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Hot water treatment of *Vitis* sp. for *Xylella fastidiosa*

Scientific Panel on Plant Health (PLH)

Abstract

Following a request from the European Commission, the EFSA Panel on Plant Health (PLH) reviewed Italian technical guidelines and the ANSES (Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail) opinion on the use of hot water treatment (HWT) on *Vitis* sp. planting material, assessing its efficacy in the elimination of the xylem-invading bacterial pathogen, *Xylella fastidiosa*. HWT is a robust and reliable technique used to destroy life stages of pests (insects, nematodes) and to inactivate pathogens (phytoplasma, bacteria, fungi) in dormant plant propagation materials (grapevine and other crops). An effective HWT sanitizes the planting material without affecting plant survival and development. For grapevine, HWT to eliminate the Grapevine flavescence dorée phytoplasma (FD) from planting materials is among the special requirements for the introduction and movement of *Vitis* sp. to protected zones in the EU. The conditions of 50°C for 45 min, prescribed and recommended to sanitize grapevine planting material against FD, are considered by the Panel to be also effective against *X. fastidiosa* and its subspecies. Despite uncertainties on variable thermotolerances of the bacteria, a HWT treatment of 50°C for 45 minutes can effectively account for different thermotolerances. It should be noted that the quality of the HWT is subject to the proper application of the operating procedures to guarantee vigorous growth and pathogen freedom of planting material.

Keywords: dormant cuttings, Grapevine flavescence dorée, Pierce's disease, plant propagating material, sanitation

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1. Introduction

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

The purpose of this mandate is to request, pursuant to Article 29 of Regulation (EC) No 178/2002, scientific advice in the field of plant health as regards the regulated harmful organism *Xylella fastidiosa* (Wells et al.)

Specifically, the EFSA Scientific Opinion on *X. fastidiosa* published in January 2015 refers to thermotherapy as an effective treatment of dormant plants to control *X. fastidiosa* in grapevine plants for planting. This practice is already applied to control other pathogens in *Vitis* plant propagating material.

In the meantime, given the uncertainty of the host range of the Apulian strain of *X. fastidiosa* and the ongoing pathogenicity tests, *Vitis* has been included in Annex I of the recently adopted Commission Implementing Decision (EU) 2015/789 as a specified plant known to be susceptible to the European and non-European isolates of *X. fastidiosa*. This implies that any movement out of the demarcated areas can take place only if the *Vitis* plants for planting have been grown in a site where a series of conditions are met (Article 9(2)), in order to prevent any infection with the bacterium and contact with the insect vectors.

The Italian plant health Authorities have submitted to the Commission technical guidelines for hot water treatment against *X. fastidiosa* for the safe movement of grapevine germplasm. The guidelines, annexed to the letter, details the scientific background of this practice, the equipment needed, as well as pre-treatment and quality of the propagating material, treatment conditions, precautions during and after the hot water treatment, including during transport.

Furthermore, the French plant health Authorities have submitted to the Commission an Opinion (annexed to the letter) of their national Agency for Food, Environmental and Occupational Health & Safety (ANSES) as regards the efficacy of hot water treatments of plant propagating material against *X. fastidiosa*.

Consequently, EFSA is requested to review such information, assess the efficacy of such treatment on *Vitis* sp. plant propagating material against *X. fastidiosa* and validate accordingly the guidelines submitted by the Italian Authorities.

1.2. Interpretation of the Terms of Reference

The Panel interpreted the request as focused on the application of:

- hot water treatment
- against *X. fastidiosa*
- in *Vitis* sp. planting material

The Panel reviewed the two documents provided with the mandate according to this focus.

2. Data and Methodologies

2.1. Data

At the beginning of the mandate, an extensive literature search on the use of hot water treatment on *Vitis* sp. plants against *Xylella fastidiosa* was conducted. Keywords used were 'Hot water treatment', '*Xylella*' and '*Vitis*' and numerous variants of these basic search terms. The strings were applied on the research platform ISI Web of Science. The collected references were reviewed together with those cited in the two documents provided with the mandate and with those cited in the relevant section of the pest risk assessment that the Panel produced on this pest (EFSA PLH Panel, 2015). Further references and information were obtained from citations within the reviewed references and from experts.

