF-ACTORS, 2017(*)	8.68×10^5CFU/ml	7.41×10^5CFU/ml	$4,95\times10^5$CFU/ml		4.19×10^3CFU/ml	

(*) data in the last two rows were kindly provided by the authors of the reference as personal communication

The responses of olive varieties to *X. fastidiosa* infections are currently further investigated in olive inoculation trials performed in Italy and France by the POnTE and XF-ACTORS projects (Table 4; section 3.4). In the experimental design of these trials, the varieties Leccino and Cellina di Nardò are included as tolerant and susceptible controls (Technical Report by POnTE and XF-ACTORS, 2017).

3.4. Ongoing research activity

Given the gravity of the disease caused by *X. fastidiosa* in olives and the threat it also presents to other crops and other regions in the EU, this pathogen has quickly become a priority research topic in many EU and Mediterranean countries. After the first EFSA funded pilot project (Saponari et al., 2016), two H2020 EU funded projects started in the last 2 years: POnTE and XF-ACTORS. The work plans of both projects allocate major research activities to pathogenicity tests and host range studies for the EU strains of *X. fastidiosa*, with particular focus on ST53.

Based on the pioneering work on *X. fastidiosa* conducted by Apulian researchers, more research groups from Belgium, France, Italy and The Netherlands are applying similar methodologies (e.g.

needle inoculation, vectors transmission) to study the impact of ST53 and other *X. fastidiosa* strains on various host plant species (Table 4).

Table 4: Ongoing and future inoculation trials with *Xylella fastidiosa* strains and different host plants as part of POnTE and/or XF-ACTORS work plans (aggregated information: not all the possible combinations of strain and host species will be tested)

Country	Institution	Consortium	Xylella strain	Host species
Belgium	ILVO	XF-ACTORS	- subsp *fastidiosa* LMG 17159 (ST2) - subsp *multiplex* CFBP 8070 (ST10) - subsp *pauca* CoDiRO (ST53)	*Nerium oleander*, *Prunus domestica* ssp., *Quercus petraea*, *Salix alba*, *Vitis vinifera*
France	ANSES	POnTE	- subsp *pauca* CFBP 8072 (LSV41.03) - subsp *pauca* CoDiRO - CFBP 8402 (LSV 47.29)	*Catharanthus roseus*, *Citrus sinensis*, *Coffea arabica*, *Coffea canephora*, *Medicago sativa*, *Nicotiana tabacum*
France	INRA	POnTE and XF-ACTORS	- subsp. *fastidiosa*, CFBP 7970 - subsp. *multiplex*, CFBP 8416 - subsp. *multiplex*, CFBP 8418 - subsp. *pauca*, CFBP 8402 – ST53 - subsp. *pauca*, CFBP 8072 - subsp. *sandyi*, CFBP 8077	*Citrus clementina*, *Citrus maxima*, *Coffea arabica*, *Nerium oleander*, *Olea europaea*, *Polygala myrtifolia*, *Vitis vinifera*
Italy	IPSP-CNR DiSSPA-UNIBA	POnTE and XF-ACTORS	- subsp. *pauca*, CoDiRO - ST 53 - subsp. *fastidiosa/sandyi* CO33 – ST72 (subsp.)	*Olea europaea*, *Vitis vinifera*
The Netherlands	NVWA	XF-ACTORS	- subsp. *pauca* PD 7202 - ST 53 - subsp. *pauca*, PD 7211 - ST 73	*Catharanthus roseus*, *Coffea* sp., *Nerium oleander*, *Nicotiana tabacum*, *Polygala myrtifolia*, *Prunus avium*, *Prunus domestica*

In France, INRA is currently conducting inoculation trials on olive varieties Aglanglau, Cailletier, Picholine du Gard and Sabine. A first trial was conducted between 2015 and 2016, involving *X. fastidiosa* subsp. *pauca*, CFBP 8402 –ST53 and the variety Cailletier (which is widely cultivated in Corse) but results are not yet available (Technical Report by POnTE and XF-ACTORS, 2017; personal communication from Marie-Agnes Jacques, INRA, 23 March 2017).

