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FINAL REPORT OF AN AUDIT
CARRIED OUT IN
ITALY
FROM 10 TO 14 FEBRUARY 2014
IN ORDER TO EVALUATE THE SITUATION AND OFFICIAL CONTROLS FOR XYLELLA
FASTIDIOSA.

Executive Summary

This report describes the outcome of an audit carried out by the Food and Veterinary Office (FVO) in Italy, from 10 to 14 February 2014.

The objective of the audit was to evaluate the situation and official controls for Xylella fastidiosa (Well and Raju) (hereafter "Xf"). This organism is listed as a harmful organism in Annex I, Section A, Part I of Council Directive 2000/29/EC, which means that it is not present in the EU and if found, Member States must eradicate it, or if that is impossible, inhibit its spread. It was identified in the Lecce province in the Puglia region of Italy in October 2013. As part of a complex of harmful organisms it has caused devastating die back in olive groves over a substantial area in Lecce. In view of the seriousness of this organism and the potential risk to the EU, this audit was added to the FVOs planned 2014 audit programme.

The audit found that, the competent authorities have taken significant steps since the finding of a new strain of Xf (Salento strain) in Lecce province, in October 2013. Based on regional legislation, adopted in 2013, measures are in place establishing conditions for the production and movement of plants for planting in nurseries located in Lecce province.

An extensive survey activity is still being carried out in order to delimit the spread of the disease in the province and to define infected and buffer zones. However, significant parts of the survey were not carried out in the most favourable time of the year. The survey is planned to be concluded by the end of March 2014.

No eradication or containment measures have been taken and the disease has spread very rapidly. Diseased trees are left in place, acting as a reservoir of infection. Unless action is taken, further rapid spread of the disease must therefore be anticipated.

The ELISA test for plant species other than olive is not yet fully reliable. In addition, the testing of dormant woody material (e.g. Vitis) during the winter and the limited sample sizes used also affect the reliability of the testing. In these circumstances, there is a risk of obtaining false negative results. Until this is addressed the authorities cannot say for sure that plants listed in the annexes of Decision 2014/87/EU are actually free from Xf prior to permitting their movement within the EU. This represents a potential risk of spreading the organism to other parts of Italy and to other Member States.

Although research work has been carried out and is continuing, key factors regarding the epidemiology of Xf remain to be clarified.

The report makes a number of recommendations to the competent authorities, aimed at rectifying the shortcomings identified and enhancing the implementation of control measures.

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ABBREVIATIONS AND DEFINITIONS USED IN THIS REPORT

Abbreviation	Explanation
ARECBC	Agriculture Research and Experimental Centre Basile Caramia
CNR	Research National Centre
ELISA	Enzyme-linked immunosorbent assay
EPPO	European Plant and Mediterranean Plant Protection Organisation
EU	European Union
FVO	Food and Veterinary Office
GPS	Global Positioning System
ha	Hectare
IAMB	Mediterranean Agronomic Institute of Bari
ISTAT	Italian National Institute of Statistics
IVV	Institute of Plant Virology
km	Kilometre
LOD	Level of detection
MIPAAF	Ministry of Agriculture, Foodstuffs and Forest Policies
PAO	Provincial Agricultural Offices
PCR	Polymerase chain reaction
QDSO	Quick decline syndrome of olive
RPS	Regional Plant Health Services
SA	Single Authority
t	Tonne
UB	University of Bari
UF	University of Foggia
<i>Xf</i>	<i>Xylella fastidiosa</i>

1 INTRODUCTION

This audit took place in Italy from 10 to 14 February 2014. It was added to the 2014 Food and Veterinary Office's (FVO) planned audit programme in response to the emergency situation in Italy.

The FVO team consisted of two auditors from the FVO and one expert from a European Union (EU) Member State. Representatives from the Single Authority (SA), Central Plant Health Service of the Ministry of Agriculture, Foodstuffs and Forest Policies (MIPAAF), and the Regional Competent Authority from Puglia accompanied the FVO team during the audit.

An opening meeting was held on 10 February at the headquarters of the Regional Plant Health Services (RPS) in Bari during which the objective, scope and itinerary for the audit were confirmed by the FVO team and additional information, necessary for the conduct of the audit, was requested.

2 OBJECTIVES

The objective of the audit is to evaluate the plant health situation and control measures applied for *Xylella fastidiosa* (Well and Raju)

The following table lists the sites visited and meetings held in order to achieve these objectives:

Meetings/visits		No.	Comments
	Regional	1	RPS of Puglia. During the meeting representatives of the SA were present.
	Laboratories	1	Laboratory of the Institute of Plant Virology – CNR/UB.
	Research	1	Meeting with researchers of Puglia region, who are involved in the research activity related to <i>Xf</i> . The meeting was organised by the RPS of Puglia.
Plant health control sites			
Infected olive fruit production sites		6	Copertino and Gallipoli
Places of production of plants for planting		3	Lecce province
Garden centre		1	Lecce province

3 LEGAL BASIS

The audit was carried out under the general provisions of EU legislation, in particular Articles 21 and 27a of Council Directive 2000/29/EC.

4 BACKGROUND

In October 2013, the Italian plant health authorities started an investigation into the cause of the 'Quick decline syndrome of olive' (QDSO) which, according to Italian sources, had become an increasing problem in the province of Lecce (region of Puglia) since 2010. The investigations confirmed the presence of *Xf*, a regulated harmful organism in the European Union, listed in Annex I, Part A, Section I to Council Directive 2000/29/EC, in an area of approximately 8,000 ha in the province of Lecce. Of this area 1,000 ha of olive orchards is severely affected, leading in most cases to the death of trees.

Xf has a wide host range (more than 150 species) some of which are crops of economic importance.

At least four *Xf* subspecies are known, infecting different hosts: *X. fastidiosa* subsp. *fastidiosa* is found in grapevines (*Vitis*), citrus, coffee and almond; *X. fastidiosa* subsp. *sandy* is found in oleander; *X. fastidiosa* subsp. *multiplex* is found in almond, peach, plum, oak, blueberry, pecan, etc.; *X. fastidiosa* subsp. *pauca* is found in citrus and coffee.

The bacteria is transmitted by xylem-sap-feeding sharpshooter leafhoppers (Hemiptera: *Cicadellidae*) and spittlebugs (Hemiptera: *Cercopidae*).

There is consensus among the competent authorities and researchers that a new strain of *Xf* (Salento strain) is involved in the QDSO, together with some fungi and one insect.

The RPS stated that the fungi and the leopard moth are widespread in the Puglia region however, the presence of *Xf* triggers the death of the olive trees. Taking into account the large dispersion of the symptomatic trees in one year, the RPS could not preclude the possibility that other spreading mechanisms are involved and not only the insect vectors, but this possibility needs to be investigated.

The audit team visited one vineyard and two citrus orchards in the middle of infected olive orchards. The RPS stated that the three orchards had been sampled and the results were negative. Some almond trees were present on some of the visited sites and the RPS stated that some of trees had *Xf* positive results while other trees had negative results. Most of the research work to identify the possible *Xf* host plants in the local flora, will be carried out in the area, from Spring 2014 onwards. Plants will also be brought here to assess whether they are host plants.

