Development of Effective, Efficient and Reliable Hot Water Treatments





Final Project Report to
GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: **SAR 99/4**

Principal Investigators: **John Crocker & Helen Waite**

Research Organisations: **SA Research & Development Institute**
University of Melbourne

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Development of Effective, Efficient and Reliable Hot Water Treatments

GWRDC Project - SAR 99/4

Final Project Report

John Crocker
South Australian Research and Development Institute
Lenswood Horticultural Centre
Swamp Road, Lenswood SA 5240

Helen Waite
Institute of Land and Food Resources
University of Melbourne
Dookie College, Victoria 3647

With contributions by

Graeme Fletcher, Sue McConnell and Adam Wightwick
Department of Primary Industries Victoria
Sunraysia Horticulture Centre
Box 905, Mildura, Victoria 3502.

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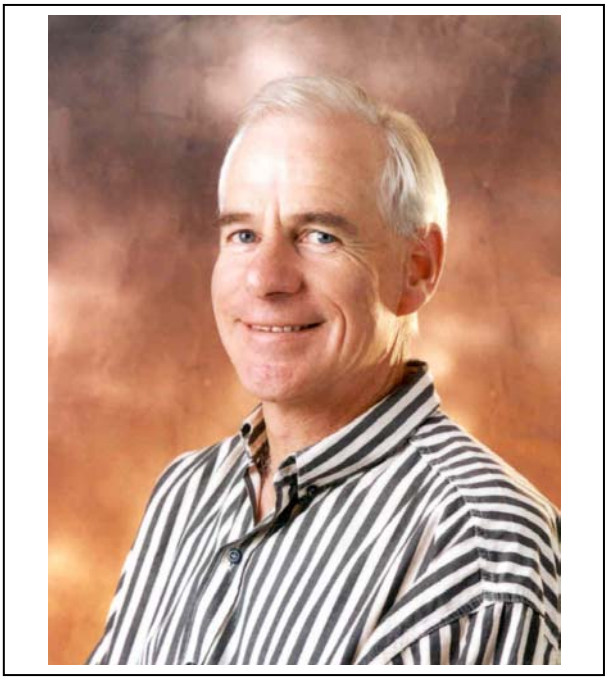
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1. Abstract

Collaborative research revealed factors influencing grapevine-cutting responses to 50^0C/30min hot water treatment (HWT). Cutting respiration measurement detected changing dormancy states. Variety and source area parameters influenced cutting performance. Cutting dehydration, over-hydration (>8h), poor water sanitation, poor hygiene and source area moisture stress may impinge on cutting performance following HWT. Hydration and HWT effects on cuttings persisted during early development, but were moderated by good nursery management. Post-HWT cold storage conditions and storage bag ventilation were critical. A bar code system tracked nursery processes. Industry collaboration, workshops and revision of AVIA & VINA HWT protocols optimised industry adoption and information relevance.

Dedication



This report is dedicated to the memory of Graeme Fletcher

HWT project team member, colleague and friend.

Graeme Fletcher was an advocate of vine improvement in Australia and was instrumental in the consolidation of the Victorian & Murray Valley Vine Improvement Association (VAMVVIA) as one of Australia's leading providers of improved propagation material. Graeme was recognised nationally for his knowledge of grapevine viruses and his work on improving vine yields and quality by clonal selection. He was a graduate of the Burnley Horticultural College, managed the Victorian Grapevine Vine Improvement Scheme and investigated the use of heat treatment to eliminate viruses and improve grapevine yield in Sultanas, Cabernet Sauvignon and Gordo. Graeme developed high yielding grapevine clones by selection and heat therapy and compared heat-treated and untreated clones of Zante Currant, Waltham Cross, Malbec, Gordo, Doradillo and Cabernet Sauvignon. Graeme investigated HWT and cool-storage from 1999 and was central to a range of other viticultural projects between 1969 and 2003, including the control of grapevine mildews and botrytis. Graeme's loss creates a personal and professional gap for many of us that won't be easily filled. He is sadly missed and will be remembered by us all for his enthusiasm, professionalism, encouragement and humour.

Executive Summary

Development of Effective, Efficient and Reliable Hot Water Treatments – SAR 99/4

Hot water treatment (HWT) is widely used by Australian vine nurseries to suppress a range of pests and pathogens in grapevine cuttings or rootlings during grapevine propagation. HWT is practiced in two forms, either as a short duration surface treatment involving immersion hot water at 55°C for 5 minutes, or as a long duration penetrating treatment, involving immersion at 50°C for 30 minutes. In a small number of cases, long duration HWT has been associated with cutting mortality where HWT was performed correctly and no single factor could be readily attributed as the cause. These incidents lead to industry concerns regarding the safety of HWT and precipitated GWRDC funded research into the HWT process.

The project 'Development of Effective, Efficient and Reliable Hot Water Treatments' (SAR 99/4) investigated key aspects of vine propagation to determine which source-vine, nursery practice and biological factors had potential to significantly influence cutting performance following HWT. Research outcomes and benefits to industry realised during SAR 99/4's four collaborative sub-programs are summarised as follows:

Program 1, SARDI: **Dormancy Measurement:** Nurseries suspect that vine cuttings become increasingly sensitive to HWT as cutting dormancy levels fall. No dormancy measurement tools were available to verify this. Three possible dormancy measurement techniques were screened and only the measurement of cutting respiration rates indicated changed source vine dormancy states. The onset of bud swell in purple-leafed flowering Prunus coincided with minimum vine respiration rates in mid-July and provided a useful indicator of increased metabolic activity within dormant source vines. *Industry Benefits*: Improved understanding of vine dormancy profiles will lead to improved cutting harvest practices. Flowering in purple-leafed Prunus provides a seasonally flexible bio-indicator to signal that cutting harvest should be concluded where possible.

Influence of Nursery Factors on Performance After HWT: Three commercial scale experiments were conducted at the Jacob's Creek Vine Nursery between 2000 and 2003. Cutting variety and a range of propagation factors were systematically varied to assess their role in determining vine performance following HWT. Cutting variety proved to be significant with Pinot Noir and Merlot recording HWT sensitivity. Delayed cutting handling and post-delivery hydration durations exceeding 8hours were not always beneficial. Seasonal variation significantly influenced optimum cutting harvest date. Maintenance of source area soil moisture prior to cutting collection may prove to be beneficial. Good nursery management practices enabled vines to recover from HWT and most treatment regimes with little impact on long-term viability. *Industry Benefits*: Results amended improved HWT protocols for use by VINA and AVIA members. Identification of varieties sensitive to HWT will allow nursery managers to be aware of the potential for suppressed root initiation and the possible impact of moisture stress on vine growth following HWT.

Program 2, *DPI – Victoria*: *The Effect of Storage Treatments on the Viability of Grapevine Cuttings:* Post-HWT cold storage conditions were evaluated for Chardonnay in 2001 and 2002. Varying chlorination dip concentrations did not prove to be significant; however, provision of storage bag ventilation holes improved cutting condition and propagation success. In 2002, ventilated storage bags had a healthy earthy aroma, while cuttings from sealed bags occasionally had a wine aroma. Cuttings from bags with vent holes at one end propagated best after 10 weeks, however, there were no significant propagation differences between ventilation treatments after 20 weeks storage. *Industry Benefits:* Where post-HWT cold-storage of cuttings 10 weeks or more is necessary, provision of adequate storage bag ventilation will reduce the incidence of poor cutting performance.

Program 3, Uni. Melbourne: Nursery Practice & Cellular Level Effects of HWT.
Experiments exploring the impact of pre HWT cutting hydration and the order of nursery operations illustrated the potential for suppressed root initiation induced by varying nursery propagation procedures. *Benefit*: This information may assist nurseries in the prevention of poor cutting performance caused by moisture stress. Studies into the treatment of cold-stored rootlings with an ethylene suppressant, '1-methylcyclopropene' (1-MCP) demonstrated lower mortality rates and improved shoot growth following 1-MCP treatment where storage atmospheres were ethylene contaminated. Benefit: This information demonstrates the risk associated with co-storage of cuttings or rootlings with ethylene producing materials and identifies the potential for 1-MCP treatment of stored rootlings to improve strike rates and provide earlier, more even bud burst.

The examination of dehydration rates in dormant cuttings demonstrated similar, constant rates of cutting moisture loss for differing varieties over a 19-day period and a corresponding linear decline in root initiation as cutting moisture levels fell. *Benefit:* This result proves that cutting suppliers and nurseries should take care to handle cuttings rapidly and prevent cutting dehydration prior to cold storage. Studies into the effects of hydration on cutting moisture content and root Initiation in Sauvignon Blanc indicated that cutting hydration durations exceeding 8 hours were not clearly beneficial. *Benefit:* This result suggests that nurseries should review their hydration protocols to avoid suppression of root development through over-hydration in sensitive varieties.

The effects of HWT and nursery processes on dormant cutting tissues were examined by measuring changes in the ratio of cutting electrical impedance to high (10.0kHz) and low (<1.0kHz) frequency currents at varying stages during Chardonnay propagation. Results indicated that cell membrane integrity was measurably and progressively affected by each propagation treatment. *Benefit:* This result offers insight to possible reasons behind HWT sensitivity in some vine varieties. The effects of HWT on the cellular ultrastructure of dormant Pinot Noir ray tissues were examined by light microscope and transmission electron microscope (TEM). Cells with secondary thickening, packed with intact or fractured amyloplasts were observed following HWT under natural light. TEM examination demonstrated increased electron density in the cytoplasm of HWT cells, possibly due to ribosome involvement in production of heat shock proteins or other metabolic processes clustered around the endoplasmic reticulum. Further investigation will be required to clarify these observations.

The effect of HWT, cold storage and harvest date on the respiration rate of dormant cutting varieties illustrated that respiration rates varied with the date of collection and supported anecdotal observations that dormancy is deepest and most prolonged in Cabernet Sauvignon and quite brief and shallow in Chardonnay. Respiration rates generally increased 1 hour after HWT and fell again within 24 hours, possibly indicating a HWT wounding response. Benefits may result from resting cuttings for 24 hours after HWT and prior to cold storage. Cold storage produced varying respirative responses dependent on variety, harvest time and treatment regimes. *Benefit:* These results assist in the understanding of poor cutting performance following HWT and cold storage by illustrating the need for adequate storage bag ventilation.

The effects of source area and HWT on the development of Pinot Noir cuttings were examined in cuttings sourced from the Barossa Valley & Riverland (SA), Margaret River (WA) and Kyneton (Vic). Results demonstrated that callus development was not affected by source area or HWT. The number of root initials varied between source areas, but not between treatments, except for cuttings sourced from Western Australia where HWT cuttings had significantly fewer roots than non-HWT. When root number and length were considered together, cuttings sourced from the Riverland developed stronger root systems in the early stages of the propagation cycle. On the basis of these results, growers may prefer to obtain cuttings from the Riverland or Barossa Valley.

Program 4: QA and technology transfer issues

Research results generated during SAR 99/4 were conveyed to vine nurseries in a series of participative workshops conducted with GWRDC and VINA support during 2000 and 2002 (note GWRDC workshop reports). Workshops provided opportunities to discuss outcomes with HWT practitioners, gain feedback on progress from industry stakeholders, solicit input on future research directions and receive requests for the resolution of specific HWT issues raised by vine nurseries. Regular publication of articles in the Australian and New Zealand Grapegrower and Winemaker, presentation of posters at industry conferences, and presentation of papers at National and International conferences ensured that all interested parties were regularly appraised of research outcomes.

SAR 99/4 generated a broad suite of results that were incorporated into an amended HWT protocol document for use by AVIA and VINA members. The new protocol document forms an integral component of VINA's 'Vine Industry Nursery Accreditation Scheme' and will be used as the basis for ongoing nursery audits and the award of nursery accreditation status to participating vine nurseries.

A cutting tracking system, Nursery QA and research tool was developed collaboratively with Tracking Labels and Consulting (TLC) during SAR 99/4. The system uses bar codes to track individual cuttings or batches and enables unit specific data collection during each critical propagation and quality control point. Final commercial testing will be conducted during 2004 and release to industry as the product 'UniTrack' is proposed prior to the 2005 cutting season. UniTrack will assist nurseries to maintain quality standards through their propagation processes, will assist with record keeping necessary to achieve nursery accreditation and will assist in information collation where legal defence is required.

Future Research

Industry stakeholders continually provided feedback on their HWT research needs during the passage of SAR 99/4. A project reference group assisted with further needs analysis and program short-listing during 2003 to arrive at a new GWRDC project proposal titled "Advances in Hot Water Treatment for Rootstocks, Rootlings and Grafted Vines" (R2774). Though this proposal was approved by GWRDC, work commencement will be delayed until a VINA voluntary contribution finding scheme can be initiated and HAL co-funding can be secured.

Acknowledgements

SAR 99/4 was made possible through funding support by GWRDC and the generous collaborative efforts of the Orlando-Wyndham Group through the 'Jacob's Creek Nursery Team' and Langhorne Creek Vineyard, Boulevarde Nurseries, Cobaw Ridge and Hanging Rock Wineries. The enthusiastic support of numerous VINA and AVIA members was vital to the success of nursery-based studies as was the outstanding support and hospitality provided by the Ceccato and Harding families. Respiration studies were assisted through access to lab space and a Varian Gas Chromatograph by Dr Andreas Klieber (University of Adelaide) and by MEA for CO_2 logger design. The support of VINA Chairman Paul Wright was invaluable and ranged from assistance with workshop delivery and bar code system design, to trial design advice, revision of HWT protocols and accommodation of HWT team members while working in SA. The 'UniTrack' bar code system would not have eventuated without the collaborative efforts and financial investment of Tracking Labels and Consulting Pty Ltd. In-kind contributions by SARDI, DPI-Victoria and University of Melbourne provided essential facilities, equipment and salary support for the project team between 1999 and 2004. Acknowledgement is also given to HWT team's family members for their support and patience during SAR 99/4 and for the accommodation provided to HWT team members while working in SA and Victoria.

3. Background

3.1 Introduction

Hot water treatment is an efficient, environmentally safe and commercially viable method of controlling or suppressing a wide range of pests and pathogens in vine cuttings, rootlings or grafted rootlings (Hamilton 1997). HWT is conducted in two forms within Australia, either as (1) a short duration or surface treatment process involving the immersion of propagules in clean hot water at 55°C for 5 minutes and (2) a long duration or penetrating treatment (HWT), involving immersion at 50°C for 30 minutes.

Short duration HWT has been used for many years to satisfy statutory quarantine requirements for the treatment of cuttings and rootlings prior to movement between wine regions in Australia. 55°C / 5 minute HWT controls *Phylloxera* and provides a level of surface suppression of root rotting fungi, nematodes and bacteria in *V. vinifera* cuttings & rootlings. Success rates are very high with correspondingly low rates of mortality following treatment application.

Long duration HWT controls the suite of organisms targeted by short duration hot water treatment in addition to suppression of phytoplasma, *Phaoacremonium* and *Agrobacterium*. Many Australian vine nurseries have adopted long duration HWT as an integral component of their vine propagation protocols and experience a high level of success overall.

In a relatively small number of cases, long duration HWT has occasionally been followed by cutting mortality. Characteristically, treatments were performed in accordance with established industry protocols and no single factor could be identified as aberrant or directly attributed to mortality following treatment.

These events were contrasted strongly by the overwhelming proportion of successful hot water treatments, but predictably, they acted to engender a degree of distrust in HWT by some vine nurserymen. This eventually resulted in an element of reluctance by a few operators who became sceptical about the value of adopting or persisting with long duration HWT in vine nursery practice.

This was understandable when Industry representatives reported isolated instances where up to 84% loss of cuttings followed HWT on batches of Merlot cuttings valued at approximately $25,000 (McCarthy pers. com.). Similar batches of Cabernet Sauvignon recorded 100% survival under precisely the same treatment conditions, further perplexing this issue in the eyes vine nursery operators.

Faced with a desire to preserve the health of Australian vineyards by supplying the highest quality, disease free propagation material, vine nurserymen, through the Australian Vine Improvement Association (AVIA) and the Vine Industry Nursery Association (VINA), sought to establish a collaborative research program to explore and resolve issues with potential to influence the survival of cuttings exposed to long duration HWT. This research program was to follow on from the initial work conducted by Helen Waite in association with AVIA (Waite and May 1999).

A meeting of interested researchers and vine nursery representatives was convened at the Sunraysia Horticultural Centre (Irymple, Vic.) in February 1999. The meeting discussed Long duration HWT issues, industry's R&D requirements and resolved how each interested R&D agency could collaborate to fully explore this issue. The meeting prioritised industry's HWT research requirements and precipitated the successful composition of a research proposal to GWRDC under the title 'Development of Effective, Efficient and Reliable Hot Water Treatments' (SAR 99/4 – Commenced November 1999).

3.2 Project Scope

The overall scope of SAR 99/4 can be briefly defined as follows:

> **"*The study of factors with potential to influence the performance of V. vinifera cuttings following nursery based exposure to long duration hot water treatment at 50^0C ($\pm$ 1^0C) for 30 minutes ($\pm$ 1 minute)".***

Industry representatives agreed that the scope of SAR 99/4 should not initially include the influence of HWT on dormant rootlings, grafted rootlings, scion wood or rootstock cuttings as this would have exponentially increased the size and complexity of the R&D program beyond the dimensions of available resources. Though this information is of importance to vine nurseries, it was decided to relegate the study of these issues to a future R&D program (see the GWRDC project application 'Advances in Hot Water Treatment for Rootstocks, Rootlings and Grafted Vines').

The scope of SAR 99/4 included the identification of critical parameters with potential to influence the successful outcome of long duration HWT within each phase of the vine propagation process. The scope also included the exploration of each parameter with a view to minimising it's potential to induce poor cutting performance following HWT. SAR 99/4 did not examine any aspect of Short Duration HWT within nursery practice. Work effort was divided between collaborating agencies as follows:

Program 1. SARDI, 'Pre-HWT issues', including the measurement of vine dormancy, influence of cutting harvest date, vine variety, handling speed following cutting harvest, cutting hydration duration, ± HWT on cutting performance.

Program 2. DPI - Victoria, 'Post-HWT issues', including measurement of the influence of post-HWT cold storage temperatures, storage conditions and storage duration on survival of treated *V. vinifera* cuttings.

Program 3. University of Melbourne, 'Nursery Practice & Cellular Level Effects of HWT', including measurement of changes to organelles and cell membranes induced in *V. vinifera* cuttings by pre-HWT hydration and HWT.

Program 4. AVIA, VINA, SARDI & Uni Melbourne, HWT Quality Assurance, Industry Adoption and Technology Transfer, including rapid, timely transfer of research outcomes to stakeholders, provision for industry feedback and input on the R&D program, revision of AVIA and VINA HWT protocols and development of a bar code based QA and trace-back system for vine nurseries.

4. Project Aims and Performance Targets

4.1 Sub-Program Details

Each sub-program within SAR 99/4 was managed separately by the relevant participating agency with the following aims and program outcomes in each case. Overall project management was coordinated by SARDI as the lead agency with Dr Michael McCarthy as the project supervisor and John Crocker as the project coordinator.

Program 1: ***Pre-HWT Issues***

Principal Investigator: John Crocker; ***SA Research and Development Institute***

Research Focus: The measurement of vine dormancy, the influence of pre-HWT factors on cutting performance and development of a bar code nursery tracking and QA system.

Program Outcomes:

- Development of an effective field-test for vine dormancy.
- Identification of major pre-HWT parameters with potential to influence cutting performance [(1)].

Program 2: ***Post-HWT Issues.***

Principal Investigator: Graeme Fletcher [(3)] Adam Wightwick and Sue McConnell; ***Department of Primary Industries – Victoria.***

Research Focus: The influence of post-HWT cold storage parameters on cutting survival.

Program Outcomes:

- The evaluation of post-HWT cold-storage temperatures as a factor influencing the development of 'Post Harvest Decay Syndrome' (PHDS) in vine cuttings.
- Determination of pathogens involved in PHDS
- Modification of cold-storage protocols to reduce PHDS and improve cutting survival following HWT

Program 3: ***Nursery Practice, Cutting Hydration and Effect of HWT on Cell Function.***

Principal Investigator: Helen Waite; ***University of Melbourne.***

Research Focus: The influence of hydration and HWT on the cellular structure and function of vine cuttings.

Program Outcomes:

- Determination of the effects associated with pre-treatment hydration and duration of hydration on *V. vinifera* cuttings.
- Determination of changes in the cells of cuttings treated by HWT, (particularly changes to cell membranes and organelles).
- Use of program outcomes to improve AVIA and VINA HWT protocols

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Program 4:	**HWT Quality Assurance, Industry Adoption and Technology Transfer, VINA, AVIA, SARDI and University of Melbourne.**
Principal Investigators:	Paul Wright (VINA) and Andrew deLaine (AVIA) [(4)]
Focus:	The effective timely transfer of research results to industry stakeholders and the development and testing of a bar code based tracking and vine nursery QA system.

Program Outcomes:

- The extension of research findings to industry through a series of annual HWT Research update seminars.
- Incorporation of R&D outcomes into improved AVIA and VINA HWT protocols [(2)].
- Facilitated Development of a Nursery Bar Code System.

4.2 Notes and Amendments to Original Project Objectives:

(1) The original objective of defining 'windows of opportunity for cutting harvest by region and variety' was modified following input by industry stakeholders. It was incorporated into a series of major, commercial-scale experiments designed to objectively assess pre-HWT parameters on the performance of cuttings following HWT. A range of parameters was evaluated including cutting harvest date and variety. This outcome modification was presented to the GWRDC board during a tour and discussion of HWT R&D progress at the Jacob's Creek Vine Nursery during August 2000.

(2) At the time of SAR 99/4 project inception, AVIA was actively developing and implementing a nursery accreditation system. Only reference to the AVIA HWT-protocols was cited in the original project proposal. By the time SAR 99/4 was being concluded, VINA was also developing and implementing an independent nursery accreditation system. Subsequently, outcomes from SAR 99/4 will be incorporated into both the AVIA and VINA nursery protocols and accreditation systems to achieve the greatest possible reach of this information to the Australian vine nursery industry.

(3) Graeme Fletcher commenced work as a member of the HWT project team following GWRDC project approval in November 1999. Graeme subsequently became ill and his condition progressively deteriorated until he was not able to continue working. Sue McConnell (Ag Vic) assumed Graeme's responsibilities and completed Graeme's work. Graeme eventually succumbed to his condition in 2004. All who had the privilege to have worked with Graeme will miss his friendly outlook, wit, experience and invaluable stewardship of the vine nursery industry.

