Phytopathol. Mediterr. (2007) 46, 5–17

REVIEW

Hot water treatment, trunk diseases and other critical factors in the production of high-quality grapevine planting material

HELEN WAITE[1] and LUCIE MORTON[2]

[1]Northern Melbourne Institute of TAFE, Cnr. Dalton Rd and Cooper St., P.O. Box 8, Epping, Victoria 3076, Australia
[2]P.O. Box 208, Broad Run, Virginia, 20137 USA

Summary. This review describes the critical factors on which successful grapevine propagation depends and discusses the steps that can be taken to improve the quality of planting material available to growers. Spasmodic occurrences of young vine decline and the failure of planting material have plagued the wine industry since the 1990s. The syndrome now described as Petri disease has been identified as the probable cause of many of the failures, but hot water treatment (HWT) of dormant cuttings (50°C/30 min), for the control of *Phaeomoniella chlamydospora* and other endogenous pathogens, has also been implicated in the losses. HWT is known to cause a temporary switch to fermentative respiration and early retarded growth in treated material, particularly in Pinot Noir, but the effects of HWT on dormant vine tissue are not yet fully understood. Poor nursery hygiene and poor storage and handling practices during propagation and planting have also been implicated in vine failure. Demand for planting material has exceeded supply and there has been little incentive for nurseries to improve their standards. The quality of planting material could be significantly improved by changing nursery practices, particularly by discontinuing the practice of soaking cuttings in water, treated or untreated, and by improving general standards of nursery hygiene and the management of cool rooms. There is a need to develop a set of universal quality standards for cuttings and rooted vines. Growers also need to be made aware of the characteristics and benefits of high quality planting material.

Key words: *Phaeomoniella chlamydospora*, grapevine propagation, nursery sanitation, cold storage.

Introduction

Spasmodic occurrences of young vine decline and failure of planting material have plagued the wine industry in Australia, New Zealand, the United States and elsewhere since the mid-1990s when the wine industry entered a period of rapid expansion that was sustained for a decade (Anonymous, 2006). During this time vine nurseries struggled to meet demand for planting material, and seconds, or vines propagated from cuttings sourced from unregistered suppliers, were sold when first-quality vines could not be supplied. Inevitably there were problems. In some cases vines failed to establish and vineyards had to be replanted within 2 years, or establishment was slow, and cropping delayed for at least 1 season. A healthy, productive vineyard begins with healthy planting material. High-quality vines establish quickly and require fewer inputs than poor-quality vines that often result in vineyards that are

Corresponding author: H. Waite
Fax: +61 3 9269 1046
E-mail: helenw-aas@nmit.vic.edu.au

difficult and costly to manage. Poor-quality vines may fail, or be slow to establish, and result in a vineyard that is uneven and less productive both in the short and long term (Jordan, 1997).

In the course of investigations it has become clear that the causes of cutting and young-vine declines and failures are numerous and complex and include infection by pathogens such as *Phaeomoniella chlamydospora* and poor nursery and planting practices. Hot water treatment (HWT), used for pest and pathogen control in dormant cuttings and rooted vines, has frequently been singled out by the industry as the cause of cutting and vine failure. However, it has been difficult to separate HWT from other possible causes of cutting and vine failure and investigations have frequently revealed that cutting and vine failure is the result of many, seemingly minor, but poor decisions during the propagating and planting process, each of which has had a small but cumulative impact on the quality of the vine. Opportunities for injury and contamination during the production of planting material are numerous, beginning with the management of mother-vine source blocks and continuing through the propagation process to storage, transport and planting in the vineyard.

Grapevines are relatively easy plants to propagate, cuttings strike readily and very little skill is required to produce a vine that appears to be of an acceptable standard. However, on closer inspection many of these apparently acceptable vines do not meet all quality criteria and fail to perform to expectations in the vineyard. The wine industry has now begun to recognise that the quality of planting material is fundamental to the establishment of healthy productive vineyards and there is an urgent need to improve the quality and consistency of the planting material available to growers (Constable and Drew, 2004; Waite, 2006).

The purpose of this paper is to review the causes of cutting and young-vine failure and the factors that impinge on the quality of planting material, and to identify the best practice to ensure that propagating and planting material is of the highest quality. Impediments to the implementation of improvements are also discussed.

Attributes of quality planting material

Before embarking on a discussion of the factors that impinge on the quality of propagating and planting material, quality parameters need to be defined. Some of the characteristics of quality planting material are obvious and can be easily distinguished by visual inspection. Unlike ornamental plants, where sales depend on the product appearing healthy, uniform and vigorous (Baker and Linderman, 1979), the appearance of grapevines is less important. Grapevines are usually sold dormant and bare rooted, and the health and vigour of the product is not always readily discernable until the vines begin to grow in the vineyard.

Dormant and green potted vines and grafted vines should have at least 3 well formed roots evenly spaced around the base, and one or two well developed shoots with healthy buds that are neither stunted nor rank (Nicholas *et al*., 1992). Vines should also be free of external wounds caused by machinery or vermin as these wounds may result in structural weakness and expose the tissue to infection by pathogens. Graft unions should be completely healed and should not break when moderate pressure is applied, and callus development should not bulge outside the stem diameter by more than 2–2.5 mm. In some cases there will be apparently good callus development, but on closer inspection it can be seen that the callus has formed from the stock, or the scion, but not both. Unless both the stock and scion produce callus tissue from the cambium, a proper union between the phloem and xylem does not occur and the scion will die when it has consumed its stored carbohydrates, or is water-stressed (Hartmann *et al*., 1990). These grafts normally break under moderate pressure.

Other quality parameters including trueness to type of both variety and clone, disease status and exposure to environmental stresses are not readily apparent from a visual inspection of dormant material and can only be determined by specialist ampelographers and pathologists and inspection of records. Consequently, high-quality planting material must not only conform to the parameters listed above, but must also be traceable to its source (mother-vine block and nursery) and accompanied by paperwork certifying its origins and disease status.

In summary, a quality vine that will perform to expectations is one that is sound, true to type, has good plant architecture, a well-healed graft union,

is free of serious viruses and other important pathogens, has not been exposed to environmental stress and is traceable to source.