Detailed information on *Xf* can be obtained from the European and Mediterranean Plant Protection Organisation (EPPO) website:

https://www.eppo.int/QUARANTINE/special_topics/Xylella_fastidiosa/Xylella_fastidiosa.htm

Information can also be obtained in the '*Statement of EFSA on host plants, entry and spread pathways and risk reduction options for Xylella fastidiosa Wells et al.*' which is available on the EFSA website:

<http://www.efsa.europa.eu/en/efsajournal/pub/3468.htm>

According to the official data for 2013 (source ISTAT – Italian National Institute of Statistics), Lecce province has an area of olive production of 92,500 ha (about 7% of the total Italian surface) and the total production is 322,000 t. In Lecce province, there are 67,738 olive farms with an average area of 1.48 ha.

The RPS stated that the total suspected infected area is around 8,000 ha with around 600,000 olive trees. In this area, all the olive trees show symptoms and therefore the level of infection is to be considered 100%. There is a core area of 1,000 ha where the level of mortality is extremely high. From 2012 to 2013, an extensive sudden death of olive trees took place and an 'explosion' of the disease from a relatively small area to 8,000 ha, occurred. In the area there are very old trees many of them of more than 200 years old and even 500 years old. The decay symptoms and consequent mortality, is more prevalent in old trees. The young trees show fewer symptoms and the RPS explained that the fungi have had no time to affect the young trees. The symptoms start being more obvious by the end of April when the impact of the water supply of the tree (water stress) is more important.

The effects on olive yields are also dramatic. The audit team visited one organic olive producer located in the core infected area. The farm has 5 ha and most of the trees, of the two main regional varieties, were between 200 and 300 years old. The owner informed the audit team that symptoms started to appear two to two and half years ago. The olive production was 5,000 kg in 2011, 2,000 kg in 2012 and as little as 400 kg in 2013. Depending on the variety, the average yield per tree

before the outbreak was around 80 kg. Most of the trees observed were virtually dead with only few shoots growing.

In total there are 120 registered nurseries in Lecce province producing plants for planting (many produce several types) of which, 12 produce vegetable plants, 12 citrus plants, 34 fruit trees, 74 ornamental plants, 18 forestry trees and 51 *Vitis*. Eight nurseries also sell plants directly to final consumers.

Regarding host plants that cannot be moved outside Lecce province as required by Deliberation 521, there are 65 nurseries which had in stock 99,740 olive plants, 6 nurseries 141 almond trees and 26 oleander nurseries stocking 37,171 plants.

The 51 *Vitis* nurseries occupy a total area of 172 ha producing on average 9 million plants per year. However in 2013/2014, the production went down to 6,700,000 plants, due to unfavourable weather conditions and only 40 nurseries traded *Vitis* plants. This represents 10% of the Italian production of *Vitis* plants for planting.

5 FINDINGS AND CONCLUSIONS

5.1 ORGANISATIONAL ASPECTS OF PLANT HEALTH CONTROLS

Legal requirements

Article 1(4) of Council Directive 2000/29/EC provides that Member States shall ensure a close, rapid, immediate and effective cooperation between themselves and the Commission in relation to matters covered by this Directive and that, to this end, each Member State shall establish or designate a Single Authority, which shall be responsible, at least, for the coordination and contact in relation to such matters.

Article 2(1)(g) of Council Directive 2000/29/EC requires that the responsible official bodies in a Member State shall either be the official plant protection organisation established under the IPPC, or any other State authority established at national level or at regional level, under the supervision of the national authorities. Article 2(1)(i) of the same Directive requires Member States to ensure that their public servants and qualified agents have the qualifications necessary for the proper application of the Directive.

Findings

5.1.1 Competent authorities

The SA stated that there have been no changes made to the organisational aspects of plant health controls in Italy, described in the FVO country profile for Italy (http://ec.europa.eu/food/fvo/last5_en.cfm?co_id=IT), and in the reports of previous FVO audits, in particular that of a general audit carried out in Italy from 1 to 12 March 2010 (Ref: DG(SANCO) 2010/8601). In summary:

- The SA, within the meaning of Article 1(4) of Council Directive 2000/29/EC is the Central Plant Health Service of the Ministry of Agriculture, Foodstuffs and Forest Policies (MIPAAF);
- The Regional Plant Health Services (RPS), which are usually part of the Agriculture Department of each Region, are responsible for the implementation of plant health controls within their region.

Meetings of the National Plant Health Committee, which is chaired by MIPAAF, are held at least once a month. This body provides the main platform for the co-ordination between the MIPAAF and RPSs. It allows for the consultation of new legislative drafts before being forwarded to the

State-Region Conference as well as the exchange of information on various topics related to plant health.

The RPS of Puglia is composed of Provincial Agricultural Offices (PAO), where the plant health inspectors are based. At the moment, only the PAO of Lecce province is involved in the implementation of the official *Xf* control measures.

Due to the enormous tasks to be carried out in Lecce province especially those related to surveys, the RPS has established agreements with a number of private and non-governmental organisations (plant protection consortia). The financial support for the agreements was provided by the MIPAAF. After a specific training, the consortia technicians become plant health agents nominated by MIPAAF. They are not entitled to issue official acts but they are authorised to collect official samples. Currently they give support to the inspectors in the conduct of survey and are supervised by them. The PAO Lecce province has 39 staff (14 inspectors and 25 agents) involved in the *Xf* related activities.

5.1.2 Guidelines and training

The RPS had issued guidelines and technical information for staff involved in the controls of *Xf*. Regular training events were organised. During the visits, the FVO team noted that all of the staff met during the audit were fully familiar with the harmful organism and the requirements of the regional legislation (see section 5.2. below). The guidelines are continuously updated in light of new developments.

5.1.3 Communication with stakeholders

The SA and RPS stated that there is wide publicity about *Xf* and they had issued a wide range of general and technical information to stakeholders. Information on *Xf* aimed at farmers, technicians and public officials is provided in several ways: specific meetings, plant protection bulletins and information through the media (television and local newspapers). A leaflet on the topic is currently being prepared.

During the audit, the FVO team met with plant producers and traders and noted that they were fully familiar with the regional legislation as well as the symptoms and control measures for *Xf*. Many of these stakeholders had participated in several meetings where the situation and control strategy for *Xf* had been discussed (see section 5.4 below). There is widespread coverage by the regional media and there is considerable awareness of the problem.

5.1.4 Laboratories and testing

At the time of the audit there were four laboratories involved in the *Xf* testing. The laboratories belong to the following institutions:

- Institute of Plant Virology (IVV) – Research National Centre (CNR)/Department of Plant and Soil Sciences and Food - University of Bari (UB). This laboratory belongs to the Research Public Network;
- Mediterranean Agronomic Institute of Bari (IAMB);
- Agriculture Research and Experimental Centre 'Basile Caramia' (ARECBC) – Locorotondo, Bari;
- Department of Agro Environmental Sciences, Chemistry and Plant Protection - University of Foggia (UF).

The audit team visited the IVV laboratory. The IVV is a research laboratory. It is the reference laboratory for *Xf* detection and has issued the test protocols. It is used for confirmation of the doubtful results from other laboratories and for confirmation of any new findings. The other three

laboratories are considered as designated laboratories. They are funded by the competent authorities for undertaking sampling and research.

Initially, the IVV was the only laboratory that could carry out conventional PCR and they first detected *Xf* in olive plant material from Lecce province by PCR. Immediately thereafter they started the adaptation and validation of the ELISA test for mass detection purposes.

The audit team was informed that the laboratory has processed around 500 samples, of which 180 with PCR, 300 with ELISA and around 50 for confirmation of new findings (PCR).

The IVV has issued a work instruction '*Diagnostic detection of Xylella fastidiosa in olive samples*' which is used by all the laboratories. Recently, PCR and ELISA ring tests were carried out with the other three laboratories, the results were consistent.