(4) Andrew deLaine ceased employment as Executive Officer of AVIA shortly after SAR 99/4 commenced. Andrew's position was not replaced until January 2004, when renowned ampelographer and viticulturalist Dr George Kerridge was appointed as AVIA Executive Officer on a part-time basis.

Due to the relative complexity of SAR 99/4, research methodology, reporting of results, statistical analysis and discussion of outcomes will be best reported as a series of discrete studies within each program area. This will maintain continuity within each topic studied and will allow overall program conclusions to be integrated appropriately.

5.0 Project Methodologies, Data Analysis and Discussion of Outcomes

5.1 - Program 1: Pre-HWT Issues, SARDI

P1.1 Development of a Measurement tool for Vine Dormancy

John Crocker, SARDI[1]

[1]South Australian Research and Development Institute, Lenswood Horticultural Centre, Swamp Road, Lenswood SA 5240.
E-mail, crocker.john@saugov.sa.gov.au

Summary

Nurseries suspect that vine cuttings become increasingly sensitive to HWT as cutting dormancy levels fall. No dormancy measurement tools were available to verify this. Measurement of vine trunk and cane diameter fluctuations, variations in cane moisture content and measurement of cutting respiration rates were screened as possible dormancy measurement techniques. Only the measurement of cutting respiration rates indicated changed source vine dormancy states. The onset of bud swell in red-leafed flowering Prunus coincided with minimum vine respiration rates in mid-July and provided a useful indicator of increased activity within dormant source vines.

Aim

To screen a range of physical and physiological parameters and their respective measurement techniques as potential tools for the assessment of vine dormancy state in source area vines.

Background

The commercial vine nursery sector in Australia widely perceives that fully dormant *V. vinifera* cuttings tolerate long duration HWT with a lower possibility of mortality than quiescent or physiologically active cuttings (P. Wright and H. Waite, personal communication). Subsequently, many vine nurserymen prefer to harvest vine cuttings within a window of opportunity ranging between 10 days after full leaf fall and 4 weeks before bud swell (prior to the advent of cane weeping). While the external absence of leaves or vegetative growth on cutting source vines suggests that vines are dormant within this window of opportunity, the validity of these beliefs can only be tested through objective measurement of the state of internal or physiological dormancy.

Lavee and May (1997) provide a concise review of vine dormancy understanding from morphological, physiological and biochemical perspectives. Lavee and May cite Vegis (1965) who defines dormancy as “the temporary cessation of growth, while metabolic processes, including respiration, continue”, while ‘complete dormancy’ is viewed as a state where “the water content of the organs is reduced to such low levels that metabolism appears to cease until rehydration”.

Traditional techniques of dormancy assessment in vine cuttings relate the time taken from the imposition of ideal growing conditions to the appearance of budburst (Antcliff and May, 1961), while Pouget (1963) defined a value called ‘DD50’ as ‘the time taken for 50% of buds to burst’ under ideal conditions. Many other similar studies have been undertaken, however, they all provide a retrospective assessment of vine dormancy that would possibly

allow a practitioner to understand why an unfavourable HWT result occurred, but they would not facilitate a pre-emptive decision on the likely safety of HWT prior to treatment.

As there were no readily available tools to directly quantify the degree of vine dormancy in real time, at any given stage during the nursery cutting harvest window, studies were undertaken to identify and screen a range of possible measurement techniques for this task.

The criteria used to initially select candidate techniques were as follows:

- The test must operate in real time, not retrospectively.
- It must directly or indirectly measure a factor generally associated with changing internal physiological activity within the vine.
- The test must be robust, easily achieved and have potential for adaptation as field technique to be carried out by nurserymen.
- The field test should not rely upon equipment that would be too expensive or too complex for general nursery use.

Four vine physiological events were initially selected as possible dormancy measurement techniques, namely:

P 1.11, Technique 1. 'The measurement of variations in pre-budburst trunk and vine cane diameter in Vitis Vinifera cv Chardonnay and Pinot Noir.

P1.12, Technique 2. 'The measurement of fluctuations in cane moisture content prior to budburst'.

P1.13, Technique 3. 'The measurement of changing cutting respiration rate as a response to changing metabolic activity during the various phases of vine dormancy'.

Technique 4*. 'The measurement of fluctuations in cutting tissue sugar: starch ratio caused by mobilisation of starch reserves as metabolic activity increases following the conclusion of dormancy'.

** Ongoing investigations into changing sugar: starch ratios were discarded after initial assay trials in Autumn/Winter 2000. Starch: sugar assays were deemed to be unsuitable for eventual adaptation as a routine test to be carried out by vine nurseries due to the cost of assay reagents, the complexity and level of technical skill required to perform assays and the range of specialised equipment required to complete assays on cutting samples.*

Sugar: Starch ratio assays may prove to provide a viable lab based dormancy test that should not be discounted and may be worthy of further investigation in a different context

Research during SAR 99/4 subsequently focussed on the remaining three test options with the aim of selecting at least one technique for further investigation and development. The results of testing and evaluation of each measurement option during SAR 99/4 are as follows:

Unless otherwise stated, data analysis and statistical interpretation was carried out using GenStat version 6, Statistix version 7.1 and/or the imbedded statistics and graphing functions within Microsoft Excel 2000.

References:

(See Appendix 3, pp. 146 to 149 for a compiled list of references)

P 1.11 - Technique 1:

Measurement of Variations in Pre-budburst Vine trunk and Cane Diameter in *Vitis vinifera* cv Chardonnay and Pinot Noir.

John Crocker[1]

[1]South Australian Research and Development Institute, Lenswood Horticultural Centre, Swamp Road, Lenswood SA 5240.
E-mail, crocker.john@saugov.sa.gov.au

Abstract

Collaboration between SARDI and Phytech, Israel facilitated the measurement of *Vitis vinifera* trunk and cane diameter fluctuations between autumn and spring, 2000 to 2001 at the Lenswood Horticultural Centre, South Australia. Prior data from the Golan Heights suggested that the measured onset of trunk and cane diameter increases offered potential as a tool for indicating the end of full dormancy in cutting source vines. Observations from Lenswood indicated that trunk and cane diameters often fluctuated in synchrony between adjacent vines and often in association with rain events, however, observations achieved in Israel could not be replicated at Lenswood. While the measurement of trunk and cane diameter fluctuations is recommended for investigation from an irrigation measurement context, this technique was not adopted as a dormancy measurement tool in vines.

Aim

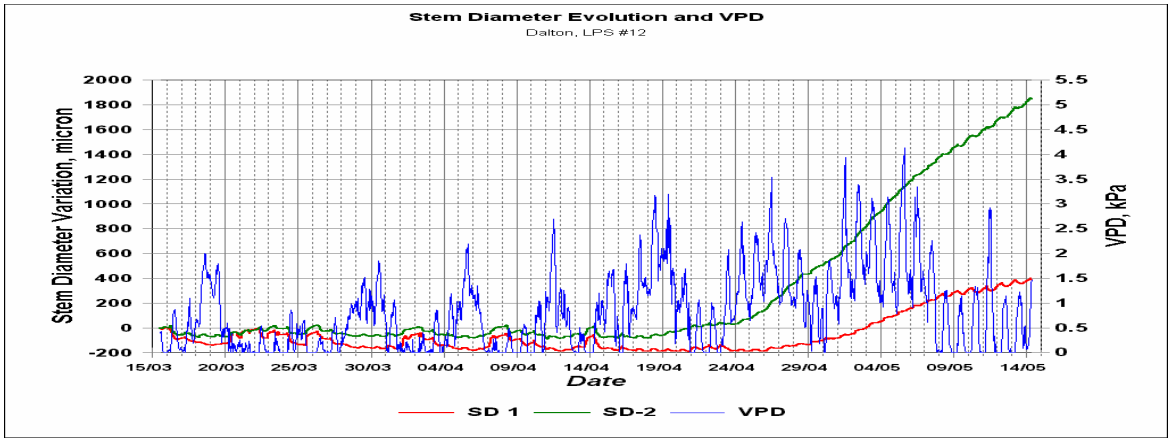
This experiment tests the supposition that accurate, continuous measurement of vine trunk and cane diameters during winter and spring has the potential to act as a vine dormancy measurement tool by revealing periods of declining or static dimensions followed by periods of rapidly increasing dimensions associated with the commencement of pre-budburst sap flow.

Background

Fluctuating vascular pressure associated with changing sap flow in vine trunks and canes following the end of full dormancy may be associated with micron-scale fluctuations in the dimensions of vascular tissue. It is proposed that the detection of cane dimensions transitioning from quiescence to rapid expansion has the potential to provide an easily measured parameter indicating the type of physiological changes generally associated with broken dormancy in vines.

In 2000, few commercial manufacturers distributed systems capable of measuring and continuously monitoring a diverse range of vine physiological parameters *in situ*. Dr. Michael McCarthy's prior experience with Phytech of Israel and his experience with the evaluation of Phytech's Phytomonitoring® systems at SARDI's Nuriootpa Research Centre suggested that Phytech may be capable of providing an 'off the shelf' solution for this task (see http://www.phytech.co.il/)

Correspondence with Dr. Uri Ton, Phytech's Director of Research and Development, indicated that Phytech could supply the necessary equipment and wished to collaborate in this study. Dr Ton provided sample data collected form Cabernet-Sauvignon (1999, Dalton Winery, Golan Heights, Israel) that clearly indicated a gross increase in cane diameter after mid-April, correlating with the expected commencement of sap flow prior to budburst in this region. (Note Graph 1, below).



Graph 1; Evolution of stem (cane) diameter (SD1, SD2) and Vapour Pressure Deficit (VPD) in Cabernet Sauvignon, Golan Heights – Israel. Courtesy of Dr. Uri Ton (1999).

Dr Ton's data suggested that a significant rise in stem diameter could be measured in association with the commencement of spring growth in Cabernet Sauvignon. Dr. Ton subsequently organised the supply and delivery of two complete Phytomonitoring units to SARDI's Lenswood Horticultural Centre at no cost to the project. Dr Ton also incorporated assistance with the installation and commissioning of each unit on-site at Lenswood while he was in Australia during July 2000.

Method

Both Phytomonitoring units were installed in 10 year old own-rooted vines at the Lenswood Horticultural Centre in South Australia. One unit was placed in Pinot Noir D5V12 and the second unit was installed in Chardonnay I10V1. Both blocks were cordon trained with vertical foliage management. The Phytomonitoring units were configured to continuously record trunk and cane diameter fluctuations from 3 replicate vines. Soil moisture, solar radiation and weather parameters were also recorded.

Trunk diameter sensors were installed on the main vine trunk 300mm below the primary cordon branch (see Photo 2, below). The outer friable bark layers were removed until the epidermis was reached prior to sensor installation. Trunk and cane sensors were initially shrouded in heavy-duty aluminium foil to provide a degree of thermal buffering and mechanical protection. The aluminium foil shrouds were later replaced on all sensors with construction sarking to provide additional mechanical protection and shroud durability. Cane diameter sensors were installed immediately prior to the first node on the first cane above the left-hand primary cordon branches.



Photo.1; Original Phytomonitor installation.

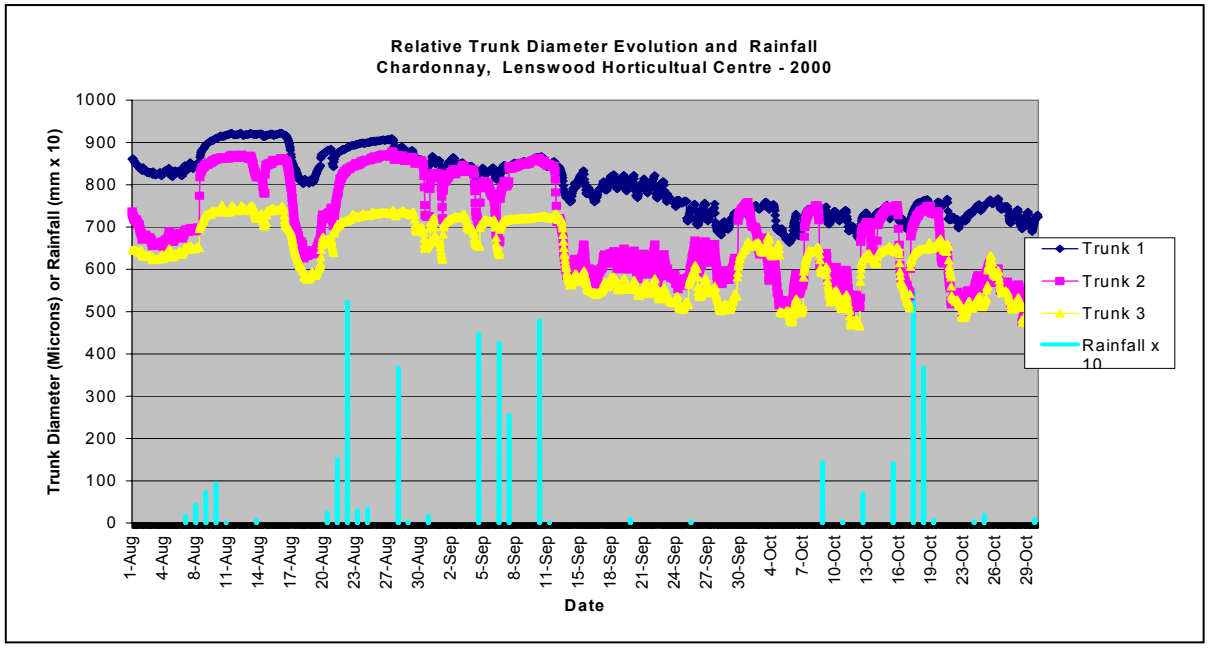


Photo. 2; New Sensor shrouds.

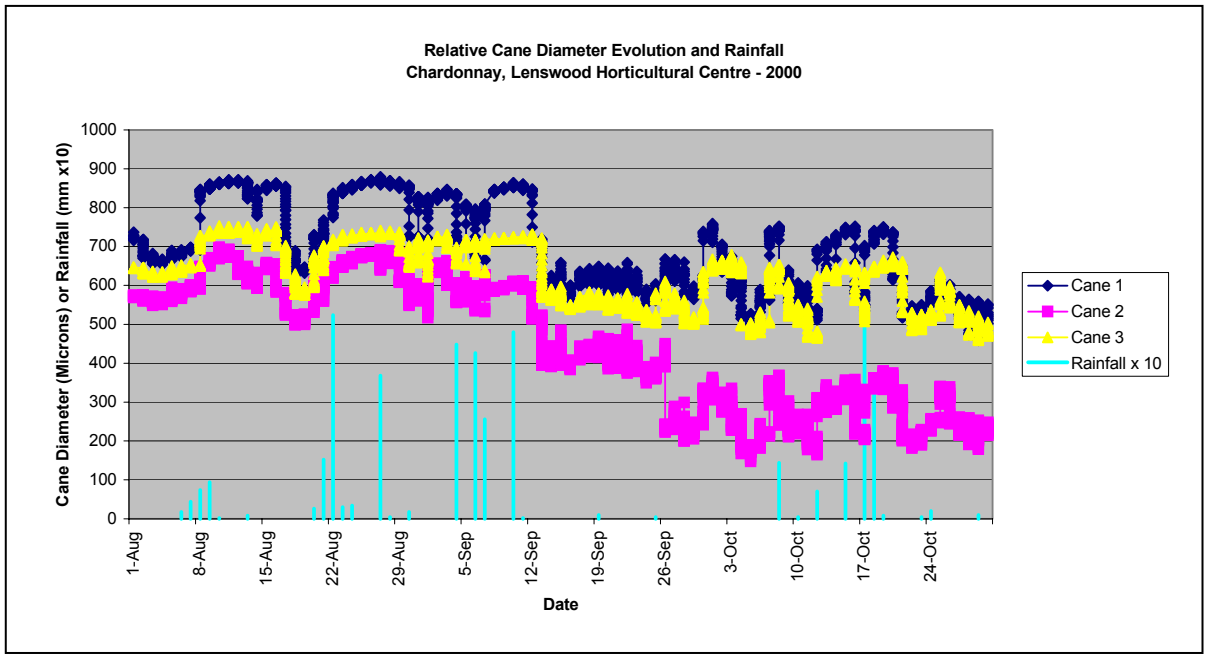
Data was captured at hourly intervals from the 1st of August 2000 to the 30th of October 2000 and again from 9th of April 2001 to 26th of October 2001. Both Phytomonitoring units were downloaded directly at weekly intervals by interfacing with a laptop computer in the field.

Results

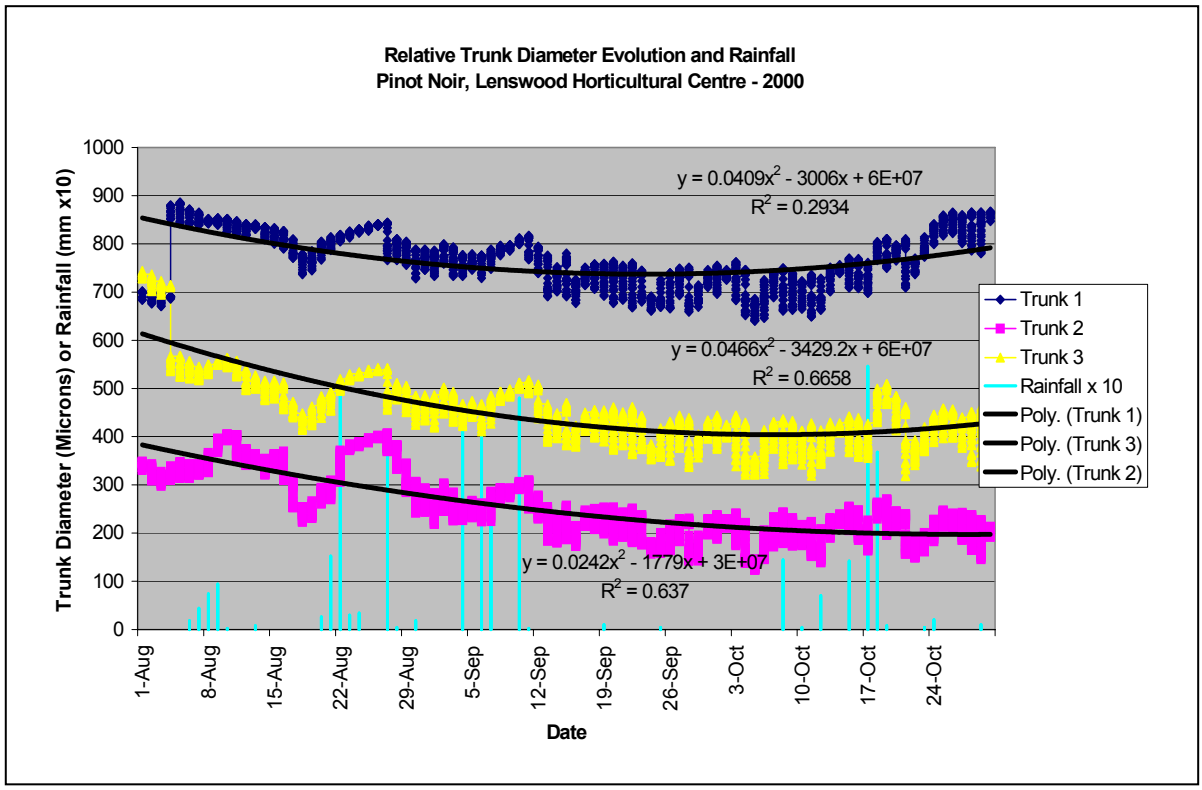
Graphs 2 and 3. (below) summarise trunk diameter changes in Chardonnay and Pinot Noir (respectively) during 2000. Rainfall events are included for reference.



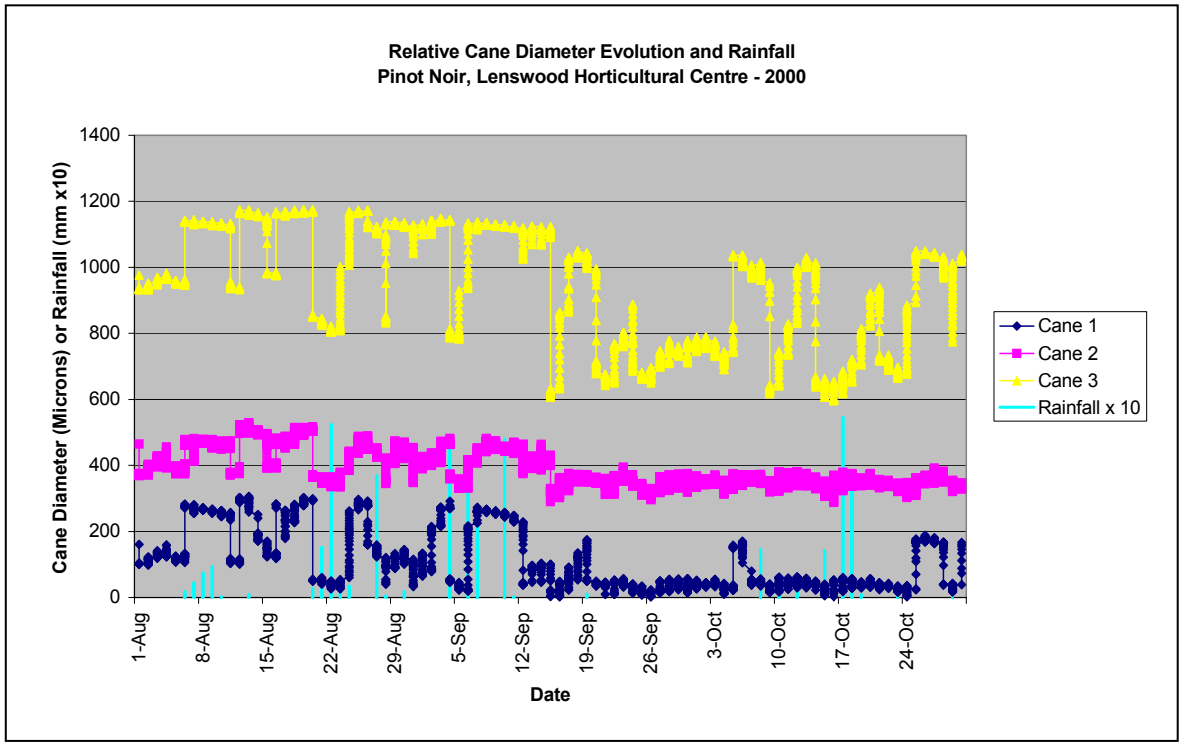
Graph 2; Relative trunk diameter fluctuations (microns) and rainfall (mm x10), Chardonnay I10V1, Lenswood Horticultural Centre, August to October 2000.