Cutting selection and source block management

Good quality cuttings that are free of disease, true to type and moderately vigorous are critical to the production of quality plants (Hartmann *et al*., 1990). Anecdotal evidence that grapevine cuttings taken from vines grown in warm climates are superior to cuttings taken from vines grown in cool climates and better able to withstand HWT is supported by the work of Crocker *et al*. (2002) who found that in south-eastern Australia cuttings sourced from well-managed vineyards and rootstock plantings in warm climates (MJT 21°C) performed better in propagation than cuttings from vineyards in cool climates (MJT 18°C), or vineyards that had suffered from water stress in the growing season prior to cutting collection.

Inadequate or unbalanced nutrition of mother vines also affects the quality of cuttings although there is some debate regarding the effects of nitrogen fertilization of mother vines. Samish and Spiegel-Roy (1957) found that contrary to the findings of other researchers, relatively high nitrogen levels in cuttings of 41B rootstocks did not negatively affect the rooting or development of cuttings, but they did note that these cuttings had longer internodes and were less dense compared to treatments with N, P, K and Zn. Cuttings from vines that had received a complete NPK fertilizer or Zn, as compared to cuttings from vines that had received none or only one of the other 3 elements, had the highest strike rates (68 and 64% compared to 47–56%) and the highest percentage of A grade cuttings (57 and 58% compared to 35–40%).

The most reliable sources of superior grapevine cuttings are those that are established and managed specifically to supply registered disease-free cuttings to propagators. Cuttings sourced from unregistered vineyards are frequently inferior and of unknown disease status and type. Purchase of vines propagated from unregistered source areas not only carries a serious risk of introducing diseases such as viruses, crown gall and Petri disease into the vineyard, but also carries a risk of establishing a vineyard that is not of the desired variety or clone.

Hot water treatment

Hot water treatment (HWT) has been used successfully to disinfest plant material, including seeds and storage organs, since the 19th century (Birchfield and van Pelt, 1958; Baker, 1962) and remains the only effective means of controlling a number of important pests and pathogens in grapevine propagating and planting material for which there are no other practical chemical or biological controls. HWT of 1-year-old rooted vines as a control for phylloxera and later root-knot nematodes, was developed in the early part of the 20th century (Lear and Lider, 1959; Suatmadji, 1982; Stonerod and Strik, 1996). Since then it has also been applied to cuttings as well as 1-year-old rooted vines to control and prevent the dissemination of Pierce's disease (Goheen *et al*., 1973), phytophthora (Von Broembsen and Marais, 1978), crown gall (Burr *et al*., 1989; Ophel *et al*., 1990; Bazzi *et al*., 1991; Burr *et al*., 1996), phytoplasmas (Caudwell *et al*., 1997), mealy bug (Haviland *et al*., 2005) and endogenous fungal pathogens including *Phaeomoniella chlamydospora* (Crous *et al*., 2001; Laukart *et al*., 2001; Fourie and Halleen, 2004) in planting material, and it has been in widespread use since the mid-1990s. However HWT is a significant stress and can result in the loss of treated material if not applied correctly. Poorly functioning plant and equipment can result in uneven temperature distribution in the treatment tanks, creating hot spots that damage even the most robust material (Anonymous, 1998).

Reports that some *Vitis vinifera* varieties are more sensitive to HWT than others began to surface in the mid-1990s when HWT was integrated into standard nursery practice. Nurseries reported that Pinot Noir and Chardonnay were particularly sensitive to HWT compared to Cabernet Sauvignon that was least affected by HWT. This anecdotal evidence was supported by subsequent research that demonstrated clear differences in the response of different *V. vinifera* varieties to HWT. Pinot Noir is the most sensitive variety, Chardonnay, Reisling and Merlot are moderately sensitive and Cabernet Sauvignon the least sensitive (Waite *et al*., 2001; Crocker *et al*., 2002). Very few problems have been reported with rootstock cuttings, but there have been some recent reports from nurseries that 1103 Paulsen is somewhat sensitive to HWT. Hot water treated cuttings, particularly

those of sensitive varieties, are generally slower to establish than cuttings that have not been hot water treated (HWTed). Bud and root development in HWTed cuttings is delayed in the early part of the growing season until mid summer when the cuttings are observed to recover. By the end of the first growing season HWTed cuttings have made up the difference in growth and are indistinguishable from untreated cuttings (Waite, 1998; Waite, 2002).

There is also a mounting body of evidence to suggest that tolerance to HWT is affected by the climate in which the cuttings are grown. Cuttings grown in cool climates in Australia and New Zealand are more susceptible to injury in HWT than cuttings grown in warm climates (Crocker *et al*., 2002; Graham, 2006), but there is also parallel evidence indicating that pathogens, particularly *P. chlamydospora,* are similarly affected and are controlled at 45–47°C for 30 min rather than the standard treatment (50°C for 30 min) that results in unacceptable losses of cuttings (Graham, 2006). Conversely there is also evidence indicating that both cuttings and their accompanying pathogens grown in warm climates are less susceptible to HWT, and that the treatment temperature may need to be as high as 51–53°C to ensure adequate pathogen control (Armengol *et al*., 2006).

HWT protocols vary. There are 2 different HWT regimes in common use, a short duration regime, usually 54°C for 5 min, for the control of external pests and pathogens and long duration regimes, generally 50°C for 30–45 min for the control of internal pests and pathogens. The short-duration HWT controls nematodes on rooted cuttings and phylloxera on cuttings and rooted vines (Lear and Lider, 1959; Meagher, 1960), but the heat does not penetrate the tissue sufficiently to control endogenous pathogens. Long-duration HWT allows heat to penetrate the wood for long enough to control endogenous pathogens including *Xylella fastidiosa* (Goheen *et al*., 1973), *Agrobacterium vitis* (Burr *et al*., 1989; Ophel *et al*., 1990; Burr *et al*., 1996), phytoplasmas (Caudwell *et al*., 1997), *Cylindrocarpon* spp. (Halleen *et al*., 2006) and *P. chlamydospora* (Fourie and Halleen, 2004), without killing the vine tissue.