The ELISA and PCR tests are components of the 'EPPO standard diagnostic protocol PM 7/24, EPPO Standards, Diagnostic protocols for regulated pests, *Xylella fastidiosa*'.

The audit team observed the test procedure from the reception room to the issue of the final result and observed the different procedures for PCR and ELISA.

Samples arrive at the laboratory in closed plastic bags, with a label indicating: sample specific code, GPS coordinates, inspector identification, plant species and date of sampling. Olive samples are composite samples of four twigs of 20cm each collected from a cardinal point of the canopy (N, E, S, W). The plant material should not be dried. About 8 leaves in total are sampled from the four twigs, from which petioles are taken for analysis. The initial stage of preparation of the plant material is carried out in the same manner regardless whether the sample is going to PCR or ELISA.

For PCR and ELISA, the experts stated that the amplified DNA electrophoresis revelation gives a very good signal for olive, followed by oleander and almond. The positive controls used are the commercial lyophilised *Xylella fastidiosa* subsp. *fastidiosa* from an ELISA kit and plant extract from positive olive trees. The negative control used is plant extract from negative olive trees from the region and a 'no template control'.

The experts informed the audit team that the level of detection (LOD) of both methods is not yet defined. They also advised that it is very difficult to detect the bacteria in host plants other than olive, as their reactions are extremely weak.

The audit team noted that the laboratory has a skilled, competent and very motivated team and has the required equipment in order to carry out the analysis. There is good sample preparation and use of different positive and negative controls for PCR and ELISA. However, there is a risk of cross-contamination in the laboratory because the sample preparation and the amplified DNA electrophoresis are carried out in the same room, and the final step of DNA extraction (pellet suspension into ultra pure water) and the PCR mix preparation, are done under the same hood.

It was also noted that, until now, it was not possible to isolate the *Xf* strain, however this is very important in order to verify its pathogenicity on plants other than olive, oleander and almond (e.g. *Vitis*, citrus, etc.), or to verify if these others plants could be tolerant-host plants or non-host plants. The ELISA kit appears to be reliable for olive, but it still remains to be validated for plant species other than olive. Therefore, with the exception of olive plant material, the full reliability of the ELISA test carried out for other plants is not yet ensured.

There are scientific literature references stating that if *Xf* is present in dormant woody cuttings, the concentration of the bacteria in these tissues during winter is low and the distribution is heterogeneous. Therefore concentration of bacteria in the sample may be lower than the LOD of the ELISA test. Furthermore, poly-phenolic compounds, mainly present in the bark could inhibit the PCR. These issues may lead to false-negative test results. As the impact of these factors was not

examined during the method development, the tests carried out on dormant plant material (e.g. *Vitis*) during the winter, cannot be considered fully reliable.

5.1.5 Research

The RPS stated that agreements were established with several research institutions. The main actions to be developed in the near future will cover three main areas:

- genome sequencing
- pathogenicity tests
 - graft transmission to olive;
 - artificial inoculations of olive with isolated bacterial and fungal cultures or their combination,
- epidemiology
 - extensive survey of the natural flora to detect the presence of *Xf* and identify its host range (symptomatic and latent hosts);
 - collection and identification of potential vectors;
 - transmission trials to olive and other hosts by placing of potted bait plants (olive, almond, grapes) in heavily infected olive groves. These will be kept *in situ* throughout the year and checked monthly by PCR and symptom appearance.

The RPS stated that priorities will be given to the identification of vectors, identification of the host plants and isolation of the *Xf* strain.

The audit team met a large group of researchers, from several entities, that are involved in research activities related to *Xf*. These entities are: CNR, UB, IAMB, ARECBC and UF. They have been working with the official services for many years on research related to other plant pests and plant production.

The researchers stated that, at the moment, key elements of the epidemiology of the bacteria are missing. The pest was only detected four months ago and more time is required to achieve consistent results.

One of the key question that needs to be clarified is the role of olive as source of inoculum. There are indications that grafting is responsible for disseminating the bacteria, on the other hand, pruning seems not to spread *Xf*. Many species may be symptomless carriers of the bacteria but, the role of (non-symptomatic) latent infections is not known. The level of infection in oleander seems to be lower than in olive.

Regarding the vector, the spittlebug *Philaenus spumarius* is the only vector identified until now . It will be next year before there will be a clear idea of the possible vectors that are spreading the disease in Puglia. Apparently, *Xf* multiplies in the vector and it loses its infectiousness only during the moulting phase. The spittlebug is a poor flyer but, it is a very good 'hitch-hiker' and has been found inside many vehicles. Insects were still found in December but the peak of the population is expected to be sometime May/June. It is assumed that the insect feeds on several host plants, but it is not known when they fly to the olive trees. It is not known which is their host preference and which are the crucial elements of the insect's life cycle and behaviour from the point of view of *Xf* transmission.

The fungi associated to the QDSO are of the genera *Phaeocremonium* (in particular: *P. parasiticum*, *P. rubrigenum*, *P. aleophilum*, and *P. alvesii*). *P. parasiticum* and *P. alvesii* were detected for the first time in Italy. There is evidence that the fungi on their own can cause the decay of trees.

The symptomatology is different in old and young olive trees. In the old trees (more than 50 years old) the QDSO is very clear and practically in every case, the associated fungi and galleries of the leopard moth, *Zeuzera pyrina* are present besides *Xf*. On younger trees (20 years old) the QDSO does not yet exist, only a few branches show symptoms if *Xf* is present. Studies are going to be carried out to see if fungi are also present, but in younger trees the effect of *Xf* on its own remains to be seen.

The researchers stated that although nobody can be totally sure that *Vitis* is not a host of this particular strain, vineyards located in the middle of the heavily infected olive orchards have never shown any symptoms associated with this bacteria. Furthermore, *Xf* was not detected in the samples taken from those plants at the end of November 2013. In addition, tests carried out in the nursery stock material in the period of November 2013 – February 2014 (see section 5.4.2) did not detect the presence of *Xf*. Also *Citrus* orchards located in the heavily infected area have not shown any symptoms and tests for the presence of *Xf*, always gave negative results.

Conclusions on organisational aspects of plant health controls

There is a clear structure and division of responsibilities between the competent authorities responsible for developing and implementing control measures against *Xf* in Italy. There is a good awareness of the disease amongst staff responsible for performing surveys and controls, stakeholders and the general public.

The ELISA test for the plant species other than olive is not yet fully reliable. In addition, it is not proven, whether the testing of dormant woody material (e.g. *Vitis*) during the winter is reliable or not. Until this is addressed the Italian authorities cannot conclude for sure that plants listed in the annexes of the Decision 2014/87/EU are actually free from *Xf* prior to permitting their movement within the EU.

Although research work is progressing, at the moment, the following factors remain to be clarified: the host range for this particular strain, the epidemiology (timing, vectors involved, factors promoting/inhibiting the infection) or the full range of vectors (life and preferred host species).

5.2 REGIONAL LEGISLATION

The SA stated that there have been no changes to the national plant health legal framework for the organisation and implementation of controls from that described in the FVO country profile.

