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CURRENT TOPICS - 9TH SPECIAL ISSUE ON GRAPEVINE TRUNK DISEASES

Identifying practices likely to have impacts on grapevine trunk disease infections: a European nursery survey

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Summary. A questionnaire covering all aspects of grapevine propagation including cultural and sanitation practices in mother blocks and harvest and transport of cuttings from mother blocks to nurseries, nursery operations and field nursery management, was mailed to all Management Committee members of the European COST Action FA1303 "Sustainable Control of Grapevine Trunk Diseases" for distribution to the identifiable nurseries in each European country. The main objective was to develop understanding of the current propagation practices and to identify those likely to have the greatest impacts on the quality of planting material, especially with regard to the control measures used against fungal trunk pathogen infections. The questionnaire was sent to 666 vine nurseries, and 146 replies were received (21.9% response rate) The study identified several risks factors which could increase infection by fungal trunk pathogens during the propagation processes, as well as a clear need for further research into the effects of treatments on grapevine viability, including hot water treatment, and the potential of biological agents and other strategies such as ozonation to control grapevine trunk diseases in nurseries.

Key words: black-foot, Botryosphaeria dieback, Petri disease, young vine decline, *Vitis vinifera.*

Introduction

The European Cooperation in Science and Technology (COST) Action FA1303 "Sustainable Control of Grapevine Trunk Diseases" (funded by the European Union), was initiated in 2013. The main aim of this initiative was to develop a network of European expertise to improve the understanding of grapevine trunk diseases (GTDs), by acquiring knowledge of pathogen epidemiology, vine/pathogen interactions and the ecology of wood-inhabiting microorganisms. The goal was to develop new management protocols and biocontrol methods (Fontaine and Armengol, 2014). This COST Action is a collaborative programme involving leading multidisciplinary researchers and institutions within Europe, working together to develop recommendations for the management of GTDs and to establish Europe as a world leader in GTD research to safeguard vineyards. In an effort to increase knowledge and awareness of the problems caused by GTDs, four Working Groups have been established to disseminate the outcomes of research and knowledge gathering to end-users, including grape growers, authorities in the viticulture sectors and the general public. One of the objectives of Working Group 4 (WG4) "Disease Management" is to recommend protocols that may prevent and reduce the impacts of pre-existing GTD pathogen infections in nurseries.

Defects in young vines originate from a number of sources and frequently involve interactions between several factors including: i) cutting quality (Stamp, 2001); ii) nursery practices (Waite and Morton, 2007; Waite *et al*., 2015); and iii) fungal trunk infections (Gramaje and Armengol, 2011). In the case of the European Union (EU) countries, there is

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a certification scheme elaborated by the European and Mediterranean Plant Protection Organization (EPPO), which provides detailed guidance on the production of pathogen-tested material of grafted grapevine varieties and rootstocks (OEPP/EPPO, 2008). Planting material produced according to this certification scheme is derived from nuclear stock plants that have been tested and found free from some viruses and phytoplasmas, and checked for the presence of other pathogens such as *Phaeoacremonium minimum* (Gramaje *et al*., 2015) and *Phaeomoniella chlamydospora*. This certification scheme lacks detail, however, particularly in the area of general nursery sanitation and stress management. Consequently practices are variable within and between nurseries (Gramaje *et al*., 2012), wastage rates are high, and the quality of the resulting planting material is inconsistent (Stamp, 2001; Smart, 2013). In addition, many wounds are produced on plant material during cutting and graft preparation, such as those resulting from disbudding, grafting, improperly matched or healed graft unions, or the rooting process. The large numbers of cuts and wounds make the propagation material very susceptible to infection by fungal trunk pathogens (Gramaje and Armengol, 2011).

Despite the significant body of research investigating the effects of nursery practices in cuttings and rootlings since the early 2000s (Crocker *et al*., 2002; Waite and May, 2005; Waite and Morton, 2007; Gramaje *et al*., 2009a; Gramaje and Armengol, 2012), further research is needed to develop propagation procedures that are reliable and results in the production of planting material free of GTDs and results in high quality vines. Nursery surveys have been previously conducted in Australia (Waite *et al*., 2013b) and Spain (Gramaje *et al*., 2012) and have identified a number of nursery practices, such as hydration or poor sanitation, that favour disease transmission and impact on the quality of planting material. To facilitate the planning of a relevant and targeted Europe-wide research programme (Kelley *et al*., 2003), a systematic survey of grapevine nurseries across Europe was undertaken to develop understanding of the current propagation practices and to identify those likely to have the most impact on the quality of planting material, especially with regards to the control measures used against grapevine trunk fungal pathogen infections.

Materials and methods

Survey type

An emailed survey designed to ensure that respondents remained anonymous was sent to all Managing Committee (MC) members of the COST Action FA1303 by the leader and vice leader of the WG4, for distribution to identifiable nurseries in each European country. MC members then contacted the nurseries using several approaches, including electronic surveys, telephone interviews, hardcopy surveys and face to face interviews. After gathering the completed surveys from the nurseries, MC members sent them to the vice leader of the WG4 for collation and analysis of results.

Survey design

The survey consisted of 32 questions divided into three sections: 1) management of grapevine mother fields; 2) nursery operations; and 3) field nursery management. Section 1 covered the cultural and sanitation practices in mother blocks and harvest and transport of cuttings from mother blocks to nurseries. Section 2 covered nursery operations including the use of hydration (immersion of dormant rootstock and scion cuttings in water), cold storage, bench grafting, general sanitation practices and management strategies used to control fungal trunk pathogens (fungicides, biocontrol agents and hot water treatment). Section 3 covered field nursery practices including grafting, herbicide treatments and management of pests and diseases other than GTDs.

Each section included closed and open questions and covered all aspects of grapevine propagation. The closed format was used for both factual and subjective questions. Closed questions have a number of benefits including reduction of time taken to complete the survey and standardization of responses, but may not offer all alternative responses (Kalton and Schuman, 1982; Kelley *et al*., 2003). To ensure that vital information was not inadvertently excluded, open questions were linked with some closed questions to provide the opportunity for respondents to elaborate on their replies. The surveys were emailed to nursery operators in winter 2014–2015, and nurseries had ample time to respond the questionnaires (approx. 6 months).

Results and discussion

The survey was sent to 666 identifiable nurseries in Europe, and 146 replies were received, giving a response rate of 21.9%. The response rates by country are shown in Table 1. Although the response rate was relatively high (Hayman and Alston, 1999), the low actual numbers of responses (146) compare to the approximate total number of nurseries in the European countries surveyed (1491), and the self-selecting nature of the respondents, mean that the capacity to apply statistical tests to the data is limited and results may be biased. Therefore, we have reported proportions and summary statistics only.