Although the standard treatment of 50°C for 30 min is regarded as an effective means of controlling both endogenous and surface pests and pathogens, it is recognised that this treatment may not always completely eliminate all endogenous pathogens, notably *Agrobacterium vitis* (Burr *et al*., 1996) and *P. chlamydospora* (Fourie and Halleen, 2004). Therefore, it is recommended that propagating material with obvious signs of disease should not be used (Anonymous, 1998). Care should also be taken to ensure that material to be HWTed is fully dormant. Cuttings or rooted vines that are not fully dormant are very sensitive to HWT and are often fatally injured during treatment (Von Broembsen and Marais, 1978; Burr *et al*., 1989; Morrell and Wample, 1995).

HWT is occasionally confused with thermotherapy, a technique used for virus control; however HWT does not control viruses. Thermotherapy for virus control involves the *in vitro* propagation of shoot tip meristems from vines that have been grown at constantly high temperatures of 37–38°C for several months to eliminate viruses from the apical meristem and is generally employed to produce clean stock for germplasm plantings (Krake *et al*., 1999). Thermotherapy is not a suitable technique for routine commercial production of large quantities of planting material.

HWT plant and equipment

While early research (Lear and Lider, 1959; Meagher, 1960; Goheen *et al*., 1973; Goussard 1977; Burr *et al*., 1989) demonstrated that HWT is a safe and effective treatment for the control of pests and pathogens in cuttings and rooted vines, the application of the technique to commercial industry practice has not always been successful and problems with design, temperature control and monitoring in some early HWT plants allowed the development of hot and cold spots during treatment. However, improvements in the design and monitoring of HWT plant and training programs for HWT plant operators resolved these problems and losses caused by poorly functioning HWT plant are now almost unknown (Anonymous, 1998).

The design of HWT plant and equipment varies, but usually consists of one or more hydrating tanks that are used to pre-soak material in cold water before treatment, an insulated HWT tank with a heat source and a pump to circulate the water, and one or more cool-down tanks for plunging treated material to facilitate rapid cooling. Material that is to be treated is packed in mesh

baskets for insertion into the tanks, usually by means of a forklift or block and tackle. Baskets are slightly smaller than the tanks to allow a 300 mm gap between the walls and floor of the tank to facilitate water circulation in the HWT tank. Tanks and baskets are normally constructed of stainless or galvanized steel to prevent rust. Baskets are packed with cuttings and rooted vines in bundles of 100 laid in the direction of the water flow to facilitate even heat distribution allowing 500 ml of water for each cutting and 1 l for each rooted vine (Anonymous, 1998). Bundles tied too tightly and wrapping of bundles in hessian or similar materials and over packing dipping baskets impedes the flow of water and results in the development of hot and cold spots.

Hydration

The usual procedure in most nurseries is to presoak (hydrate) both cuttings and rooted vines in cold water before HWT (Anonymous, 1998), but hydration times and water quality vary. Hydration of cuttings is not a recognised practice in the propagation of ornamental and other fruiting plants (Hartmann *et al*., 1990) and appears to be confined to the vine nursery industry. Since soaking cuttings in water is likely to facilitate the dispersal of field-acquired pathogens such as botrytis that may occur on the surfaces of the cuttings, or are introduced in untreated water (Hartmann *et al*., 1990) it would be reasonable to expect that hydration of cuttings is likely to result in a loss of quality rather than an improvement. However there is a widely held belief in the vine nursery industry that the pre-HWT hydration is beneficial and improves the strike rate of cuttings by compensating for any dehydration resulting from delayed processing of cuttings and other practices that expose cuttings to dehydration.

The practice of hydrating cuttings and vines most likely arose from the research of Spiegel (1953–1955) who reported prolonged hydration (up to 96 h) of rootstock and *V. vinifera* cuttings improved rooting by leaching auxin inhibitors, but these inhibitors disappeared naturally as the cuttings emerged from dormancy in spring after exposure to either natural or artificial chilling obviating the need for soaking. A 15 h pre-soak was also recommended to facilitate the adsorption of Chinosol® (8-hydroxyquinoline sulphate) used to control botrytis in cold storage (Becker and Hiller, 1977; Nicholas *et al*., 1992). Cuttings must also be soaked again in clean water before grafting to ensure that the concentration of 8-hydroxyquinoline sulphate in the tissue is low enough to prevent inhibition of callusing and graft healing. Over time hydration has become disassociated from the leaching of auxin inhibitors and the use of Chinosol® and has been applied indiscriminately in the belief that it is beneficial.

Cuttings and rooted vines are often hydrated in untreated water sourced from irrigation schemes, town supplies and rainwater tanks used to collect rain falling on the roofs of buildings. Of these sources only town water has been treated to control micro-organisms, but none is actively antimicrobial. Untreated rainwater, commonly regarded in the general community as clean, is frequently contaminated with dust and bird faeces, and irrigation water with suspended colloids and animal faeces.

Crocker *et al*. (2002) reported a variable response of cuttings to hydration (+/-HWT) in an experiment to determine the effects of hydration (0, 1 and 8 h) and HWT on root initiation on 6 *Vitis vinifera* cultivars and concluded that adequate watering of mother vines between vintage and leaf fall and protecting cuttings from dehydration during processing was a better strategy for successful propagation than hydration. Waite and May (2005) investigating the effects of hydration time (0, 4 and 15 h), HWT and order of nursery operations on Chardonnay and Cabernet Sauvignon, also found that the effects of hydration on cutting establishment were variable, but that it did not have long-term effects on the success of cutting propagation when cuttings were grown in the protected environments of the glasshouse and shade house. In the light of these results it is difficult to justify the use of pre-HWT hydration when the supposed benefits are doubtful and there is a real risk of spreading pathogens such as botrytis that may not be controlled by the subsequent HWT (Hartmann *et al*., 1990). The case for abandoning hydration is further strengthened by the work of Bell *et al*. (1995) and Cole and Waite (2006) who isolated a variety of other micro-organisms from the surface and wood of cuttings that are not recognised as vine pathogens, but are suspected of being detrimental when introduced into the woody tissue of cuttings and grafted vines during

propagation. Whiteman *et al*. (2004) also found that the infection rates of *P. chlamydospora* in cuttings increased from 39% prior to nursery processing to 70% after processing and identified pre-storage and pre-grafting hydration tanks as potential sources of infection.