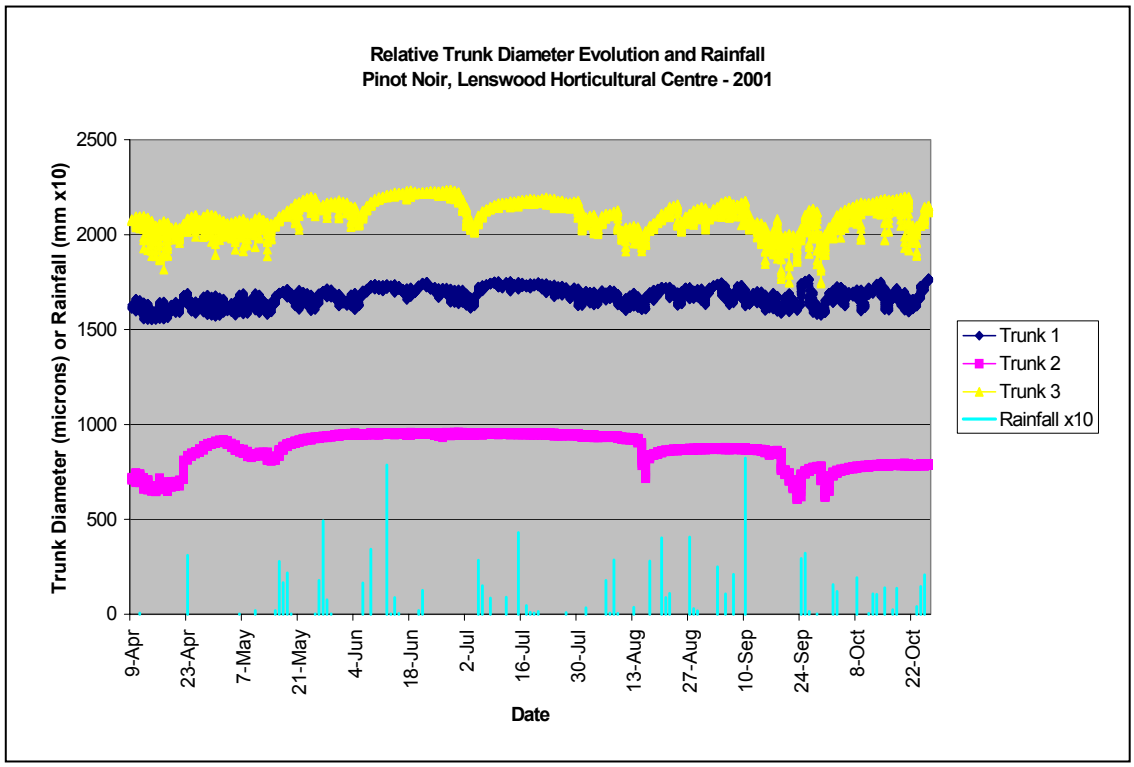
Graph 3; Relative cane diameter fluctuations (microns) and rainfall (mm x10), Chardonnay I10V1, Lenswood Horticultural Centre, August to October 2000.



Graph 4; Relative Trunk diameter fluctuations (microns) and rainfall (mm x10), Pinot Noir D3V5, Lenswood Horticultural Centre, August to October 2000.



Graph 5; Relative cane diameter fluctuations (microns) and rainfall (mm x10), Pinot Noir D3V5, Lenswood Horticultural Centre, August to October 2000.



Graph 6; Relative Trunk Diameter Evolution and Rainfall, Pinot Noir D5V12, Lenswood Horticultural Centre 2001.

Discussion

Phytomonitor data for Chardonnay I10V1 trunk and cane diameter fluctuations in 2000 (Graphs 2 and 3) demonstrated cyclic expansion and contraction in synchrony between all 3 adjacent vines. The amplitude of fluctuations observed in the cane data was generally greater than that observed in the trunk data.

Vine trunks and canes appear to expand and contract in unison between August and the end of October. In some cases, trunk and cane expansion appears to be related to rainfall events and corresponding rises in soil moisture, however, this is not always so.

Trends for Chardonnay through the 2000 season demonstrated a gradual reduction in overall trunk and cane diameter to the end of October in all three vines. A significant rise in trunk or cane diameter was not associated with budswell or shoot extension. This was contrary to the trends observed in Dr Ton's data from Cabernet Sauvignon in the Golan Heights (see Graph 1, above).

Data recorded for Pinot Noir D5V12 during 2000 demonstrated similar trends to Chardonnay through the season, although the Pinot Noir trunk data (Graph 4) suggested a slight increase in trunk diameters following budburst. A polynomial regression of the data with R^2 values of 0.64 and 0.66 for trunks 2 & 3 (respectively) confirmed this trend, although the increases were not as pronounced as those observed in Dr Ton's data. These trends were not observed in the Pinot Noir cane data during 2000 (Graph 5).

Sensor failure during summer 2000/2001 and difficulties in obtaining and calibrating replacement sensors restricted data collection to Pinot Noir trunks only during winter 2001 (Graph 6). Pinot Noir trunk diameter trends again demonstrated synchronous, but inconsistent increases following rainfall events.

Overall trunk diameters were relatively constant with no significant increase in trunk diameter evident at budburst during 2001. Data recorded from Trunk 2 during 2001 is considered to be aberrant due to sensor malfunction.

Conclusions

The measurement of fluctuations in vine trunk or cane diameter in Chardonnay and Pinot Noir during 2000 and 2001 did not result in data that indicated a sudden increase in trunk or cane diameter associated with pre-budburst sap flow, disproving the original supposition of this study (see page 14).

The only slight continuous increases in mean trunk or cane diameters observed were in Pinot Noir during 2000. This occurred after the incidence of budswell and subsequently negated the value of this technique for predictive assessment of changed dormancy states prior to the appearance of externally visible indicators in the field.

Further exploration of this technique as a possible dormancy measurement tool was not considered viable and was discontinued beyond winter 2001.

Recommendations

The measurement of trunk and cane diameter fluctuations may incidentally provide an additional tool for sensing the influence of soil moisture fluctuations directly on vines in the field. The cause of occasional inconsistencies between rising soil moisture and the observation of increasing trunk or cane diameters would need to be resolved before this technique could be implemented and should be the subject of further detailed study.

Phytech's trunk and cane diameter sensors were designed primarily for use in a glasshouse environment. They functioned well in the field at Lenswood during 2000, however the sensors subsequently failed relatively quickly, even with revised external shielding (note Photo 2). The trunk diameter sensors appeared to be slightly more durable than the cane diameter sensors. Sensor design revisions would be required to make the sensors more robust and durable in the field before they would be suitable for long -term use in an Australian viticultural context. This in no way detracts from the viability of Phytech's systems in a glasshouse context.

The project team wishes to sincerely thank Phytech for the provision and installation of Phytomonitoring units used in this study and their collaboration with this series of experiments.

P1.12 - Technique 2:

Measurement of Fluctuations in Vine Cane Moisture Content Prior to Budburst.

John Crocker[1]

[1]South Australian Research and Development Institute, Lenswood Horticultural Centre, Swamp Road, Lenswood SA 5240.
E-mail, crocker.john@saugov.sa.gov.au

Abstract

V vinifera cane moisture contents were measured between leaf fall and budswell in 2000 for differing vine varieties and rootstocks at Lenswood, Loxton and Nuriootpa Research Centres. Overall cane moisture contents from Lenswood were significantly higher than those from Nuriootpa or Loxton. Cane moisture contents from Nuriootpa rose just prior to budswell but this trend was only apparent retrospectively. This trend was not repeated at Lenswood or Loxton. Use of fluctuating cane moisture content as an indicator of changed dormancy state was not recommended.

Aim

To test the supposition that: 'Measurement of moisture contents in vine canes excised from source vines between leaf fall and budswell will reveal a period of rapid moisture content increase prior to budswell associated with changed vine dormancy state in the field'.

Background

Vegis (1965) defined 'complete dormancy' as a state where "the water content of the (plant) organs are reduced to such low levels that metabolism appears to cease until rehydration". Empirical observations of deciduous Genera from *Malus* to *Vitus* support this statement where the moisture content of lignified tissue visibly falls during dormancy. This is familiar to most in the case where vine canes begin to weep prior to budswell, or where seeds are transformed from a dormant to an active state following the imbibition of water through the seed coat.

A transverse section cut through vine canes during early June often reveals dry, light green tissue with no free moisture. Conversely, the same observation carried out in late August will reveal moist, bright green tissue that is generally associated with the familiar welling or weeping of sap from pruning wounds. The transition between the 'dry' and 'moist' states often occurs over a matter of days and appears to be largely independent of soil moisture status.

If vine tissues can progress from dry to moist states prior to budburst, it is proposed that the measurement of changed cane moisture status may provide a quantifiable parameter that can be correlated with a change in dormancy state prior to the advent of any externally observable features.

Method

Eight to twelve year old *V. vinifera* cutting source blocks were selected Lenswood, Nuriootpa and Loxton Research Centres. Source vines were chosen for uniformity of size, age and aspect, with consideration given to ease of access on a weekly basis.

Vines at Lenswood and Nuriootpa were own rooted and cordon trained, with vertical foliage management at Lenswood and sprawling canopies at Nuriootpa. Vines at Loxton were cordon trained with sprawling canopies and were grafted on Ramsey or were on their own roots (as indicated below).

The following varieties were chosen for study at each location:

1. **Lenswood**: Chardonnay I10V1, Pinot Noir D5V12, Sauvignon Blank 5385
2. **Nuriootpa**: Chardonnay I10V1, Pinot Noir D5V12, Cabernet Sauvignon G9V3
3. **Loxton**: Chardonnay I10V1/Ramsey, Chardonnay I10V1/Own Roots, Cabernet Sauvignon G9V3/ Ramsey

Sample Collection and Processing

- Cuttings were collected and processed at 7 to 10 day intervals commencing before leaf fall and ending at bud-swell at each location. Variations in the duration of sample collection reflect differences in the commencement of budswell at each site

- At each sample time, 28 x 300mm long cuttings were selected at random from source vines of each variety in each location. Cuttings were taken from a point 2 buds above the base of the current season's growth to around 2 buds before the end of the lignified section of each cane.

- Cuttings were immediately placed in a plastic bag in the field and bags were closed with a rubber band after being labelled by variety and harvest date. Notes were taken on bud state or the presence of cane weeping on each occasion.

- Cuttings were divided and sorted into 5 bundles of 5 cuttings for each variety on return to the lab at each location. All 5 bundles of each variety contained approximately the same selection of cutting sizes, maturities and overall weight. Any additional under-size or non-mature cuttings were discarded.

- Each bundle of 5 cuttings was carefully cut into 10mm segments. The segments and all fragments were placed into labelled foil trays and were accurately weighed within 5 minutes to obtain fresh weight data.

- The foil trays and cutting segments were placed into drying cabinets or drying ovens at 60^0C for approximately 24 hours before removal from the drying cabinet, cooling for 10 minutes and re-weighing.

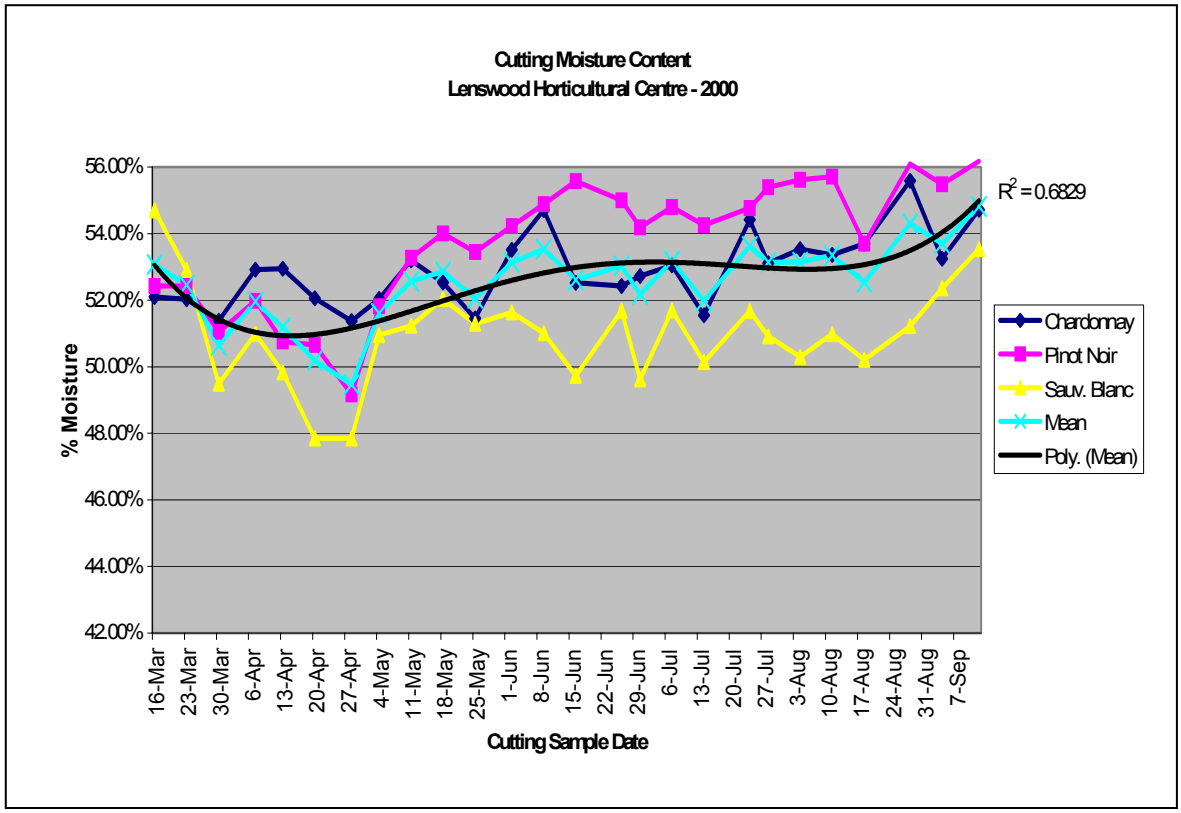
- The foil trays were returned to the drying cabinet for a further 24 hours before removing, cooling and weighing again. This was repeated until there was no change in the sample weight after any given 24-hour cycle and an accurate dry weight could be obtained for each sample.

(Experience rapidly indicated that 96 hours drying time at 60^0C was sufficient to obtain an accurate dry weight in all cases. Cutting collection was generally scheduled to allow for processing and fresh-weight recording on Thursday, drying over the weekend and dry weight recording on Monday afternoons).

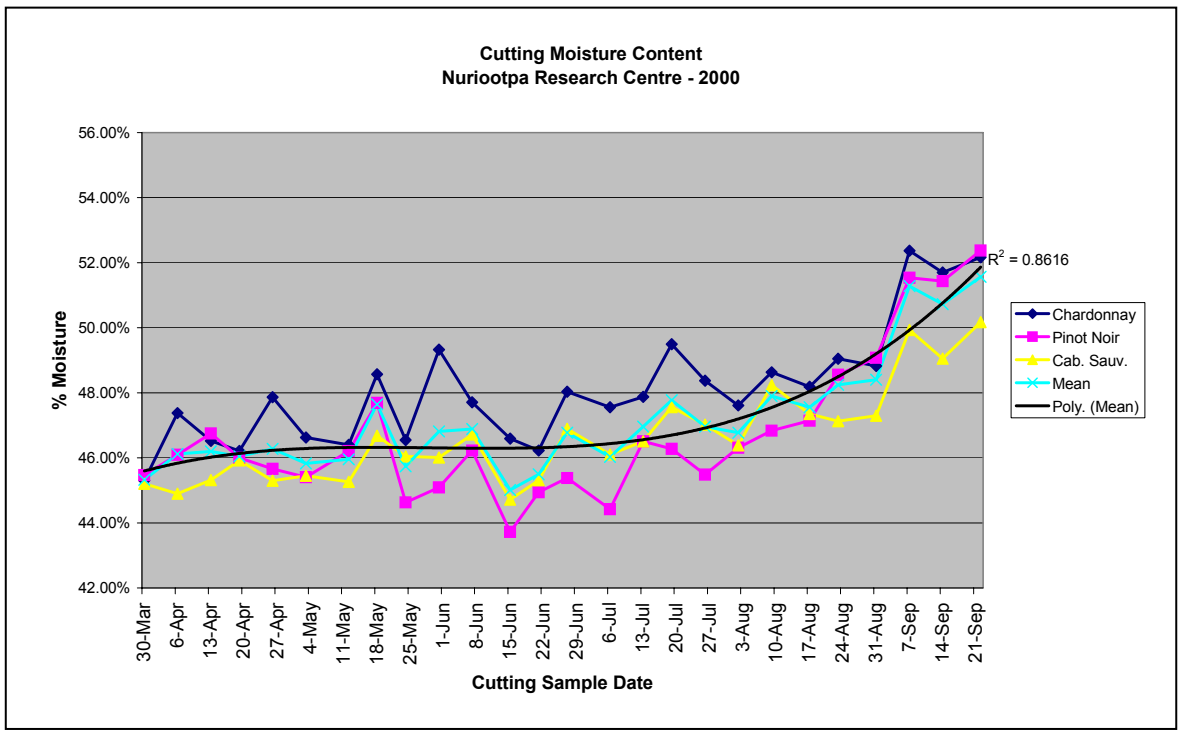
- Considerable care was taken to ensure that sampling procedures, drying equipment and data processing was functionally identical at each site. SARDI farm staff were trained in experimental protocol at each site and were actively involved in this experiment during 2000.

Results

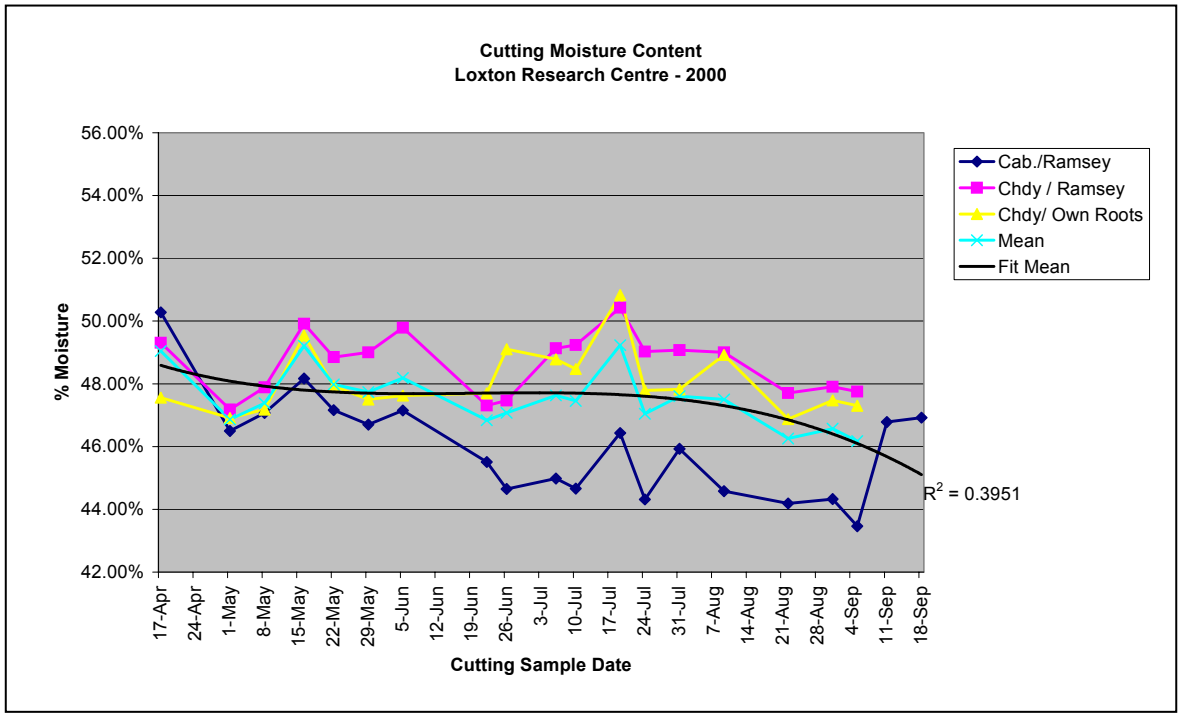
Samples were collected from Lenswood, Loxton and Nuriootpa during 2000 with data analysis and interpretation during summer 2000.



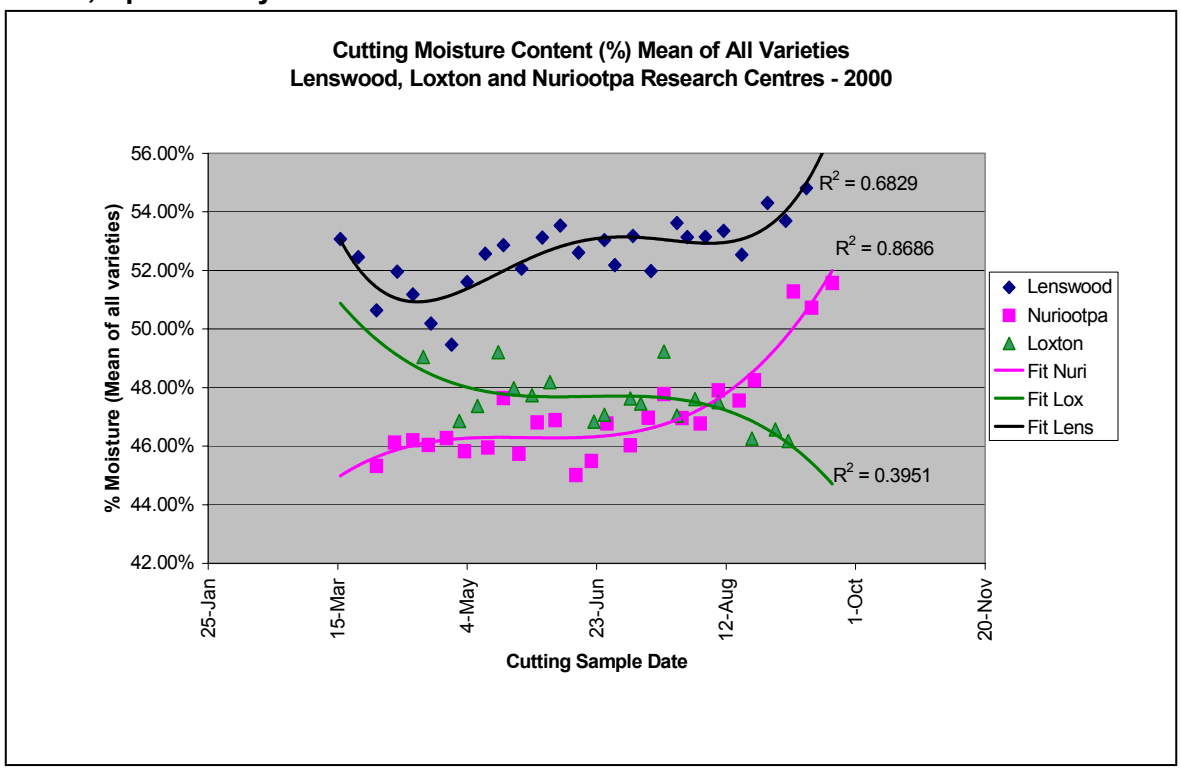
Graph 1; Variation in cutting dry weights from Chardonnay I10V1, Pinot Noir D5V12 and Sauvignon Blanc 5385 source vines, Lenswood Horticultural Centre, March to August 2000.