Survey Part 1 – Grapevine mother fields

Sources of grapevine cuttings

Rootstock and scion mother plants are replaced 15 to 25 years after planting (47.9% of respondents each). A total of 21.2% of nurseries reported that they usually replace rootstock blocks, and 25.3% replace scion mother blocks, less than 15 years after initial establishment. Only 10.2% reported that they usually replace rootstock plants, and 15.7% of respondents replace scion plants later than 25 years after initial planting. The large number of cuts and wounds made during the lifespan of mother vines make them very susceptible to infection by fungal trunk pathogens (Gramaje and Armengol, 2011). As such, mother vines in the nursery production blocks can accumulate multiple infections by different trunk pathogens. The older the block, the greater is the chance of being infected.

Harvest and transport of cuttings

The very first step in the propagation process is the harvesting of cuttings and their transport from mother vine blocks to nurseries. Well managed harvesting operations in mother vine blocks are critical

Table 1. Data of the European grapevine propagation nursery survey.

Country	Questionnaires sent to nurseries	Replies received	Response rate (%)	Total number of nurseries[a]
Algeria	6	3	50.0	7
Bulgaria	15	10	66.7	22
Croatia	13	2	15.4	15
Czech Republic	3	3	100	15
France	200	13	6.5	400
Germany	7	5	71.4	100
Greece	5	2	40	12
Hungary	73	36	49.3	250
Israel	6	2	33.3	10
Italy	90	16	17.7	120
Portugal	19	6	31.6	116
Romania	4	4	100	15
Slovenia	15	5	33.3	29
Spain	170	32	18.8	380
Switzerland	40	7	17.5	nd[b]
Total	**666**	**146**	**21.9**	**1491**

[a] Approximately according to the MC members of each country.
[b] No data.

to the maintenance of cutting quality (Daughtrey and Benson, 2005). Cuttings left lying in the vineyard are liable to suffer dehydration, contamination by soilborne organisms and frost damage. Transport to the nursery may take several days, further increasing the risk of damage through dehydration, adverse temperatures and hypoxia (Waite *et al*., 2015). A narrow majority (51.4%) of nurseries that responded to the survey stated that cuttings are usually less than 4 h in transit before they arrive at the nursery from the mother fields. However, 12.3% of respondents reported having the cuttings more than 24 h in transit before nursery processing.

Irrigation

Thirty-three nurseries reported not using irrigation systems in the mother blocks. Of those that reported using irrigation systems, the majority (73.4%) reported that they used drip irrigation rather than sprinkler irrigation. Drip irrigation is most often used once the vine root systems are established. Overhead sprinklers have been considered a irrigation good method providing the sprinklers have uniform distribution patterns and are mounted high enough to clear the foliage (Nicholas *et al*., 2001). However, this method could enhance pathogen survival and dispersal, and disease development (Koike *et al*., 2007). Recent studies have demonstrated that overhead sprinkler irrigation can trigger release of Botryosphaeriaceae spores and *Pm. minimum* ascopores in some vineyard sites in California (Úrbez-Torres *et al*., 2010; Gubler *et al*., 2013).

Trellising

The majority (61.6%) of respondents reported using trellising systems for mother vines instead of allowing mother vines to sprawl along the ground from self-supporting crowns approx. 30 cm above the soil surface (22.6% of respondents). Only 17.8% of respondents reported using both methods, rootstock vines sprawled on the ground and scion vines cultivated on trellises. These practices in mother plants can have direct effects on trunk disease incidence and thus in the quality of propagating material (Gramaje and Armengol, 2011). Mother vines on trellises provide greater shoot mass, with longer shoots of higher quality and more uniform diameter. This method can eliminate potential black-foot pathogen contamination, but it is more expensive and labour intensive (Hunter *et al*., 2004). By contrast, the shoots of mother vine sprawled on the ground differ in the extent of exposure to sunlight. This has a direct effect on the reserves and ripeness of shoots; they do not ripen uniformly and this may eventually lead to varying callus success (Hunter *et al*., 2004). The susceptibility of flat-growing shoots to soilborne pathogens increases as a result of higher temperature and humidity, possible physical damage and the difficulties associated with the application of chemical sprays and other control methods (Whiteman *et al*., 2007).

Sanitation

The majority of respondents reported not using fungicides (63%) or biological control agents (93.1%) as pruning wound protectants in mother blocks. There is a mounting body of evidence that little attention has been paid to the role of mother vine management in the production of quality propagating material, and there is a paucity of literature on the subject. Most grapevine trunk pathogens penetrate grapevines through wounds. These wounds remain susceptible to infection for at least 4 weeks (Eskalen *et al*., 2007a) and up to 4 months (Serra *et al*., 2008). Wound protection, especially pruning wound protection, is therefore an extremely important preventative treatment, especially since some pathogens can move upwards through the vines and infect the shoots that are subsequently selected for propagation (Gramaje and Armengol, 2011). Pruning wounds should therefore be treated immediately after pruning with a biological control agent, fungicide or wound sealant (Di Marco *et al*., 2004; Fourie and Halleen, 2006). A total of 53.4% of respondents reported not using any product to regularly disinfest pruning tools. Agustí-Brisach *et al*. (2015) recently demonstrated that pruning shears can spread fungal trunk pathogen inoculum; therefore, disinfesting pruning shears between vines would reduce the spread of trunk pathogens through these tools.

The majority of respondents (70.5%) reported eliminating the pruning debris from the mother field. Of those, a majority of growers usually burn the pruning material (80.6%), while the remainder usually compost pruning waste (19.4%). Burial or removal of dead tissues and trimming debris in source blocks is strongly recommended since numerous fungal fruiting bodies can be found on trimmings left in the vineyards that then become a potential sources of inoculum for new infections.