Post-HWT cooling

Following HWT it is standard practice to immerse the treated material immediately in an equal volume of cold water for a minimum of 30 min to facilitate rapid cooling and minimise heat damage to the tissue. Although it is recommended that cool-down tanks be agitated to facilitate heat exchange from the central mass of cuttings (Anonymous, 1998), cool-down tanks in nurseries are not normally fitted with pumps. This means that the large volumes (5,000–10,000 cuttings per batch) that are treated are likely to cool unevenly, possibly explaining some of the variability in the quality of HWTed cuttings and rootlings.

Water used in commercial cool-down tanks is usually chlorinated, but it is not sterile and is a potential source of *P. chlamydospora* and other microbial contaminants (Whiteman *et al*., 2004; Cole and Waite, 2006; Constable *et al*., 2006; Retief *et al*., 2006). To reduce the risk of contamination or reinfection by potential pathogens during the cool-down process it would be preferable to allow cuttings to air-cool provided the slower cooling did not affect cutting viability. The effects of rapid versus slow cooling of HWTed vine material have not been fully investigated, but the positive results of Waite *et al*. (2005) who reported that both post HWT air-cooled and water-cooled dormant cuttings of *V. vinifera* cultivars, Pinot Noir, Chardonnay, Semillon and Cabernet Sauvignon were viable and produced adequate numbers of roots at callusing to enable establishment, suggest that the expense of a commercial trial would be warranted. However, the volume of cuttings treated in commercial HWT plants is very much larger than that of the experimental batch, and cooling of cuttings in the centre of the dipping basket would be many times slower than in the cuttings in the laboratory, with potentially very different results. Consequently water-cooling is currently favoured as the best means of avoiding permanent damage to vine tissue through prolonged exposure to high temperatures (Anonymous, 1998).

Nursery sanitation

Good sanitation has long been recognised as fundamental to the propagation of healthy nursery plants (Hartmann *et al*., 1990; Daughtry and Benson, 2005); however the need for high levels of hygiene in not well recognised in the vine nursery industry, and in the course of investigations poor nursery sanitation has emerged as a primary cause of cutting and vine failure. Nursery owners and managers often have only a very limited understanding of the effects of poor hygiene on the quality of their product and the shortage of planting material during the planting boom that began in the mid-1990s meant that there has been little incentive for nurseries to raise their standards. In nurseries where hygiene is poor losses can be as high as 60%, and unacceptably high numbers of unsaleable second-quality vines are produced with faults such as poorly developed root systems, weak shoots and incompletely healed grafts (Hartmann *et al*., 1990).

When they are dissected, incompletely healed grafts often show dark staining in the wood extending away from both the rootstock and scion, an indication that contamination of the wound has occurred during the grafting process. Similar staining is also frequently observed extending upwards from the basal cut and sometimes downwards from the apical cut in both rooted cuttings and grafted vines. Pathogens such as *P. chlamydospora* are known to inhibit graft healing (Wallace *et al*., 2003) and a variety of soil and water-borne organisms including *P. chlamydospora* (Fourie and Halleen, 2004), *Enterobacter* spp., *Pseudomonas* spp. and *Rahnella* sp. have been isolated from the stained wood of rooted cuttings and grafted grapevines that have failed, or performed poorly in the field (Bell *et al*., 1995; Cole and Waite, 2006), indicating that contamination with untreated water, soil or dust has occurred during the propagation process.

The common practice in vine nurseries of holding pre-cut buds in water to prevent dehydration of the cut surface is an obvious source of wound and graft union contamination. The water itself may be a source of water-borne micro-organisms, but even if the soaking water is clean it will be contaminated by field-acquired micro-organisms and abiotic contaminants on the bark of the bud-sections dispersing into the soaking water. Therefore it would be prudent for nurseries to change

their practices so that buds are cut as required for grafting to minimise exposure to dehydration or contamination from soaking water (Cole and Waite, 2006).

In many nurseries infrastructure is not purpose-built and is old and difficult to clean. Corners of tool sheds, workshops and storage sheds are often utilised as grafting rooms, cleaning tends to be cursory and open doors and gaps in walls and windows allow the entry of vermin, birds and wind-borne contaminants including dust and spores that blow over the work areas contaminating the work benches, and cuttings (Daughtry and Benson, 2005). Protective aprons, overalls and other clothing worn by nursery workers can also be a source of contaminants unless cleaned daily. In one instance it was reported that an outbreak of botrytis in callusing boxes was traced to the clothing of workers who had been picking fruit on the day prior to working at the grafting bench and had not laundered their outer garments.

Air-conditioning systems used in uninsulated sheds to improve worker comfort may also blow contaminants onto work surfaces, tools and cuttings, particularly if floors are swept while they are operating. Sheds made of the prefabricated insulated panels that are used to construct cool rooms are more comfortable for workers and reduce the need to use fans and air conditioning systems. These sheds are relatively cheap, easy to keep clean and dramatically reduce the level of contamination in a nursery.

The effects of poor nursery sanitation are not confined to the grafting process. The high temperatures (25–28°C) and humidity (98%) in callusing boxes and callusing rooms favour the growth of pathogens, and in nurseries where sanitation is poor outbreaks of botrytis and other pathogens that can kill all the cuttings (100 or more) in a callusing box are common (Becker and Hiller, 1977). In these situations losses can be catastrophic, particularly when the problem is compounded by heater fans blowing spores through the callusing room from a point source of contamination. Sources of contamination in callusing rooms include dirty callusing boxes, contaminated or recycled callusing media, cuttings with high levels of exogenous micro-organisms and contaminated surfaces including walls and floors.

To prevent outbreaks of disease in callusing rooms it is important to ensure that the callusing room itself and everything placed in it is sanitised, the callusing medium is moist, but not wet, and cuttings have the lowest possible titre of potential pathogens. A fungicidal dip may be necessary to control botrytis and other fungal pathogens on the cuttings before packing in the callusing boxes. Wooden callusing boxes are particularly difficult to clean and sterile plastic liners are used in many nurseries to prevent contact between the cuttings and the wood of the boxes. However plastic liners do not allow air penetration and drainage of excess water and the wooden boxes remain a potential source of contamination in the callusing room. Plastic callusing boxes recommended by Becker and Hiller (1977) have the advantage of being easier to clean than wood and are usually slotted or perforated allowing better drainage and air penetration than plastic liners. Vermiculite is the preferred callusing medium because it is sterile, has a low density and can hold large amounts of water without becoming soggy. Other callusing media such as sand and sawdust that have traditionally been used as callusing media are not sterile. Sand is heavy and sawdust from some sources may contain abiotic contaminants that are toxic to vine cuttings (Hartmann *et al*., 1990).