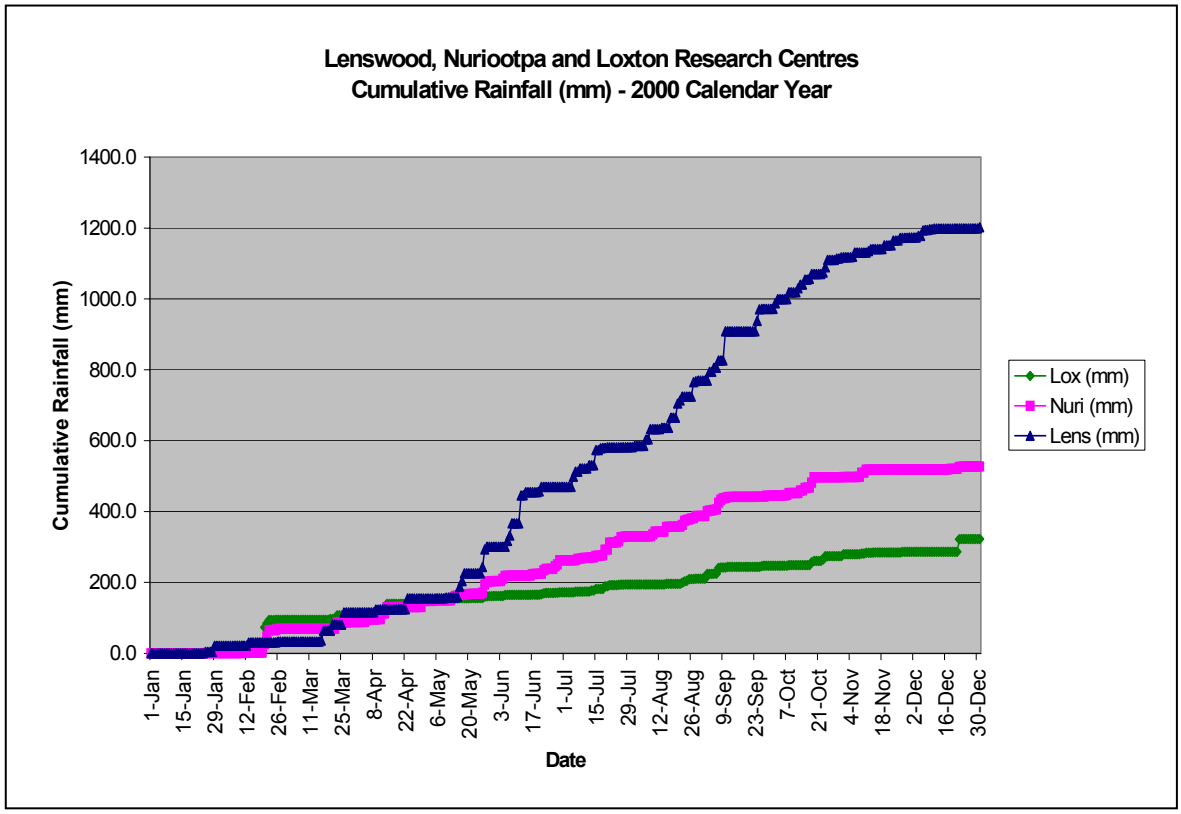
Graph 2; Variation in cutting dry weights - Nuriootpa Research Centre, March to September 2000.



Graph 3; Variation in cutting dry weights from Cabernet Sauvignon G9V3/ Ramsey, Chardonnay I10V1/ Ramsey and Chardonnay I10V1/ Own Roots, Loxton Research Centre, April to July 2000.



Graph 4; Variation in mean of cutting dry weights highlighting relative site differences between the aggregate means of all varieties harvested at Lenswood, Nuriootpa or Loxton Research Centres, March to August 2000.



Graph 5; Cumulative rainfall recorded at Lenswood, Nuriootpa and Loxton Research Centres – 2000 calendar year.

Discussion

Graph 4 plots the aggregate mean moisture content for all varieties at each location from leaf fall to bud swell. Data trends between varieties at each site were similar, however, moisture content profiles between sites were notably different. Cuttings sourced from Lenswood contained significantly more moisture overall than those from Nuriootpa or Loxton. ($F_{.05,2,322} = 6.46$), while the moisture content of cuttings from Nuriootpa and Loxton did not vary significantly from one another. Graph 5 illustrates considerable differences between the rainfall profiles recorded at each site during 2000

Lenswood

The Lenswood cutting source blocks are located at approximately 400m in a cool, temperate region receiving an average of 1050mm of predominantly winter rainfall per year in addition to supplementary drip irrigation between spring and harvest in March/April.

Graph 1 illustrates that Lenswood's cuttings appear to progressively dehydrate following vintage and reached their lowest moisture content at the time of leaf fall in mid to late April 2000. Moisture contents then slowly rise until late June and then stabilised before rising again in early August just prior to bud swell. A small rise in moisture content was associated with the commencement of sap flow on 11th of August and the appearance of budswell on the 28th of August.

A plot of the aggregate mean of all moisture data at Lenswood during 2000 fits the above description based on a fourth order polynomial curve plotted in Graph 1 with a confidence of 68.3%.

Nuriootpa

Nuriootpa's cutting source blocks are located at approximately 280m in a warm temperate climatic region receiving an average of 525mm of predominantly winter rainfall per year in addition to supplementary drip irrigation limited to 100mm per annum applied between spring and harvest in March.

Graph 2 illustrates that Nuriootpa's cuttings reached their lowest moisture content after vintage and during early April 2000, with moisture contents slowly rising until leaf fall in mid May. Moisture contents then remained constant through May until late June before continuously rising from early July until woolly bud on the 17th of August and bud burst on the 7th of September.

This description is based on a plot of the aggregate mean of all moisture data at Nuriootpa as fitted to a third order polynomial curve plotted in Graph 2 with a confidence of 86.2%. Cutting moisture contents for Nuriootpa follow a similar pattern to those observed at Lenswood, however, the fitted curve of aggregate means for Nuriootpa is transposed by approximately 3 weeks relative to Lenswood reflecting the earlier seasonal cues at Nuriootpa.

Loxton

The Loxton cutting source blocks are located at approximately 50m in an arid climatic region receiving an average of 225mm of predominantly winter rainfall per year in addition to intensive neutron probe scheduled irrigation between spring and harvest in early March. No supplementary irrigation is applied during vine dormancy.

Significant data variability was observed at Loxton during 2000 raising questions as to the validity of the Loxton data set. This may be due to variations in sample processing, but is unlikely to be due to soil moisture, seasonal or climatic factors.

Graph 3 illustrates that cutting moisture contents at Loxton generally fell following vintage until leaf fall at the end of April 2000. Moisture contents then appeared to stabilise from May through to the end of July before they continued to fall slightly towards bud swell (where data collection ended for both Chardonnay blocks). Cabernet bud swell was approximately two weeks later than Chardonnay and indicated a marked increase in moisture content from the 4th to the 18th of September. This may also have been observed in the Chardonnay samples had they continued to be collected. This description is based on a plot of the aggregate mean of all moisture data at Loxton as fitted to a fourth order polynomial curve plotted in Graph 3. This curve returned only a 39.5% confidence of fit due to data variability from this site.

Graph 3 suggests that cuttings collected from Cabernet/Ramsey source vines have consistently lower moisture contents than cuttings collected from either of the Chardonnay blocks, however, analysis of variance does not return significant moisture differences between cutting types. The plot of the aggregate mean of all moisture data for Loxton follows a very different pattern to the plots observed at Lenswood and Nuriootpa as illustrated in Graph 4. It is hypothesised, though not proven, that this may be due to Lenswood and Nuriootpa receiving modest winter rainfall during 2000 while at Loxton there was no irrigation following vintage, relatively low winter rainfall and progressive soil dehydration resulting in a progressive fall in cutting moisture levels during dormancy.

Conclusions

Analysis of cutting moisture data at Lenswood and Nuriootpa during 2000 suggests that a measurable rise in mean cutting moisture content can be generally associated with the commencement of sap flow and budswell, although this was only discernable retrospectively following data analysis and was not clearly evident from the raw data at the time of sample collection. This was most pronounced at Nuriootpa, less so at Lenswood and was not observed at Loxton.

A pronounced rise or clear peak in cutting moisture content associated with the commencement of sap flow was not observed at any site during 2000.

Retrospective data analysis at Lenswood provided an association between a fitted curve of rising moisture content and the appearance of sap flow and budswell in source area vines. Relatively large fluctuations in cane moisture content can mask trends between successive sample collections and were not reliably associated with major rainfall events.

The fitted curve for mean cutting moisture content at Nuriootpa suggests that cutting moisture begins to rise significantly at the end of July at approximately the time that changes would be expected in the dormancy status of the vine. Though this is promising, this change was not discernable at the time of data collection due to moisture content variations between successive sample periods.

While the measurement of cutting moisture content between leaf fall and bud burst provides an interesting insight into changing moisture status in the vine during dormancy, this process is very labour intensive and retrospective analysis is required to reveal data trends. This reduces the utility of this technique for source vine dormancy status assessment *in situ.*

The absence of a clear peak in cane moisture content associated with the commencement of sap flow disproves the original hypothesis that ‘Measurement of moisture contents in vine canes excised from source vines between leaf fall and budswell will reveal a period of rapid moisture content increase prior to budswell associated with changed vine dormancy state in the field’. Subsequently, the examination of cane moisture content as a dormancy measurement technique was not continued beyond 2000.

Recommendations

Studies within other program areas of SAR 99/4 revealed a strong correlation between cutting moisture content at the time of delivery to the nursery and later performance through the propagation process. Data revealing a range of cutting moisture contents from 3 different sites during 2000 provides the basis for further study into the development of a cutting moisture content standard at the point of cutting delivery to the nursery. This topic is of strong interest to the nursery industry and further study will be proposed as the subject of a future project submission.

References:

(See Appendix 3, pp. 146 to 149 for a compiled list of references)

P1.13 Technique 3:

Measurement of changing vine cutting respiration rate as a response to changing metabolic activity during the various phases of vine dormancy.

John Crocker[1]

[1]South Australian Research and Development Institute, Lenswood Horticultural Centre, Swamp Road, Lenswood SA 5240.
E-mail, crocker.john@saugov.sa.gov.au

Abstract

The rate of CO_2 evolution measured from *Vitis vinifera* cutting fragments excised from source vines at regular intervals between leaf fall and budburst in 2000, 2001 and 2002 demonstrated that cutting respiration rates progressively fall post-leaf fall, reach a minimum towards the end of July and then rise towards budburst. Dormancy state verification demonstrated that minimum respiration rate does not imply full dormancy. Increasing respirative activity post-July is proposed as a seasonal cue to indicate that vine cuttings harvested post-July become more metabolically active and potentially progressively less tolerant of 50^0C/30min hot water treatment. Budswell and flowering in *Prunus x blireana* was coincident with the commencement of increasing respirative activity in *Vitis vinifera*, providing a useful bio-indicator for this event.

Aim

To evaluate measurement of the rate of CO_2 evolution from excised vine cuttings as a viable technique for determining changing cutting metabolic states during dormancy.

Background

The rate of basal respirative metabolism as measured through the rate of CO_2 liberation can provide an indication of the status of metabolic activity in samples of living tissue. In animals, respirative activity is highest during physical activity or periods of rapid growth and is lowest during sleep or tissue quiescence. This relationship is less intuitively obvious in plant tissues, particularly defoliated deciduous cuttings, however, relatively high respirative activity in plants is also associated with growth, while low metabolic activity is observed during quiescence.

Given that the earliest definitions of dormancy consider that it is “the temporary cessation of growth, while metabolic processes, including respiration, continue”, it follows that the measurement of the rate of basal respirative metabolism should provide an indication of the physiological status of a deciduous plant at any given stage in the growing season.

Australian nurseries consider that fully dormant vine cuttings more readily tolerate long duration (*50^0C / 30* minute) hot water treatment (HWT) than physiologically active or environmentally suppressed ‘*ecodormant’* vine cuttings. Unfortunately, there are no externally visible cues to mark the transition between endodormancy and ecodormancy in source vines or vine cuttings. To compensate for this, nurseries generally aim to propagate cuttings that have been harvested approximately 4 weeks before the expected budswell date and prior to the commencement of sap flow.

Myking (1998) studied the relationship between respiration and dormancy in the buds of three hardwood deciduous species, concluding that declines in respirative metabolism could be measured following leaf fall and then increases in respirative metabolism could be measured prior to budburst. Mayking artificially forced cuttings and concluded that increased metabolic activity was associated with the resumption of growth and occurred after the time of dormancy release. He also noted that artificial imbibition (hydration) induced a doubling of respiration rate and seasonal variations in bud moisture content significantly influenced respirative activity.

If a rise in basal metabolism can be measured in dormant vines prior to the observation of sap flow or bud swell, the measurement of basal metabolism may provide a viable indication of changed metabolic state in the field that is easily achieved through readily available equipment and has the potential for development as a commercial tool. This in-turn will allow cutting harvest to be scheduled prior to the commencement of increased metabolic activity and may assist in the improvement of cutting performance following HWT.

Aim

To test the supposition that: 'Measurement of the rate of CO_2 liberation from vine cane segments excised from source vines between leaf fall and budswell will reveal a period of rising metabolic activity associated with the cessation of full physiological dormancy'.

Method - Initial studies – 2000

Reaction vessel preparation.

A supply of unused 500ml PETE 'Nippy's' fruit juice containers and lids were obtained from Knipsel Pty Ltd, Regency Park SA. Containers were pierced with a 6mm heated glass rod as near as practicable to the top of the bottle.

Each bottle was fitted with a 9mm rubber septum fixed to the bottle with 'Selly's' neutral cure silicone (Photo 1; Respiration reaction vessel). After curing, each bottle was numbered and weighed with lids fitted. Bottles were filled with water and volumetric uniformity was verified in each case (±0.05%).



Photo 1; Respiration reaction vessel



(Photo 2; Respiration sample types).

Sample processing

Initial screening studies were conducted during 2000 using cuttings sourced from own-rooted Chardonnay I10V1 vines planted in December 1990 at the Lenswood Horticultural Centre.

40 cutting samples were collected at random from source vines on 4 occasions (the 7th of July, 28th of July, 7th of August and 16th of August 2000). Sample times were chosen to coincide with the expected time of transition from full dormancy to sap flow in Lenswood's Chardonnay vines.

Cuttings were collected from a point located 2 buds above the base of the '99/2000 season's growth to around 2 buds before the end of the lignified section of each cane. Samples were labelled and placed in Zip Loc sample bags, then stored in an Esky cooler with approximately 1.0L of bagged, crushed ice. Samples were transported for processing at the laboratory of Dr Andreas Klieber (University of Adelaide, Department of Horticulture, Viticulture and Oenology) with approximately 1 hour elapsing between sample collection and processing.

Samples were removed from the Esky on arrival and were divided into 8 bundles of 5 cuttings and were equilibrated to the Lab's 22^0C ambient temperature for 30 minutes. Cuttings within each bundle were sorted to contain a similar range of diameters overall. Each cutting was trimmed to 150mm, ensuring that all cuttings included 2 nodes and associated buds. 4 bundles of 5 cuttings were retained entire, while 4 bundles were cut into 10mm segments with care taken to capture all fragments including any bark shards (Photo 2, above, Respiration sample types).

Cutting samples were sequentially processed, placed into reaction bottles and were sealed. One bottle remained empty as a control. This process was timed to allow 1 minute between successive closures for gas sample extraction at timed intervals from each reaction bottle. Each bottle was weighed once it was sealed to obtain a record of sample fresh weights in each case.

Gas samples were extracted by piercing the bottle septum with a 25G x 1" needle fitted to a 1ml Tuberculin syringe. The syringe plunger was cycled and a 1ml gas sample was extracted from the bottle on each occasion. Gas samples were collected at 30minutes, 60 minutes, 90 minutes and 120 minutes from the time of bottle closure (Photo 3, below, Reaction vessels and syringes).

Gas samples were pooled and then analysed for CO_2 content with a Varian 3300 Gas Chromatograph using the sample integration method routinely employed for gas analysis in controlled atmosphere storage experiments with post-harvest produce in Dr Klieber's laboratory. The rate of CO_2 production within each reaction bottle was calculated by difference between the CO_2 concentrations recorded for each sample period.

All reaction bottles were returned to Lenswood's post-harvest laboratory where the cutting samples were removed from the reaction bottles, were placed in labelled foil trays and were dried at 60^0C for approximately 96 hours to obtain dry weights. CO_2 production rates were recorded as Δ %CO_2 /g dry-weight/h x 1000 to obtain a uniform basis for comparison.



Photo 3; Reaction vessels and syringes



Photo 4; Data logger & CO_2 sensors

Method – 2001

Though CO_2 analysis with the Varian 3300 Gas Chromatograph (GC) provided a useful tool for initial concept examination, the time taken to transport, prepare and analyse samples was prohibitive if more frequent or more detailed studies were to be undertaken. A custom built CO_2 measurement and data logging system was designed in association with Measurement Engineering Australia (MEA) to provide a rapid on-site CO_2 measurement tool at Lenswood…see http://www.mea.com.au/.

Sample analysis was initially performed on the GC at Adelaide University from 28th of May 2001 until the 25th of July 2001 when the new CO_2 measurement equipment became available from MEA. Samples from the 8th of August 2001 (onwards) were measured by the new equipment at Lenswood.

The new CO_2 measurement system was configured to be relatively portable and allow replicated 'desk top' measurement of CO_2-levels with minimal equipment or sample preparation (see Photo 4, above). Three Vaisala, 0 to 5000ppm GMP222 CO_2 sensors and a Vaisala HMP45A temperature/RH sensor were coupled to a Starlogger 6004C data logger to allow continuous data collection and storage. MEA's Magpie® software provided real-time data observation and facilitated data export for analysis.

The lids of the 500ml PETE reaction vessels were modified to allow entry of the Vaisala CO_2 sensors. A rubber o-ring was fitted to each sensor and was positioned to allow the sensor to rest at the same position within the top 50% of the reaction vessel and provide a uniform gas volume within each reaction vessel. The o-ring created a gas-tight seal between the sensor and the bottle lid when the lid was screwed into place.

Cutting samples were collected at random from Chardonnay I10V1, Pinot Noir D5V12 and Sauvignon Blanc 5385 source vines grown at the Lenswood Horticultural Centre. 20 cuttings of each variety were collected at approximately fortnightly intervals from the end of May until mid September 2001.

Cuttings were collected from a point located 2 buds above the base of the '00/2001 season's growth to around 2 buds before the end of the lignified section of each cane. Samples were labelled and placed in Zip Loc sample bags, then stored in an Esky cooler with approximately 1.0L of bagged, crushed ice.

Samples were initially transported to the University of Adelaide for processing as described for 2000 (see 2000 method, above), or were transported a short distance for processing at the Lenswood Horticultural Centre laboratory after the 25th of July.

At Lenswood, samples of 1 variety were removed from the Esky on arrival at the laboratory and were equilibrated to the Lab's 22^0C ambient temperature for 30 minutes. Each cutting was trimmed to 150mm, ensuring that all included 2 nodes and associated buds (as illustrated in Photo 2, above). The cuttings were sorted into 3 bundles of 5 trimmed cuttings containing a similar range of diameters overall. Each bundle of 5 cuttings was then cut into 10mm segments with care taken to capture all fragments including any bark shards.

Cutting samples were sequentially loaded into each of 3 reaction bottles. The CO_2 sensors were then inserted, the bottles were sealed and the time of closure was noted in each case. The CO_2 monitoring system then recorded reaction vessel CO_2 concentrations at 30-second intervals until a CO_2 concentration of 5142ppm was reached (the limit of sensitivity). The time taken to reach 5142ppm in each reaction vessel was recorded together with the sample fresh weight.

The process outlined above was repeated for the next variety as measurement of the first and subsequent varieties were concluding.

Cutting samples were removed from the reaction bottles, were placed in labelled foil trays and were dried at 60^0C for approximately 96 hours to obtain dry weights. CO_2 production rates were recorded as Δ %CO_2 /g dry-weight/h x 1000 to obtain a uniform basis for comparison. The mean respiration rates for each sample date were plotted and regression curves were fitted to the data.

Observations were also made to correlate changes in the respirative state of source vines with externally visible seasonal indicators on non-related deciduous and evergreen plant species. If the bud-swell or full bloom date of another plant species could be shown to coincide with measured changes in the respirative rate of source vines, a simple bio-indicator could be adopted to flag this change where no externally visible signs are present on the source vines.

Method 2002

The 2001 sample preparation and measurement process developed for use at Lenswood was repeated in 2002. Additional samples of Chardonnay and Pinot Noir were collected from comparable source vines grown at the Orlando-Wyndham Group's Langhorne Creek Vineyard. This provided data to assess the ability of the respirative measurement technique to detect regional differences associated with the end of full dormancy in two widely contrasting microclimates.