Survey Part 2 – Nursery process

Hydration

Repeatedly soaking cuttings, pre-cut buds and also rooted vines in water, in the belief that this reverses the effects of dehydration and promotes root initiation, is a widespread practice in grapevine nurseries. Narrow majorities of nurseries stated that they never soak rootstock (50.6%) and scion cuttings (51.3%) on arrival at the nursery and before cold storage. However, more than 25% of the nurseries usually soak rootstock and scion cuttings for more than 8 h in water at this stage of the propagation process, and 10 respondents reported soaking rootstock and scion cuttings for more than 24 h. A substantial number of nurseries (48.6%) reported soaking rootstock cuttings for more than 24 h in water after cold storage and before grafting (Figure 1). In the case of scion cuttings, a majority of respondents (64.9%) reported soaking them for more than 8 h after removal from cold storage, with 30.1% of respondents reported soaking planting material for more than 24 h (Figure 1). A substantial number (41.7%) frequently (many times during the season) clean hydration tanks during the season, and a further 14.3% of the respondents reported that they occasionally (sometimes during the season) clean hydration tanks. Only 24.6% clean the tanks at the beginning of the season and only 14.4% of the respondents reported that they clean the hydration tanks at the end of the season. Soaking cuttings for a long time in water could threaten the phytosanitary status of grapevine planting material. Trunk disease pathogens have been detected in soaking water sampled from hydration tanks in commercial grapevine nurseries, which is evidence that hydration tanks are potential sources of cross contamination (Retief *et al*., 2006; Aroca *et al*., 2010; Gramaje *et al*., 2011; Agustí-Brisach *et al*., 2013; Waite *et al*., 2013a). The value of soaking has been questioned by researchers for more than a decade (Crocker *et al*., 2002; Fourie and Halleen 2006; Waite *et al*., 2015), but previous survey results indicate that the practice is still widespread (Gramaje *et al*., 2012; Waite *et al*., 2013b).

Cold storage

Cuttings that are not to be processed immediately after grading can be stored in a clean coolroom at 1–2°C in clean packaging with several small, well-spaced, 7–10 mm holes that allow air to reach the cuttings without danger of dehydration (Waite *et al*., 2015). Cold storage delays root initiation and bud burst in cuttings and enables nurseries to extend the

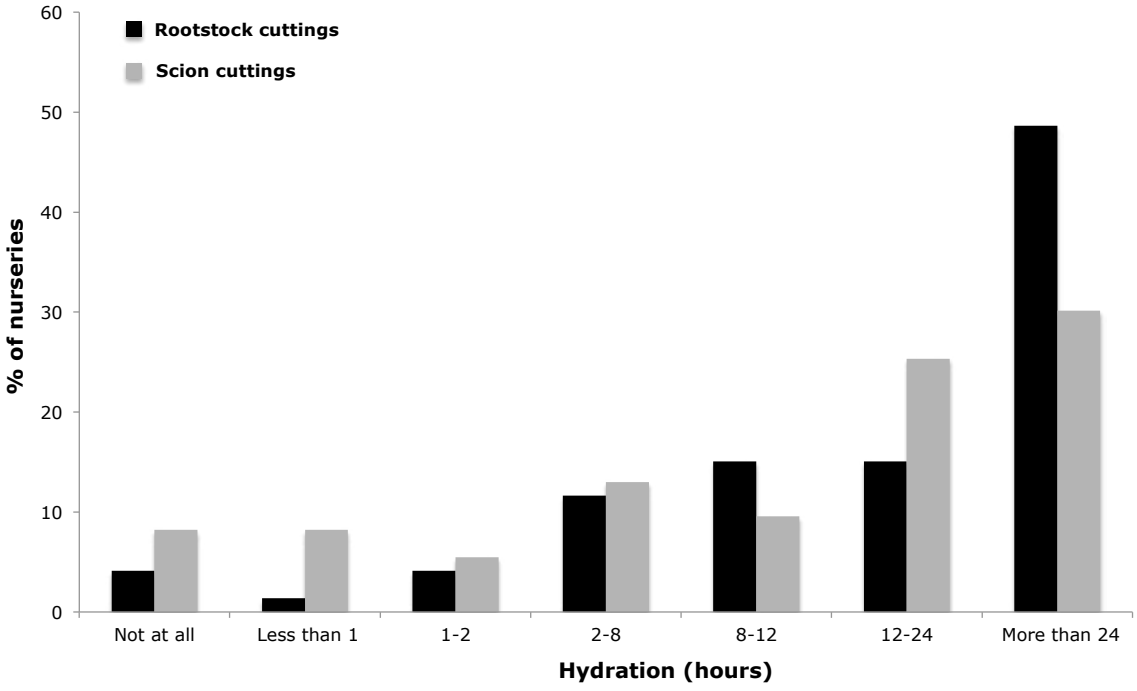

Figure 1. Duration of the hydration process of rootstock and scion cuttings after cold storage.

propagating season by several weeks, making better use of labour. This practice has largely replaced the storing cuttings in sand or sawdust callusing pits. In this survey, the majority of nurseries (76.7%) reported cleaning bins, boxes or crates before use in the cold storage phase. Only 20.5% of respondents reported regularly cleaning on site cool rooms during the season, while 34.2% reported cleaning coolrooms at the beginning of the season and 28.7% reported cleaning cool rooms at the end of the season. The majority of respondents (65.7%) reported storing rootstock and scion cuttings in cool rooms for 1–3 months. However, a substantial number (39.0%) reported storing 1-year-old vines in cool rooms for 4–6 months, and 32.9% of the nurseries reported storing 1-year-old vines for more than 6 months. The sources of microbial contaminants in cold storage include the cuttings or vines themselves, contaminated packaging materials, other products in the cool rooms and the cool room surfaces (Waite and Morton, 2007). Reports from industry and investigations of failed cuttings and planting material have implicated cold storage in cutting and vine failures. Failures in cold storage are usually the result of proliferation of cold-adapted microorganisms and poor storage conditions. *Botrytis cinerea* is tolerant of a wide range of temperatures and has been reported to grow, albeit very slowly, at 0°C (Becker and Hiller, 1977), and this fungus is the most common cause of microbial decay in storage. Anoxic conditions may also develop in cold storage with some adverse effects on cuttings. Moderate exposure to anoxia can result in enhanced bud burst (Halaly *et al*., 2008; Ophir *et al*., 2009), but very low oxygen levels, accumulation of toxic fermentation metabolites and the growth of anaerobic microorganisms (Phillips, 1996) can result in fatal tissue damage when material is stored for 3–6 months in bags with limited head spaces (Chen *et al*., 2011).

Use of chemical and biological control

Different management strategies are commonly used in the nursery industry to control fungal trunk pathogens during the propagation processes. These include drenching or dipping propagating material in contact fungicides or biological control agents at various stages, with the aim of limiting superficial fungal growth during storage and callusing. Hydration tanks containing drenches for soaking are therefore an important focus for management strategies (Gramaje and Armengol, 2011).