The following regional legislation has been issued:

- Resolution 2023 issued by the Regional Council of the Apulia Region on 29th October 2013 on *Emergency measures for the prevention, control and eradication of the quarantine pest Xylella fastidiosa associated to the QDSO (quick decline syndrome of olive)*.
- Deliberation 521, issued on 20th November 2013 by the Agriculture Department of Puglia Region on Directive 2000/29/EC – Legislative Decree n. 214/2005 and subsequent amendments - DGR 2023/2013: *Implementing provisions relating to the exercise of nursery activity in Lecce province*.
- Deliberation 562, issued on 5th December 2013 by the Agriculture Department of Puglia Region on Directive 2000/29/EC – Legislative Decree n. 214/2005 and subsequent amendments - DGR 2023/2013 – Deliberation 521/2013: *Further implementing provisions related to the exercise of nursery activity in Lecce province*.
- *Further Technical requirements on the marketing of Vitis propagating material for the season 2013/14*, issued by the RPS on 30th December 2013, ref. A0030/109838.

Following the development of *Xf* in Italy, the affected region of Puglia adopted their own legislation

establishing control measures for *Xf*.

The regional control measures provide for the following:

- surveys to detect *Xf*;
- definition of the infested zone, buffer zone and safety zone and details of the measures to be taken for each zone;
- provisions for nurseries
- other provisions

Regarding the nurseries and the control of plants for planting, two additional legal Deliberations were issued, as well as a note with technical requirements for *Vitis*. These include a prohibition on movement of all known host plants from Lecce province.

Details are included in sections 5.3 and 5.4 below.

The SA notified the Commission and other Member States of the adoption of the regional legislation at the same time as their adoption.

Conclusion

The RPS took prompt action on drafting and issuing regional legislation for the control of *Xf*. However, Resolution 2023 was never fully implemented since no infected and buffer zones have been defined (see section 5.4.3 below). The legislation was notified to the Commission and other Member States, in line with relevant EU legislation.

5.3 SITUATION OF *XYLELLA FASTIDIOSA* IN ITALY

Legal requirements

Article 16 (1) of Directive 2000/29/EC requires that, each Member State shall immediately notify in writing the Commission and the other Member States of the presence of any harmful organisms listed in n Annex I, Part A, Section I whose presence was previously unknown in its territory.

The Commission Implementing Decision 2014/87/EU, which entered into force on 13th February 2014, requires that Member States shall conduct official annual surveys for the presence of *Xf* in their territory. Those surveys shall be carried out, as appropriate, taking into account the biology, growing conditions and growing periods of the plants subject to the survey, the climatic conditions, the biology of the specified organism and the characteristics of the potential vectors. The results of the surveys shall be notified to the Commission and to the other Member States.

Findings

5.3.1 Development of Xylella fastidiosa in Italy

In Autumn 2013, the RPS Puglia started an investigation into the cause of the QDSO, which, since 2010, had become an increasing problem in the province of Lecce. It was known that leopard moth (*Zeuzera pyrina*) and xylem fungi (mainly *Phaeocremonium* sp.) were present in Lecce province. In 2013 it was noted that young shoots were dying in pruned trees. Extensive investigations were carried out including testing water and fertilizers, for nematodes etc.

Taking into account the pattern of the spread of disease, one of the researchers suspected the possible presence of *Xf*. The IVV laboratory confirmed by PCR the presence of *Xf*, on 15th October 2013. The SA notified the Commission and other Member States of the presence of *Xf* on 21st October 2013.

The RPS stated that the QDSO is caused by three agents: *Xf*, the xylem fungi (mainly *Phaeocremonium* sp.) and the leopard moth (*Zeuzera pyrina*). In Lecce province, symptoms of

QDSO start with the desiccation of terminal shoots distributed at random which expand to the rest of the canopy, thus resulting in the collapse and death of the trees. In the affected orchards, all the plants are symptomatic. Selective pruning has been attempted, however, this is not effective and the trees continue to decline rapidly.

Xf was found initially in the area of Gallipoli (around 8,000 ha, of which 1,000 ha of olive trees are severely affected) and, to date, it was subsequently found in seven other sites, to the north (near Santa Barbara, San Donato de Lecce, Galugnano, Squinzano (2) and Surbo (2)).

The SA stated that, with the exception of Lecce province, in Italy there is no evidence of the occurrence of *Xf*. Further investigations are in progress in the other Italian regions.

The intensive survey carried out in the neighbouring provinces (Brindisi and Taranto) indicates the absence of further outbreaks. However, taking into account the time of the year, not all potential host plants have been tested.

The RPS informed the FVO team that research, including genetic analysis of the *Xf* strains present in Italy, had shown that this strain (Salento strain) was a particular one different from any other previously known, even if showing a certain similarity with *X. fastidiosa* subsp. *pauca*. Later studies have confirmed that the strain is very close to that detected in Central America.

The FVO team noted that the first occurrences of the disease in the region was notified in writing immediately to the Commission and to other Member States following confirmation of presence of the organism. However, the seven additional outbreaks near Lecce city have not been notified to the Commission and to the other Member States.

5.3.2 *Insect vectors*

The RPS stated that in the infected olive orchards, entomologists of the University of Bari have carried out the collection of insects at 2-week intervals (November-December 2013) on weeds using a traditional insect sweeping net. The insects were identified and some individuals subjected to molecular tests for the presence of the bacteria. The predominant insects found were spittlebugs, specifically *Philaenus spumarius*. PCR tests on *P. spumarius* showed that around 60% of the insects collected in November had the bacteria, whereas negative results were obtained for the insects collected in December.

This results show the vectoring ability of this spittlebug, and identify it as a likely vector of *Xf*. In the same period, yellow sticky traps and an automatic live insect catcher (equipped with a black light/UV lamp) were also used to monitor insect populations. They confirmed spittlebugs as the prevailing insects at that time of the year.

5.3.3 *Host plants*

The RPS stated that, immediately after the finding of *Xf* in olive trees, samples from other plant species were tested in order to confirm the possible presence of *Xf*. However, it was not possible to test many plants because during the Winter many plant species do not have leaves or are not present in the fields.

Plants were sampled in the *Xf* contaminated area, under natural inoculum pressure except in the case of *Vinca rosea* (pink periwinkle), which was exposed to potential insect vectors under controlled conditions. For annual plants, the sampling was carried out from November to January. Samples were tested by ELISA and/or PCR.

So far, *Xf* was confirmed in: *Olea europea* (olive), *Nerium oleander* (oleander), *Prunus dulcis* (almond) and *Vinca rosea*. The infection in periwinkle was symptomless, for the remaining plants, symptoms (leaf scorching) were visible.

In the case of *Quercus* sp. (oak), *Malva sylvestris* (mallow), *Sorghum halepense* (Johnson grass)

and *Portulaca oleracea* (purslane) the initial PCR tests were positive for *Xf*, however it was not possible to confirm its presence at a later stage. An explanation for this was not given. These species are considered as potential host plants. Symptoms of leaf scorching were present in *Quercus* sp.. The other three plants species (herbaceous) seem to be symptomless carriers of *Xf*.

The bacteria was not detected in: *Vitis* spp., *Citrus* spp., *Pistacia lentiscus*, *Pittosporum* spp., *Calendula arvensis*, *Papaver rhoes*, *Senecio vulgaris*, *Cynodon dactylon*, *Merculliaris annua*, *Clematis vitalba*, *Sonchus oleraceus*, *Stellaria media*, *Daucus carota*, *Capsella bursa pastoris*, *Urtica dioica*, *Oxalis pes-caprea*, *Fumaria officinalis*, *Trifolium* spp., *Geranium pusillum*, *Smilax aspera*, and *Myrtus communis*.

The RPS stated that the laboratory tests remain necessary also for the symptomless olive trees because of the frequent pruning carried out by many olive growers with the aim of eliminating the symptomatic branches, thus masking symptoms.