[1] Submitted by European Commission, ref. SANCO E2/PdR/pm (2015) 2433474

2.2. Methodologies

The assessment was conducted in line with the principles described in the EFSA Guidance on transparency in the scientific aspects of risk assessment (EFSA Scientific Committee, 2009). The present document is structured according to the Guidance on the structure and content of EFSA's scientific opinions and statements (EFSA Scientific Committee, 2014). Uncertainties are identified and discussed with regard to their impact on the final conclusions.

With respect to the EFSA Guidance on risk assessment terminology (EFSA Scientific Committee, 2012) the Panel decided to use the term 'hot water treatment' for referring to the heat treatment of grapevine planting material, instead of using the term 'thermotherapy' (Waite and Morton, 2007).

3. Assessment

Hot water treatment (HWT) is a robust and reliable technique used for the elimination of pests (insects, nematodes) and pathogens (phytoplasma, bacteria, fungi) from plant propagation material (grapevine and other crops). For grapevine, it comprises the submersion in water of dormant, woody planting material (grafts and cuttings, with or without roots) for a given temperature and time (Waite and Morton, 2007; Gramaje et al., 2014). The mechanism at the base of the process of HWT is the application of heat to the material in order to denature the pathogens and kill insects and nematodes.

The hot water is necessary to obtain an even and rapid distribution of the hot temperature to the material treated. An effective HWT sanitizes the planting material without affecting plant survival and development (Waite and Morton, 2007). Temperature and time of HWT are correlated such that, within the limits of temperatures effective for pathogen elimination (>45°C) (Goheen et al., 1973; Caudwell et al., 1997; Sanderlin and Melanson, 2008) and for phytotoxic responses (<60°C) (Goussard, 1977), lower temperatures can be compensated by longer exposure times to efficiently eliminate pathogens.

HWT of 50°C for 45 min is indicated in Annex IV, Part B, Art. 32 of Council Directive 2000/29/EC[2], to eliminate the Grapevine flavescence dorée phytoplasma (FD), as a special requirement for the introduction and movement of grapevine planting material to the listed protected zones. The phytosanitary standard detailing the HWT conditions of 50°C for 45 min against FD is provided by EPPO (2012). This same temperature/time regime is considered effective against *X. fastidiosa*, the causal agent of Pierce's decline, in both the Italian guidelines and the ANSES opinion (for details see the section 'Documentation provided to EFSA'). Also outside the EU, similar HWT are in use, such as in Australia, where a HWT of 50°C for 30 min is mandatory for imported dormant grapevine cuttings, against both FD and Pierce's disease (DAFF, 2013).

Unlike the FD, which lacks a cell wall and reproduces in the plant phloem, *X. fastidiosa* is a xylem-limited bacterium which multiplies and spreads within the plant xylem vessels (that extend deeply towards the centre of the lignified stem, closer to the pith than phloem) (EFSA PLH Panel 2014, 2015). These differences in morphology and tissue specificity of the two pests could imply different heat tolerances and time requirements to reach the effective denaturing temperature in the invaded tissue.

Inactivating of xylem-inhabiting bacteria in *Vitis* sp. plants subjected to HWT is reported for *X. fastidiosa* causing Pierce's decline (Goheen et al., 1973), for *Xylophilus ampelinus* (previously named *Xanthomonas ampelina*) causing the bacterial blight (Roberts, 1993) and for *Agrobacterium vitis* causing the grapevine crown gall disease (Burr et al., 1989, 1996; Bazzi et al., 1991). When applied to eliminate *A. vitis* from dormant grapevine cuttings, HWT produced substantial reductions in viability but did not completely inactivate all *A. vitis* bacteria. At treatment temperatures of 55°C for 30 min or extended treatment time (50°C for 90 min, in Burr et al., 1996) viable *Agrobacterium* cells could still be isolated from the treated cuttings. In contrast, HWT on dormant cuttings or rooted plants eliminated *X. fastidiosa* (Goheen et al., 1973) and likewise *X. ampelinus* (Roberts, 1993). Already a 20 min submersion time at 50°C effectively eliminated all *X. fastidiosa* bacteria from grapevine (Goheen et al., 1973) and neither viable bacteria could be isolated from the treated cuttings nor did

[2] 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. Official Journal of the European Communities L 169/1, 30.06.2014, p. 1–181.

the plants cured by HWT develop symptoms of the disease in the following two years. A HWT of 45°C was effective against *X. fastidiosa* when applied for an extended time (45°C for 3 hours, also suggested in the Italian guidelines) and with similar effectiveness as the 50°C treatment. In contrast, a HWT to eliminate *X. fastidiosa* from pecan (*Carya illinoinensis*) scion wood at 46°C applied for only 30 min did not guarantee a complete sanitation from the pathogen (Sanderlin and Melanson, 2008; Melanson and Sanderlin, 2015). Even taking into account possible differences in thermotolerance of bacteria species, 50°C can be considered an effective denaturing temperature for bacteria and phytoplasmas alike.