In this survey, only 11.6% of nurseries reported not using fungicides at any of the stages in the propagation process. Of the remaining 88.4% of nurseries, 75% used fungicides more than twice, but only 12% used fungicides at all stages of the cycle: in hydrating tanks, in callusing boxes, as dips for cuttings before storage, as dips for vines after grafting, and as dips for 1-year-old vines before storage and before despatch to customers. The use of fungicides in the various stages of the propagation processes is shown in Figure 2. The use of more than one type of fungicide was reported. Chinosol (8-hydroxyquinoline sulphate) was the most commonly used fungicide, followed by thiophanate methyl, captan, mancozeb and thiram. However, the use of Chinosol is not absolute proof against the growth of *Pa. chlamydospora* and *Pm. minimum* (Gramaje *et al*., 2009b). In addition, this compound has been reported to inhibit callusing and graft heal-

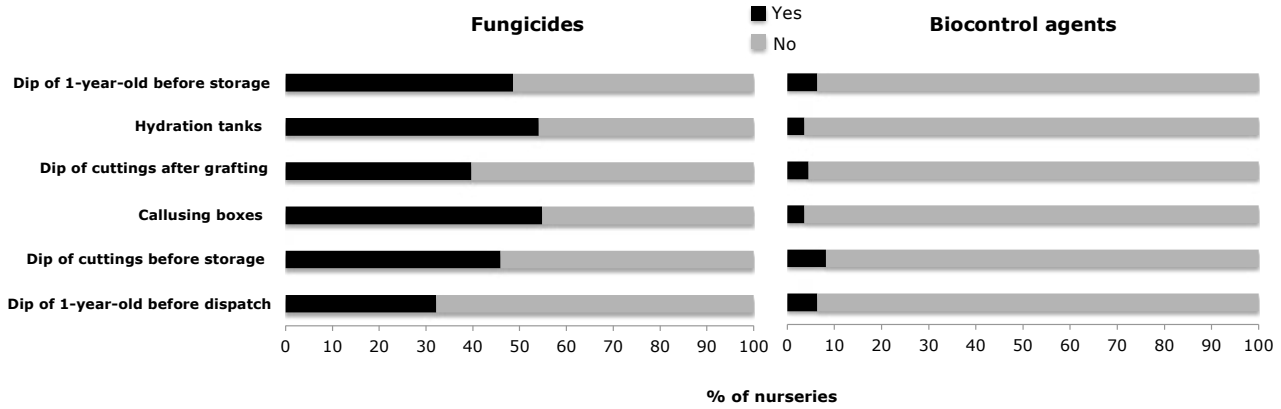

Figure 2. Treatment applications of fungicides or biocontrol agents in any of the stages of grapevine propagation processes.

ing (Becker and Hiller, 1977). The potential of benzimidazole fungicides, such as benomyl, carbendazim and thiophanate-methyl, to reduce infection by Botryosphaeriaceae spp. (Billones-Baaijens *et al*., 2014), black-foot (Rego *et al*., 2006; Alaniz *et al*., 2011) and Petri disease pathogens (Fourie and Halleen, 2004, 2006; Eskalen *et al*., 2007b; Gramaje *et al*., 2009b) in grapevine nurseries has been demonstrated in previous research. A recent study carried out in Hungarian nurseries revealed that thiophanate methyl and thiram applied to cuttings at four different stages in the propagation process resulted in significantly reduced *Pa. chlamydospora* and *Pm. minimum* infection levels of nursery plants (Kun and Kocsis, 2014).

In contrast, only 11 nurseries use biological control agents at any of the stages in the propagation processes: in hydrating tanks (3.6%), in callusing boxes (3.6%), as dips for cuttings before storage (8.2%), as dips for vines after grafting (4.5%), and as dips for 1-year-old vines before storage (6.4%) and before despatch to customers (6.4%) (Figure 2). The biological products reported contained a *Trichoderma* sp., which was the most commonly used biocontrol agent (four nurseries), a *Bacillus* sp. (three nurseries), *Glomus intraradices* (one nursery) and an organic alga (one nursery). Two nurseries did not reveal the name of the biological control agents. The efficacy of biological products has been demonstrated in a number of studies. In South African nurseries, the incidence of *Pa. chlamydospora* and *Phaeacremonium* spp. in rootstock cuttings was reduced by soaking the planting material in *Trichoderma* formulations (Fourie and Halleen, 2004, 2006). In Italian nurseries, at rooting before planting, dipping the basal ends of plants in a commercial formulation of *T. harzianum* reduced the lengths of *Pa. chlamydospora* necrosis, improved the quantitative and qualitative characteristics of the root systems, and increased the percentage of certifiable vines produced (Di Marco and Osti, 2007). In French nurseries, dipping young plants in *T. atroviride* I-1237 resulted in decreased necrosis length caused by *Diplodia seriata* and *Pa. chlamydospora* (Mounier *et al*., 2014). Inoculation of roots with the mycorrhizal fungus *Glomus intraradices* reduced both the number of root lesions, and disease severity caused by black-foot pathogens, while increasing root dry weight (Petit and Gubler, 2006). In a recent study, the arbuscular mycorrhizal fungi *Acaulospora laevis* and *Funneliformis mosseae* increased root dry weight of three grapevine rootstocks and altered rootstock susceptibility to *Ilyonectria* spp (Jones *et al*., 2014).

Hot water treatment

Routine hot water treatment (HWT) of all cuttings prior to propagation or before despatch to grape growers, in conjunction with other management strategies, has also been considered as an effective method to control GTD pathogens in some countries such as Australia (Waite and Morton, 2007), South Africa (Fourie and Halleen 2004, 2006) and Spain (Gramaje *et al*., 2009a). However, HWTed material is susceptible to stresses caused by inappropriate handling practices (Gramaje and Armengol, 2012), and because HWT does not always provide 100% control of trunk disease pathogens (Rooney and Gubler, 2001; Gramaje *et al*., 2010), its use remains controversial (Waite *et al*., 2013b). Negative effects of HWTs that have been reported previously, include delayed callusing and rooting of cuttings (Waite and May, 2005), delayed development or death of buds in cuttings and rooted vines (Caudwell *et al*., 1997; Laukart *et al*., 2001; Gramaje *et al*., 2009a), and failed or incomplete healing of graft unions as well as fermentation in cold storage (Waite and Morton, 2007).