The FVO team noted that taking into account the time of the year, it was not possible to carry out testing in many herbaceous and deciduous plant species, in order to establish if they are host plants of *Xf*. Furthermore, species that have had an initial PCR positive result but later it was not possible to confirm the presence of *Xf*, are considered as potential host plants by the RPS.

5.3.4 Surveys in Puglia

The RPS stated that under Resolution 2032, surveys are being carried out in the region to detect the presence of *Xf* on olive trees and other host plants on farmed land, in nurseries, urban areas or any other area deemed necessary.

Visual inspections of the host plants are being carried out to verify the presence of symptoms of the bacteria followed appropriate by laboratory tests to confirm the presence of *Xf*.

After completion of the surveys, the Resolution also foresees the definition of the focal zone, establishment zone, buffer zone and safety zone and details of the measures to be taken for each zone:

- infected zone: area or place where the presence of the *Xf* has been officially established and it is deemed technically possible to eradicate it;
- establishment zone: area where the spread of *Xf* is such that it is no longer technically possible to eradicate it and action must therefore be taken to ensure it is contained;
- buffer zone: area around the infected or establishment zone, in which the presence of the pathogen has not yet been detected;
- security zone: area around the buffer zone further ensuring containment of the pathogen

The SA stated that, the survey priorities have changed since the initial detection of *Xf* in Lecce province. Three stages can be distinguished:

- initially the survey targeted the infected area (Gallipoli) to assess the plant health situation.
- in the second stage, surveys were carried out in the neighbouring provinces of Brindisi and Taranto, in order to determine whether they were free from the pest. With the same objective and at the same time, a survey was also carried out in the *Vitis* production area of Otranto (Lecce province). These surveys were mainly targeted surveys on olive trees and looked for symptoms along the roads.
- the third stage of the surveys started at the beginning of 2014 and are based on a regional grid. For Lecce province, it should be finished by the end of March 2014.

The RPS drafted a survey protocol for the region of Puglia. The protocol describes the survey and sampling method in detail and establishes two types of surveys: the grid survey and the

programmed survey.

The programmed survey is based on satellite images where olive orchards and other host crops are identified and risk assessed. Satellite images of the situation both before and after the presence of the disease, are used. As a result of this study, more precise and updated field maps can then be used for the surveys. The protocol also establishes priorities for action.

The grid survey is based on a 2.75x3.5 km grid, where at least 13 samples from seven plant species are collected in each grid unit: five samples of olive, two of oleander, two of mallow, one of Bermuda grass and one of Johnson grass. It is also foreseen to collect three samples of insect vectors per each grid unit.

The official surveys are carried out by the regional phytosanitary inspectors and phytosanitary agents coordinated by RPS and researchers. Generally, the survey teams consist of two agents one collecting the sample and other one filling in the forms.

The phytosanitary agents that carry out the official survey have to map the site under observation, check for symptoms, collect samples from both plants with symptoms and randomly from asymptomatic plants and deliver the samples to the laboratories for diagnosis.

All samples should be placed in appropriate bags, sealed and identified with a code. For each sample the agent fills in a form with relevant information: date; location, GPS coordinates, plant species, age of tree, phenological phase, presence of symptomatology, name of property (if available). This information is uploaded in a file which is later forwarded to the laboratory.

Once delivered to the designated laboratories, samples are primarily tested by ELISA and only if confirmation is needed, they are tested by PCR. After processing, the laboratory fills in the results and sends the file to the RPS.

At the moment, the survey focuses on olive and oleander which showed to be more susceptible to *Xf* infection, and also because they are ever green plants. During the Spring 2014, there is an intention of surveying also other plant species that grow during this season.

The numbers of samples to be collected for each grid unit may be adapted according the occurrence of the QDSO. The samples shall consist of leaves and branches and possibly parts of the trunk of the risk species.

Until the time of the audit, 3,562 samples (from survey stages 1, 2 and early 3) of different plant species had been processed in the laboratories (see table 1, below).

The SA informed the audit team that for the grid survey it was decided to start with returning to the heavily infected area of Gallipoli in order to define the infected area and the buffer zone. Subsequently the rest of the Lecce province will be surveyed. In parallel, the grid survey on Brindisi and Taranto has already started. The survey in Lecce province should be finished by the end of March 2014.

The RPS stated that after the survey is concluded in Lecce province, the grid survey will be extended to other areas of Puglia following a priority:

- 1) the road linking Bari to Taranto, where an important area of olive and stone fruit nurseries is located;
- 2) a large olive production area along the coast north of Bari;
- 3) the olive production areas of Mattinata and Gargana.

Table 1 - Number of samples collected during the Xf survey (31/12/2013)

Species	No of samples	Negative	Positive	Not confirmed
Olive	1,757	1,731	21	5
Mallow	557	-	-	-
Oleander	433	432	-	1
Vitis	174	174	-	-
Johnson grass	170	169	-	1
Almond	155	-	-	3
Oak	133	-	-	2
Citrus	131	131	-	-
Bermuda grass	22	22	-	-
Total	3,562	3,529	21	12

The audit visited a survey point and discussed with the phytosanitary agents the survey procedures. It was confirmed that the agents had a specific training to carry out surveys. The training addressed several topics: symptom recognition, sampling, laboratories, other harmful organisms and insect vectors. They mentioned that the symptoms are very difficult to identify especially in the winter when the olive trees have lost the dead leaves. They were using grid maps to identify the areas to be surveyed. At the time of the visit (Winter) the agents were collecting samples of olive (8), oleander (3) and mallow (2). In the Spring, more plant species will be surveyed. The first survey step is to check the olive orchards to see if there are symptoms and in such case, samples are collected from symptomatic trees. If no symptoms are found, samples will be collected from symptomless trees.

In addition, mallow, Johnson grass and Bermuda grass were given priority in the ongoing survey. All of these plants will be sampled regardless of whether or not they have symptoms.

The audit team noted that sampling of almond and the potential host oak is not included in the protocol. Since the surveys are not completed, the infected zone(s), buffer zone(s) and safety zone(s) have not yet been established, as required in the regional legislation. Taking into account the time of the year (Winter), several plant species have not been surveyed.

Conclusions on the situation of Xf in Italy

The detection in Italy of Xf in October 2013 was notified to the Commission and other Member States. However, further outbreaks detected outside the initial area were not notified, which is not in line with point 1 of Art. 16 of Council Directive 2000/29/EC.

There is consensus among the competent authorities and researchers that, in Puglia a new strain (Salento strain) of Xf infects olive trees and other hosts and causes the QDSO. Therefore, literature references from other subspecies or strains should be considered cautiously. General references of the literature on Xf to the specific situation in Italy need to be confirmed. The newly identified 'Salento strain' seems to be genetically close to one present in Central America.

So far, one insect *Philaenus spumaris* was identified as vector of Xf.

Regarding host plants, Xf was confirmed in olive, oleander, almond and pink periwinkle, however many plant species have not yet been tested in order to confirm if they are Xf host plants. Tests carried out to date, on numerous species (including *Vitis sp.*) cannot be considered as fully reliable concerning the non-host status of the species involved (see section 5.1.4).

A survey protocol is in place and is followed. The surveys are carried out by trained and skilled technicians and the sampling for laboratory analysis was carried out appropriately. However, the survey of *Xf* in Lecce province is still to be completed, therefore no infected area(s), buffer zone(s) or safety zone(s) have been defined. In addition, the survey has been carried out only in a few number of plant species that were available for sampling and testing during the Winter. Some hosts and potential hosts, like almond and oak, are not included in the survey protocol and will not be surveyed.

