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European and Mediterranean Plant Protection Organization
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PM 10/18(1)

Phytosanitary treatments
Traitments phytosanitaires

Hot water treatment of grapevine to control Grapevine flavescence dorée phytoplasma

Specific scope

This Standard describes a long-duration hot water treatment of grapevine material against flavescence dorée phytoplasma. It is also useful against its vector, *Scaphoideus titanus*, and other grapevine pathogens. A short-duration hot water treatment of grapevine against *Viteus vitifoliae* is described in PM 10/16 *Hot water treatment of grapevine to control* Viteus vitifoliae.

Specific scope

First approved in 2012-09.

Introduction

Grapevine flavescence dorée phytoplasma is the causal agent of flavescence dorée disease in *Vitis vinifera* (grapevine). It is listed in Annex II/A2 of the EU Plant Health Directive (2000/29/EC) and as an EPPO A2 pest recommended for regulation as a quarantine pest. The principal vector of flavescence dorée is the leafhopper *Scaphoideus titanus* (Auchenorrhyncha: Cicadellidae), which was introduced into Europe from North America.

There are two types of hot water treatment often used as prophylactic and quarantine measures for grapevine propagation material throughout the world: (1) a short-duration hot water treatment (52°C for 5 min) for the control of external pests such as *Viteus vitifoliae*; (2) a long-duration hot water treatment (see below) for control of both external and internal (endogenous) pests and pathogens (Metlitskiy, 2002) such as flavescence dorée. This hot water treatment is recommended in EPPO Standard PM 4/8 *Pathogen-tested material of grapevine varieties and rootstocks.*

Commodities/regulated articles

Dormant wood of both scions and rootstocks prior to grafting or rooted grafted vines of *Vitis vinifera* (VITVI) for planting.

Grapevine flavescence dorée phytoplasma (PHYP64) and its vector *Scaphoideus titanus* (SCAPLI.)

The hot water treatment recommended for phytoplasma elimination is immersion at 50°C for 45 min. Alternative schedules are given in Caudwell *et al.* (1990) and Groupe de travail National (2006). The alternative schedules are mentioned in this paper only as an exception with specific premises.

Treatment conditions

Hot water treatment is a significant stress and can result in the loss of treated material if not applied correctly. Precautions should be taken prior to, during and after the treatment as described below. The treatment schedule is summarized in Table 1.

Pre-treatment

Plant material to be treated should contain the best possible amount of reserves, that is, the plant material should be fully

Table 1 Treatment schedule

Pre-treatment		Hot water treatment		Post-treatment	
Time (h)	Air T (°C)	Time (min)	Water T (°C)	Time (h)	Air T (°C)
12–24	Room temperature	45	50	12–24	Room temperature

lignified. Plants should have completed their vegetative cycle at the moment of pruning or uprooting and stay in full dormancy. Material should be kept at optimal temperature and humidity after cutting or uprooting. Cuttings or rooted vines that are not fully dormant are very sensitive to hot water treatments and may not survive treatment.

Grapevine wood, scions and rootstocks prior to grafting should be held in cold storage (1–5°C and high relative humidity) to maintain dormancy and enhance quality. However, grapevine plant material should be taken out of cold storage 12–24 h prior to treatment and stored at room temperature in a humid and aerated chamber. Although it used to be common practice, soaking the material in cold water is no longer recommended as it creates a risk of spreading pathogens (Waite & Morton, 2007).

The roots should be washed prior to treatment. Canes should not be cut or treated with fungicides just before treatment.

Treatment

The hot water treatment should be done immediately before grafting, at the end of the storage period. Treatment before or during storage in a refrigerating chamber is strongly inadvisable.

The temperature (50°C) after immersion and the treatment duration (45 min) should be respected. Note that after immersion of the plants the temperature of the water may decrease below 50°C; the duration should be recorded only once the temperature of the water is back to 50°C.

No fungicide should be added to the soaking water.

The water in the tank should be changed regularly according to the frequency of treatments, but at least once a day.

Post-treatment

Long-term storage after treatment should be avoided as it might cause superficial mould and also a temporary delay in vegetative revival (Boudon-Padieu & Grenan, 2002; Metlitskiy, 2002).

After treatment, plant material should be left to return to room temperature for 12–24 h in a humid and aerated atmosphere before storage in a cold chamber for a short time, or before grafting. Direct contact with cold water should be avoided as it may result in increased mortality or infection by pathogens (Boudon-Padieu & Grenan, 2002; Waite & Morton, 2007).