The time required to reach the effective 50°C temperature in the internal tissues (pith, the central zone of the stem, which is surrounded by the xylem tissues) varies with the section diameter of the plant material. Pith temperature measurements of vine cuttings subjected to HWT showed that this temperature is reached within 2–10 minutes, depending on the anatomical region, such as nodes or internodes, and on the thickness of the cutting. Burr et al. (1989) observed that the largest regions (cross-sectional area of 297 mm^2) reach this temperature in around 10 min. A treatment of 50°C for 45 minutes is already recommended for HWT on grapevine dormant cuttings and rooted plants in Council Directive 2000/29/EC and in the EPPO standard (EPPO, 2012). Such treatment of 50°C HWT for 45 min is considered sufficient for the elimination of the xylem-invading bacterium *X. fastidiosa* as it provides additional treatment time to cover for differences in the diameter of the dormant planting material.

Additional considerations

***Xylella fastidiosa* subsp. *pauca* strain CoDiRO.** *X. fastidiosa* subsp. *fastidiosa* infecting grapevine and *X. fastidiosa* strain CoDiRO infecting olives in Apulia (considered a new genetic variant within subsp. *pauca* (Cariddi et al., 2014; EFSA PLH Panel, 2015)), belong to the same bacterial species. *X. fastidiosa* subsp. *fastidiosa* and other plant pathogenic bacteria (such as *X. ampelinus* and *A. vitis*) can invade, replicate and move in the xylem of *Vitis* sp., whereas *X. fastidiosa* subsp. *pauca* has hitherto not been reported to naturally infect this host. Despite the uncertainties of grapevine being a host for *X. fastidiosa* subsp. *pauca* strain CoDiRO, a similar xylem infection pattern in *Vitis* sp. can be assumed for all subspecies of *X. fastidiosa*, should they be proven pathogenic to *Vitis* sp. Hence, despite missing experimental data on thermotolerance of *X. fastidiosa* subspecies other than subsp. *fastidiosa* and the uncertainties on *Vitis* sp. being a possible host for *X. fastidiosa* subsp. *pauca* strain CoDiRO, the HWT conditions prescribed and recommended to sanitize against FD (EPPO, 2012) and Pierce's decline (Purcell et al., 2013) can by analogy be considered effective also against other *X. fastidiosa* subspecies, including *X. fastidiosa* subsp. *pauca*. strain CoDiRO.

HWT. Temperature and time prescribed for HWT (50°C/45 min) are efficient for general sanitation of the material treated. However, treatments at the lower temperatures (45°C) are generally of poorer effectiveness to eliminate other pathogens (Gramaje et al., 2009), surface contaminants and insects, nymphs or overwintering eggs, including those laid by potential vectors (Caudwell et al., 1997). In the EUPHRESCO (European Phytosanitary Research Coordination II) project PROPSCAPH (Evaluating the risk of spread of *Scaphoideus titanus* with propagation material), a 90% reduction of the number of nymphs hatching from eggs laid by *Scaphoideus titanus*, the vector of FD, was observed after HWT (50°C/45 min). The risk of vector spread via overwintering eggs was then considered significantly reduced (Linder et al., 2010). Such effect on overwintering insect eggs is potentially advantageous against *X. fastidiosa* vectors too.

Technical Guidelines. It is beyond the scope of this opinion to assess the safety of the proposed HWT to the planting material. This lies with the nurseries to follow the proper production process from selection of mother plants to cold storage, HWT and post-treatment operations and the manufacturers of the equipment. The technical guidelines by the Italian Authorities and the ANSES opinion are in line with the EPPO standard (EPPO, 2012) and prescriptions and brochures by other organisations for HWT (Caudwell et al., 1997; Boudon-Padieu and Grenan, 2002; Waite and Morton, 2007). The guidelines provide recommendations for the entire process that are to be considered to limit plant losses from HWT and to guarantee pathogen freedom. When all precautions are followed and the prescribed pre- and post-treatment protocols respected, HWT can be effectively applied to fully dormant tissues minimizing the plant losses resulting from HWT (Mannini, 2005; Mannini and Marzachì, 2007; EPPO, 2012; Gramaje et al., 2014).

