

The Director General

Maisons-Alfort, 3 February 2017

OPINION of 26 October 2016 revised on 11 January 2017¹

of the French Agency for Food, Environmental and Occupational Health & Safety

relating to "Changes to the time/temperature combination in the hot water treatment of plant material"

ANSES undertakes independent and pluralistic scientific expert assessments.

ANSES's public health mission involves ensuring environmental, occupational and food safety as well as assessing the potential health risks they may entail.

It also contributes to the protection of the health and welfare of animals, the protection of plant health and the evaluation of the nutritional characteristics of food.

It provides the competent authorities with the necessary information concerning these risks as well as the requisite expertise and technical support for drafting legislative and statutory provisions and implementing risk management strategies (Article L.1313-1 of the French Public Health Code).

Its opinions are made public.

This opinion is a translation of the original French version. In the event of any discrepancy or ambiguity the French language text dated 3 February 2017 shall prevail.

On 18 December 2015, ANSES received a formal request from the Directorate General for Food (DGAL) to undertake the following expert appraisal: Request for an Opinion on "Changes to the time/temperature combination in the hot water treatment of plant material".

1. BACKGROUND AND PURPOSE OF THE REQUEST

The plant health regulations relative to the Grapevine Flavescence Dorée (FD) leafhopper state that the hot water treatment (HWT) of grapevine plant material must be carried out with a time/temperature combination of 45 minutes at 50°C (Annex IV, Part B. Art 32 of Directive 2000/29/EC). Furthermore, hot water treatment with the time/temperature combination of 45 minutes at 50°C has also been validated by EFSA for sanitising plant material regarding *Xylella fastidiosa* (X.f.). It is included in the amendment of the Community Decision on *Xylella fastidiosa* adopted on 23 November 2015.

However, according to some professionals, performing this hot water treatment hampers renewed growth of the plant material and delays bud break, which is harmful to the wine-growing sector.

In view of this, FranceAgriMer and the French Federation of Vine Nurseries (FFPV) have co-financed a study programme by the French Wine and Vine Institute (IFV). This study shows that a

¹ The section "*Agrobacterium tumefaciens* and *Agrobacterium vitis*" (page 4) has been replaced.

time/temperature combination of 35 minutes at 50°C sanitises grapevine plant material concerning FD without the harmful effects on renewed growth or delayed bud break.

In view of all these factors, this expert appraisal was expected to determine:

1- the effectiveness of hot water treatment on Flavescence Dorée (FD) and *Xylella fastidiosa* (X.f.) with a time/temperature combination of 35 minutes at 50°C,

2- the effectiveness of hot water treatment on Flavescence Dorée (FD) with time/temperature combinations of 35 minutes at 50°C and 45 minutes at 50°C on:

- the organisms responsible for yellows diseases in general,
- *Xylophilus ampelinus*,
- phylloxera,
- *Agrobacterium tumefaciens* and *Agrobacterium vitis*,
- virus-vector nematodes,
- insects, particularly during the egg, larval and nymph stages of their development.

2. ORGANISATION OF THE EXPERT APPRAISAL

The expert appraisal was carried out in accordance with French Standard NF X 50-110 "Quality in Expert Appraisals – General Requirements of Competence for Expert Appraisals (May 2003)".

It falls within the sphere of competence of the Expert Committee (CES) on "Biological risks for plant health". ANSES entrusted the expert appraisal to the Working Group on "Hot water". The methodological and scientific aspects of the work were presented to the CES between 19 January 2016 and 12 September 2016. The work was adopted by the CES on "Biological risks for plant health" at its meeting on 12 September 2016.

ANSES analyses the links of interest declared by the experts prior to their appointment and throughout the work, in order to avoid potential conflicts of interest with regard to the matters dealt with as part of the expert appraisal.

The experts' declarations of interests are made public via the ANSES website (www.anses.fr).

3. ANALYSIS AND CONCLUSIONS OF THE CES

- **The effectiveness of hot water treatment on Flavescence Dorée (FD) and *Xylella fastidiosa* (X.f.) with a time/temperature combination of 35 minutes at 50°C**
 - **Comparison of different time/temperature combinations on the effectiveness of the treatment regarding Flavescence Dorée based on the analysis of the work of the French Wine and Vine Institute (IFV)**

It is clear from the work of the IFV that treatment for 35 minutes at 50°C was effective in eliminating FD in a first test performed in 2010, with heavily infected plants. In the absence of symptoms in the second year, similar results were obtained in an independent trial conducted in 2011, albeit with a very low infection rate as shown by the untreated controls. As no signs of FD were observed following hot water treatments (HWTs), either with a shorter time at 50°C, or with a lower temperature (48°C) for 35 minutes, these results can be assumed to demonstrate that hot water treatment against FD of 35 minutes at 50°C is effective with a reasonable safety margin.