Cold storage

In recent years cold storage has emerged as a critical factor in the production of quality grapevine planting material. Cold storage at 1–2°C is utilised to hold cuttings and rooted vines until they are required for processing or planting in the vineyard. Stored material remains in a dormant, or near-dormant state and growth is suppressed, extending the propagating and planting season, and giving nurseries and growers greater flexibility in planning and in the management of labour and resources (Deans *et al*., 1990; Hartmann *et al*., 1990).

Both cuttings and 1-year-old rooted vines are placed in cold storage for periods varying from a few weeks to many months. However, 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 micro-organisms and poor storage conditions. Botrytis 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 is the most common cause of microbial decay in storage. However other micro-organisms can also cause the decay of cuttings and vines in cold storage (Becker and Hiller, 1977). The sources of microbial contaminants in cold storage include the cuttings or vines themselves, contaminated packaging materials, other products in the cool room and the cool room surfaces.

In one case investigated by the author the growth of a field-acquired *Penicillium* sp. on vines in cold storage was so abundant that the vines were completely covered in the blue spores and failed to grow when planted in the vineyard. In another case the source of botrytis contamination that caused the deaths of cuttings held in cold storage was traced to table grapes infected with botrytis that had been stored in the cool room prior to the storage of the cuttings. Only rootstock cuttings that had been hot water treated and stored in perforated bags were affected. Cuttings that had not been HWTed,or were stored in bags that had not been perforated, were not affected, and cuttings from the same batch as the affected cuttings stored in another cool room were also unaffected. In the affected cool room the floor had been swept but the room had not been sanitised before the cuttings were placed inside. The botrytis spores raised by the sweeping had been dispersed around the cold room by the cooling fans and contaminated the cuttings by means of the perforations in the storage bags. The mucilage that had oozed from the ends of the cuttings following HWT provided the substrate for the botrytis to grow. The very large sclerotia, resembling blackened callus tissue, that had formed at the ends of the cuttings indicated that the botrytis was a cold-adapted strain (Becker and Hiller, 1977) that had probably existed in the cool room for some time.

In addition to problems caused by botrytis and other pathogenic micro-organisms, several other problems are experienced with cuttings and rooted vines in cold storage. From time to time there are reports of cuttings or rooted vines that are found to be fermenting when they are removed from storage. When the packages are opened there is a strong 'winey' aroma and the affected material either fails, or is slow to establish in the field. There are a number of potential causes of fermentation in cold storage including inappropriate packaging, the activity of micro-organisms (Bouard, 1982) and HWT (Waite *et al*., 2004).

Reports from nurseries that hot water treated cuttings and vines are particularly prone to fermentation in storage prompted an investigation by Waite *et al*. (2004) into the effects of HWT and hydration on cutting respiration to determine if HWT or hydration cause cuttings to ferment. Ethanol is a by-product of fermentative respiration that occurs when living tissue is deprived of oxygen, and both ethanol and acetaldehyde (also a by-product of fermentation) are thought to be toxic to plant tissue suffering from anoxia (Kennedy *et al*., 1992; Perata and Alpi, 1993). Dormant 2-bud cuttings of Cabernet Sauvignon and Pinot Noir were randomly assigned to 4 treatments; HWT for 30 min at 50°C, hydration for 8 h, hydration plus HWT and untreated control. The following day the cuttings were enclosed in test tubes and incubated at room temperature for 4 h. The atmospheres in the test tubes were then sampled with a syringe and gas chromatography was used to measure ethanol concentration in the samples. Following the initial sampling the cuttings were removed from the test tubes, packed in perforated plastic bags and placed in cold storage at 5°C for 4 weeks when the sampling process was repeated using the same protocols. Significant concentrations of ethanol (0.1–0.197 mg g^{-1} h^{-1} in HWTed cuttings compared to 0.001–0.005 mg g^{-1} h^{-1} in controls) were recorded in the atmospheres of all hot water treated cuttings at the initial sampling regardless of hydration, but not in the hydration-only cuttings or the untreated controls, indicating that the HWT cuttings had become fermentative, but not the hydrated or untreated cuttings.

When examined again 4 weeks later, cold storage no cuttings from any treatment showed evidence of significant ethanol production, indicating that the HWT cuttings had resumed aerobic respiration. It was concluded that the elevated short-term respiration rate of the HWT cuttings caused by the high treatment temperature and the saturation of the tissue with water relatively low in oxygen most likely interacted to create an oxygen deficit in the tissue, initiating the onset of fermentation (Pouget, 1963; Gray, 1999; Waite *et al*., 2004). Although the tissues of higher plants are able to live in anaerobic conditions for a few hours, or pos-

sibly longer, recovery may not be possible if the period of anoxia is prolonged (Kennedy *et al*., 1992; Perata and Alpi, 1993). The practice of packaging HWTed cuttings and vines in sealed plastic bags within a few hours of treatment may prolong the anaerobiosis and cause the accumulation of ethanol and acetaldehyde in the bags, further exacerbating the damage already caused to the tissue. The superior performance of cuttings stored in vented bags compared to sealed bags reported by Crocker and Waite (2004) has resulted in a recommendation to nurseries that vented bags should be used for cold storage of cuttings and rooted vines.