The 2002 program concentrated on Chardonnay and Pinot Noir source vines. Samples and bio-indicator observations were collected from the Lenswood Horticultural Centre at fortnightly intervals between the 1st of May 2002 and 23rd of September 2002.

Additional samples and bio-indicator observations were collected on alternate fortnights from comparable own-rooted Chardonnay I10V1 and Pinot Noir D5V12 source vines grown at the Orlando-Wyndham Group's Langhorne Creek Vineyard between the 11th of April 2002 and the 22nd of August 2002.

The dormancy states of cuttings from Lenswood and Langhorne Creek were verified at the time of minimum respirative activity (between early June and early July 2002). Cuttings from both sites were collected, trimmed to 250mm, grouped into 3 replicate bundles of 7 cuttings per variety (Photo 5). They were then labelled and hydrated for 8 hours in clean chlorinated water and before being packed into new vermiculite, prior to callusing by incubating for 21 days at 26^0C in darkness (Photo 6). After callusing, cuttings were assessed for root and bud growth activity.



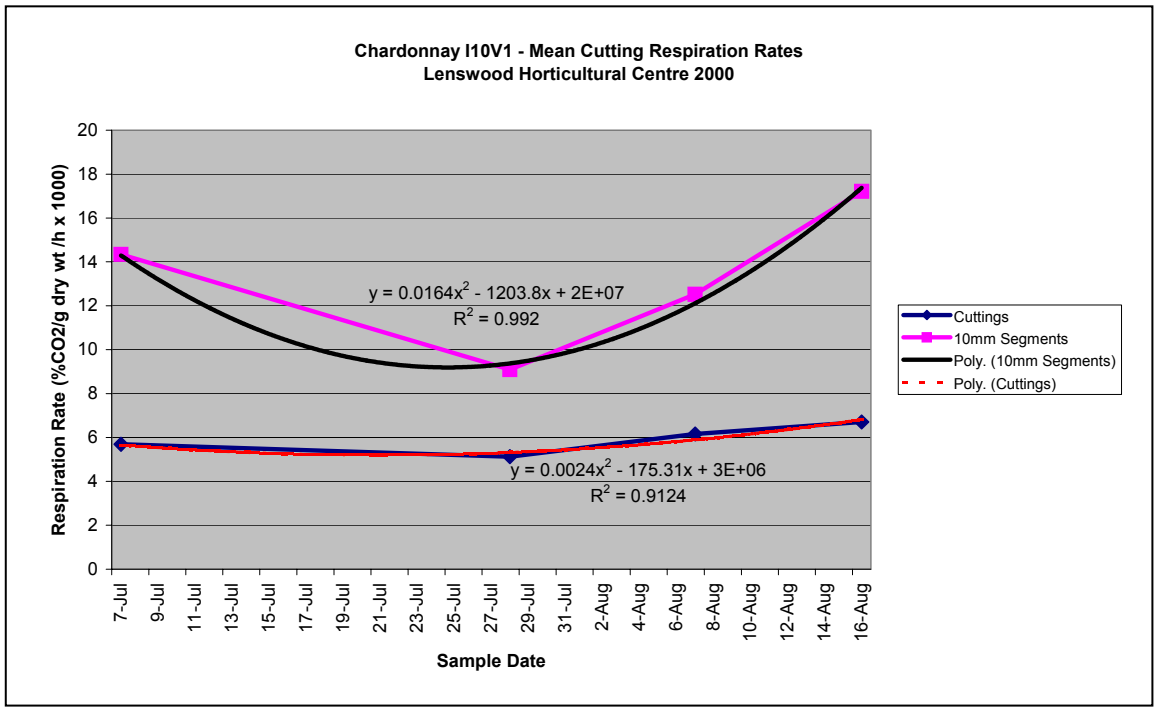
Photo 5; Dormancy verification – cuttings



Photo 6; Incubation in vermiculite

Results 2000

Experiments conducted during 2000 were designed as a feasibility study to provide general information on the use of respirative measurement as an indicator of changed dormancy state in source vines. Results obtained during 2000 are summarised in Graph 1, below.



Graph 1; Chardonnay cutting respiration rates, Lenswood Horticultural Centre 2000

Discussion 2000

Sample type

Graph 1 demonstrates that the respiration rates recorded for 150mm two bud cuttings were clearly and consistently lower than those recorded for 150mm two bud cuttings sectioned into 10mm segments. This is due to the entire cuttings having a comparatively low surface area of cane tissue involved in the respirative response associated with wounding or crushing of cane tissues during cutting. This response is generally considered to be the product of polyphenolic compound oxidation, increased substrate access for glycolytic enzymes through cellular disruption and the initiation of meristematic activity associated with callus formation and wound healing (Bidwell 1979).

In either case, the data presented in Graph 1 illustrates the maximum respirative rate possible from each sample type at the four sample dates processed in 2000. As the samples were all collected and processed in a uniform manner and were allowed to equilibrate to a lab ambient temperature of 22^0C prior to measurement, it is considered that this data represents changing rates of source vine basal metabolic activity at each sample date and is not considered to be simply associated with external climatic variation. The use of 150mm two bud cuttings sectioned into 10mm segments offered the highest rates of CO_2 production and became the preferred option for future respirative studies.

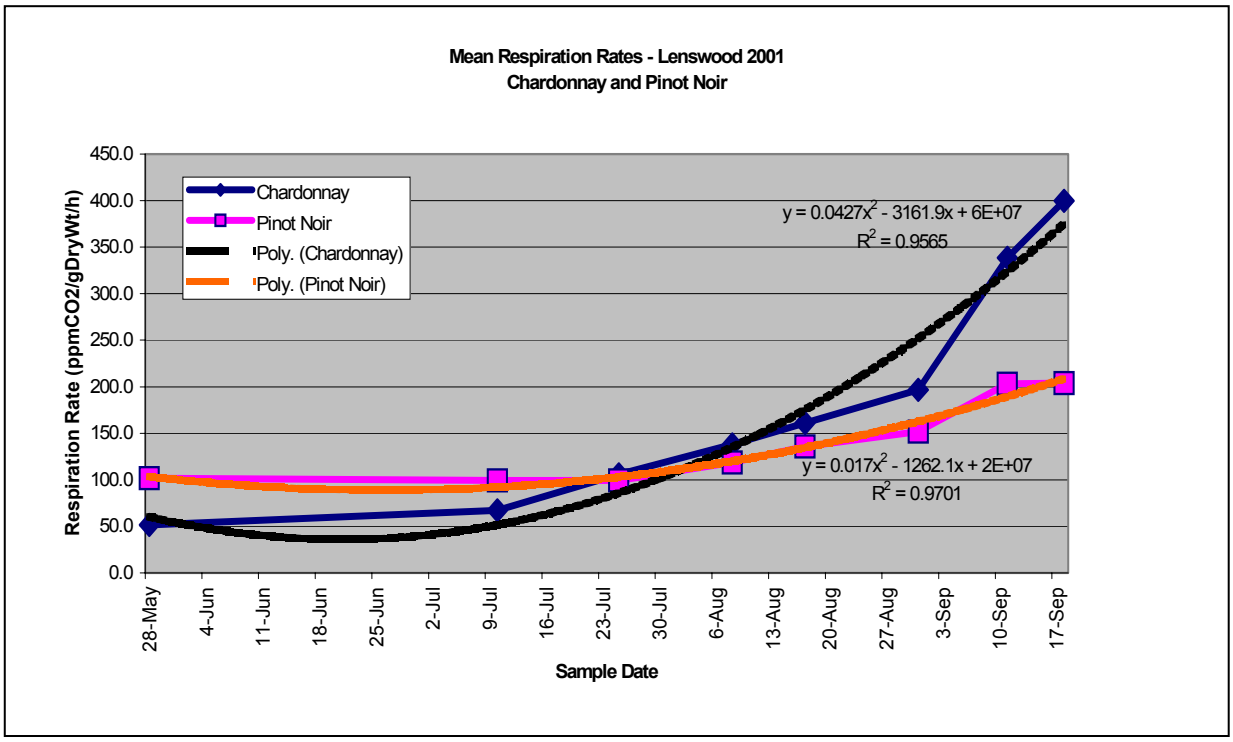
Respiration Rates

A plot of sample means as illustrated in Graph 1, demonstrates that basal respirative rates in Chardonnay declined between the 7th and 28th of July 2000 before commencing a continuous rise to the 16th of August 2000. This trend was reflected in both sample types. A second-order polynomial trend line can be plotted for the sample means of the '10mm segment' data with a 99.2% fit.

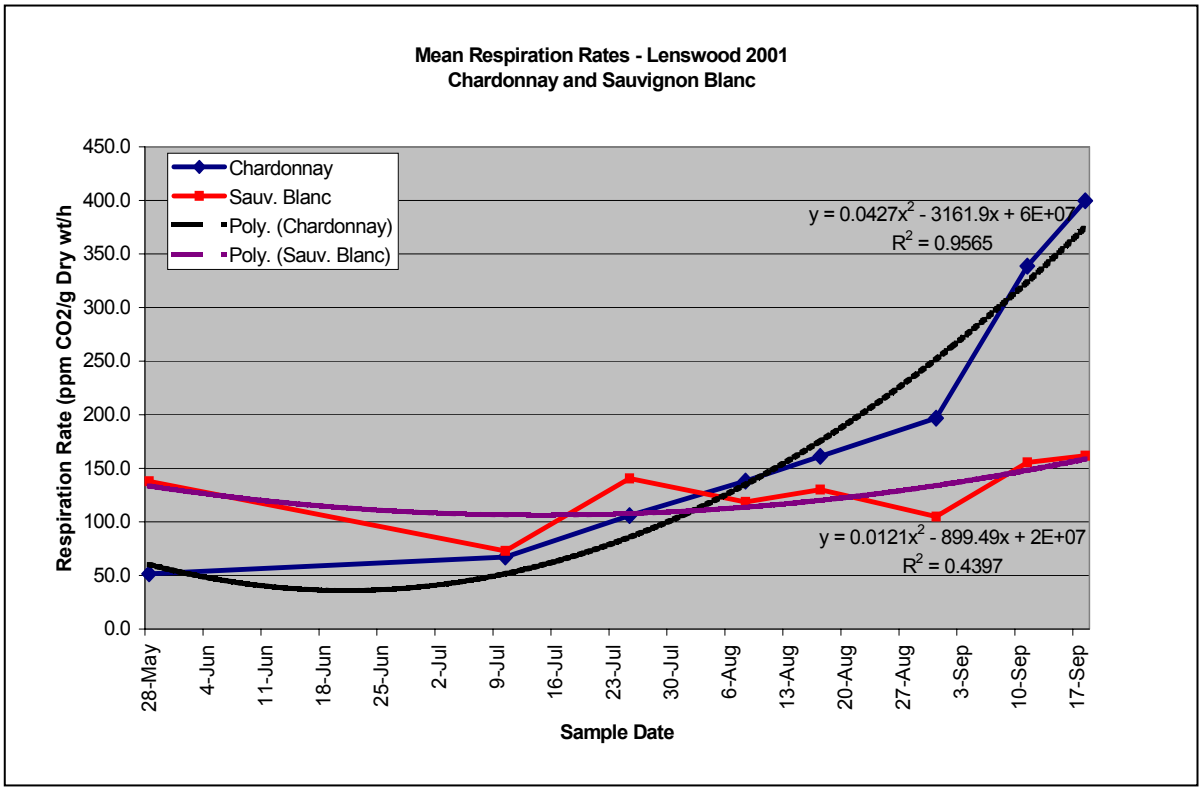
Extrapolation from the fitted trend line suggests that respirative rates in Lenswood's Chardonnay source vines reached their lowest level on the 24th of July 2000 before progressively increasing towards budswell. This data suggests that the measurement of respirative metabolism in cane samples has the ability to reveal changed metabolic states in cutting source vines during dormancy where no externally visible cues could be observed on the cutting source vines. Data collected during 2000 suggested that the measurement of respirative parameters has merit as a tool for detecting changes in the metabolic state of vines and warranted further examination.

Results 2001

Experiments during 2001were designed to refine the relatively small number of observations obtained during 2000 and capitalise on the ease of sample analysis afforded by ready access to the MEA CO_2 measurement equipment at Lenswood. Graphs 2 and 3 summarises data collected between the 28th of May 2001 and 18th of September 2001. For clarity, respiration rates for Pinot Noir and Sauvignon are plotted separately against those observed for Chardonnay.



Graph 2; Chardonnay I10V1 vs. Pinot Noir G9V3 mean cutting respiration rates observed at Lenswood during 2001.



Graph 3; Chardonnay I10V1 vs. Sauvignon Blanc 5385 mean cutting respiration rates observed at Lenswood during 2001.

Discussion 2001

The plot of sample means for Pinot Noir and Chardonnay illustrated in Graph 2, demonstrates that basal respirative rates for Chardonnay and Pinot Noir appeared to be relatively constant between the 28th of May and 10th of July 2001, however the absence of data during this interval (due to GC accessibility issues) leaves this assumption open to question. The data for both Chardonnay and Pinot Noir indicates a progressive rise in respiration rates from the 10th of July and 25th of July 2001 (respectively). This trend continued until sampling concluded after budburst on the 18th of September 2001.

Second-order polynomial trend lines plotted for the means of Chardonnay and Pinot Noir data provide a 95.6% and 97% fit respectively. Extrapolation from the fitted trend line suggests that respirative rates in Lenswood's Chardonnay source vines reached their lowest level on the 20th of June before progressively increasing towards budswell. Similarly, extrapolation from the Pinot Noir trend line suggests that respirative rates in Lenswood's Pinot Noir source vines reached their lowest level on the 25th of June before progressively increasing towards budswell. Again, care must be exercised with this interpretation due to the absence of data during June.

The earliest externally visible signs of bud movement for Chardonnay and Pinot Noir were observed on the 8th of August 2001 and was preceded by cane weeping on the 1st of August. Chardonnay reached budburst by the 3rd of September, while budburst was observed in Pinot Noir by the 18th of September 2001. This sequence is consistent with historical phenological observations at Lenswood, however the difference in budburst date between Chardonnay (earlier) and Pinot Noir (later) is generally only 2 to 5 days (see http://www.geoffweaver.com.au/Vintagecharts/PHENDATES.pdf for historical phenological data pre-1999). Differences of 12 days or more in budburst date are rare but are not unknown at Lenswood.

The plot of sample means for Sauvignon Blanc 5385 illustrated in Graph 3, demonstrated considerably more variance than either the Chardonnay or Pinot Noir data. Subsequently, a second order polynomial trend line could only be plotted with a 44% fit. The data and extrapolation from fitted trend line suggest that Sauvignon Blanc respirative rates began to rise after the 10th of July, though they remained variable until budburst.

Externally visible signs of metabolic activity in Sauvignon Blanc commenced around the 17th of August with cane weeping and progressed to the first signs of bud movement by the 18th of September2001.

Data collected during 2001 indicated that the measurement of respirative metabolism in cane samples has the ability to reveal changed metabolic states in cutting source vines during dormancy at a time when no externally visible cues could be observed on the cutting source vines. The changes are consistent with expected patterns of metabolic activity and reveal a quiescent phase preceding a progressive increase in respirative metabolism prior to budburst.

Observations of deciduous flowering plants in the Adelaide Hills revealed that the earliest signs of bud movement in streetscape specimens of Manchurian Pear (*Pyrus ussurensis)* occurred in early August 2001, though careful observation was required to detect this change. Bud movement was more apparently visible at this time as blossom on purple leafed ornamental flowering plums (*Prunus x blireana)* growing throughout the Hills (see photo 6, below).



Photo 5; Manchurian pear

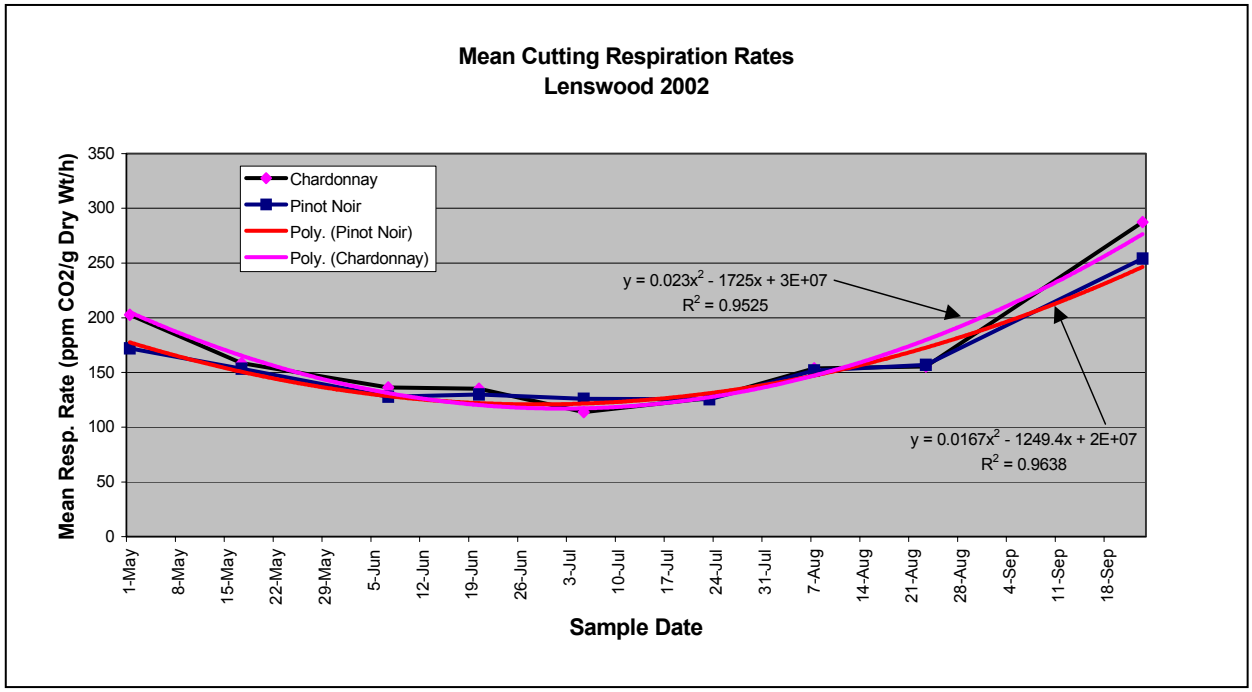


Photo 6; Purple leafed flowering plum

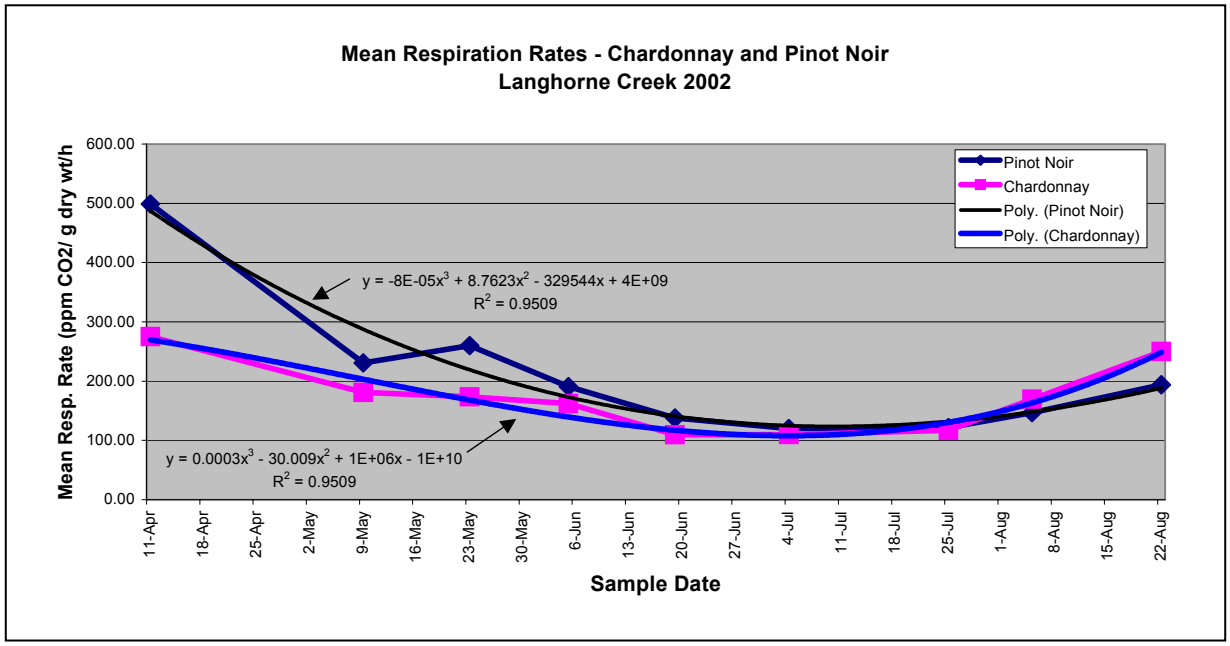
Both species provide externally visible cues to changed metabolic state in deciduous plants at a time when changes in *V. vinifera* are not visible in the field but is measurable through the commencement of increased respirative metabolism. It is likely that flowering plums responds to the same environmental parameters that drive dormancy in *Vitus*. It is also probable that flowering dates in *Prunus x blireana* and increasing metabolic activity in *V. vinifera* will vary in unison and in accordance with seasonal variations. Subsequently, the purple leafed flowering plum may provide an inexpensive, readily observed bio-indicator for the commencement of heightened metabolic activity in cutting source vines.