In this survey, the majority of respondents (85.6%) reported that they knew about HWT, but most had never used it (71.2%). Forty-two respondents reported that they currently used, or had used, HWT. Of those, 47.6% applied HWT before grafting, 16.7% at the end of the propagation processes and before selling plants to growers, and 35.7% at both of these stages. The temperature and time combinations for HWTs varied among nurseries. Treatment at 50ºC for 45 min was the most commonly used combination (nine nurseries), followed by 55ºC for 30 min, 53ºC for 45 or 40 min, 52ºC for 45 min, 50ºC for 50 or 30 min (two nurseries for each combination), and 53ºC for 40 or 45 min and 50ºC for 40 min (one nursery each combination). In Spain, Gramaje *et al*. (2008, 2010) fixed 53°C for 30 min as the most effective treatment to reduce conidial germination and mycelial growth of black-foot and Petri disease pathogens. The effect of this treatment was further evaluated in dormant rootstock cuttings and grafted plants after one growing season (Gramaje *et al*. 2009a; Gramaje and Armengol, 2012) and in a long term study (Gramaje *et al*., 2014). The results demonstrated that it is possible to hot water treat grapevine planting material in Spanish nurseries using protocols with temperatures up to 53°C. However, in the relatively cool climate of New Zealand, unacceptable damage to vine tissue has been reported after treatment at 50–53ºC for 30

min (Graham, 2007; Bleach *et al*., 2009; Billones-Baaijens *et al*., 2014). However, treatment at the slightly lower temperature of 48ºC for 30 min has been found to be safe and effective against some pathogens (Graham, 2007; Bleach *et al*., 2009).

Thirty-three respondents gave reasons for not currently using HWT. The reliability and efficacy of HWT was questioned by most nursery operators, who suggested that significant losses are still being attributed to HWT, that it is an ineffective treatment for eliminating trunk disease pathogens, that logistics involved in these treatments are too difficult and that lack of studies into commercial batches are barriers to adoption of HWT. The results of this European survey are in disagreement with those obtained in Australia by Waite *et al*. (2013b). In that survey, the majority of respondents (80%) reported that they currently used, or had used, short and/or long duration HWTs. A majority of growers agreed that long duration HWTs provided some levels of control for internal and external pests and diseases. The number of respondents who did not believe in the efficacy of HWT was very small (Waite *et al*., 2013b).

Grafting and callusing

A total of 57.5% of respondents reported not using any products to regular disinfest pruning tools or grafting machines. Viable propagules of *C. luteo-olivacea*, *Pa. chlamydospora* and *Phaeoacremonium* spp. have been detected in water used for washing pruning shears and grafting machines during the process of propagating grafted plants (Aroca *et al*., 2010; Gramaje *et al*., 2011). The vast majority (93.1%) of respondents reported using omega-cut grafting machines in preference to other grafting machines.

For callusing, cuttings and grafted vines are generally packed into crates or boxes containing moist, but not wet, sterile media, such as vermiculite, perlite, moss, or sawdust. Cuttings are then incubated for 2–3 weeks, depending on temperature and variety, until callus rings form around the cutting bases and graft unions (Gramaje and Armengol, 2011). Callusing is slower at lower temperatures (26–27 °C), but slow growing callus forms stronger unions than callus that develops at higher temperatures (28–29 °C) (Waite *et al*. 2015). In this survey, the time that grafted plants were left in callusing room was more than 20 d for 41.1% of the respondents, between 15 and 20 d for 36.9% of respondents and less than 15 d for 22% of respondents. The mean temperatures for callusing and rooting were more than 28ºC for 44.5% of respondents, between 25 and 28ºC for 43.8%, and less than 25ºC for 11.6% of the respondents.

High temperatures and humidities in callusing boxes and callusing rooms favour the growth of some pathogens. In nurseries where sanitation is poor, outbreaks of *Botrytis* and other pathogens frequently kill all the cuttings in individual callusing boxes (Becker and Hiller, 1977). Halleen *et al*. (2003) isolated high percentages of several *Phaeoacremonium* spp. and *Pa. chlamydospora* from callused cuttings prior to planting in South African nurseries. In Australian nurseries, Wallace *et al*. (2004) reported reduced percentages of certifiable vines due to callus inhibition by *Pa. chlamydospora* infections. Edwards *et al*. (2007) also detected *Pa. chlamydospora* in water from hydration, hot water and cooldown tanks, and in callusing media, by using PCR. Larignon *et al*. (2009) demonstrated that *Pa. chlamydospora* contamination is possible during the callusing stages in French nurseries by bringing inoculated plants into contact with healthy plants. Aroca *et al*. (2010) were able to detect viable propagules of *Phaeoacremonium* spp. and *Pa. chlamydospora* from washing callusing media by filtering the water samples and culturing the filtrate on appropriate media. Agustí-Brisach *et al*. (2013) recently detected *Ilyonectria liriodendri* and *Dactylonectria madrodydima*-complex in callusing peat from two nurseries in Spain by multiplex nested-PCR. If cuttings are left in the callusing boxes for more than 3 weeks, excessive amounts of callus tissue can form that impedes the formation of new xylem and phloem across the graft unions. Callus tissue should not protrude outwards from the graft unions by more than 2–3 mm (Hartmann *et al*. 2001). Callusing cuttings at temperatures above 29°C, or high-density packing of cuttings, can prevent the dissipation of metabolic heat with fatal consequences for the cuttings (Waite *et al*., 2015).

In this survey, the majority of respondents (54.8%) reported using sawdust as a substrate for the callusing stage, 11.6% of the respondents use peat and perlite, 10.9% use other substrates, and 6.2% use vermiculite and 4.9% use and water. According to Hartmann *et al*. (2001), vermiculite could be an adequate callusing medium because it is sterile, has low density and can hold large amounts of water without becoming saturated. Other callusing media (such as sawdust) that have traditionally been used are not sterile. Sawdust from some sources may contain

abiotic contaminants that are toxic to vine cuttings (Hartmann *et al*., 2001). Previous studies have shown that peat is likely to be infected by trunk pathogens in grapevine nurseries (Aroca *et al*., 2010; Agustí-Brisach *et al*., 2013).

As an alternative to planting in field nurseries, callused cuttings and grafted vines can be planted into biodegradable pots filled with a standard commercial potting mixture, sandy loam soil, or peat/perlite. Plants are then grown in glasshouses or shade houses to be ready for spring/summer delivery and planted by early summer in the year of propagation. Forty-two respondents reported that they currently used this method of growing plants. Of those, 30.9% believed that this method of production increased the phytosanitary quality of planting material, 26.2% thought that it decreased the phytosanitary quality of the vines, 19.1% thought that it had no effect and 23.8% of the respondents were not sure about its effect. Although potted grapevine plants do perform well, their use is more restricted than dormant field-finished product. Potted products are more difficult to check for defects and can rapidly become stressed by remaining too long in the pots before planting (Nicholas *et al*., 2001; Stamp, 2003), or by exposure to harsh field conditions before they are properly acclimatized.