The survey results so far indicate that *Xf* is confined to the Lecce province in Puglia region. However, taking into account the time of the year, the range of hosts covered in the survey and the test constrains (see 5.1.4) there is a risk of declaring free areas, which may not be really free. Surveys will be carried out during 2014 in other Italian regions.

5.4 CONTROL MEASURES

Legal requirements

Xylella fastidiosa is listed as a harmful organism in Annex I, Part A, Section I to Council Directive 2000/29/EC. Article 16(1) of the same Directive requires that, *inter alia*, following the actual or suspected appearance of any harmful organisms listed in Annex I, Part A, Section I [of that Directive] whose presence was previously unknown in its territory, it shall take all the necessary measures to eradicate, or if that is impossible, to inhibit the spread of the harmful organism concerned.

The Commission Implementing Decision 2014/87/EU, regulates the movement of plants for planting out of the province of Lecce. All movement of plants for planting is banned with the exception of seeds and the plants listed in the Annexes I and II of the Decision. The said plants can leave the province of Lecce only after sampling and being subject to tests concerning the presence of *Xf* and for which the result is negative. In addition, plants listed in Annex II have to be grown under complete physical protection and have to be officially certified under a certification scheme.

ISPM 31 - International Standards for Phytosanitary Measures N°31, Methodologies for sampling of consignments, Food and Agriculture Organisation.

Findings

The RPS stated that Resolution 2023 establishing control measures and measures for nurseries in the infected zone(s), buffer zone(s) and safety zone(s) had been in place since 29th November 2013. However, since the survey in Lecce province is not finished, those zones were never defined and the Resolution has not been fully implemented. Priority was given to the implementation of measures allowing the movement of planting material of certain species outside Lecce province. Therefore two additional Deliberations for nurseries were issued in November and December 2013 requiring specific control measures for plants for planting. These include a movement prohibition of all known host plants from Lecce province and, a specific monitoring and testing regime for planting material considered as non-hosts of *Xf*.

5.4.1 Eradication measures

The regional Resolution 2023 requires the following mandatory control measures in the infected zone(s), buffer zone(s) and safety zone(s):

- grubbing-up of infected plants;
- burning of small vegetation (offcuts) from pruning;
- desiccation of the woody portion *in situ* before it is moved;
- monitoring to establish the spread of infections;

- ban on moving any infected plant material outside the infected zone;
- phytosanitary interventions using insecticides to control vectors;
- keeping cultivated and non-cultivated areas, including roadsides, free of weeds;
- insecticide treatment of host plants (especially oleander);
- thorough cleaning of drains and irrigation channels;
- adoption of preventive measures in cooperation with the municipalities, local health authorities and authorities managing parks and protected areas, in order to implement intervention plans in urban areas, parks, and public and private gardens;
- compliance with any other measures recommended by the RPS.

The SA stated that, so far, no eradication or containment measures have been taken, neither in the heavily infected area of Gallipoli nor in the seven outbreak sites identified outside this area. Before implementing any measure in Gallipoli, the SA intends to delimit the infected zone, which is still to be completed. To contain the bacteria, a buffer zone will also have to be established and it will then become clear which trees have to be eradicated, taking into account the complexity of the situation.

In addition, for the seven outbreaks (most of them detected 4 months ago) which the RPS considers to be limited in extent, it is not yet identified which trees are positive and which ones are not, therefore no measures have been taken.

The SA also stated that, it is essential to have more information about the epidemiology of the bacteria, otherwise an eradication campaign may prove inefficient or may promote the spread of the bacteria. In any case, after the surveys are completed by the end of March, the mandatory control measures of Resolution 2023, will have to be implemented.

The RPS stated that for one of the outbreaks outside Gallipoli, located near Squinzano, a letter was sent out just before the FVO audit to the PAO of Lecce province requiring an investigation by the plant health inspectors in order to identify the owners and collect further samples to identify the infected trees and delimit the outbreak area.

The audit team visited one of the seven outbreak sites (near Santa Barbara) where an olive tree with decline symptoms was identified in the targeted survey. A sample was collected at the beginning of November which was positive for the presence of *Xf*.

The RPS could not find an explanation as to how the bacteria moved from Gallipoli 20 km away to this site (assuming that Gallipoli is the initial source of *Xf*). Three possibilities were advanced by the RPS to explain the spread: tornados in the area, 'hitch-hiking' of the vector insect or movement of infected plants.

The audit team noted that, at the time of the visit, no eradication or containment measures had been taken at the site and none of the olive trees surrounding the infected one had been sampled and tested.

Additionally, the audit team visited five sites within the heavily *Xf* contaminated area of Gallipoli.

The audit team noted that no eradication and containment measures were taken in the heavily *Xf* infected area of Gallipoli. The disease has progressed dramatically in recent times. Extensive mortality was observed in the core infected area.

Conclusion on eradication measures

Recently the disease has spread very rapidly and no eradication or containment measures have been taken. Diseased trees are left in place, acting as a reservoir of infection. Unless action is taken, further rapid spread of the disease must therefore be anticipated.

5.4.2 Control measures in the nurseries

Resolution 2032, requires that nurseries located in the infected zone must comply with the following mandatory measures:

- suspension of issuing the EU plant passport for host plants of *Xf*;
- prohibition on the movement outside the outbreak area of any plant propagating material of the host species of *Xf* and in particular of olive, almond, oleander and oak;
- immediate destruction of the whole lot of plants infected with *Xf*;
- mandatory cleaning and elimination of weeds;
- compulsory insecticides sprays against potential *Xf* insect vectors;
- obligation to carry out the weeds control and insecticide treatments around the nursery;
- fulfilment of any further measures prescribed by RPS;

In addition, Resolution 2032 requires that nurseries located in the buffer and safety zone must comply with the following mandatory measures:

- thorough removal and elimination of weeds;
- application of insecticides in the nursery to control the vectors;
- compliance with any other measures recommended by the RPS.

Regarding the plant propagation material movement, the previous requirements were complemented by the requirements below.

Deliberation 521, issued implementing provisions relating to the nursery activity in Lecce province, confirms the above mentioned provisions for the nurseries located in the infected zone, furthermore, the Deliberation:

- bans the movement of plants and plants for planting of olive, fruit (incl. *Vitis*), ornamental, vegetable and forestry species, originated in nurseries located in an area limited by the roads Lecce-Leverano-Porto Cesareo and Lecce-Maglie-Santa Maria de Leuca, outside of that same area;
- the movement of the same plant species is banned outside Lecce province, for nurseries located outside the limits of the two provincial roads above mentioned. However, the movement of plants for planting of *Vitis* is allowed from nurseries located in this second area;
- all nurseries located in the province of Lecce are subject to official controls and laboratory testing to confirm the absence of *Xf*.

Deliberation 562, issued on 5th December 2013 defines further the provisions for the movements plants outside Lecce province

- Annex 1 lists the plant species (150 species) that are referred to in the scientific literature as being potentially hosts of *Xf*. Such plants are subject to the provisions of Deliberation 521 except if they are produced during the entire cycle of vegetation in complete physical protection and they are under a certification scheme with specific protocols for sampling and testing, in such case the plants can be moved outside Lecce province after a negative result for the presence of *Xf*;
- plants not listed in Annex 1 may be moved outside Lecce province if they are subjected to the provisions of Annex 2 (with the exception of non-host plants of the families: Coniferae,

Cactaceae and Areceaceae which have free movement);

- Annex 2 requires that the nurseries have to fulfil specific requirements and that the plants have to be subject to a *Xf* test prior to marketing in 1% of the plants of the same species and same lot, up to 100 plants. The sample may be constituted by one plant or a maximum of three plants per sample.

