Competent Authority response to the report recommendations, received 5 June 2014

ANNEX

Response of the Competent Authorities of Italy to the recommendations of Report ref. DG(SANCO)/2014-7260 -MR of a/an audit carried out from 10 February 2014 to 14 February 2014 in order to evaluate the situation and official controls for Xylella fastidiosa..

N°	***Recommendation***	***Action Proposed by the Competent Authority***
1	Immediately report to the Commission and other Member States any additional outbreak of Xylella fastidiosa outside the known infected area, as required by point 1 of Article 16 of Council Directive 2000/29/EC	The local and national authorities have always notified promptly the situation of *Xylella fastidiosa* in Apulia and any changes occurred. In the monthly meetings of the Standing Committee on Plant Health were carried out in near real time updates on the evolution of the investigation and the scientific research results.
2	Validate the ELISA test for plant species other than Olea europea and confirm the reliability of ELISA and PCR tests on dormant woody plant material (e.g. Vitis sp.) in order to guarantee that, the tests detect the 'Salento strain' of Xylella fastidiosa 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.	Implementation of the ELISA and PCR assays was done by testing a larger number of samples collected in the contaminated area. Specifically, 400 samples of citrus, 146 samples of Cactaceae, 226 samples of conifers, 86 samples of Arecaceae, were double checked by ELISA and PCR between March and April 2014. Validation of the tests was possible. Positive controls were represented by sap extracts, of the different matrices, spiked with aliquots of the suspension of the pure culture recently (March, 2014) obtained on BCYE and PWG media (Cariddi et al., 2014- doi: 10.4454/JPP.V96I2.024). In both tests the positive controls were correctly identified in all plant matrices, suggesting that no cross-reaction or PCR-inhibition occurred. In addition, more investigations have been done on almond trees. The identification of the bacterium in this host plant was limited to few samples tested in October 2013, using leaf-petioles of senescent leaves. Starting in January 2014, the leaves from the new shoots have been tested monthly, along with the xylem tissues recovered from 1-2y old branches. The results so far gathered consistently showed that leaf tissues (young leaves) have not been colonized by the bacterium yet, while the xylem tissues gave always clear cut positive reactions in both assays. These data

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		proved that the bacteria can survive in the xylem tissue during the winter season (plant dormancy), and that it takes time before it can move and colonize the new tissues (young shoots and leaves). Concerning the ELISA and PCR performed on the vines in the dormant stage, as it was already remarked, several scientific reports, as well the EPPO diagnostic protocol, use grape canes to perform the diagnostic assays. As shown by other reports the bacteria can be acquired and transmitted by insect vectors even from plants in dormant stage. The abovementioned data recovered from almond trees and the tests on sentinel olive trees grown in open field in the contaminated area, showed that the bacteria can be detected during the winter season either on vegetating trees (olive) and on dormant trees (almond). If no symptoms are recorded on the canopy, samples consisting in 4-5 cuttings from mature shoots are collected from the 4 quadrant of the canopy.
3	Ensure, that all identified and potential host species of the 'Salento strain' of Xylella fastidiosa 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.	The surveys began in November - December, period in which some species had winter rest. A group of reliable plant species was selected to avoid that the analysis would give an unreliable result and to be definitely able to detect the presence of the bacterium in the territory. In the first phase of survey, in fact , the activities were mainly focused in checking the spread of *X. fastidiosa* in the territory of the Apulia region in order to understand the territorial extent in the province of Lecce but also the entire region. It was necessary to detect and demarcate the infected areas in order to take the necessary measures. A different assessment was made in relation to grapevine, for which the protocols already provided for the possibility of being able to make reliable analyzes of material in quiescence excluding cases of infection of grapevine.