Exposure to ethylene during storage has also been implicated in a number of cases of failed planting material. Dormant bare rooted vines are normally purchased from nurseries in mid to late winter and held in cold storage until spring planting. Growers normally rent space in cool rooms at a location convenient to the vineyard, and the vines are often stored with pome fruits that generate ethylene. Ethylene is known to cause abnormalities in growth and premature senescence of plant material including cut flowers and fruit and may interact with ethanol and acetaldehyde to stimulate respiration, further increasing the need for oxygen, problematic for material stored in sealed bags with a low oxygen environment (Reid and Pratt, 1970; Rychter *et al*., 1979; Bouard, 1982). The effect of ethylene on vines in cold storage was indirectly assessed in an experiment that examined the effects of the ethylene inhibitor 1-Methylcyclopropene (1-MCP) on dormant 1-year-old vines held in a commercial apple cool room with a mean atmospheric ethylene concentration of 0.66 ppm for 12 weeks (Crocker and Waite, 2004). Shoot development in the vines treated with 1-MCP began earlier and was more advanced than in the untreated group throughout the trial. Bud burst in the untreated group was generally delayed by one week compared to the vines that had been treated with 1-MCP. The highly specific action of 1-MCP and the delayed and stunted shoot development observed in the untreated vines in the trial indicated that the untreated vines were most likely affected by the ethylene present in the cool-room atmosphere. The poorer growth of the untreated vines conformed to the known effects of ethylene on plants and although the concentration of ethylene in the cool room was variable, it was always within the range known to affect plants; consequently it is strongly recommended that nurseries and growers avoid storing cuttings and vine in cool rooms that may be contaminated with ethylene generated by other produce, or with the exhaust gases of forklifts.

Vineyard handling and planting practices

Correct handling and storage of grapevine planting material despatched from nurseries is also a critical factor in maintaining vine quality. Problems arise when transport and handling procedures result in dehydration of the vines, or the temperature inside the packages increases and causes the vines to die from heat stress. The insulating properties of plastic packaging impede heat dissipation from the vine mass and a dangerous temperature rise can occur very quickly if packages are not refrigerated during transport, or packages of vines awaiting planting are stored in the open or in sheds without refrigeration. This problem is compounded by metabolic heat that is generated in response to the initial rise in temperature (Breidenbach *et al*., 1997; Palliotti *et al*., 2004). The vines may also begin to ferment as the available oxygen is consumed and the atmosphere in the bags becomes anoxic.

Green potted vines present different management issues. Like dormant vines, exposure to high temperatures during transit can be fatal, but most problems occur as a result of the soil in pots being allowed to dry out. If the potting mix is allowed to dry out it will become hydrophobic and will not rewet easily, and the vines will suffer severe water stress in spite of regular watering. Green potted vines should be placed in a holding area that has dappled shade and is protected from wind until they are planted out. Depending on the weather they may need to be watered 2–3 times each day. If it has not been applied at the nursery, it may be useful to treat the vines with an anti-transpirant to reduce water loss from the leaves.

Once dormant vines are removed from the cool room they should be planted within 36-48 h, particularly if the weather is warm. Bales or boxes of vines should not be exposed to high temperatures, agrochemicals or fuel, and care should be taken to prevent dehydration during the trimming and handling process. If dehydration is a potential problem it is preferable to spray the vines with clean

water rather than soak them. Anecdotal evidence suggests that trimmed vines should not be returned to the cool room. If vines are not able to be planted for a few days they can be "heeled in" by placing bundles upright in a trench and loosely covering the root with moist soil, sand or well rotted sawdust, making sure that there are no large air pockets around the roots of the vines.

Concluding remarks

It has now become clear that the success of grapevine propagation and the viability of the resulting vines are dependent on a wide range of factors, from the management of the mother vines right through the propagation process to the establishment of the new vines in the vineyard. Although the factors that impinge on grapevine propagation are numerous, they fall into 3 main categories; 1) the intrinsic variability of the propagating material resulting from variations in genotype and the growing environment of the mother vines, 2) nursery production practices and 3) storage and handling practices. It has also become clear that the success of propagation hinges on the application of the best practice at every stage of the propagation cycle. Cutting and vine failures are usually the result of a series of important, but seemingly minor, cumulative insults the effects of which are not recognised as detrimental.

Of the three areas that may affect the quality of grapevine planting material, the greatest amount of information available to the industry is about nursery practices, since the fundamental techniques of propagation and nursery management are universal, regardless of the species being propagated (eg: Hartmann *et al*., 1990). The vine nursery industry has been quick to adopt bench grafting as the most efficient way of producing large quantities of vines, but nurseries have been slow to adopt concomitant practices, particularly good sanitation, that are vital to the production of high-quality planting material.

While the information on storage and handling of cuttings and vines is not as comprehensive as the information on propagation practices and sanitation, sufficient information is available in plant propagation texts (e.g. Hartmann *et al*., 1990) and in journals and magazines that publish research results (e.g. Deans *et al*., 1990; Crocker and Waite, 2004) to develop a set of protocols that will be reliable in the majority of situations and result in the production of high quality planting material and improve the economic viability of nurseries.

However, we do not yet fully understand the effects of the growing environment and nursery practices on propagating material that is inherently highly variable, and it is therefore not possible to predict the outcome of any given propagation cycle with complete certainty. The results of preliminary research investigating the physiological responses of cuttings and rooted vines to various protocols including HWT and cold storage have not yet identified the factors that mediate the response of grapevine propagating material to propagation protocols, and much work remains to be done. It has been established that HWT causes cuttings to become temporarily anaerobic and also results in long-term changes to respiration rates that persist through at least 14 weeks of cold storage (Waite *et al*., 2004). Transmission electron microscopy has also shown varying effects of HWT and cold storage on cell ultrastructure in ray tissue of Pinot Noir and Cabernet Sauvignon (Waite and Jaudzems, 2005). However, an understanding of the molecular basis of these and other responses to propagation protocols will be critical to the development of models that can be used to develop nursery protocols that accommodate the innate variability of propagating material and are reliable in every circumstance.

Vine propagation tends to be based on traditional practices that are sometimes at odds with the production of quality planting material, and although modern nursery practices are being taken up by leading nurseries, there is room for improvement in the nursery industry as a whole, particularly in the areas of nursery hygiene and handling practices.

The consequences of using inferior planting material are serious in both the short and long term (Jordan, 1997), but there has been insufficient value placed on high-quality planting material and consequently there has been little incentive for nurseries to improve the quality of their product. However, the high cost of ongoing vine failures has resulted in both the nursery and wine industries recognising that the quality of planting material

generally available is in urgent need of improvement (Constable and Drew, 2004; Waite, 2006).