Further Technical requirements on the marketing of Vitis propagating material for the season 2013/14, was issued by the RPS on 30th December 2013, ref. A0030/109838. This legislation is applicable to the marketing of *Vitis* in 2013/2014 and requires that all field lots of rootstock or mother plants are subject to tests for the presence of *Xf* before marketing.

The SA stated that priority was given to the nurseries, in particular *Vitis* nurseries, in order to allow the movement of plants outside Lecce province.

The RPS also stated that no contingency plans have been established for *Xf*, in particular if findings occur in nurseries. Such plans may be prepared in the near future.

After the detection of *Xf*, the RPS, as early as November 2013 started a specific monitoring of all nurseries, with particular attention to those in proximity to the infected areas. It consisted of a documentary check of the production of plant propagating material, visual inspections and sampling for testing to verify the absence of *Xf* on plants not listed as host plants. All inspection activities are carried out by phytosanitary inspectors belonging to the PAO.

Table 2 lists the results of traded main species listed in Annex I and II of Decision 2014/87/EU, for which samples have been collected in the nurseries. All results were negative for the presence of *Xf*.

Table 2 – Number of samples for the main species listed in the annexes of EU Decision 2014/87/EU produced in Lecce province nurseries (Dec. 2013 – Jan. 2014).

Species	Annex Dec 2014/87/EU	No of samples
<i>Vitis</i>	I	1,758
<i>Euphorbia pulcherrima</i>	I	227
<i>Solanaceae</i>	II	85
<i>Cyclamen</i>	I	83
<i>Myrtus communis</i>	I	55
<i>Compositae</i>	II	53
<i>Chamelaucium uncinatum</i>	I	40
<i>Punica granatum</i>	I	35
<i>Mandevilla vogue</i>	I	34
<i>Cucurbitaceae</i>	II	32
<i>Polygala myrtifolia</i>	I	31
<i>Cruciferae</i>	II	29
<i>Viola L</i>	I	29
<i>Grevillea sp.</i>	I	27
<i>Callistemon citrinus</i>	I	16
<i>Citrus sp.</i>	I	13
<i>Umbrelliferae</i>	II	10

<i>Ficus sp.</i>	I	9
<i>Laurus nobilis</i>	I	9
<i>Schinus molle</i>	I	8
<i>Magnolia grandiflora</i>	I	7
Other plant species	I	78

In addition, samples from other species were also collected in nurseries before marketing. All results were negative. Table 3, lists the test results for other main species produced in Lecce province nurseries.

Table 3 - Number of samples for other species produced in nurseries (Dec 2013 – Jan 2014).

Species	No of samples
<i>Olive</i>	33
<i>Quercus</i>	6
<i>Oleander</i>	5
<i>Prunus</i>	3

The audit team visited one *Vitis* nursery located in the Otranto area (Lecce province), outside the area limited by the roads Lecce-Leverano-Porto Cesareo and Lecce-Maglie-Santa Maria de Leuca. The nursery has a total area of 15 ha and, in 2013 produced around 280,000 plants, 70% of which for wine production and 30% for table grape production. The great majority of the plants for wine production stay in Italy but the production of plants for table grape is also sold to other Member States and to third countries. The nursery markets rootstock cuttings and grafted rooted vine plants.

The RPS stated that for *Vitis*, the control of the nurseries is regulated by national legislation transposing EU rules for *Vitis* propagating material. For 2014, it was not possible to apply fully the above mentioned technical requirements issued by RPS. In most of the cases, the plants had already been uprooted and the sampling for *Xf* testing had to be done when the plants were in the warehouse. It is foreseen that for the season 2014/15, the situation will be corrected and the sampling will be carried in field lots.

The nursery was subject to official controls during the vegetation period and no symptoms were observed.

During the season, 66 samples were drawn in the warehouse from two lots at the end of 2013. The samples were negative for the presence of *Xf*. The *Xf* sampling is carried by staff of the ARECBC.

The audit team visited one of these nurseries with 0.6 ha of physical protection (screen houses), located in the restricted area limited by the roads Lecce-Leverano-Porto Cesareo and Lecce-Maglie-Santa Maria de Leuca. The total production is around 6,000,000 plants per year and the main species produced are: peppers, tomato and squash. Most of the production is going to retailers and later to small producers, but the larger farmers buy directly from the nursery. The plants are generally sold in the region (including Brindisi and Taranto) where the production of fresh vegetables is very important.

The RPS stated that the production of young vegetable plants is subject to a national certification scheme. Under this scheme it is mandatory to produce the plants in insect proof screen houses and, monitor and sample them for harmful organisms. The sampling was adapted for this type of plant material and it follows a specific procedure. In addition to these requirements the testing for *Xf*, was

added. In total, 30 samples were taken in the nursery in December 2013, all results were negative.

The audit team visited a nursery producing ornamental plants also located in the restricted area limited by the roads Lecce-Leverano-Porto Cesareo and Lecce-Maglie-Santa Maria de Leuca. The total area of the nursery is 3 ha of which 1.8 ha are under physical protection. The main species produced are: *Chamaelaurium uncinatum*, *Grevillea* sp., *Myrtus communis*, *Polygala myrtifolia* and *Boronia crenulata* (listed in the annex I of the Decision 2014/87/EU). The original plant material was provided by other nurseries in Italy and mother plants stay in the screen house. The production is around 100,000 plants per year and is destined to a trade centre in Bari from where the plants are sold.

The RPS stated that after the entry into force of Deliberation 521, the producer (located inside the restricted area) was not allowed to sell plants. Only after the entry into force of Deliberation 562, trade was allowed for plants not listed in Annex 1 and only after a negative test result for *Xf*. The producer added that this was damaging for the reputation of the company and many orders were cancelled.

In December 2013, three lots had been sampled and tested for *Xf* in the ARECBC laboratory and the results were negative.

The RPS stated that a list of plants originating in the nurseries of Lecce province and sold last year to other regions of Italy and other Member States had been completed recently. The list includes several consignments of host plants (olive, oleander, stone fruit trees) dispatched to several destinations outside Lecce province. The list will be made available to the SA for further distribution.

In the three visited nurseries the FVO team could confirm the implementation of control measures and noted that:

- the owners were fully aware of the *Xf* problem in Lecce province and had participated in meetings with the RPS for information and implementation of the regional legislation;
- the official inspectors from PAO Lecce province stated that they had participated in the *Xf* training course organised by the RPS and the number of inspections in the nurseries had increased after the detection of *Xf*;
- prior to marketing, the procedure for sampling of *Vitis* rootstock cuttings and ornamental plants, requires that a sample is drawn for *Xf* tests on 1% of the plants belonging to the same species, from a lot clearly identified, up to a maximum number of 100 plants per lot. Each sample is composed of three plants;
- prior to marketing, the procedure for sampling of young watermelon plants, requires that a sample of at least 20 to 25 leaves is drawn for *Xf* tests from several plants belonging to a homogeneous lot of plants with a maximum of 100,000 plants;
- from the sampled plant material, in addition to the laboratory tests for certification purposes, an ELISA test will also be carried out for *Xf*;
- after filling in the forms, the samples are taken to the laboratory of ARECBC for ELISA screening and the results are communicated via e-mail or fax to the producer.