- **Comparison of different time/temperature combinations on the effectiveness of the treatment against Flavescence Dorée based on an analysis of the literature**

The numerous studies carried out by Caudwell et al. show the range of HWT combinations that are effective for destroying FD. Caudwell states that a temperature of 50°C is particularly effective for durations of 30 to 60 minutes. This obviously encompasses the HWT “35 minutes at 50°C” which is the subject of this Request.

The work of Goheen et al. gives a range of effectiveness of HWT against Pierce's disease (PD), caused by *X. fastidiosa*. This range, also used by Caudwell, is similar to that effective against FD. These studies indicate that the two pathogens are effectively controlled by HWT of 35 minutes at 50°C.

- **Effectiveness of hot water treatment with time/temperature combinations of 35 minutes at 50°C and 45 minutes at 50°C against the following organisms: yellows in general, *Xylophilus ampelinus*, phylloxera, *Agrobacterium tumefaciens* and *Agrobacterium vitis*, virus-vector nematodes, and insects, particularly during the egg, larval and nymph stages of their development.**

- ***Grapevine yellows in general***

To treat grapevines against the yellows reported in France, HWTs for 35 and 45 min at 50°C are effective against FD and also apparently against Palatinate grapevine yellows, but ineffective or only partially effective (because not supported by conclusive tests) against the *Candidatus* *Phytoplasma solani* causing black wood of grapevines and the Aster yellows phytoplasma.

- ***Xylophilus ampelinus***

To the extent that HWT at 50°C for 20 minutes sanitises grapevine cuttings regarding *X. ampelinus*, longer HWTs for 35 min or 45 min at 50°C will also be effective in destroying the bacterium. There is some uncertainty concerning the effectiveness of these treatment parameters regarding their extrapolation to grapevine cuttings and plants infected by a natural process, where the bacteria could be sensitive to different temperatures by reason of their location or their physiology. This uncertainty is relatively low, however.

- ***Phylloxera***

We can extrapolate with a low degree of uncertainty that HWTs for 35 and 45 minutes at a temperature of 50°C are effective against phylloxera, apparently at all stages of the insect's development.

- ***Agrobacterium tumefaciens* and *Agrobacterium vitis***²

A. vitis and *A. tumefaciens* biovar 1 have different biologies and do not respond to HWTs in the same way. The extrapolation of experimental data suggests with moderate uncertainty that HWT for 35 min and especially for 45 min at 50°C could be effective for sanitising plants against *A. vitis*. On the other hand, even in the absence of experimental data on infected plants, it is very unlikely that HWT for 35 min at 50°C would sanitise plants infected by *A. tumefaciens* biovar 1. The absence of data makes it impossible to assess the effectiveness of HWT for 45 min at 50°C against this type of *Agrobacterium*.

- ***Virus-vector nematodes***

The nematodes associated with grapevines are quickly destroyed by HWT at 50°C for a duration of a few minutes and therefore, the time/temperature combination (50°C for 35 or 45 min) proposed in the Request should have no difficulty in eliminating this type of organism if it happened to be present in grapevine plants. Virus-vector nematodes are ectoparasites that are not transmitted by plants. It is therefore not necessary to recommend an HWT to eliminate them.

- ***Insects during their egg, larval and nymph stages***

Regarding insects, HWTs have been tested on few organisms. HWT at 50°C for 45 min is not totally effective for rendering all the eggs of *Scaphoideus titanus* non-viable. A substantial reduction in the production of larvae has been observed, however. In the case of *Planococcus ficus*, the extrapolation of HWT tests suggests with moderate to low uncertainty that HWTs at 50°C for 35 min or 45 min result in complete mortality of cochineal eggs.

■ **Conclusion of the report**

Applying hot water treatments to grapevine wood generally sanitises the organs treated regarding grapevine pests. However, pests differ in their sensitivity to the treatments.