Results 2002

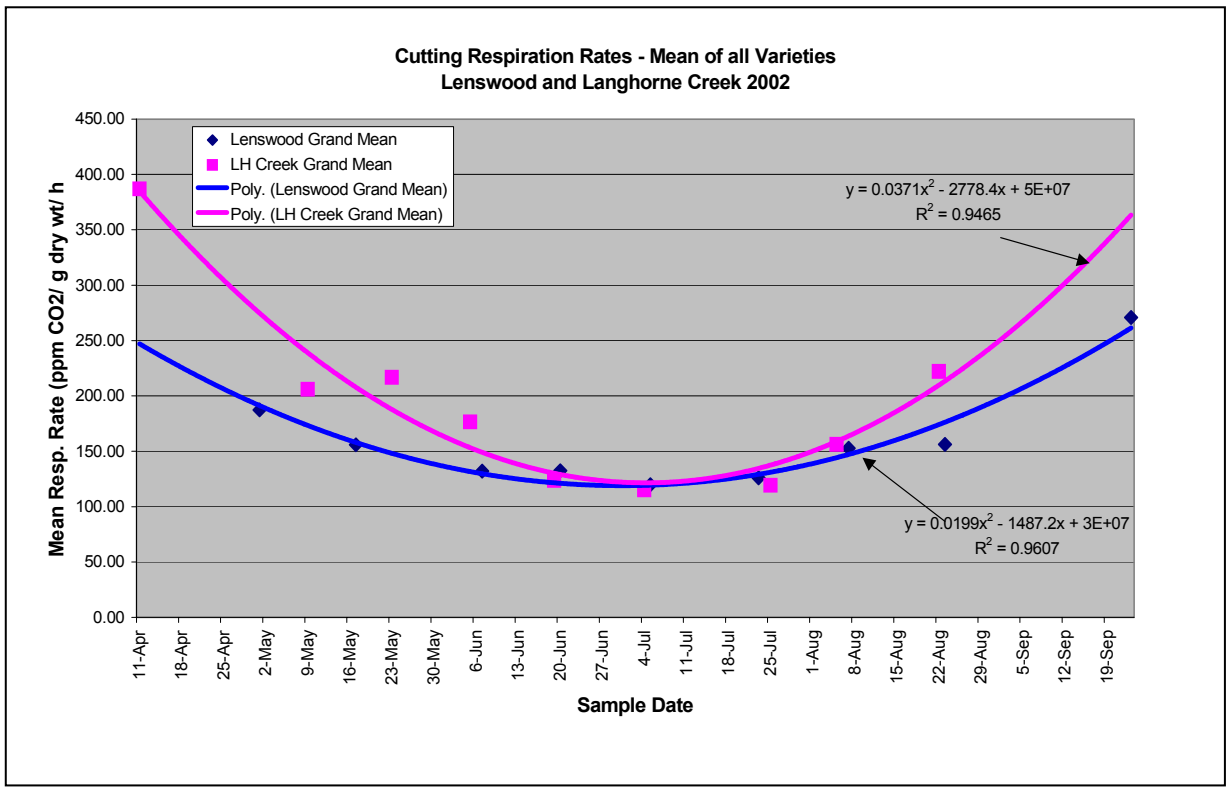
Experiments during 2002 were designed to refine previous data and compare the rate of respirative metabolism measured in Pinot Noir and Chardonnay cuttings harvested from Lenswood and Langhorne Creek. Graph 4 summarises data collected between the 1st of May 2002 and 23rd of September 2002 at Lenswood, while Graph 5 summarises data collected between the 11th of April 2002 and 22nd of August 2002 at Langhorne Creek. The grand means of all observations from each site are plotted on Graph 6, where the fitted trend lines provide a comparison between the changes in the overall respirative rates from leaf fall to budburst at two contrasting locations.



Graph 4; Mean cutting respiration rates, Lenswood 2002



Graph 5; Mean cutting respiration rates, Langhorne Creek 2002



Graph 6; Comparison of Mean respiration rates, all varieties, Lenswood and Langhorne Creek 2002

Discussion 2002

Data collected at Lenswood between early May and early July 2002 (Graph 4) confirms that cutting respirative rates progressively decline between leaf fall and full dormancy in both Chardonnay and Pinot Noir.

Trend lines provide 95% and 96% fit (respectively) and indicate that the lowest level of respirative activity occurred around the 26th of June for Pinot Noir and 3rd of July 2002 for Chardonnay. Respirative activity in both varieties progressively increased towards budswell (23rd August 2002) and rapidly increased between budswell and budburst (23rd September 2002).

Data collected at Langhorne Creek between early April and early July 2002 (Graph 5) also confirms that cutting respirative rates progressively decline between leaf fall and full early July in both Chardonnay and Pinot Noir. Trend lines both provide 95% fit and indicate that the lowest level of respirative activity occurred around the 27th of June for Chardonnay and 11th of July 2002 for Pinot Noir. Respirative activity in both varieties progressively increased towards budswell in mid August.

Bud movement in *Prunus x blireana* at Lenswood was observed again towards the end of July 2002 with full bloom by mid August (Photo 7) and coincided with the end of lowest respirative activity in Pinot Noir and Chardonnay. Correspondingly, the first signs of bud movement in commercial almond orchards towards the end of July at Langhorne Creek also coincided with the end of lowest metabolic activity in Pinot Noir and Chardonnay source vines and culminated in full bloom in almonds by mid August (Photo 8).



Photo 7; *Prunus x blireana* budswell



Photo 8; Full bloom - almonds

Verification of cutting dormancy status by 'forcing' (callusing) vine cuttings at 26^0C for 21 days between early June and early July 2002 illustrated that cuttings from Lenswood (Photo 9) and cuttings from Langhorne Creek (Photo 10) had passed the point of true *endodormancy* at the time that respirative metabolism reached a minimum. It has long been known that endodormant cuttings have not received their chilling requirement and are unable to initiate root growth when forced without a period of cold-treatment.

This was clearly not the case with the cuttings from Lenswood and Langhorne Creek (as illustrated below) and corroborated observations by Myking (1998) in *Betula pendula* and *Prunus padus*, where he suggested that the measurement of rising respiration rates in deciduous buds indicates the resumption of growth rather than the end of true dormancy.

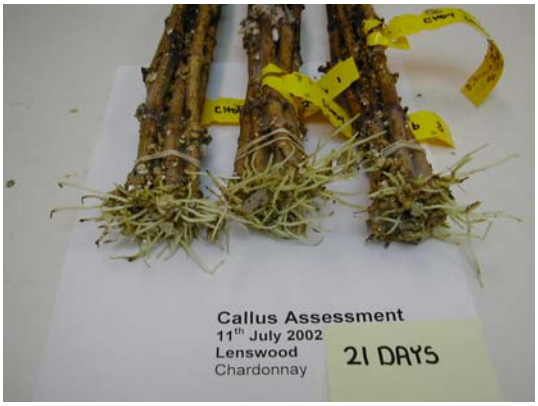




Photo 9; Chardonnay, Lenswood **Photo 10; Chardonnay, Langhorne Ck**

Conclusions

When viewed from the context of cutting performance following HWT, the widely held belief that "fully dormant' cuttings survive HWT more readily than non-dormant cuttings" may not be entirely correct as cuttings harvested before the end of July may have already broken dormancy and are in a phase of declining respirative activity. Cuttings harvested after the end of July are considered by Australian nurseries to become progressively less suitable for propagation/HWT and have been shown by these experiments to be undergoing a period of increasing respiratory activity. Cutting moisture status at the time of collection also plays an important role in this interaction and is discussed in detail as a component of the Jacob's Creek Trials (see P1.2 pp 40 to 69).

The commencement of increasing metabolic activity in source vines should not signal an end to cutting collection, but rather it should serve as a seasonal waypoint indicating that cutting collection should be concluded as soon as practicable given that cutting respiration rates are beginning to rise.

The measurement of cutting respiratory metabolism between leaf fall and budswell has been shown to provide a clear indication of changing metabolic conditions within source vines where no externally visible cues can be observed. This measurement technique is relatively simple, robust, uses established technology, employs low cost components and has potential for development as a commercial tool following further investigation. Relatively minor procedural modifications will also allow this technique to be adapted for use in unrelated deciduous fruit and ornamental species.

The coincidence of budswell in *Prunus x blireana* and the commencement of increased respirative activity in source vines provide a readily observable, seasonally variable bio-indicator of changed metabolic state in cutting source vines. *Prunus x blireana* trees are hardy, inexpensive (<$15) and would be ideally suited for establishment in the vicinity of cutting source areas as a cutting harvest waypoint for the Australian vine nursery industry.

References:

(See Appendix 3, pp. 146 to 149 for a compiled list of references)

P1.2 Program 1, Section 2:

Quantifying the impact of pre-HWT parameters on cutting performance following 50^{0}C/30min Hot Water Treatment - 'The Jacob's Creek Trials'.

John Crocker[1], Paul Wright[2] and Philip Deverell[3]

[1] South Australian Research and Development Institute, Lenswood Horticultural Centre, Swamp Road, Lenswood SA 5240.
E-mail, crocker.john@saugov.sa.gov.au

[2] Chairman – Vine Industry Nursery Association, Springton Rd, Mt Pleasant, SA 5235 (Formerly, Manager – Jacob's Creek Vine Nursery, Orlando Wyndham Group)
E-mail plwright@senet.com.au

[3] Manager – Jacob's Creek Vine Nursery, Orlando Wyndham Group, PO Box 943, Rowland Flat SA 5352.
E-mail, deverelp@orlando.com.au

Abstract

The influence of cutting variety, harvest date, handling speed, hydration duration, source area irrigation and 50^{0}C/30 min HWT were assessed for impact on root initiation, shoot growth and survival in three commercial scale experiments between 2000 and 2003. Cabernet Sauvignon, Sauvignon Blanc, Chardonnay and to a lesser extent, Shiraz, were relatively tolerant of HWT and varying nursery practices. Pinot Noir, Merlot and to a lesser extent, Riesling, were sensitive to HWT and varying nursery practices. Cutting harvest date influenced early root initiation and shoot growth. June/July harvests were associated with better performance in wetter seasons and August/September harvests performed better where winter rainfall was low or late. 5-day delayed handling of cuttings suppressed Pinot Noir development. Post-delivery cutting hydration durations beyond 8 hours were not always beneficial. HWT suppressed early root initiation and shoot development in sensitive varieties. The influence of post-vintage source area irrigation was promising but inconclusive. Avoidance of moisture stress and good nursery management practices enabled vines to recover from most treatment regimes with little detectible impact on long-term viability.

Aims

To evaluate parameters with potential to influence the performance of cuttings following exposure to 50^{0}C/30min hot water treatment (HWT) and progressively modify vine nursery protocols to reduce the probability of poor cutting performance following HWT.

Background

Long duration hot water treatment of grapevine cuttings at 50^0 C for 30 minutes (HWT) offers vine propagators a simple, cost effective, chemical free method of controlling specific pests and diseases in planting material on a commercial scale. If correctly executed, long duration hot water treatment minimises the probability of transmitting a wide range of pests and pathogens in planting material including crown gall, Phomopsis, phytoplasmas, nematodes mealy bug and Phylloxera, however, HWT will not eliminate viruses or improve poor quality propagating material.

HWT is used by many nurseries to satisfy local and interstate quarantine requirements and forms a cornerstone of many quality assurance programs. If HWT is carried out in accordance with industry protocols it is a very useful and reliable process, however, in isolated instances, nurseries have experienced losses following HWT with no single clearly identifiable cause.

Cuttings arriving at our vine nurseries have been acted upon by a wide range of factors between the time of collection and the point of delivery. Post-delivery handling, hydration, cold storage, re-hydration, HWT, callusing and shadehouse management further impact upon cuttings and collectively determine the performance of the finished vine.

The 'Jacob's Creek Trials' were established to systematically evaluate critical nursery procedures and determine their potential to influence the performance of vine cuttings following HWT.

The Orlando-Wyndham Group offered collaborative support through the Jacob's Creek Vine Nursery to mount an R&D program in conjunction with GWRDC and SARDI. The Jacob's Creek Vine Nursery under the management of Paul Wright and later Philip Deverell, offered an ideal site for this work as it is one of only a few nurseries operating in Australia with clearly defined, documented, SOP's for every stage of the propagation process.

The Jacob's Creek trials also offered a platform to facilitate the development of a bar code based QA and vine tracking system for Australian vine nursery operations. The tracking system was initially conceived as a research tool to enable individually treated cutting batches to be traced at any point through a commercial nursery operation. The bar code system was rapidly recognised as a commercially relevant data storage and tracking platform. The development and specifications of this system are outlined in this report under P4.3, pp 140 to 144.

Acknowledgement

The Jacob's Creek Vine Nursery is well known throughout the Australian vine nursery industry for its high nursery management standards, its benchmark nursery hygiene practices and the production of uniform, high quality vines. It offered a unique research opportunity to consistently and repetitively vary single factors within the vine propagation process on a commercial scale. Paul Wright, Philip Deverell, Tim Scheiner and 'The Jacob's Creek Team' were pivotal to the successful execution of the research protocols outlined below, providing greatly appreciated support and input to for this research program between 2000 and 2003. The Orlando-Wyndham Group's collaboration significantly reduced overall program operating costs while providing a platform for the efficient generation of industry relevant research results.

Method

1.21 2000/2001 – Trial 1 'Scoping study'.

A program reference group composed of Paul Wright, Philip Deverell, Andrew deLaine (EO AVIA), Wayne Farquhar (EO SAVI), Dr Michael McCarthy (SARDI), John Crocker (SARDI) and Helen Waite (University of Melbourne) was convened to short-list factors within the vine propagation process that were considered to have potential for significant impact upon the performance of cuttings following HWT. The group determined that nursery experience and prior research had broadly questioned the role of the following key factors and considered that these factors warranted further investigation on a commercial scale, namely:

- Cutting harvest date.
- Post-delivery cutting handling speed
- Cutting variety
- Duration of post delivery pre-HWT hydration
- Presence or absence of HWT

The following treatment levels were adopted for each factor;

- Cutting harvest date: Early (leaf fall +10 days), Standard (June/July) and Late (obvious sap flow)

- Post-delivery cutting handling speed: Rapid Handling (hydration and cold storage within 24 hours), Delayed Handling (cuttings remain on shed floor for 5 days)

- Cutting variety: Cabernet Sauvignon G9V3 (hardy), Chardonnay I10V1 (variable) and Pinot Noir MV6 (sensitive)

- Duration of post delivery pre-HWT hydration: No hydration, 1 hour, 8 hours, 16 hours and 40 hours hydration

- Presence or absence of HWT

The treatment levels were chosen as representative of broadly practiced propagation protocols, or having been implicated as significant in past instances of poor cutting performance following HWT. They provided a means of evaluating the scope of cutting responses to nursery practices within propagation protocols employing HWT and would serve to refine the range of treatments included in future experiments.

A factorial experiment containing 108 treatment combinations was required to allow fully replicated evaluation of parameters in isolation and in combination. A minimum treatment size based on a standard commercial bundle of 100 cuttings was adopted. 5 replicate bundles received each treatment combination, requiring a total of 54,000 cuttings to be handled within the experimental protocol. Cuttings were all commercially harvested from comparable source areas located within the Barossa Valley. Figure 1 (below) provides a summary of the 2000/2001 scoping study trial structure.

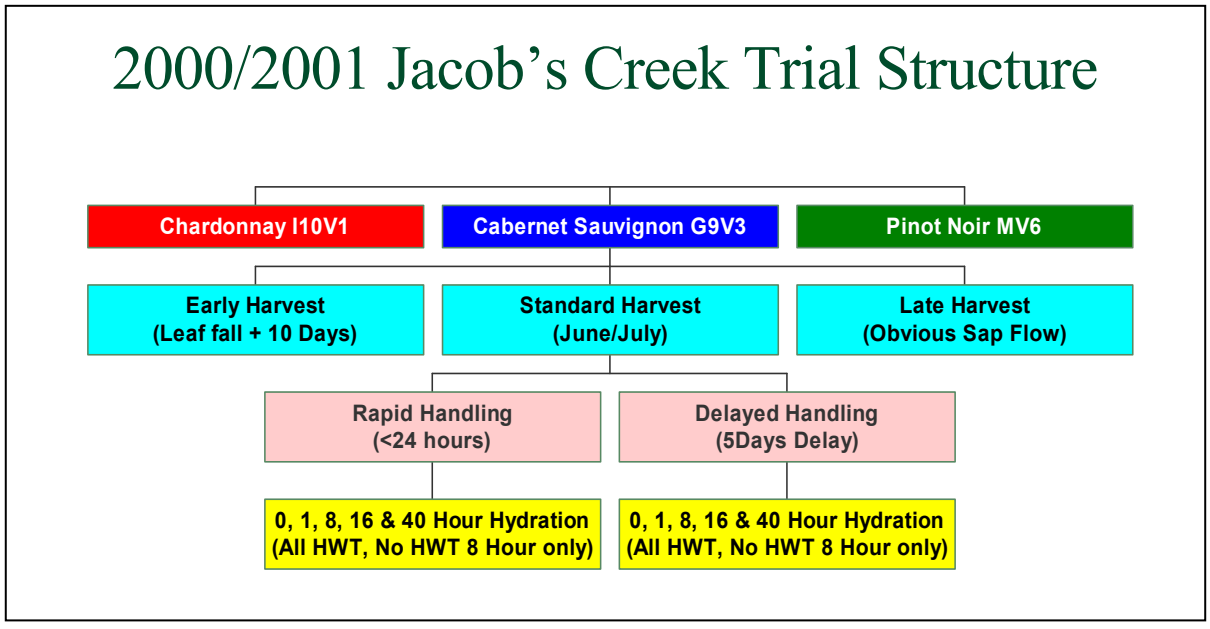


Fig. 1; 2000/2001 Scoping study, generalised trial structure.

Cutting handling and treatment protocols were generated in consultation with Paul Wright to enable all treatments to be processed by Jacob's Creek Nursery staff within normal working hours and as a component of normal nursery production runs. An extract from the 2000 treatment and handling protocol is presented in Figure 2, (below):

SARDI, Orlando Wyndham Group Hot Water Treatment Trial
Treatment Program, 2000 season.

Day	Date	Time	Variety	Batches (GRN)	Treatment	Activity	Recorded	Completed
Tuesday	06-Jun-00	8.00am	Chardonnay		Early Chardonnay	Harvest 6000 'early' cuttings		
Tuesday	06-Jun-00	3.00pm	Chardonnay		Early Chardonnay	Deliver cuttings to Jacob's creek Sort cuttings into 12 batches of		
Tuesday	06-Jun-00	3.30pm	Chardonnay		CHE (all)	500 (5 bundles of 100 in each batch) Generate GRN's for each batch		
Tuesday	06-Jun-00	4.00pm	Chardonnay		CHE (all)	of 500, print labels and apply to bundles		
Tuesday	06-Jun-00	4.15pm	Chardonnay	10, 11, 12, 13, 14, 15	CHED (all)	Leave on shed floor for 5 days of air storage		
Tuesday	06-Jun-00	4.30pm	Chardonnay	9, 6, 5 & 4	CHER 0, 1 & 8; CHER 8-NOHWT	Load into hydration crates		
Wednesday	07-Jun-00	8.00am	Chardonnay	6, 5 & 4	CHER 1,8 & CHER 8-NOHWT	Place into hydration bath		
Wednesday	07-Jun-00	9.00am	Chardonnay	6	CHER 1	Remove from hydration bath		
Wednesday	07-Jun-00	9.30am	Chardonnay	9 & 6	CHER 0 & 1	Bag & place into cold room		
Wednesday	07-Jun-00	3.45pm	Chardonnay	7 & 8	CHER 16 & 40	Load into hydration crates		
Wednesday	07-Jun-00	4.00pm	Chardonnay	7 & 8	CHER 16 & 40	Place into hydration bath		
Wednedsay	07-Jun-00	4.00pm	Chardonnay	5 & 4	CHER 8 & CHER-8-NOHWT	Remove from hydration bath		
Wednesday	07-Jun-00	4.30pm	Chardonnay	5 & 4	CHER 8 & CHER-8-NOHWT	Bag & place into cold room		
Thursday	08-Jun-00	8.00am	Chardonnay	7	CHER 16	Remove from hydration bath		
Thursday	08-Jun-00	8.30am	Chardonnay	7	CHER 16	Bag & place into cold room		
Friday	09-Jun-00	8.00am	Chardonnay	8	CHER 40	Remove from hydration bath		
Friday	09-Jun-00	8.30am	Chardonnay	8	CHER 40	Bag & place into cold room		
Friday	09-Jun-00	4.00pm	Chardonnay	10, 11, 12, 13, 14, 15	CHED 0, 1,8, 8-NOHWT, 16 & 40	Place into hydration crates		
Monday	12-Jun-00	8.00am	Chardonnay	11, 12 & 13	CHED 1 & 8,CHED 8-NOHWT	Place into hydration bath		
Monday	12-Jun-00	9.00am	Chardonnay	11	CHED 1	Remove from hydration bath		
Monday	12-Jun-00	9.30am	Chardonnay	10 & 11	CHED 0 & 1	Bag & place into cold room		
Monday	12-Jun-00	4.00pm	Chardonnay	12 & 13	CHED 8 & 8-NOHWT	Remove from hydration bath		
Monday	12-Jun-00	4.00pm	Chardonnay	14 & 15	CHED 16 & 40	Place into hydration bath		
Monday	12-Jun-00	4.30pm	Chardonnay	12 & 13	CHED 8 & 8-NOHWT	Bag & place into cold room		
Tuesday	13-Jun-00	8.00am	Pinot Noir		Early Pinot Noir	Harvest 6000 'early' cuttings		
Tuesday	13-Jun-00	8.00am	Chardonnay	14	CHED 16	Remove from hydration bath		
	13-Jun-00	8.30am	Chardonnay	14	CHED 16	Bag & place into cold room		
Tuesday	13-Jun-00	3.00pm	Pinot Noir		Early Pinot Noir	Deliver cuttings to Jacob's creek Sort cuttings into 12 batches of		

Fig. 2; Extract from 2000/01 cutting handling protocol.

6000 cuttings were harvested in each instance and in accordance with the following schedule for each variety (Figure 3, below). All cuttings were bundled in the field (Photo 1) and were transported to Jacob's Creek in covered stainless steel bins (Photo 2, below). Bundles were labelled with polyethylene tags on delivery to the Jacob's Creek Nursery. Tags were printed with a unique bar code and an alphanumeric code linking variety replicate number and treatment details.

Variety	Early Harvest Date	Standard Harvest Date	Late Harvest Date
Chardonnay	6th June 00	4th July 00	22nd August 00
Pinot Noir	14th June 00	11th July 00	29th August 00
Cabernet Sauvignon	20th June 00	18th July 00	15th September 00

Fig. 3; Cutting harvest schedule 2000

The cuttings received preliminary treatments on delivery to Jacob's Creek (handling speed and hydration duration), prior to cold storage at 0.5^0C to 2^0C in plastic lined bulk bins under high humidity. All cuttings were accumulated in cold storage until HWT could be performed in bulk during early November 2000.