In addition, the audit team noted that oleander plants and olive trees present in the nurseries had not been sampled for the presence of *Xf*.

Regarding the control measures, there are inconsistencies between the regional and the EU legislation, for instance in the regional legislation, the movement of non-host plants of the families: Coniferae, Cactaceae and Arecacea, have free movement. The EU Decision does not foresee such possibility, as plants for planting not listed in the Annexes may not be moved outside Lecce.

Conclusion on control measures in the nurseries

The sampling followed the national requirements for each type of plant material and was in line with the Italian established rules. However, the sample sizes used do not provide a level of security as envisaged e.g. in ISPM 31 for pest freedom in a consignment. This is especially important for *Vitis* and ornamentals, since the tests for woody plant material may produce false negative results (see section 5.1.4).

In the visited nurseries, it was noted that host plants (olive trees and oleander plants) present in the place of production have not been tested for the presence of *Xf*.

The customer list for olives and other high-risk plants traded from Lecce province, at least for the last season, had been received by the RPS, but had not been made available to other regions of Italy and Member states that have received such material, in order to enable a trace back or targeted surveillance.

There are inconsistencies between the regional legislation and the EU Decision regarding the plants listed in the relevant Annexes. The regional legislation allows for the movement of plants not covered by the EU Decision.

5.4.3 Movement control

The RPS stated that with exception of plants for planting originating in the registered nurseries subject to testing, no movement controls have been established for host plants.

The audit team visited a garden centre with an area of 0.7 ha located in the restricted area limited by the roads Lecce-Leverano-Porto Cesareo and Lecce-Maglie-Santa Maria de Leuca. The garden centre trades several plant species including host plants (olives, almonds and oleander). It also produces some plant species namely oleander and oak, the remaining plants are sourced in other nurseries of Italy.

The owner was aware of *Xf* and of the regional legislation regarding the movement of plants. He stated that generally he informs clients that the host plants cannot leave the restricted area defined in Deliberation 521.

The garden centre is inspected by the plant health inspectors and subject to sampling for *Xf*.

The audit team noted that there are no guarantees that the *Xf* host plants will remain in the restricted area defined by the regional legislation.

Conclusion on the movement of plants

Provisions are in place banning the sale of host and listed plants outside Lecce province. However, these provisions are not implemented and enforced by the RPS, this is not in line with Art. 1 of Decision 2014/87/EU.

6 OVERALL CONCLUSIONS

The competent authorities have taken significant steps since the finding of a new strain of *Xf* (Salento strain) in Lecce province, in October 2013. Based on regional legislation, adopted in 2013, measures are in place establishing conditions for the production and movement of plants for planting in nurseries located in Lecce province.

An extensive survey activity is still being carried out in order to delimit the spread of the disease in the province and to define infected and buffer zones. However, significant parts of the survey were not carried out in the most favourable time of the year. The survey is planned to be concluded by the end of March 2014.

No eradication or containment measures have been taken and the disease has spread very rapidly.

Diseased trees are left in place, acting as a reservoir of infection. Unless action is taken, further rapid spread of the disease must therefore be anticipated.

The ELISA test for plant species other than olive is not yet fully reliable. In addition, the testing of dormant woody material (e.g. *Vitis*) during the winter and the limited sample sizes used also affect the reliability of the testing. In these circumstances, there is a risk of obtaining false negative results. Until this is addressed the authorities cannot say for sure that plants listed in the annexes of Decision 2014/87/EU are actually free from Xf prior to permitting their movement within the EU. This represents a potential risk of spreading the organism to other parts of Italy and to other Member States.

Although research work has been carried out and is continuing, key factors regarding the epidemiology of Xf remain to be clarified.

7 CLOSING MEETING

A closing meeting was held on 14 February 2014 at the headquarters of the Regional Plant Health Service of Puglia, with representatives of the SA and the Regional Service visited during the audit.

During the meeting, the preliminary findings and conclusions of the FVO team were presented, which were, subject to certain clarifications, provisionally accepted by the authorities.

8 RECOMMENDATIONS

The Single Authority in Italy is recommended to:

N°.	Recommendation
1.	Immediately report to the Commission and other Member States any additional outbreak of <i>Xylella fastidiosa</i> outside the known infected area, as required by point 1 of Article 16 of Council Directive 2000/29/EC
2.	Validate the ELISA test for plant species other than <i>Olea europea</i> and confirm the reliability of ELISA and PCR tests on dormant woody plant material (e.g. <i>Vitis</i> sp.) in order to guarantee that, the tests detect the 'Salento strain' of <i>Xylella fastidiosa</i> in each sample where the bacterium is present, as a means to inhibit the spread of the harmful organism in line with point 1 of Art. 16 of Council Directive 2000/29/EC.
3.	Ensure, that all identified and potential host species of the 'Salento strain' of <i>Xylella fastidiosa</i> are included in the surveys, and samples of them are tested for the presence of the pathogen, as a means to inhibit the spread of the harmful organism in line with point 1 of Art. 16 of Council Directive 2000/29/EC.
4.	Ensure the delimitation of the areas infected by the 'Salento strain' of <i>Xylella fastidiosa</i> , the creation of buffer zones around them and the implementation of eradication and containment measures as appropriate in the infected and buffer zones, in line with point 1 of Art. 16 of Council Directive 2000/29/EC.
5.	Ensure that official controls cover every kind of movement of planting material out of Lecce province, as a means to prevent further spread of the harmful organism, in line with Art. 1 of Decision 2014/87/EU. In particular, movement controls related to garden

N°.	Recommendation
	centres.
6.	Ensure that the sites of production of woody planting material (especially <i>Vitis</i> sp. and ornamentals) are continuously monitored, sampled and tested for the presence of the 'Salento strain' of <i>Xylella fastidiosa</i> , during the vegetation period, in order to guarantee that point 1 of Art. 16 of Council Directive 2000/29/EC is fulfilled.
7.	Ensure that the sample size used for testing is adequate to guarantee the pest freedom envisaged in Art. 1 of Decision 2014/87/EU, using ISPM 31 as a reference.
8.	Ensure that the customer list for olives and other high-risk plants traded from Lecce province in the previous seasons, is made available immediately to other regions of Italy and the Member States that have received such material, in order to enable a targeted surveillance, as a means to inhibit the spread of the harmful organism in line with point 1 of Art. 16 of Council Directive 2000/29/EC.
9.	Ensure that the regional legislation is immediately adapted to be in line with Decision 2014/87/EU.
10.	Ensure that the survey is carried out at the most appropriate period for the detection of <i>Xylella fastidiosa</i> , as required in point 1 of Art. 2 of Decision 2014/87/EU.

The competent authority's response to the recommendations can be found at:

http://ec.europa.eu/food/fvo/rep_details_en.cfm?rep_inspection_ref=2014-7260

ANNEX 1 - LEGAL REFERENCES

Legal Reference	Official Journal	Title
Dir. 2000/29/EC	OJ L 169, 10.7.2000, p. 1-112	Council Directive 2000/29/EC of 8 May 2000 on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community
Dec. 2014/87/EU	OJ L 45, 15.2.2014, p. 29-33	2014/87/EU: Commission Implementing Decision of 13 February 2014 as regards measures to prevent the spread within the Union of <i>Xylella fastidiosa</i> (Well and Raju)

ANNEX 2 - STANDARDS QUOTED IN THE REPORT

International Standard	Title
ISPM No°31	International Standards for Phytosanitary Measures N°31, Methodologies for sampling of consignments, Food and Agriculture Organisation