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		In any case, we have already made specific investigation in infected areas on different species such as citrus fruits, Cactaceae, Arecaceae, Coniferales and other species listed in Annex 1 of the Decision 2014/87. The activities of identification of host plants is constantly changing and the regional authority keeps on monitoring the area on potentially host plants. During the growing season, in the most opportune periods, all plants in the area that can potentially be host will be tested. So far the susceptible host plants of the *X. fastidiosa* strain CoDiRO (Salento strain) are restricted to: olive, oleander, almond and Vinca spp. In depth surveys have been done and are still on-going, to ascertain if other crops (grapes, citrus, stone fruits, etc.) or alternative hosts (ornamental, weeds and other perennial plants) can sustain the bacterial infections and can serve as host/source of inoculum. Specifically, till now samples from more than 100 species have been collected in the contaminated olive groves or in the neighbour areas, and tested always negative, except for the 4 host plants listed above. This list could appear very limited if compared to the complete list of known *X. fastidiosa* host plants, however, two aspects have to be underlined: - the list of known host plants refers to all *X. fastidiosa* strains; it is known that each strain has specific host range (i.e. classification of the subspecies is related to the specific hosts); - the molecular characterization of the bacterial population spreading in the contaminated area of Apulia, showed high genetic identity, suggesting that only one strain/genotype has been introduced, and that its introduction is relatively recent with no evidence of mutation or genetic recombination. This latter evidence can help to explain the restricted host range identified in

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		Salento.
4	Ensure the delimitation of the areas infected by the 'Salento strain' of Xylella fastidiosa, 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.	At the end of the first phase of survey of the entire region and in particular the Province of Lecce, demarcated areas were identified and officially established with the Regional Agriculture Service Act no. 157 of April 18, 2014, a copy of which is attached.
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 centres.	In order to ensure official controls on movement of plants intended for planting, the regional authority has reorganized the control procedures of nurseries by phytosanitary inspectors and has intensified inspections on movement of all species and in particular on the known host species of *X. fastidiosa*. The same procedure is used for the garden centers. The information and continuous updates provided to the nurserymen are frequent and therefore they have the full aware of the procedures to be followed.
6	Ensure that the sites of production of woody planting material (especially Vitis sp. and ornamentals) are continuously monitored, sampled and tested for the presence of the 'Salento strain' of Xylella fastidiosa, during the vegetation period, in order to guarantee that point 1 of Art. 16 of Council Directive 2000/29/EC is fulfilled.	In order to ensure compliance with paragraph 1 of Art. 16 Directive 2000/29/EC, specific monitoring are constantly carried out at the sites of production of woody plants, with particular reference to Vitis, Citrus and ornamentals. Inspections at the sites of production of Vitis and Citrus have been intensified in order to obtain further evidence on their exclusion from the list of host plants. Approximately 400 samples of Citrus and about 200 samples of grapevine taken from the most infected area (Gallipoli) were tested in order to verify the presence of *X. fastidiosa*.
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.	Studies to determine the distribution of the bacterium in the olive trees was done by ELISA and quantitative PCR assays. The studies included trees with different ages and different symptoms severity.

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		The bacterium was consistently detected in symptomatic or asymptomatic leaves collected from branches showing withering and dieback. While in the samples collected from branches without symptoms, the % of the infected samples varied. Based on this evidence, inspectors were instructed to inspect the trees prior sampling, and to collect cuttings (with alive tissues) maily from the branches showing suspicious or clear dieback symptoms and in any case also from asymptomatic plants and branches. The sample usually consists of 4-5 cuttings taken from different parts of the same plant from which is extracted the sample for laboratory analysis. The amount of branches collected has been determined on the basis of experimental tests, in order to have a representative sample allowing a high reliability.
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.	During the period of the FVO audit, the inspectors of the regional Service were concluding the investigation of sales and the results have been communicated in a note dated February 19, 2014 n. prot. 142014 to the Central Plant Health Service and to the other Regional Plant Health Services. The Central Plant Health Service sent the communication to the Member States concerned on 20-26/02/2014).
9	Ensure that the regional legislation is immediately adapted to be in line with Decision 2014/87/EU.	The Implementing Decision 2014/87 is dated February 13, 2014 and was published on February 15, so later than FVO audit and therefore it was not possible to adapt the regional legislation. This adjustment was made by an act of the Regional Agricultural Service n. 31 of 27.2.2014, copy of which is attached.
10	Ensure that the survey is carried out at the most appropriate period for the detection of Xylella fastidiosa, as required in point 1 of Art. 2 of Decision 2014/87/EU.	The finding of *X. fastidiosa* was made in October 2013, so the first investigations were carried out to immediately ascertain the presence and spread of the pathogen. In any case, the analysis of

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		some species such as olive and oleander allowed to detect the presence of the pest even in this period. With the preparation of the Action Plan have been defined the procedures and criteria by which the monitoring activities will be carried out in 2014 in accordance with article 2 of the Decision 2014/87/EU and certainly in the most appropriate period for the detection of *Xylella fastidiosa*.