In the Italian guidelines, details of the equipment design and process description are provided, to minimize temperature differences within the tank and to guarantee the rapid distribution of the warm temperature to the plant material. Those are likely implemented in the equipment available to conduct HWT on an industrial scale. As provided in the Italian guidelines, in brochures and in technical literature (Mannini, 2005; Mannini and Marzachì, 2007; Mannini and Bagnulo, 2009; Linder et al., 2010), the detailed description of the material to be treated, the batch size, water flow, mounting of materials into the basket and water tank are available to guide a successful HWT process.

There is also a need to assess the correct calibration of the HWT equipment and to ensure that it is operated properly, e.g. through accreditation by independent testing authorities or the application of harmonised standards (Waite, 2005).

Production of *Vitis* sp. planting material. HWT to sanitize propagation material is only one element in the nursery production of grapevine planting material (e.g. cuttings, budwood, rootstocks, etc.). To assure the quality of the propagation material and to guarantee freedom of pathogens, which is supported by EU phytosanitary measures and procedures related to grapevine germplasm movement (Council Directive 2000/29/EC; Council Directive 68/193/EEC and its amendments[3]), nurseries are regularly inspected, plantations are monitored for symptoms and harmful organisms are kept at a minimum. Thus propagation materials are taken from certified sources, and there are comprehensive crop management practices in place to guarantee the quality of the product and to ensure pathogen freedom. Within the context of the good production practices for nursery materials, HWT is a robust and reliable measure to guarantee a health status of the material treated and to safeguard freedom from many pathogen species.

3.1. Uncertainties

Only a few experimental publications exist on the effect of HWT to eliminate *X. fastidiosa* from grapevine (Goheen et al., 1973), and from pecan scion wood (Sanderlin and Melanson, 2008; Melanson and Sanderlin, 2015). For grapevine, an effective elimination of *X. fastidiosa* the causative agent of Pierce's decline in grapevine planting material was reported (Goheen et al., 1973). Uncertainty exists on possible differences in thermotolerance of *Xylella* subspecies and strains and on the effectiveness of the same treatment conducted in real conditions instead of experimental conditions. However, taking into account the duration and the temperature of the treatment and the information available on *X. fastidiosa* subsp. *fastidiosa* and other plant pathogenic bacteria invading grapevine, these uncertainties are sufficiently addressed by the prescribed protocol

Grapevine varieties respond differently to HWT and some, like Pinot noir, are more susceptible than others (Waite et al., 2001). The general lack of data and publications therefore limits the possibility to assess the negative impact of HWT on different grapevine genotypes. However, following the prescribed procedures this negative impact of HWT can be considered limited.

4. Conclusions

Based on the Italian technical guidelines and the ANSES opinion reviewed by the Panel as well as on the literature currently available on HWT for controlling *X. fastidiosa* and other grapevine pathogenic bacteria, it is concluded that the standard HWT of 50°C for 45 min already in place to eliminate FD from dormant planting material is also efficient for controlling *X. fastidiosa* in grapevine.

Within the context of the good production practices for nursery materials, HWT is considered as a robust and reliable measure to guarantee the health status of the material treated and to safeguard freedom from pathogens and pests. The quality of the entire process, however, relies on the proper application of operating procedures to guarantee vigorous growth and pathogen freedom of planting material.

[3] Council Directive 68/193/EEC of 9 April 1968 on the marketing of material for the vegetative propagation of the vine. Official Journal of the European Communities L 93/15, 17.4.68, p. 93–103.
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Commission Directive 2005/43/EC of 23 June 2005 amending the Annexes to Council Directive 68/193/EEC on the marketing of material for the vegetative propagation of the vine. Official Journal of the European Union L 164/37, 24.6.2005, p. 37–45.

Documentation provided to EFSA

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2. Avis relatif à l'efficacité du traitement à l'eau chaude sur le matériel végétal vis-à-vis de *Xylella fastidiosa*. Saisine n° «2015-SA-0099». Maisons-Alfort (France), le 13 mai 2015. Prepared by ANSES (Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail).

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Abbreviations

CoDiRO	Complesso del Disseccamento Rapido dell'Olivo
EPPO	European and Mediterranean Plant Protection Organization
EU	European Union
FD	Grapevine flavescence dorée phytoplasma
HWT	hot water treatment
PLH Panel	EFSA Scientific Panel on Plant Health