In Italy, research conducted by IPSP-CNR DiSSPA-UNIBA is ongoing to investigate the susceptibility of olive varieties to ST53, based on inoculation trials and "sentinel trials" on four new experimental plantations established in the infected area in southern Apulia. The following (more than 60) olive varieties/selections are included in at least one of these experiments: Arbequina o.p., Arbosana, Ascolana tenera, Ayvalik, Barnea, Bella di Spagna, Biancolilla, Canino/caninese, Carolea, Cassanese, Cellina di Nardò, Changlot Real, Changlot × Dolce Agogia, Chemlal de Kabylie, Chetoui, Cipressino, Coratina, Empeltre, Frantoio o.p., Frantoio × Arbosana, Galega, Giarraffa, Gordal Sevillana, Hojiblanca, Itrana, JA5 (*O. europaea* subsp. *europaea* var. *sylvestris*), Kalamon, Koroneiki o.p., Koroneiki × Empeltre, Lastovka, Leccino, Manzanilla de Sevilla, Mastoidis, Megaritiki, Memeçik, Meski, Moraiolo, Morrut, Nocellara del Belice, Nocellara etnea, Nocellara messinese, Nociara, Nolca, Ogliarola barese, Ogliarola salentina, *O. europaea* subsp. *cerasiformis*, Olivastro, Ottobratico, Pasola, Picholine, Picholine marrocaine, Picula, Picudo, PicualxJeac9, MSAC43 (*O. europaea* subsp. *europaea* var. *sylvestris*), OLM28 (*O. europaea* subsp. *europaea* var. *sylvestris*), OLM40 (*O. europaea* subsp. *europaea* var. *sylvestris*), Pendolino, Roggianella, San Agostino, Simone, Termite di Bitetto, TN2 (*O. europaea* subsp. *guanchica*), Urano, TN10 (*O. europaea* subsp. *guanchica*), Toffahi, Uovo di Piccione, Uslu.

In addition more than 200 olive varieties have been grafted in 2016 on infected olive trees to observe the grafts responses to the ST53 infection (Boscia et al., 2017).

Conclusions

EFSA was requested by the European Commission to produce a report on the susceptibility of olive varieties to the Apulian strain of *X. fastidiosa* subsp. *pauca* strain CoDiRO, ST53. A systematic literature search identified 21 references providing results of primary research studies on olive plants susceptibility to ST53.

The main conclusions that can be drawn at this phase are that:

- Given the recent introduction of *X. fastidiosa* into the EU, up to now only limited information on olive varieties tolerant or resistant to *X. fastidiosa* subsp. *pauca* ST53 is available.
- For the same reason, there are no studies yet to assess the impact of *X. fastidiosa* subsp. *pauca* ST53 on yield and quality of olive fruits of the tolerant olive varieties.
- From experimental infectivity studies and from surveys in olive orchards, converging lines of evidence indicate tolerance of the olive variety Leccino to *X. fastidiosa* subsp. *pauca* ST53 infections. While the variety Leccino can become infected with the pathogen, it develops milder symptoms compared to those observed on susceptible varieties (e.g. Cellina di Nardò, Ogliarola salentina). Also the size of the *X. fastidiosa* subsp. *pauca* ST53 bacterial populations measured in Leccino infected plants is lower compared to susceptible olive varieties.
- Preliminary results show that tolerance or resistance traits can also be found in other olive varieties. In observations conducted in a single heavily-infected multivarietal olive orchard, the average size of *X. fastidiosa* subsp. *pauca* ST53 bacterial populations in infected plants of the olive rootstock variety FS-17® was shown to be only about half the size of bacterial populations in infected Leccino plants grown in the same plot.
- The occurrence of the disease and the detection of the pathogen in infected plants of these varieties show that the vector transmission still occurs. It is therefore important that the growth of tolerant olive varieties is accompanied by the implementation of good agriculture practices and integrated pest management for vectors control.
- New research from the Horizon 2020 research projects POnTE and XF-ACTORS and nationally funded projects are now in place to study the level of susceptibility of many olive varieties to *X. fastidiosa* subsp. *pauca* ST53 infections. It is expected that additional evidence of available sources of resistance will be produced in the coming years from these enhanced research efforts.