Survey Part 3 – Nursery field

Grafting and other treatments

Field grafting for production of grapevine plants has been practiced since ancient times. In modern-day grapevine production systems, however, the most usual method of propagation in commercial nurseries is by bench grafting dormant one-bud *V. vinifera* cuttings on to 300–400 mm dormant hardwood cuttings of selected rootstocks and propagating them as individual plants (Nicholas *et al*., 2001). The principal benefit of mechanical grafting machines, notably the omega machine, is that they allow industrial rates and yields with untrained personnel (Birebent, 2015). In this survey, only 25.3% of the respondents reported that they currently grafted, or had grafted vines in the field. Of those, 22 nurseries (59.4%) thought that the method of grafting in the field improved the phytosanitaty quality of the plants. Recently, Birebent (2015) hypothesized that the advent of mechanisation in grafting could have resulted in poor quality vines that are almost always infected with GTD pathogens. Recent observations carried out in French vineyards showed that grafting of cv. Mourvèdre directly onto rootstocks in the field gave a rate of esca disease of 0.51% in comparison with 12% dead plants and 24% with esca of a neighbouring plot planted with omega bench grafted vines (Birebent, 2015). This report author also noted that the average level of expression of esca among several susceptible varieties was ten times greater on the omega grafts (8.8%) than on manual grafts (0.64%), made by Chip-bud, T-bud, cleft grafts, and splice grafts.

A narrow majority (57.5%) of nurseries that responded to the survey reported using herbicides to control weeds in the nursery fields. *Cadophora luteo-olivacea* and *D. macrodydima*-complex were isolated frequently in Spain from roots of weeds in nursery fields (Agustí-Brisach *et al*., 2011), although the epidemiological relevance of this finding in grapevine disease development seems to be minor. A large majority of respondents (82.2%) reported that they usually apply treatments against pests and diseases other than grapevine trunk diseases.

Respondents also had opportunity to make general comments at the end of the survey responses. Of the 19.8% of respondents (29 nurseries) who exercised this option, 13 commented about the lack of knowledge and training on the principles and methods to improve the phytosanitary quality of grapevine planting material, especially in West-European countries. Ten respondents requested more research into control methods such as hot water treatment, ozonation, chemicals or *in vitro* rootstock production.

Conclusions

This survey has provided a useful insights into the grapevine nursery industry in Europe and the factors that may influence the quality of planting material, especially with regards to the control measures used against GTDs, regardless of the small actual number of respondents compared to the total number of nurseries in Europe. Very low response rate was found from France, Italy and Spain, which represent the majority of European nurseries.

Our results have revealed wide variations in practices both within and between nurseries, and have detected those likely to have an impact on the quality of planting material. Pruning wound protection in mother blocks is uncommon and can increase shoot infection by trunk pathogens before cuttings

enter the propagation process in nurseries. Nurserymen do not regularly disinfest pruning tools in mother blocks or during the propagation processes, leading to possible vine to vine contamination by GTD pathogens. Planting material is soaked for very extended periods after cold storage which can severely compromise sanitation by providing multiple opportunities for microorganisms from the tissues and bark of infected cuttings to contaminate the hydration water, and from there infect propagation wounds on uninfected cuttings. One-year-old vines are usually stored for very extended periods which can result in fatal tissue damage. Grafted plants are frequently left in callusing boxes for long periods, during which the high temperatures can have fatal consequences for the cuttings.

Previous research has demonstrated that infection by GTD pathogens occurs during the grafting processes in nurseries (Whiteman *et al*., 2007; Retief *et al*., 2006; Aroca *et al*., 2010; Gramaje *et al*., 2011; Agustí-Brisach *et al*., 2013). Nursery forcing operations can expose vines to stress, and grafting can induce damaging changes in plant physiology (Bavaresco and Lovisolo, 2000). Among the bench grafting techniques, the omega graft is the most widely practiced in vine nurseries due to its high level of graft success. To date, there has been little research on grafting and graft quality. Further studies are required to confirm the importance of the graft on the development of GTDs.

The consistent use of fungicides, especially benzimidazole compounds, questions the efficacy of the active ingredients authorized for use against grapevine diseases in nurseries, and allows for debate on whether fungal trunk pathogens are developing resistance to these compounds. In contrast, despite increased availability of registered biocontrol products in some European countries, their adoption and use has been limited due to entrenched belief that biocontrol agents are less effective than conventional pesticides. The results of this survey also show that reliability and efficacy of HWT continues to be questioned by a majority of nursery operators. Thus, there is clear need for further research into the effects of treatments, such as HWT, on grapevine viability, and the potential for use of biological agents and other newly developed strategies such as ozonation (Pierron *et al*., 2015), to control GTDs in nurseries.

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Literature cited

Agustí-Brisach C., D. Gramaje, M. León, J. García-Jiménez, and J. Armengol, 2011. Evaluation of vineyard weeds as potential hosts of black-foot and Petri disease pathogens. *Plant Disease*, 95, 803–810.

Agustí-Brisach C., D. Gramaje, J. García-Jiménez and J. Armengol, 2013. Detection of black-foot disease pathogens in the grapevine nursery propagation process in Spain. *European Journal of Plant Pathology* 137, 103–112.

Agustí-Brisach C., M. León, J. García-Jiménez and J. Armengol, 2015. Detection of Grapevine Fungal Trunk Pathogens on Pruning Shears and Evaluation of Their Potential for Spread of Infection. *Plant Disease* ttp://dx.doi.org/10.1094/PDIS-12-14-1283-RE.

Alaniz S., P. Abad-Campos, J. García-Jiménez and J. Armengol, 2011. Evaluation of fungicides to control *Cylindrocarpon liriodendri* and *Cylindrocarpon macrodidymum* in vitro, and their effect during the rooting phase in the grapevine propagation process. *Crop Protection* 30, 489–494.

Aroca A., D. Gramaje, J. Armengol, J. García-Jiménez and R. Raposo, 2010. Evaluation of grapevine nursery process as a source of *Phaeoacremonium* spp. and *Phaeomoniella chlamydospora* and occurrence of trunk disease pathogens in rootstock mother vines in Spain. *European Journal of Plant Pathology* 126, 165–174.

Bavaresco L. and C. Lovisolo, 2000. Effect of grafting on grapevine chlorosis and hydraulic conductivity. *Vitis* 39, 89–92.

Becker H. and M.H. Hiller, 1977. Hygiene in modern bench grafting. *American Journal of Enology and Viticulture* 28, 113–118.

Birebent M. 2015. Grafting rootstocks for sustainable vines. Old techniques for the viticulture of the future? In: Conférence La Vigne au 2ème siècle – Retour vers le futur, February 2015, Piolenc (Vaucluse, France).

Billones-Baaijens R., A. Allard, Y. Hong, E.E. Jones, H. Ridg-

way and M.V. Jaspers, 2014. Management of *Botryosphaeria* species infection in grapevine propagation materials. *Phytopathologia Mediterr*anea 53, 589.