The slow adoption of best practice in the vine nursery industry is partly due to the severe shortage of planting material during the 10-year wine-industry boom that began the mid-1990s when nurseries were under a great deal of pressure and could sell any vine they produced several times over, regardless of quality. Growers were often ignorant of the consequences of planting inferior material and paid scant regard to the quality of the vines they were purchasing, and often accepted seconds when first-quality vines were not available.

Other barriers to the adoption of best practice in the vine nursery industry include inadequate and inappropriate infrastructure and equipment, a poor understanding of the biology of grapevines and their associated micro-organisms, and the absence of universally accepted written standards for propagating and planting material. The nursery industry in some countries including Australia and the USA lacks clear guidelines that can be use as a tool to drive structural and procedural improvements. In addition to providing a quality benchmark for nurseries, a properly codified and internationally accepted set of standards is appropriate for an increasingly internationalised wine industry that is dominated by large multinational wine producers that maintain vineyards and source vines in several countries. Properly codified grapevine standards could also be a useful basis for dispute settlement between growers and nurseries when vines fail in the field. Although a check-list covering nursery practices and quality parameters for planting material has been developed by the authors for use by nurseries and growers (Waite, unpub.) and some vine improvement groups supplying nurseries with cuttings have written standards for cuttings, these documents are not widely published. New Zealand Winegrowers have also recently commissioned a report proposing the implementation of grafted grapevine standards for that country (Anonymous, 2005) and, with the agreement of the authors, these documents could be used as a starting point for the development of a set of universal standards that could be applied across the wine producing world to raise the standard of planting material and ensure to long-term viability of the wine industry.

Literature cited

Armengol J., D. Gramaje, D. Salazar, I. Lopez-Cortes, A. Gimenez-Jaime, A. Crespo, E.H. Albaranez and J. Garcia-Jimenez, 2006. A hot-water treatment to control *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum* in Spanish grapevine nurseries. *Phytopathologia Mediterranea* 42, 126 (abstract).

Anonymous, 1998. *National Vine Accreditation Scheme*. Australian Vine Improvement Association Inc., Victoria, Australia.

Anonymous, 2005. *Proposal for Implementation of the Grafted Grapevine Standards*. New Zealand Winegrowers, Auckland, New Zealand, Nimmo-Bell and Co. Ltd.

Anonymous, 2006. Statistiques. International Organization of Vine and Wine, (n.d.) Retrieved December 5, 2006 from the International Organization of Vine and Wine web site: http://news.reseau-concept.net/pls/news/p_entree?i_sid=3989677561458 97807364039 &i_type_edition_id=20508&i_section_id=20515&i_lang=33

Baker K.F., 1962. Thermotherapy of planting material. *Phytopathology* 52, 1244–1255.

Baker K.F. and R.G. Linderman, 1979. Unique feature of the pathology of ornamental plants. *Annual Review of Phytopathology* 17, 253–277.

Bell C.R., G.A. Dickie, W.L.G. Harvey and J.W.Y.F. Chan, 1995. Endophytic bacteria in grapevine. *Canadian Journal of Microbiology* 41, 46–53.

Bazzi C., E. Stefani, R. Gozzi, T.J. Burr, C.L. Moore and F. Anaclerio, 1991. Hot-water treatment of dormant grape cuttings; its effects on *Agrobacterium tumefaciens* and on grafting and growth of vine. *Vitis* 30, 177–187.

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

Bell C.R., G.A. Dickie, W.L.G. Harvey and J.W.Y.F. Chan, 1995. Endophytic bacteria in grapevine. *Canadian Journal of Microbiology* 41, 46–53.

Birchfield W. and H.M. van Pelt, 1958. Thermotherapy for nematodes of ornamental plants. *Plant Disease Reporter* 42, 451–455.

Bouard J., 1982. Qualité des sarments et multiplication végétative. In: *Il colloque international sur la multiplication de la vigne*. Bordeaux, France, 2-4 Septembre 1982, Fédération Française des Syndicats de Producteurs de Plants de Vigne, France, 44–51.

Burr T.J., K. Ophel, B.H. Katz and A. Kerr, 1989. Effect of hot water treatment on systemic *Agrobacterium tumefaciens* Biovar 3 in dormant grape cuttings. *Plant Disease* 73, 242–245.

Burr T.J., C.L. Reid, D.F. Splittstoesser and M. Yoshimura, 1996. Effect of heat treatments on grape bud mortality and survival of *Agrobacterium vitis in vitro* and in dormant grape cuttings. *American Journal of Enology and Viticulture* 47, 119–123.

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.

Cole M. and H. Waite, 2006. An investigation of the role of endogenous and exogenous bacteria in grapevine propagation and development. Report MU01/01. Grape and Wine Research and Development Corporation, Adelaide, SA, Australia.

Constable F. and C. Drew, 2004. Review of vine health parameters, implementation priorities and capabilities for vine improvement groups and accredited nurseries. Report to Grape and Wine Research and Development Corporation, Scholefield Horticultural Services Pty. Ltd. Adelaide, SA, Australia, 8–12.

Edwards J., F. Constable, T. Wiechel and S. Salib, 2006. Comparison of molecular tests - single PCR, nested PCR and quantitative PCR (SYBR®-green and TaqMan®) - for detection of *Phaeomoniella clamydospora* spores during grapevine nursery propagation. *Phytopathologia Mediterranea* 46, 58–72.

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* Annual Technical Issue 461a, 33–37.

Crocker J. and H. Waite, 2004. Development of effective, efficient and reliable hot water treatments. Grape and Wine Research and Development Corporation, Project SAR99/4.

Crous P.W., L. Swart and S. Coertze, 2001. The effect of hot-water treatment on fungi occurring in apparently healthy grapevine cuttings. *Phytopathologea Mediterranea* 40, S464–S466.

Deans J.D., C. Lundberg, P.M. Tabbush, M.G.R. Cannell, L.J. Sheppard and M.B. Murray, 1990. The influence of dessication, rough handling and cold storage on the quality and establishment of Sitka Spruce planting stock. *Forestry* 63, 129–141.

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

Goheen A.C., G. Nyland and S.K. Lowe, 1973. Association of a Rickettsialike organism with Pierce's disease of grapevines and Alfalfa Dwarf and heat therapy of the disease in grapevines. *Phytopathology* 63, 341–345.