The various data from the literature and the recent trials by the IFV show that treating grapevine wood and young plants with hot water for 35 minutes at 50°C is effective for destroying FD phytoplasma. The literature data indicate that the same is true for *X.f.*

However, none of the literature data testify to the overall effectiveness of a treatment which, whether in its duration or in the application temperature, does not comply with the minimum conditions of 35 minutes and 50°C. Reducing the duration of the treatment from 45 to 35 minutes can therefore only be considered if the 50°C setpoint temperature of the treatment is sufficiently controlled to prevent the actual temperature from dropping below 49°C for 35 minutes. In addition, for the duration of the treatment to be really effective, it must be counted from the moment that the bath is at the setpoint temperature, i.e. after stabilisation of the temperature following the immersion of the plants.

² Cancels and replaces the section in the Opinion of 26 October 2016: *A. vitis* and *A. tumefaciens* biovar 1 have quite different biologies and do not respond to HWTs in the same way. It is very unlikely that an HWT (50°C/35 min) would be effective for sanitising grapevines against *A. tumefaciens*, and considerable uncertainty remains concerning the effectiveness of HWT (50°C/45 min). It therefore seems that an HWT of 30 min at 50°C reduces infection with crown gall but does not fully sanitise grapevines regarding *A. vitis*. Since higher temperatures are more effective, it might be assumed with only moderate uncertainty that a longer exposure, 35 min and especially 45 min, would be more effective, but to our knowledge there have been no trials concerning the effectiveness of HWTs for 35 min or 45 min at 50°C against *A. vitis*.

As regards grapevine yellows, the data collected from the literature and the experimental results provided by the IFV show a diversity of responses to the treatment among the yellows that takes the form of different tolerances to temperatures, particularly between FD and the black wood of grapevines. Treatment at 50°C for 35 min is ineffective for eliminating the phytoplasma responsible for black wood of grapevines. None of the studies are sufficiently robust to show that treatment lasting 45 minutes would be more effective. The other yellows are not found on grapevines in France. Palatinate grapevine yellows (*Vergilbungskrankheit*) is found on alder. In view of its taxonomic proximity to FD, we may conclude that its sensitivity to HWT is similar to that of FD, with considerable uncertainty due to the lack of experimental data. For the phytoplasma of Aster yellows, the scientific literature does not provide data to show that HWT is effective.

As regards *Xylophilus ampelinus*, HWT at 50°C for 35 min or 45 min is effective for eliminating the bacterium. There is some uncertainty concerning the effectiveness of these treatment parameters regarding their extrapolation to grapevine cuttings and plants infected by a natural process, where the bacteria could be sensitive to different temperatures by reason of their location or their physiology. This uncertainty is relatively low, however.

Regarding phylloxera, extrapolating the literature data indicates that HWTs at 50°C for 35 and 45 minutes are effective against phylloxera, apparently at all stages of the insect's development. Moderate uncertainty remains, because the precise parameters (50°C for 35 min or 45 min) have not been tested.

Regarding *Agrobacterium*, *A. vitis* and *A. tumefaciens* biovar 1 have different biologies and do not respond to HWTs in the same way. The extrapolation of experimental data suggests with moderate uncertainty that HWT for 35 min and especially for 45 min at 50°C could be effective for sanitising plants against *A. vitis*. On the other hand, even in the absence of experimental data on infected plants, it is very unlikely that HWT for 35 min at 50°C would sanitise plants infected by *A. tumefaciens* biovar 1. The absence of data makes it impossible to assess the effectiveness of HWT for 45 min at 50°C against this type of *Agrobacterium*.

Virus-vector nematodes are ectoparasites that are not transmitted by plants in the absence of substrate. A recommended HWT is therefore unnecessary under the terms of this Request. Nevertheless, virus-vector nematodes associated with grapevines are sensitive to HWT under the conditions proposed.

Regarding insects, HWTs have been tested on few organisms. HWT at 50°C for 45 min is not totally effective for rendering all the eggs of *Scaphoideus titanus* non-viable. A substantial reduction in the production of larvae has been observed, however.

In the case of *Planococcus ficus*, the extrapolation of HWT tests suggests with moderate to low uncertainty that HWTs at 50°C for 35 min or 45 min result in complete mortality of cochineal eggs.

4. AGENCY CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety endorses the CES's conclusions.

Roger GENET

KEYWORDS

Hot water, bacteria, insects, grapevine yellows, nematodes, Flavescence Dorée, *Xylella fastidiosa*, *Agrobacterium*, *Xylophilus*, *Phylloxera*