Bleach C.M., E.E. Jones and M.V. Jaspers, 2009. Hot water treatment for elimination of *Cylindrocarpon* species from infected grapevines. *Phytopathologia Mediterr*anea 48, 183.

Caudwell A., J. Larrue, E. Boudon-Padieu and G.D. Mclean, 1997. Flavescence dorée elimination from dormant wood of grapevines by hot-water treatment. *Australian Journal of Grape and Wine Research* 3, 21–25.

Chen S., M. Zhang and S. Wang, 2011. Effect of initial hermetic sealing on quality of 'Kyoho'grapes during storage. *Postharvest Biology and Technology* 59, 194–199.

Crocker J., H. Waite, P. Wright and G. Fletcher, 2002. Source area management: avoiding cutting dehydration and good nursery management may be the keys to successful hot water treatment. *The Australian and New Zealand Grapegrower and Winemaker* 461a, 33–37.

Di Marco S. and F. Osti, 2007. Applications of Trichoderma to prevent *Phaeomoniella chlamydospora* infections in organic nurseries. *Phytopathologia mediterranea*, 46, 73–83.

Di Marco S., F. Osti and A. Cesari, 2004. Experiments on the control of esca by Trichoderma. *Phytopathologia mediterranea*, 43, 108–115.

Daughtry M.L. and D.M. Benson, 2005. Principles of plant health management for ornamental plants. *Annual Review of Phytopathology* 43, 141–169.

Edwards J., F. Constable, T. Wiechel and S. Salib. 2007. Comparison of themolecular tests-single PCR, nested PCR and quantitative PCR (SYBR®Green and TaqMan®) for detection of *Phaeomoniella chlamydospora* during grapevine nursery propagation. *Phytopathologia Mediterranea* 46, 58–72.

Eskalen A., J. Feliciano and W.D. Gubler, 2007a. Susceptibility of grapevine pruning wounds and symptom development in response to infection by *Phaeoacremonium aleophilum* and *Phaeomoniella chlamydospora*. *Plant Disease* 91, 1100–1104.

Eskalen A., S. Rooney-Latham and W.D. Gubler, 2007b. Identifying effective management strategies for esca and Petri disease. *Phytopathologia Mediterranea* 46, 125–126.

Fontaine F. and J. Armengol, 2014. Sustainable control of grapevine trunk diseases (COST Action FA1303). *Phytopathologia Mediterranea* 53, 584–585.

Fourie P.H. and F. Halleen, 2004. Proactive control of Petri disease of grapevine through treatment of propagation material. *Plant Disease* 88, 1241–1245.

Fourie P.H. and F. Halleen, 2006. Chemical and biological protection of grapevine propagation material from trunk disease pathogens. *European Journal of Plant Pathology* 116, 255–265.

Graham A. 2007. Hot water treatment of grapevine rootstock cuttings grown in a cool climate. *Phytopathologia Mediterranea* 46, 124.

Gramaje D. and J. Armengol, 2011. Fungal trunk pathogens in the grapevine propagation process: potential inoculum sources, detection, identification, and management strategies. *Plant Disease* 95, 1040–1055.

Gramaje D. and J. Armengol, 2012. Effects of hot-water treatment, post-hot-water-treatment cooling and cold storage on the viability of dormant grafted grapevines under field conditions. *Australian Journal of Grape and Wine Research* 1, 158–163.

Gramaje D., J. Armengol, D. Salazar, I. López-Cortés and J. García-Jiménez, 2009a. Effect of hot-water treatments above 50°C on grapevine viability and survival of Petri disease pathogens. *Crop Protection* 28, 280–285.

Gramaje D., A. Aroca, R. Raposo, J. García-Jiménez and J. Armengol, 2009b. Evaluation of fungicides to control Petri disease pathogens in the grapevine propagation process. *Crop Protection* 28, 1091–1097.

Gramaje D., S. Alaniz, P. Abad-Campos, J. García-Jiménez and J. Armengol, 2010. Effect of hot-water treatments *in vitro* on conidial germination and mycelial growth of grapevine trunk pathogens. *Annals of Applied Biology* 156, 231–241.

Gramaje D., J. García-Jiménez and J. Armengol, 2008. Sensitivity of Petri disease pathogens to hot-water treatments in vitro. *Annals of Applied Biology* 153, 95–103.

Gramaje D., L. Mostert and J. Armengol, 2011. Characterization of *Cadophora luteo-olivacea* and *C. melinii* isolates obtained from grapevines and environmental samples from grapevine nurseries in Spain. *Phytopathologia Mediterranea* 50, 112–126.

Gramaje D., J. García-Jiménez and J. Armengol, 2012. Fungal trunk pathogens in Spanish grapevine nurseries: a survey of current nursery management practices in Spain. *Phytopathologia Mediterranea* 51, 411–412.

Gramaje D., F. Mañas, M.L. Lerma, R.M. Muñoz, J. García-Jiménez and J. Armengol, 2014. Effect of hot-water treatment on grapevine viability, yield components and composition of must. *Australian Journal of Grape and Wine Research* 20, 144–148.

Gramaje D., L. Mostert, J.Z. Groenewald and P.W. Crous, 2015. *Phaeoacremonium*: from esca disease to phaeohyphomycosis. *Fungal Biology* 119, 759-783.

Gubler W.D., S. Rooney-Latham, S.J. Vasquez and A. Eskalen, 2013. Esca (Black Measles) and Petri disease. In: Grape Pest Management. University of California. Agriculture and Natural Resources. Publication 3343.

Halaly T., X. Pang, T. Batikoff, O. Crane, A. Keren, J. Venkateswari, A. Ogrodovitch, A. Sadka, S. Lavee and E. Or, 2008. Similar mechanisms might be triggered by alternative external stimuli that induce dormancy release in grape buds. *Planta* 228, 79–88.

Halleen F., P.W. Crous and O. Petrini, 2003. Fungi associated with healthy grapevine cuttings in nurseries, with special reference to pathogens involved in the decline of young vines. *Australasian Plant Pathology* 32, 47–52.

Hartmann H.T., D.E. Kester, F.E. Davies and R. Geneve, 2001. *Hartmann and Kester's Plant Propagation: Principles and Practices*. 7th ed. Prentice-Hall, Englewood Cliffs, NJ.

Hayman P.T. and C.L. Alston, 1999. A survey of farmer practices and attitudes to nitrogen management in the northern New South Wales grains belt. *Australian Journal of Experimental Experimental Agriculture* 39, 51–63.

Hunter J.J., C.G. Volschenk, D.J. Le Roux, G.W. Fouché and L. Adams, 2004. *Plant Material Quality, a compilation of research*. Research Reports, ARC Infruitec-Nietvoorbij, Stellenbosch, South Africa.