Cuttings were progressively removed from cold storage and were equilibrated to ambient temperature before being subjected to Hot water treatment at 50^0C/30 minutes in a certified HWT tank (Photo 3). HWT was immediately followed by cool-down in a clean chlorinated water bath for 30 minutes. Cuttings that were not subjected to HWT were equilibrated to ambient temperature without further processing. HWT and cool down tanks were regularly emptied and cleaned in accordance with Jacob's Creek Nursery SOP's. Chlorine levels were monitored and maintained between treatments.



Photo 1; Cutting bundles – 1 per replicate



Photo 2; Cutting transport bins



Photo 3; Certified HWT & cool down tanks



Photo 4; Potted, tagged, cuttings

Following HWT and cool-down, processed cuttings were packed in layers into plastic lined bulk bins, with new hydrated vermiculite spread between successive layers. A layer of clean, dry pearlite was placed on the top of each bin before it was covered and placed into a callus room in darkness at 26^0C for 21 days.

On removal from the callus room, sub-samples of 20 cuttings were chosen at random from each 100-cutting bundle. Each sub-sample was assessed by Jacob's Creek staff for presence or absence of callus, approximate callus coverage, presence or absence of root initials and presence or absence of bud development. The same team of staff members carried out all assessments to maintain data continuity.

Assessed cuttings together with the remaining 80 cuttings from each treatment bundle were individually labelled with a bar-code tag and were potted into disposable paper 'plant bands' in an open potting mix. Potted cuttings were then packed into foam broccoli boxes at 42 pots/box, watered in and transported to the glasshouse (Photo 4; above).

Potted cuttings were grown for 35 days in the glasshouse before being assessed. The first 5 buds from the apical bud downwards on each cutting were given a growth score from 0 to 4, with '0' being given for no bud development and 4 being given for shoot growth >50mm. Three trays of 42 potted cuttings were assessed for each treatment combination (i.e. 3 x 42 potted cuttings of the total 500 cuttings per treatment).

Potted cuttings were then moved into a shadehouse where they were progressively hardened before being moved into full sunlight under overhead irrigation. Hardened cuttings were packed into transportation crates and were shipped to the Jacob's Creek field nursery site near Ramco SA by mid December 2000. Potted cuttings were carefully sorted on arrival and were planted in treatment order into mounded sandy soil with in-row drip irrigation.

The young vines were grown through summer and autumn in the field nursery until they reached dormancy where they were undercut, harvested and bundled as young rootlings during winter 2001. The bundled cuttings were transported back to Jacob's Creek for final assessment where they were sorted into treatment lots and then graded into '1st grade', '2nd grade' or 'Dead' in accordance with Jacob's Creek grading SOP's.

2001/2002 – Trial 2 Method

Results obtained during 2000/01 were presented to Australian vine nurserymen in a series of HWT Research Summary Workshops convened with GWRDC support in conjunction with VINA and AVIA at venues from Busselton WA to Griffith NSW (See GWRDC Workshop Tour Report submitted 29th June 2001). Consultation with vine nurserymen at each workshop in conjunction with discussion of results by the HWT project reference group resulted in a refined treatment list for 'Jacob's Creek Trial 2' conducted during 2001/2002.

Jacob's Creek Trial 2 was a commercial scale factorial experiment with treatments summarised in Figure 4, below:

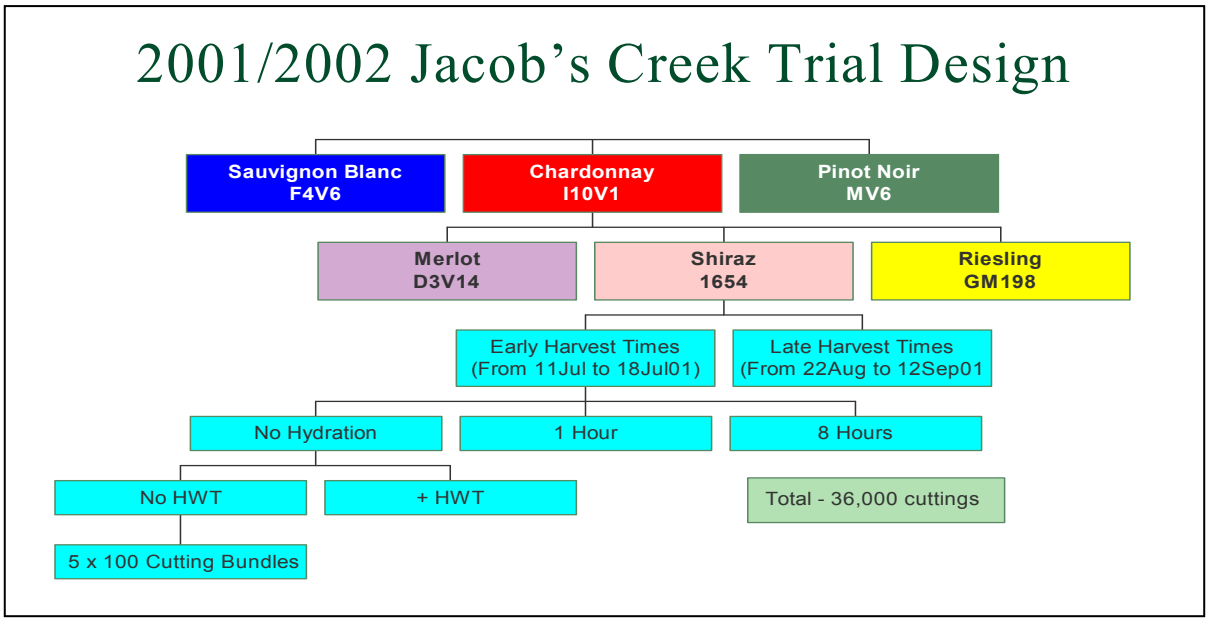


Fig. 4; 2001/2002 Jacob's Creek Trial 2 treatment summary.

Treatment protocols for 2001/2002 are summarised as follows:

All cutting treatments and assessments were conducted by Jacob's Creek staff. Propagation procedures followed standard Jacob's Creek Nursery propagation SOP's.

- **Cutting Source Location** – Orlando-Wyndham Group, Langhorne Creek Vineyard, Langhorne Creek SA
- **Cutting Varieties** – Chardonnay, Pinot Noir, Merlot, Riesling, Shiraz and Sauvignon Blanc
- **Treatment Units** – Each treatment combination comprised 5 replicate bundles of 100 cuttings each. The factorial trial design resulted in 72 unique treatment combinations and required a total of 36,000 cuttings
- **Cutting Harvest Dates** – Two harvest times were adopted, equivalent to 'Standard' and 'Late' from Trial 1. The cutting harvest schedule was as follows…

Variety	Standard Harvest	Late Harvest
Chardonnay	11th July 2001	22nd August 2001
Pinot Noir	11th July 2001	22nd August 2001
Merlot	11th July 2001	19th August 2001
Riesling	11th July 2001	19th August 2001
Shiraz	11th July 2001	19th August 2001
Sauvignon Blanc	18th July 2001	12th September 2001

- **Labelling** – All cutting bundles received a unique bar code label identifying variety, harvest time and treatment regime
- **Handling Speed** – All cuttings were packed into covered stainless steel bulk bins for transport and were handled rapidly (within 24 hours of delivery to Jacob's Creek).
- **Hydration Duration** – Post-harvest, pre-cold storage hydration durations were either 0, 1 hour or 8 hours
- **Post-hydration Cold Storage** – All cuttings were aggregated in high humidity cold storage at 0.5 to 2.0^{0}C until required for hot water treatment in early November 2001
- **Equilibration** - All cuttings were permitted to equilibrate to ambient temperature when removed from cold storage prior to HWT
- **HWT** – Half of all cuttings received HWT, while the other half remained untreated
- **Cool Down** – All HWT cuttings were immediately cooled following HWT by immersion in clean chlorinated water
- **Callusing** – Treated cuttings were packed into plastic lined bulk bins between layers of new hydrated vermiculite. Cuttings were callused in darkness at 26^{0}C for 21 days.
- **Assessment 1** – On removal from the callus room, 20 cuttings/bundle were chosen at random and received a score defining their level of callus development and root initiation.
- **Potting and Labelling** – Callused cuttings were individually labelled with bar coded tags and were potted into disposable plant bands.
- **Pot Management –** potted cuttings were initially placed in a glasshouse before being moved to a shadehouse and progressively hardened to full sunlight. All irrigation was applied overhead.
- **Field Nursery** – Hardened cuttings were transported to the Jacob's Creek field nursery site near Ramco SA and were planted in treatment order by mid December 2001. Cuttings were grown with in-mound drip irrigation until harvest as dormant rootlings in July 2002.
- **Harvest Assessment –** Harvested rootlings were transported from the field nursery to Jacob's Creek where they were graded as 1st Grade, 2nd Grade or Dead.

2002/2003 – Trial 3 Method

Results from Trial 2 were presented to Australian vine nurseries in a second round of HWT research Summary Workshops. Meetings were supported by GWRDC funds and were convened in association with VINA at venues from Busselton WA to Griffith NSW. Outcomes of the second workshop series were summarised in a Workshop Tour Report submitted to GWRDC on the 29th of June 2001.

Discussion of Trial 2 results and consultation with vine nursery operators at the research summary workshops produced a refined treatment list for the third and final Jacob's Creek Trial conducted during 2002/2003.

Trail 3 aimed to validate data collected during Trials 1&2, while providing a platform to test the assumption that "*Post-vintage, pre-cutting collection source area soil moisture status plays a dominant role in cutting performance following HWT*".

Jacob's Creek Trial 3 was a commercial scale factorial experiment with treatments summarised in Figure 5, below:

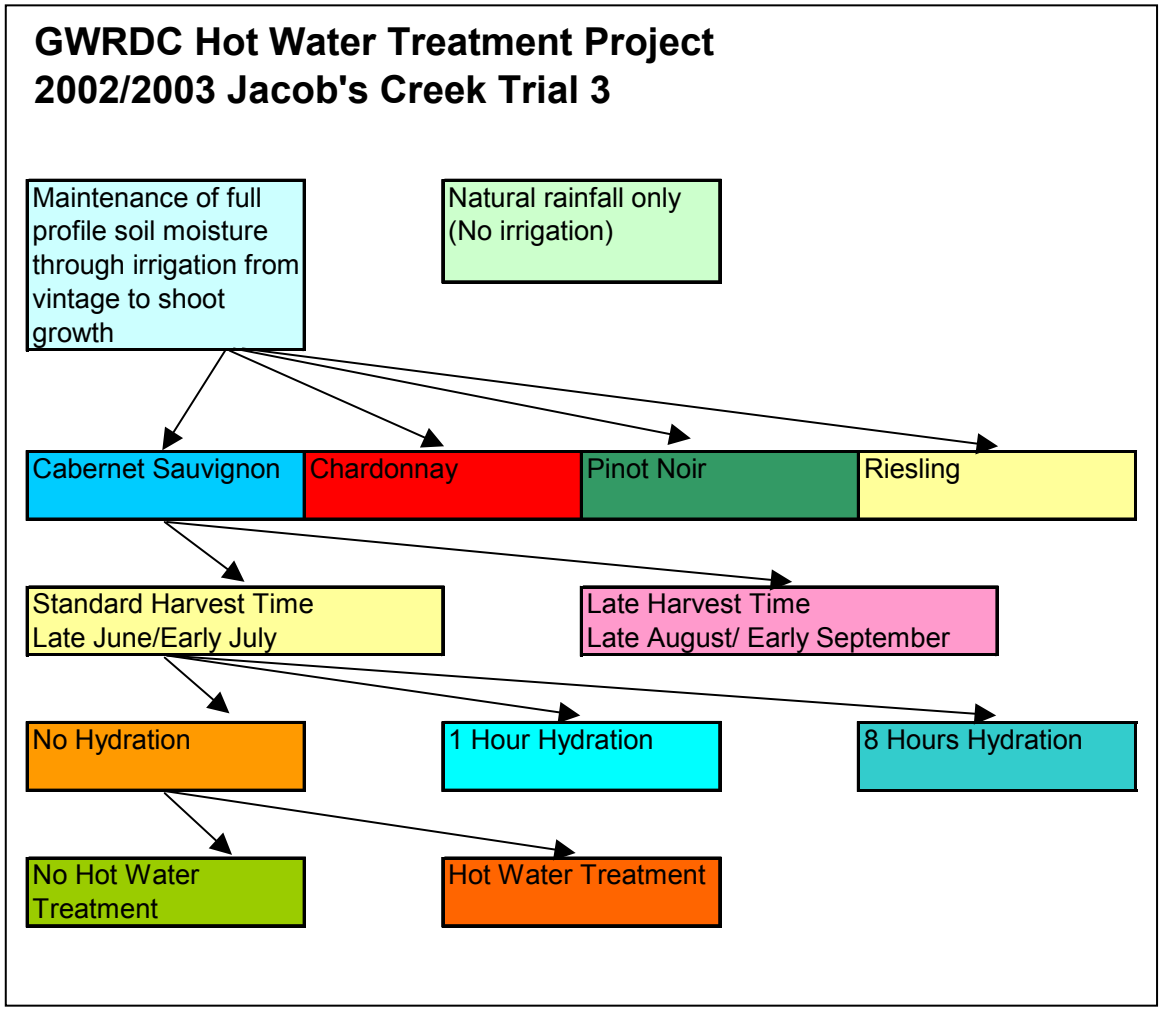


Figure 5; 2002/2003 Jacob's Creek Trial 3 treatment summary.

Trial 3 generated 96 individual treatment combinations. Each treatment combination required 5 replicate bundles of 100 cuttings or a total of 48,000 cuttings overall.

The Orlando Wyndham Group assisted with trial establishment at their Langhorne Creek Vineyard in 2002 by modifying their source area irrigation system to deliver water to half of the source area vines reserved for Trial 3. The remaining source vines received rainfall only. Post-vintage, pre-cutting harvest irrigation treatments were applied to the 'Supplementary Irrigation 'source blocks to ensure soil moisture levels remained adequate and the 'Irrigated' plots did not become water deficient.

Irrigation was scheduled through soil moisture monitoring. Langhorne Creek received 142.2mm of rainfall between the 1st of April 2002 and harvest of the last cuttings on the 11th of September 2002. Records of irrigation application to trial plots are not available due to circumstances beyond the author's control.

The 2002/2003 Cutting Harvest schedule was as follows:

Variety	Standard Harvest	Late Harvest
Chardonnay	10th July 2002	21st August 2002
Pinot Noir	10th July 2002	21st August 2002
Riesling	10th July 2002	28th August 2002
Cabernet Sauvignon	17th July 2002	11th September 2002

All other cutting treatments and handling procedures for 2002/2003 were the same as described for the 2001/2002 season on page 46 of this report. Detailed examination of shoot development was not undertaken during Trial 3.

Results – Jacob's Creek Trial 1, 2000/2001

Please note: When interpreting the data highlighted by the Jacob's Creek trial series it is important to note that varietal responses recorded as variations in the number root initials following callusing are distinctly transient and only serve to provide a research tool and a window of opportunity for the measurement of responses that would otherwise be concealed. Where a treatment regime is described as performing 'less well' or 'poorly', the description relates to relative early root initiation performance and not to the ultimate survival of the cutting. In most cases, good nursery practice and the avoidance of moisture stress allowed vines recover from initially low levels of root initiation.

2001 trial data was analysed by Michelle Lorimer of Biometrics SA unless otherwise stated.

1. Presence or Absence of HWT

The presence or absence of root initials after callusing cuttings in darkness for 21 days at 26C provided a unique window of opportunity to observe the physiological impact of HWT on vine cuttings. Root initiation differences may be observed at this point of cutting development, however, these differences are often masked by cutting recovery at later stages of growth, particularly where nursery management practices are good and cutting growth conditions are optimal.

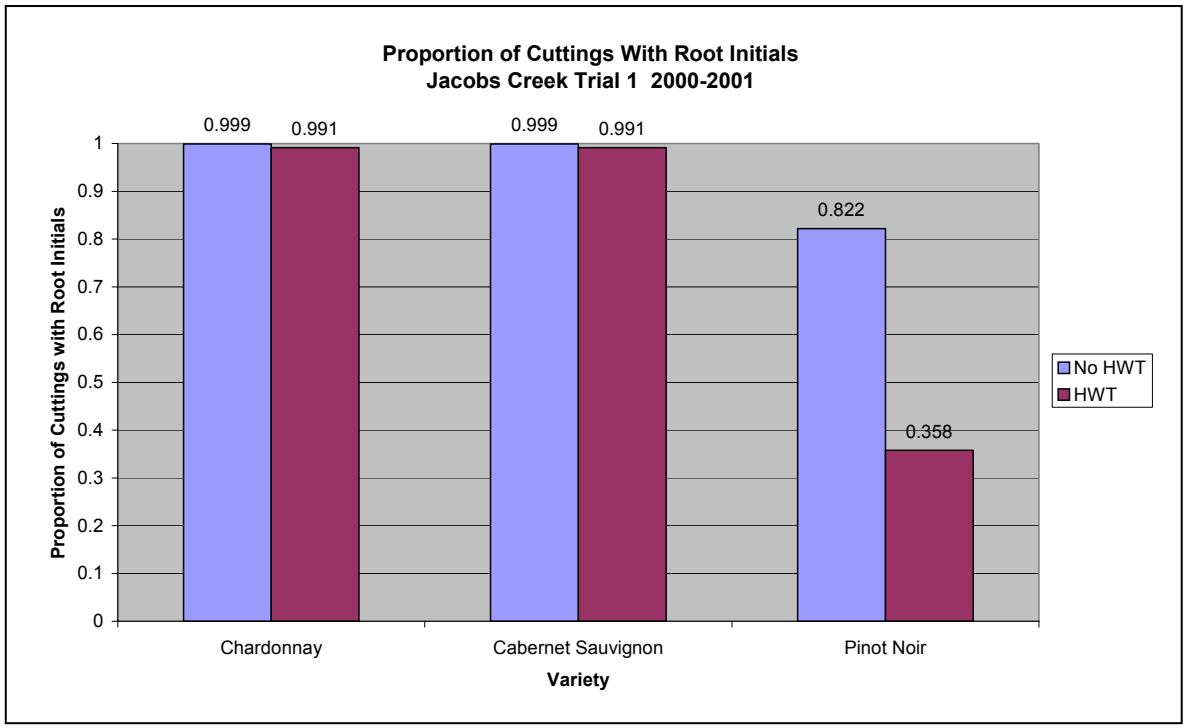
The 2000/01 Jacobs Creek trial provided useful insight into different varietal susceptibilities to long duration HWT for Chardonnay, Cabernet Sauvignon and Pinot Noir. Where 'Standard' harvest date cuttings of each variety (collected between the 4th and 18th of July 2000) were handled rapidly and were hydrated for 8 hours prior to HWT (control treatments received no HWT), logistic regression revealed that there were significant differences between varieties at the 5% level ($p<0.001$) and there was a significant difference between the presence and absence of HWT ($p<0.001$). The data lead to the following observations:

- There was no significant difference in the proportion of Chardonnay or Cabernet Sauvignon cuttings with root initials, regardless of HWT.

- Pinot Noir cuttings had a significantly lower proportion of root initials than Chardonnay or Cabernet Sauvignon.

- HWT resulted in the formation of significantly fewer root initials in treated Pinot Noir cuttings vs. untreated cuttings.

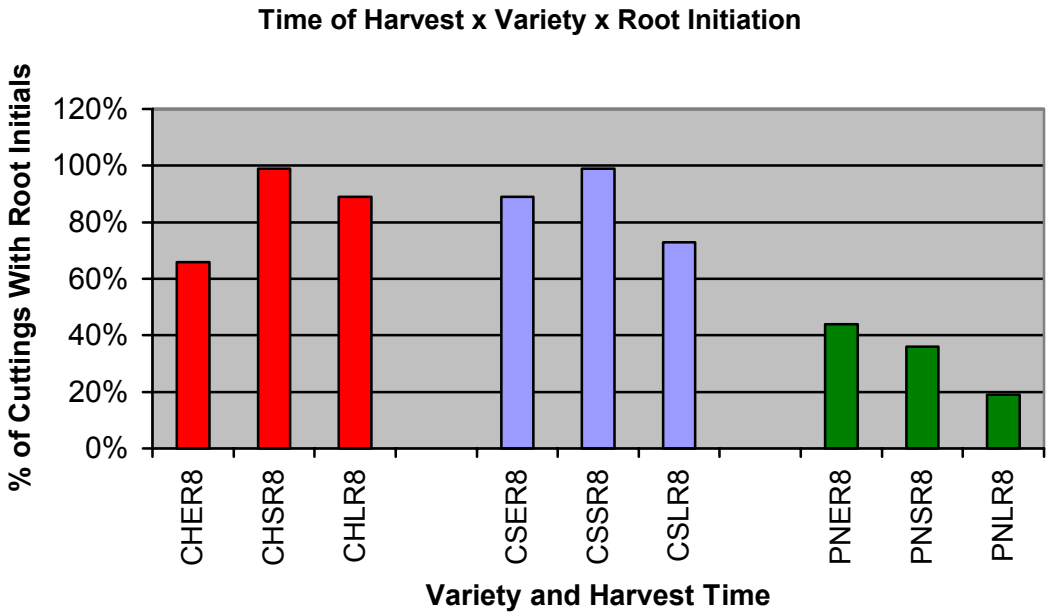
Graph 1; below, depicts the proportion of cuttings with root initials ± HWT for Cabernet Sauvignon, Chardonnay and Pinot Noir (1.0 = 100%).



Graph 1; Proportion of cuttings with root initials – Standard harvest time, rapid handling, and 8 hours hydration.

2. Harvest date and Handling Speed ±HWT

Logistic regressions performed on 8 hour hydrated cuttings for each combination of harvest date and handling speed revealed that Chardonnay and Cabernet Sauvignon both returned significantly more cuttings with root initials than Pinot Noir ($p<0.001$). Graph 2, (below) illustrates this for 8 hour hydrated, rapidly handled cuttings (No HWT). Red = Chardonnay, Blue = Cabernet Sauvignon and Green = Pinot Noir.



Graph 2; Influence of harvest date on the presence of root initials in Chardonnay, Cabernet Sauvignon and Pinot Noir for early harvest cuttings (left columns), standard harvest (middle columns) and late harvest (right columns).