Equipment

Equipment should be specially designed to maintain exactly the required temperature throughout the plant material by an efficient mixing system. Adequate equipment is described in Boudon-Padieu & Grenan, 2002; Groupe de Travail National (2006), ICA (2007).

Hot water tanks in which treatment is to occur should:
- be purpose-built;
- be constructed from inert material;
- have a means of circulating and heating water in order to maintain a consistent uniform temperature and have appropriate thermal insulation with a lid to limit heat loss and
- have appropriate measurement and recording equipment (see below).

An open-mesh cage or similar device for immersion of grapevine material in the tank should:
- be constructed from an inert material;
- allow adequate circulation of hot water around the grapevine material;
- have a clearance from the tank on all sides (e.g. 150 mm) to facilitate water circulation;
- have a mesh lid or other device to ensure all material remains fully immersed during treatment.

Temperature sensing and recording systems should have a combined overall accuracy of not more than ±0.5°C in the range 50–55°C and a resolution of up to 0.2°C (i.e. the combined sensing and data recording systems should be accurate to within 0.5°C of the true temperature and must be able to be read in increments of 0.2°C or less). This should be checked regularly.

Ideally, three sensors should be used for each tank. One sensor should be located at a depth of 100 mm from the base of the tank, another at 100 mm from the surface, and the third inserted into the centre of the load mass (Metlitskiy, 2002). In practice, the third sensor may not be necessary if the circulation of water is appropriate.

Transportation

The treated material should be placed for transport in aerated containers with water supply (providing high humidity). If external temperatures are high (increasing the risk of fermentation or desiccation of the material), it may be necessary to place containers in a refrigerated compartment during transport.

Efficacy of treatment

Management of flavescence dorée includes the eradication of infected plants that serve as sources for infection, as well as control of the vectoring leafhopper *S. titanus.*

Hot water treatment has been proposed since 1966 by Caudwell to treat dormant woody plant material against phytoplasmas. Later work showed the effectiveness of the treatment against these pathogens (Caudwell *et al.*, 1990; Tassart-Subirats *et al.*, 2003), although some other experiences indicate that *Stolbur phytoplasma* (Bois noir) was more difficult to completely eradicate than flavescence dorée (Borgo *et al.*, 1999; Bianco *et al.*, 2000; Mannini & Marzachi, 2007).

Hot water treatment against flavescence dorée phytoplasma is considered a reliable technique and is compulsory for the basic propagation material in France (Ministère de l'Agriculture France, 2003). It is recognized as a phytosanitary treatment in EU Directive 2000/29/EC (EU, 2000) and EPPO Standard PM 4/8, as well as by other organizations (Frison & Ikin, 1991; ICA, 2007).

This hot water treatment is also effective in eliminating eggs of the leafhopper *S. titanus* if used on 1-year-old grapevine propagation material (Schaub, 2010). Older (2-year) wood may have higher levels of *S. titanus* eggs, which are not fully controlled by this hot water treatment.

This hot water treatment is also effective against *V. vitifoliae* (see also Short-duration hot water treatment, PM 10/16) and eliminates, or reduces the incidence or level of infestation of, most well known fungal pathogens and endophytes responsible for grapevine diseases, including *Stolbur phytoplasma* (Bois noir causing blackwood disease), *Agrobacterium vitis*, *Xilophilus ampelinus*, and fungal pathogens responsible for trunk diseases such as *Phaeomoniella chlamydospora*, *Botryosphaeria obtusa*, *Phomopsis viticola* and *Neonectria liriodendra* (but not *Botryosphaeria parva* and *Phaeoacremonium aleophilum*).

However, such disinfection does not prevent reinfestation if the treated material is planted in infected soil.

Some reluctance concerning the use of hot water treatment exists in some countries because of its possible negative effect on the vitality of woody propagation material. It was hypothesized that some varieties may be more susceptible than others. However, there are no clear results from the literature, as different authors have different results for the same varieties (Frausin *et al.*, 1999; Moretti *et al.*, 2002; Tassart-Subirats *et al.*, 2003; Waite & Morton, 2007). Climatic conditions in which the grapevine material is cultivated after treatment also play a role. Negative effects on vitality may be more directly linked to inappropriate material, or non-respect of the pre- and post-treatment requirements.

Enquiries

Institut Français de la Vigne et du Vin (IFV), Domaine de l'Espiguette, F-30240 Le Grau du Roi, FR, www.vignevin.com
EPPO Secretariat, Paris

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