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Glossary and Abbreviations

CoDiRO: Complesso del Disseccamento Rapido dell'Olivo (italian translation of the OQDS)

DistillerSR: Systematic review software (Evidence Partners, Ottawa, Canada) used for literature data extraction.

Form: In this statement, the term refers to the data collection form used in DistillerSR. It includes information collected from the bibliographic references and represents a unique combination of plant species (and variety, if present), infection determination, location and *Xylella* identification.

MLST: Multi Locus Sequence Typing

Susceptibility: Susceptibility is the sum total of the qualities which make a plant a fit host for the pathogen (Robinson, 1969).

Systematic literature review: A systematic literature review is a type of literature review of existing evidence pertinent to a clearly formulated question, which uses pre-specified and standardised methods to identify and critically appraise relevant research, and to collect, report and analyse data from the studies that are included in the review (EFSA, 2010).

Resistance: Resistance is a term used to describe a host plant response to infection. This strategy inhibits or limits the infection (Roy and Kirchner, 2000), by preventing the infection, or limiting subsequent pathogen growth and development within the host through avoidance or clearance of infection (Miller et al., 2005).

Tolerance: Tolerance is a term used to describe a host plant response to infection. In a tolerant plant the infection is not inhibited, but its negative fitness consequences are reduced or offset (Roy and Kirchner, 2000) by reducing the additional mortality due to infection or restoring the reproductive ability (fecundity) of infected individuals (Horns and Hood, 2012).

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Appendix A – Systematic literature search methodology

A.1. Literature search

The review question (i.e "which olive variety is susceptible/tolerant to *Xylella fastidiosa* ST53?") was broken down into key elements using the PECO conceptual model (EFSA, 2010), being P (Population of interest) the population of *Olea europaea* varieties in the Apulia region, E (Exposure) the exposure to infection by *X. fastidiosa*, C (Comparator) the control/not-infected plants, and O (Outcomes/Condition of interest) the plant infection or susceptibility. No more details were added at this phase in order to avoid excluding informative papers.

During the search process two main elements were considered: the sources of information (databases) to be consulted (Table A.1) and the development of search strategies (Table A.2).

Table A.1: Sources of information

Database	Time coverage	Platform
Web of Science Core Collection	1975-present	Web of Science
CABI: CAB Abstracts	1910-present	Web of Science
BIOSIS Citation Index	1926–present	Web of Science
Chinese Science Citation Database	1989–present	Web of Science
Current Contents Connect	1998–present	Web of Science
Data Citation Index	1900–present	Web of Science
FSTA	1969–present	Web of Science
KCI-Korean Journal Database	1980-present	Web of Science
Russian Science Citation Index	2005-present	Web of Science
MEDLINE	1950–present	Web of Science
SciELO Citation Index	1997–present	Web of Science
Zoological Record	1864–present	Web of Science
Scopus	1960 - present	Elsevier
Pubmed	1946-present	NCBI

The search strategies were designed combining separated searches (splitting) on the two main aspects relevant to the scope: pathogen and host OR pathogen and location (i.e. exposure (E) and population of interest (P)).

The defined search strategies (Table A.2) were run in all the selected information sources (Table A.1) on 27 January 2017. No language, date or document type restrictions were applied in the search strings.

Table A.2: Search strings used in all the selected sources of information

Search strings	Databases		
	Web of Science	Scopus	PubMed
"Xylella" AND "oliv*"	85	44	16
"Xylella" AND "ital*"	113	46	22
"Xylella" AND "Apulia"	40	22	5
"Xylella" AND "Puglia"	25	2	0
"CoDiRO" OR "ST53"	30	14	5
"Olive quick decline syndrome"	30	17	7

The collected records were downloaded and imported into EndNote® X8 bibliographic management software. Duplicate entries were removed by the same software, leaving 95 final records which were uploaded on DistillerSR together with the full texts in portable document format (pdf).

Four additional references were included at a later stage because i) they were published after the 27 January 2017 (3 titles), or ii) they were unpublished reports (1 title).

Finally, one reference was excluded by the Duplicate Detection feature of DistillerSR (Figure 1).