Goussard P.G., 1977. Effect of hot-water treatments on vine cuttings and one-year-old grafts. *Vitis* 16, 272–278.

Graham A., 2006. Hot water treatment of grapevine rootstock cuttings grown in a cool climate. *Phytopathologia Mediterranea* 46, 124 (abstract).

Gray N.F., 1999. *Water Technology an Introduction for Environmental Scientists and Engineers*. Arnold, Hodder Headline Group, London, UK.

Halleen F., P.H. Fourie and P.W. Crous, 2006. A review of black foot disease of grapevine. *Phytopathologea Mediterranea* 45, S55–S67.

Hartmann H.T., D.E. Kester and F.T. Davies, 1990. *Plant Propagation Principles and Practices,* 5th ed. Prentice Hall International Inc., Simon and Schuster, Englewood Cliffs, New Jersey, USA.

Haviland D.R., W.J. Bentley and K.M. Daane, 2005. Hot-water treatments for control of *Planococcus ficus* (Homoptera: Pseudococcidae) on dormant grape cuttings. *Journal of Economic Entomology* 98, 1109–1115.

Jordan D., 1997. Quality of vineyard planting material: second only to influence of site. *The Australian Grapegrower and Winemaker* 402, 20–21.

Kennedy R.A., M.E. Rumpho and T.C. Fox, 1992. Anaerobic metabolism in plants. *Plant Physiology* 100, 1–6.

Krake L.R., N. Steele Scott, M.A. Rezaian and R.H. Taylor, 1999. *Graft-transmitted Diseases of Grapevine*. CSIRO Publishing, Collingwood, Australia, 6 pp.

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

Lear B. and L.A. Lider, 1959. Eradication of root-knot nematodes from grapevine rootings by hot water treatment. *Plant Disease Reporter* 43, 314–317

Meagher J.W., 1960. Root-knot nematode of the grapevine control in rooted stocks by hot water treatment. *The Journal of Agriculture, Victoria* 58, 419–445.

Nicholas, P.R., A.P.Chapman and R.M. Cirami, 1992. Grapevine propagation. In: *Viticulture vol. 2, Practices* (B.G.Coombe, P.R. Dry, ed.), Winetitles, Adelaide, 1–22.

Ophel K., P.R. Nicholas, P.A. Magarey and A.W. Bass, 1990. Hot water treatment of dormant grape cuttings reduces crown gall incidence in a field nursery. *American Journal of Enology and Viticulture* 41, 325–329.

Perata P. and A. Alpi, 1993. Plant responses to anaerobiosis. *Plant Science* 93, 1–17.

Pouget R.,1963. Action de la temperature sur les échanges gazeux des sarments de vigne dans des conditions d'aérobiose et d'anaérobiose: consequences sur la glycolyse. *Compte Rendue de l'Académie d'Agriculture Française,* 593–600.

Reid M.S. and H.K. Pratt, 1972. Effects of ethylene on potato tuber respiration. *Plant Physiology* 49, 252–255.

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

Rychter A., H.W. Janes, C. Chin and C. Frenkel, 1979. Effect of ethanol, acetaldehyde, acetic acid and ethylene on changes in respiration and respiratory metabolites in potato tubers. *Plant Physiology* 64, 108–111.

Samish R.M. and P. Spiegel-Roy, 1957. The influence of the nutrition of the mother vine on the rooting of cuttings. *Records of the Agricultural Research Station. Rehovot, Israel* 8, 93–100.

Stonerod P. and B. Strik, 1996. Hot-water dipping eradicates phylloxera from grape nursery stock. *HortTechnology* 6, 381–383.

Suatmadji R.W., 1982. Control of root knot nematodes, *Meloidogyne javanica*, in rootstocks of grapevine, *Vitis vinifera*, by immersion in nematicide solutions at different temperatures and in hot water. *Nematologia Mediterranea* 10, 119–125.

Von Broembsen S. and P.G. Marais, 1978. Eradication of *Phytopthora cinnamomi* from grapevine by hot water treatment. *Phytophylactica* 10, 25–27.

Waite H., 1998. The effects of hot water treatment on the mortality of grapevine propagules. *The Australian Grapegrower and Winemaker* 414a, 114–116.

Waite H., 2002. *The Effects of Hot Water Treatment on Grapevine Propagating and Planting Material*. Master of Applied Science (Horticulture) Thesis, University of Melbourne, Victoria, Australia.

Waite H., 2006. Who is responsible for the quality of planting material in Australian vineyards? Looking to the future. *The Australian and New Zealand Grapegrower and Winemaker* 507, 40–42.

Waite H., J. Crocker, G. Fletcher, P. Wright and A. Delaine, 2001. Hot water treatment in commercial nursery practice – an overview. *Australian Grapegrower and Winemaker* Annual Technical Issue 449a, 39–43.

Waite H., M. Cole, G. Jaudzems and J. Faragher, 2004. Recent advances in grapevine propagation research. *The Australian and New Zealand Grapegrower and Winemaker* Annual Technical Issue 485a, 39–40.

Waite H., S. Beggs, H. Dark, P. Kwak and G. Murrells, 2005. The effects of different post hot water treatment cool down protocols on dormant cuttings of *Vitis vinifera cvs*. *Phytopathologea Mediterranea* 44, 119–120.

Waite H. and G. Jaudzems, 2005. Changes in ray cell ultrastructure in dormant Pinot Noir cuttings following hot water treatment and cold storage. *The Australian and New Zealand Grapegrower and Winemaker* Annual Technical Issue 497a, 117–120.

Wallace J., J. Edwards, I.G. Pascoe and P. May, 2003. *Phaeomoniella chlamydospora* inhibits callus formation by grapevine rootstock and scion cultivars. In: *Proceedings 8th International Congress of Plant Pathology,* February 2–8, Christchurch, New Zealand, 363 (abstract).

Whiteman S.A., M.V. Jaspers, A. Stewart and H.J. Ridgway, 2004. Identification of potential sources of *Phaeomoniella chlamydospora* in the grapevine propagation process. *Phytopathologia Mediterranea* 43, 152–153.

Accepted for publication: March 13, 2007