Jones E.E., S. Hammond, C. Blond, D.S. Brown and H.J. Ridgway, 2014. Interaction between arbuscular mycorrhizal fungi and rootstock cultivar on the susceptibility to infec-

tion by Ilyonectria species. *Phytopathologia Mediterranea* 53, 582–583.

Kalton G. and H. Schuman, 1982. The effect of the question on survey responses: a review. *Journal of the Royal Statistical Society* 145, 42–73.

Kelley K., B. Clark, V. Brown and J. Sitzia, 2003. Good practice in the conduct and reporting of survey research. *International Journal for Quality Health Care* 15, 261–266.

Koike S.T., P. Gladders and A.O. Paulus, 2007. *Vegetable Diseases, A Colour Handbook*. Manson Publishing Ltd., UK.

Kun A., and L. Kocsis, 2014. Efficacy of treatments against Phaeomoniella chlamydospora and Phaeoacremonium *aleophilum* during nursery propagation. *Phytopathologia Mediterranea* 53, 592.

Larignon P., M. Coarer, K. Girardon, F. Berud and O. Jacquet, 2009. Propagation of pioneer fungi associated with esca disease by vegetative material in French grapevine nurseries. *Phytopathologia Mediterranea* 48, 177.

Laukart N., J. Edwards, I.G. Pascoe and N.K. Nguyen, 2001. Curative treatments trialed on young grapevines infected with *Phaeomoniella chlamydospora*. *Phytopathologia Mediterranea* 40, S459–S463

Mounier E., F. Cortes, M. Cadious and E. Pajot, 2014. The benefits of *Trichoderma atroviride* I-1237 for the protection of grapevines against trunk diseases: from the nursery to the vineyard. *Phytopathologia Mediterranea* 53, 591–592.

Nicholas P.R., A.P. Chapman and R.M. Cirami, 2001. Grapevine propagation. In: Viticulture, Vol. 2, Practices. B. (G. Coombe and P.R. Dry, eds). Winetitles, Adelaide, Australia, pp. 1–22.

OEPP/EPPO, 2008. Certification scheme. No. PM 4/8 (2): Pathogen-tested material of grapevine varieties and rootstocks. Bull. *OEPP/EPPO Bulletin* 38, 422–429.

Ophir R., X. Pang, T. Halaly, J. Venkateswari, S. Lavee, D. Galbraith and E. Or, 2009. Gene expression profiling of grape bud response to two alternative dormancy release stimuli expose possible links between impaired mitochondrial activity, hypoxia, ethylene-ABA interplay and cell enlargement. *Plant Molecular Biology* 71, 403–423.

Petit E. and W.D. Gubler, 2006. Influence of *Glomus intraradices* on black foot disease caused by *Cylindrocarpon macrodidymum* on *Vitis rupestris* under controlled conditions. *Plant Disease* 90, 1481–1484.

Phillips CA. 1996. Review: modified atmosphere packaging and its effects on the microbiological quality and safety of produce. *International Journal of Food and Science Technology* 31, 463–79.

Pierron R.J.G., M. Pages, C. Couderc, S. Compant, A. Jacques and F. Violleau, 2015. In vitro and in planta fungicide properties of ozonated water against the esca-associated fungus *Phaeoacremonium aleophilum*. *Scientia Horticulturae* 189, 184–191.

Rego C., L. Farropas, T. Nascimento, A. Cabral and H. Oliveira, 2006. Black foot of grapevine, sensitivity of *Cylindrocarpon destructans* to fungicides. *Phytopathologia Mediterranea* 45S, 93–100.

Retief E., A. McLeod and P.H. Fourie, 2006. Potential inoculum sources of *Phaeomoniella chlamydospora* in South African grapevine nurseries. *European Journal of Plant Pathology* 115, 331–339.

Rooney S.N. and W.D. Gubler, 2001. Effect of hot water treatments on eradication of *Phaeomoniella chlamydospora* and *Phaeoacremonium inflatipes* from dormant grapevine wood. *Phytopathologia Mediterranea* 40S, 467–472.

Serra S., M.A. Mannoni and V. Ligios, 2008. Studies on the susceptibility of pruning wounds to infection by fungi involved in grapevine wood diseases in Italy. *Phytopathologia Mediterranea* 47, 234–246.

Smart R. 2013. Trunk diseases…a larger threat than phylloxera? *Wine and Viticulture Journal* 28, 16–18.

Stamp J.A. 2001. The contribution of imperfections in nursery stock to the decline of young vines in California. *Phytopathologia Mediterranea* 40S, 369–375.

Stamp J.A. 2003. Pathogenic Status of High Quality Grapevine Nursery Stock. *Wine Business Monthly* 10, 30–35.

Úrbez-Torres J.R., M. Battany, L.J. Bettiga, C. Gispert, G. McGourty, J. Roncoroni, R.J. Smith, P. Verdegaal and W.D. Gubler, 2010. *Botryosphaeriaceae* species spore-trapping studies in California Vineyards. *Plant Disease* 94, 717–724.

Waite H. and P. May, 2005. The effects of hot water treatment, hydration and order of nursery operations on cuttings of *Vitis vinifera* cultivars. *Phytopathologia Mediterranea* 44, 144–152.

Waite H. and L. Morton, 2007. Hot water treatment, trunk diseases and other critical factors in the production of high-quality grapevine planting material. *Phytopathologia Mediterranea* 46, 5–17.

Waite H., D. Gramaje, M. Whitelaw-Weckert, P. Torley and W.J. Hardie, 2013a. Soaking grapevine cuttings in water: a potential source of cross contamination by micro-organisms. *Phytopathologia Mediterranea* 52, 359–368.

Waite H., P. May and G. Bossinger, 2013b. Variations in phytosanitary and other management practices in Australian grapevine nurseries. *Phytopathologia Mediterranea* 52, 369–379.

Waite H., M. Whitelaw-Weckert and P. Torley, 2015. Grapevine propagation: principles and methods for the production of high-quality grapevine planting material. *New Zealand Journal of Crop and Horticultural Science* DOI: 10.1080/01140671.2014.978340.

Wallace J., J. Edwards, I.G. Pascoe and P. May, 2004. *Phaeomoniella chlamydospora* inhibits callus formation by grapevine rootstock and scion cultivars. *Phytopathologia Mediterranea* 43, 151–152.

Whiteman S.A., A. Stewart, H.J. Ridgway and M.V. Jaspers, 2007. Infection of rootstock mother-vines by *Phaeomoniella chlamydospora* results in infected young grapevines. *Australian Plant Pathology* 36, 198–203.

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