A.2. Screening for relevance

The collected references were screened for relevance in two sequential steps:

1. Title/abstract screening of all the references
2. Full text screening of those references which were not excluded during the previous step

Specific inclusion/exclusion criteria were applied at each step (Tables A.3 and A.4 respectively).

The two reviewers worked in parallel: whenever the software identified a discrepant reply by the two reviewers on a given reference, the review process was stopped until agreement was reached.

A.2.1. Title/abstract screening

The first step required the reviewers to reply to two questions (Table A.3) having considered the title and abstract (if available) of the reference but not the full text. The aim of this step was to guarantee the inclusion in the data extraction only of papers presenting original research data (i.e. primary research studies) on *Xylella*. Literature reviews on the topic were therefore excluded.

Table A.3: Inclusion criteria for the title /abstract screening.

Question text	Type of answer	Answer text	Exclusion criteria
Is *Xylella* the topic of the study?	Only one of the possible alternative answers can be selected	Yes	Included
		No	Excluded
Is it a primary research study?	Only one of the possible alternative answers can be selected	Yes	Included
		No	Excluded

A negative reply to one of the two questions, if confirmed by the second reviewer, excluded the reference. In case the information provided in title/abstract was insufficiently clear, the reviewers accepted the reference and passed it to the full text screening for further consideration.

A.2.2. Full text screening

All papers which passed the title/abstract screening were subjected to the full text screening. This step required the reviewers to reply to three questions (Table A.4): the first two having a descriptive scope (neutral) and the third question having an inclusion/exclusion role. The descriptive questions were added at this phase in order to collect information about the type of reference but not to exclude a reference. For example, not only peer-reviewed journals were accepted, since some of the recent findings on *X. fastidiosa* could first be presented in national technical magazines or reports. Regarding the third question, only papers describing infections of the pathogen observed in the plant (i.e. *in vivo*) were retained and brought to the data extraction phase. All studies describing *in vitro* results were therefore excluded.

Table A.4: Inclusion criteria for the full text screening.

Question text	Type of answer	Answer text	Exclusion criteria
Is an English abstract present?	Only one of the possible alternative answers can be selected	Yes	Neutral
		No	Neutral
Which is the type of the publication?	Only one of the possible alternative answers can be selected	Peer-reviewed article	Neutral
		Article	Neutral
		Book	Neutral
		Conference proceedings	Neutral
		Abstract	Neutral
		Technical papers / Report	Neutral
		Genbank / MLST database / any sequence database	Neutral
		Other	Neutral
Is *Xylella* presence	Only one of the	Yes	Included

investigated *in vivo* in the plant?	possible alternative answers can be selected	No	Excluded

A.3. Data extraction

For each reference one or more data extraction forms were filled in to extract all data reported in the study. Each form represents a unique combination of plant species (and variety, if present), infection determination, location and *Xylella* identification. A form can only include more than one plant variety or more than one *Xylella* strain when aggregated data were used (see footnote of Table 1 in the statement).

The two reviewers worked in sequence: one reviewer performed the data extraction and the second conducted assessment and quality control of the filled forms.

A.3.1. Data extraction form

Botanical identification of the plant
Plant family
Plant species (latin name)
Cultivar/variety

Infection determination
Experimental/Survey
Year

Location
Country of origin of the plant
Location: name of the site/area
Latitude: in WGS84, in decimal format
Longitude: in WGS84, in decimal format

***Xylella* identification**
Species
Subspecies
Strain/ MLST
Isolate

Methods applied
Description of method/s used.

Possible selection of one or more of the following techniques:

- Observation of symptoms
- Culture method
- Pathogenicity test with mechanical inoculation
- Pathogenicity tests with vectors
- Optical microscope
- Fluorescence microscope
- Electron microscopy tests
- Immunoblotting (IB)
- Immunofluorescence method
- Enzyme-linked immunosorbent assay (ELISA)
- Polymerase chain reaction (PCR)
- Molecular markers
- Real time PCR
- Quantitative PCR (qPCR)
- Sequence analysis
- Southern blot

- Northern blot
- Western blot

Observation of Xylella movement into the plant
Localized / Systemic (if reported)

Indication about the Xylella resistance status of the plant
Tolerant / Resistant (if reported)

Comments

Appendix B – List of references retained by systematic literature search

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