Notable differences existed in the performance of some treatment combinations namely:

- Late harvest and delayed handling +HWT produced a significant interaction with Pinot Noir ($p=0.005$) where only 6% of cuttings could be expected to have root initials
- Late harvest and rapid handling +HWT produced a significant interaction with Pinot Noir ($p=0.001$) where only 19% of cuttings could be expected to have root initials

3. Callus formation

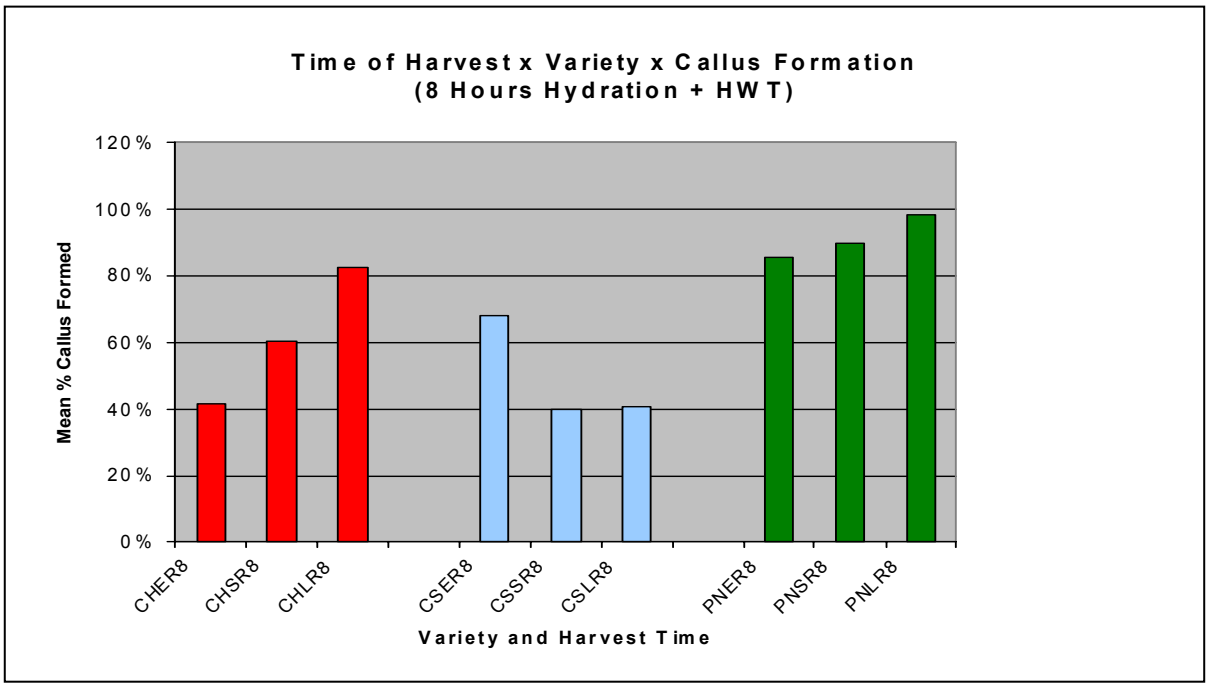
The assessment of the percentage of each cutting end covered with callus tissue after 21 days in the callus room can provide a further indication of cutting responses to applied treatments. Prolific callus growth (100% cover) may occasionally be associated with tissue stress and the early development of few root initials, tough this varies between varieties.

From the log-linear model it was concluded that the interaction between variety, HWT and callus amount was not significant at the 5% level ($p=0.61$). However, the callus amount and variety interaction, and the callus amount / HWT interactions were both highly significant ($p<0.001$).

For Standard Harvest, rapidly handled, 8 hour hydrated cuttings not exposed to HWT the data leads to the following conclusions:

- Chardonnay and Cabernet Sauvignon 'No HWT' cuttings are most likely to have lower levels of callus formation than Pinot Noir.
- Most 'No HWT' cuttings of Pinot Noir have a high level of callus formation.
- Chardonnay and Pinot Noir 'No HWT' cuttings both recorded increasing levels of callus formation with later harvest dates, however this relationship did not hold for Cabernet Sauvignon.

Graph 3; (below), illustrates this observation in the absence of HWT for Early, Standard and Late harvest date cuttings and an 8 hour hydration durations:



Graph 3; Mean % callus formation for Early, Standard and Late harvest date cuttings, rapid handling, 8 hour hydration, No HWT, Chardonnay (red), Cabernet Sauvignon (blue) and Pinot Noir (green).

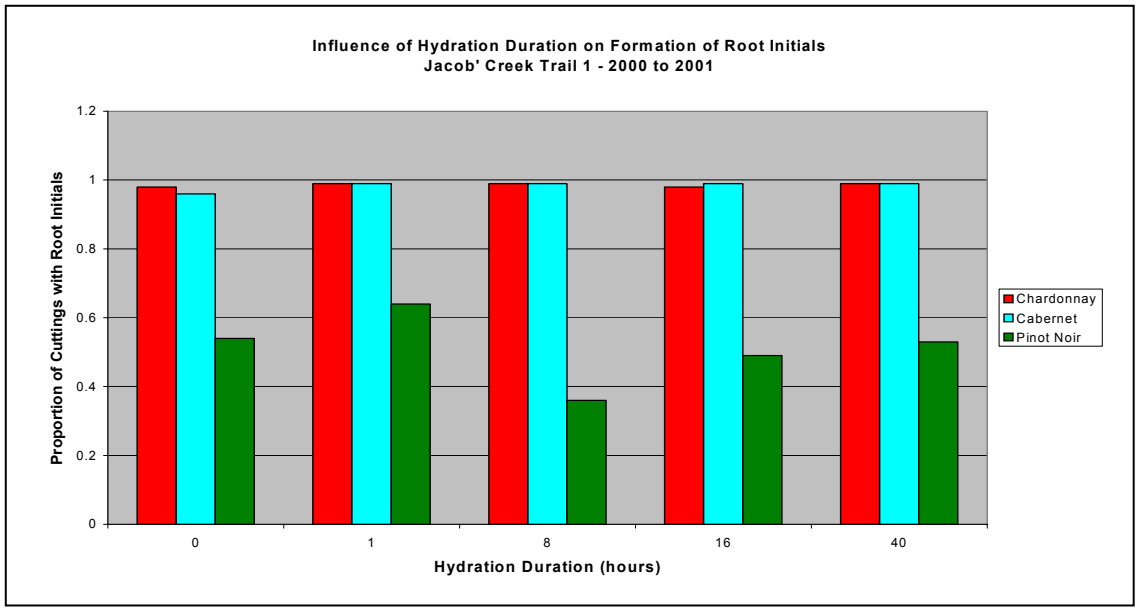
When cuttings were exposed to HWT, this situation changed significantly. HWT exposure appears to increase callus formation in Chardonnay and Pinot Noir:

- Cuttings of Chardonnay have a higher level of callus coverage (51% to 75%) when exposed to HWT than untreated Chardonnay cuttings.
- Most cuttings of Cabernet Sauvignon continue to have between 0% to 25% callus coverage when exposed to HWT
- 64% of Pinot Noir cuttings return increased callus coverage of between 76% and 100% when exposed to HWT

4. Hydration and root formation

For standard harvest, rapidly handled cuttings, logistic regression returned a significant interaction between hydration duration and the presence of root initials ($p<0.001$), however, one sample proved to be an outlier (aberrant) and was excluded from the analysis. With the outlier removed, analysis failed to prove a significant interaction between hydration duration and variety, however, the associated probability of $p=0.061$ was close to the 5% rejection level.

Analysis of the main effects of variety and hydration duration returned highly significant differences between treatments ($p<0.001$). Graph 4; below summarises the predicted proportion of cuttings with root initials under each hydration regime (0.99 = 99%)



Graph 4; Proportion of cuttings with root initials for varying hydration durations

Graph 4 leads to the following conclusions for standard harvest date, rapidly handled cuttings:

- Most cuttings of Chardonnay and Cabernet Sauvignon have root initials and the proportion of Chardonnay and Cabernet Sauvignon cuttings with root initials does not vary significantly for any of the hydration treatments.
- Pinot Noir had significantly fewer cuttings with root initials than either Chardonnay or Cabernet Sauvignon
- The proportion of Pinot Noir cuttings with root initials varied from 64% for 1-hour hydration and 36% for 8 hours hydration.

5. Shoot growth

Exposure to HWT caused a significant reduction of early shoot development in Pinot Noir. This response was evident during analysis of shoot growth data and was clearly visible in the glasshouse 28 days after potting (Photo 5). With good shadehouse and field nursery management practices, Pinot Noir cuttings recovered from all initial growth inhibition symptoms within 3 months of being planted in a field nursery (Photo 7). The same initial growth inhibition response was not observed for Chardonnay or Cabernet Sauvignon for any of the treatment combinations applied during 2000 (Photo 6).

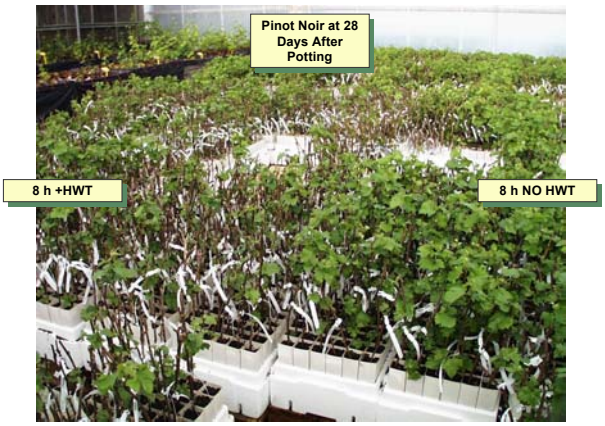


Photo 5; Pinot shoot growth at 28 days ± HWT

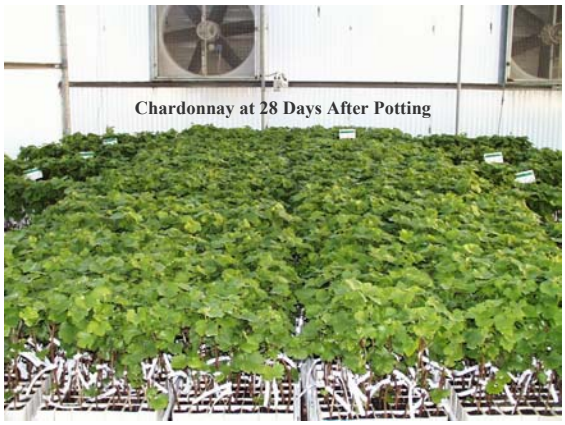


Photo 6; Chardonnay growth



Photo 7; Pinot Noir, Field Nursery + 3 months

6. Harvest and grading of rootlings

(Analysis of vine rootling data was conducted with by Statistix v.7')

Rootlings were sorted in treatment order into batches consisting of 1st grade, 2nd grade or dead following harvest from the Ramco field nursery in 2001. Individual cuttings were identified by their respective bar code tags and data was collated accordingly.

Analysis of variance indicated that HWT did not significantly influence final survival rates for any of the 8 hour hydrated treatment combinations (5% level, p=0.7891). This indicates that the influence of HWT in 8 hour hydrated cuttings as observed following callusing and initial growth may be short lived where management practices are good and cuttings can recover.

In the absence of HWT and for all hydration levels, analysis of variance indicated that there are significant differences (5% level, p=0.0096) between the long-term survival rates of cuttings propagated under varying regimes. Standard harvest date/rapidly handled Cabernet Sauvignon cuttings returned the highest mean survival rate, while early harvest date/rapidly handled cuttings of Pinot and Chardonnay performed similarly, but significantly less well than the Cabernet.

This data indicates that the effects of harvest date and handling speed can vary between varieties and can persist beyond the vine nursery to have an impact on long-term cutting survival.

Conclusions – Jacob's Creek Trial 1, 2000/2001

Jacob's Creek Trial 1 reinforced many of the observations summarised by Waite and May (1999) and provided additional insight into the performance of cuttings ± HWT and under the influence of a range of varying propagation regimes. Trail 1's commercial scale provided excellent data coherence and the application of treatments by Jacob's Creek staff ensured that the results were commercially relevant.

Trial 1 data analysis provided the following overall conclusions…

- Cabernet Sauvignon and Chardonnay cuttings were relatively hardy, recording few significant initial growth variations under a variety of propagation regimes.

- Pinot Noir cuttings demonstrated initial sensitivity to HWT when observed 21 days after the commencement of callusing and 28 days after potting with significantly fewer root initials than Chardonnay or Cabernet. Good vine nursery and field nursery management practices allowed Pinot cuttings ± HWT to visually recover from any initial sensitivity within 3 months of planting in the field nursery.

- Harvest date influenced initial root formation with 'Standard harvest dates (4^{th} to 18^{th} of July 2000) providing the best level of initial root development in Chardonnay and Cabernet, while the 'Early' harvest date (14^{th} June 2000) returned the best initial root development in Pinot Noir.

- Hydration times beyond 8 hours were not always beneficial.

- The influence of cutting variety and nursery protocol variations could still be observed when rootlings were graded after harvest from the field nursery. Standard harvest date, rapidly handled Cabernet and Early harvest date, rapidly handled Pinot and Chardonnay cuttings returned the highest field nursery survival rates of approximately 98%. Late harvested, delayed handling cuttings of Pinot, Chardonnay and Cabernet returned the lowest post-field nursery survival rates of approximately 92%.

Results generated during Jacob's Creek Trial 1 support the hypothesis that a proportion of the previously unexplained failures of Pinot Noir following HWT may have been due to initial varietal sensitivity of Pinot to HWT compounded by aberrations in cutting quality or subsequent nursery management practices.

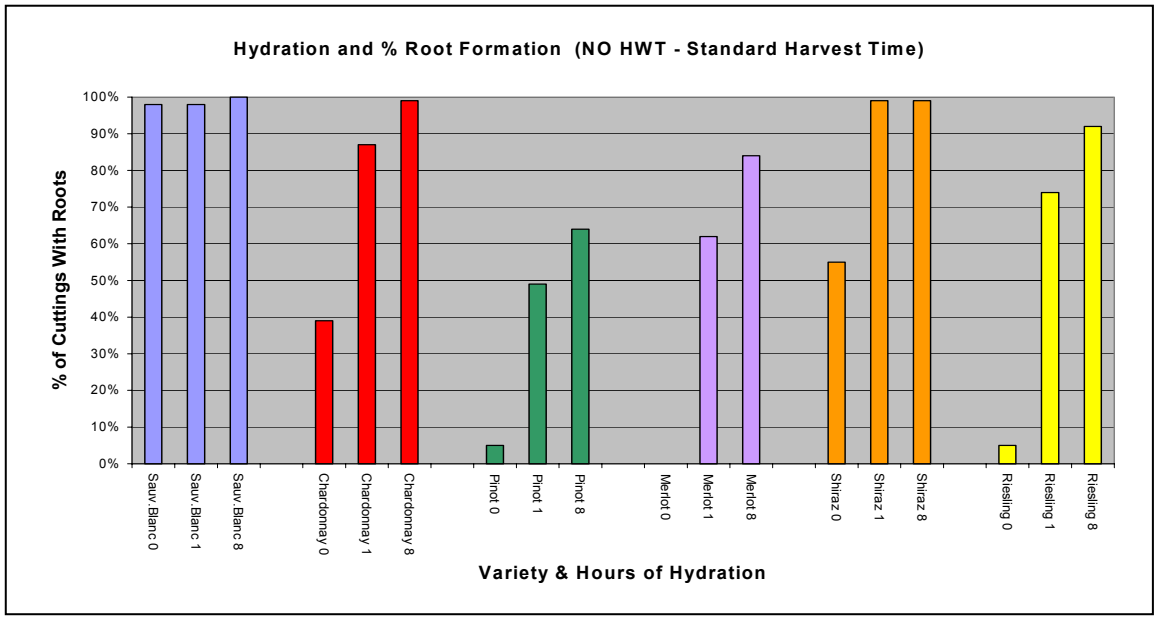
The observation that Pinot Noir is sensitive to HWT and initially forms significantly fewer roots than Cabernet or Chardonnay supports the hypothesis that moisture stress following potting or transplant to the field nursery may predispose HWT Pinot cuttings to failure if they are not managed appropriately. This was further supported by empirical observations from an unrelated vine nursery during 2000 where HWT Pinot Noir demonstrated poor performance of following delayed handling and post-harvest cutting dehydration. This ultimately resulted in a marked, detrimental impact on initial growth and high levels of subsequent cutting failure in the field nursery.

Results – Jacob's Creek Trial 2, 2001/2002

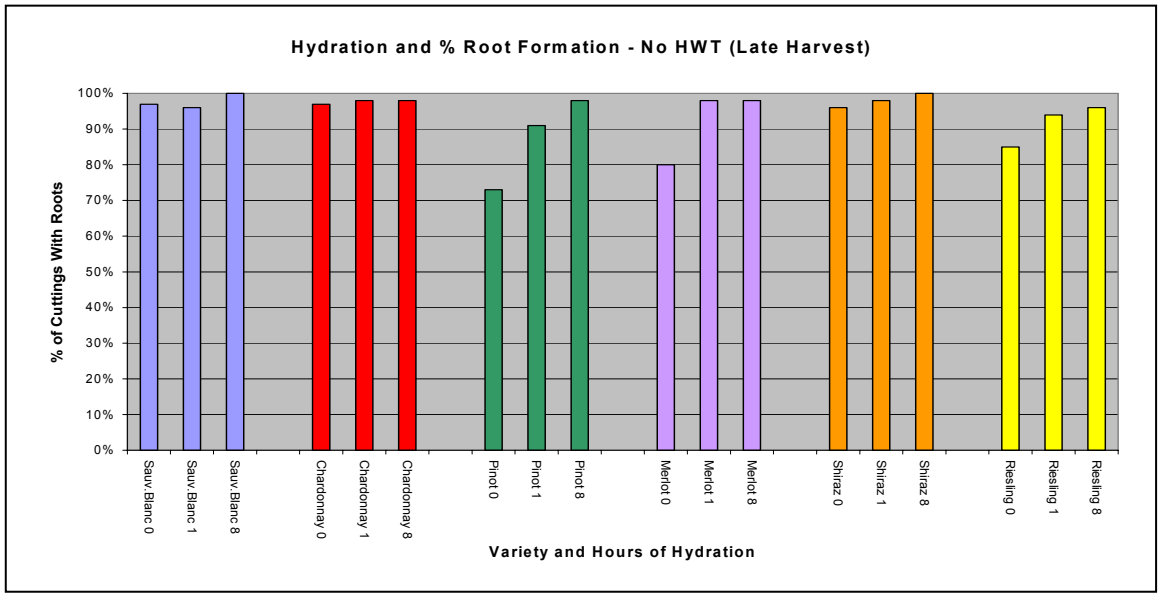
Notes: (1) All 2001/2002 trial data was subjected to ANOVA using Statistix 7. (2) Reference to 'Standard' harvest date cuttings infers that cuttings were harvested on the 11^{th} of July 2001 for all varieties except Sauvignon Blanc, which was harvested on the 18^{th} of July 2001. Reference to 'Late' harvest date cuttings infers that cuttings were harvested on the 22^{nd} of August 2001 for Chardonnay and Pinot Noir, the 19^{th} of August for Merlot, Riesling and Shiraz and the 12^{th} of September for Sauvignon Blanc. (3) Reference to 'Root initials' describes the number of root primordia observed per cutting after the cutting has been incubated in a callus room for 21 days at 26^{0}C.

1. Harvest date, hydration and proportion of cuttings with root initials

In the absence of HWT, analysis of Trial 2 root initiation observations demonstrated that Standard harvest date cuttings of all varieties and hydration regimes returned cuttings with significantly fewer root initials than the equivalent group of Late harvest date cuttings ($p<0.001$ at 5%). Note Graphs 5 and 6 below.



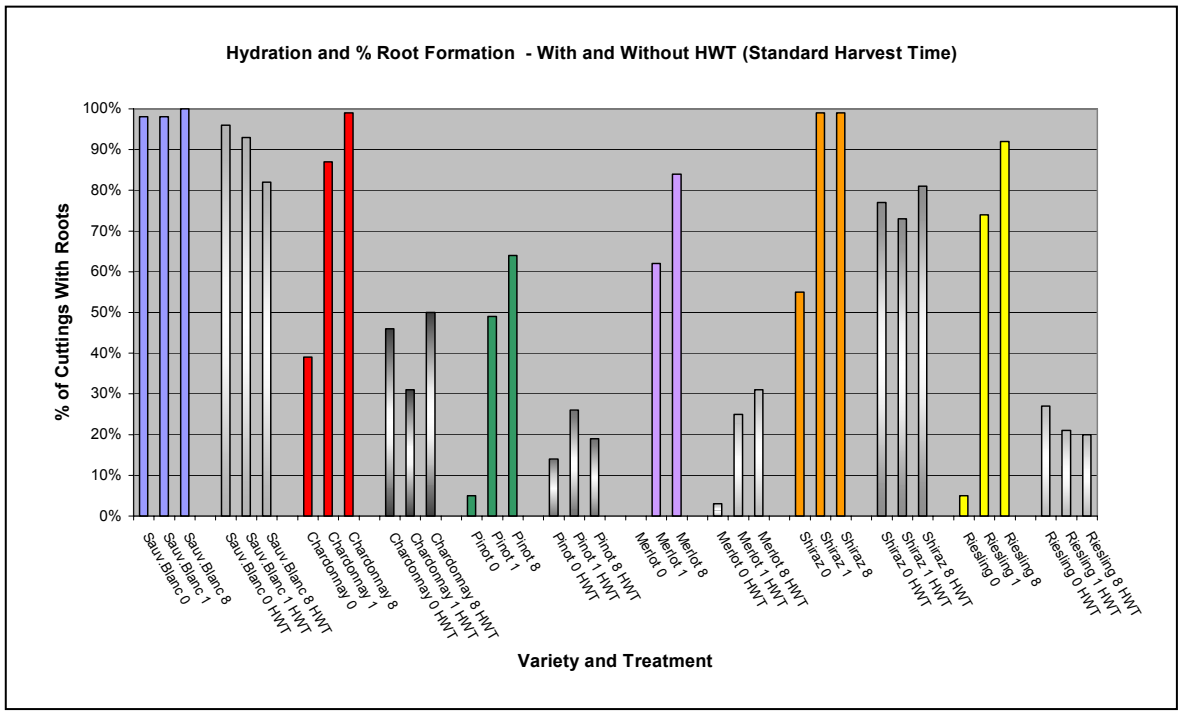
Graph 5; Percentage of cuttings with root initials, Standard harvest date, no HWT



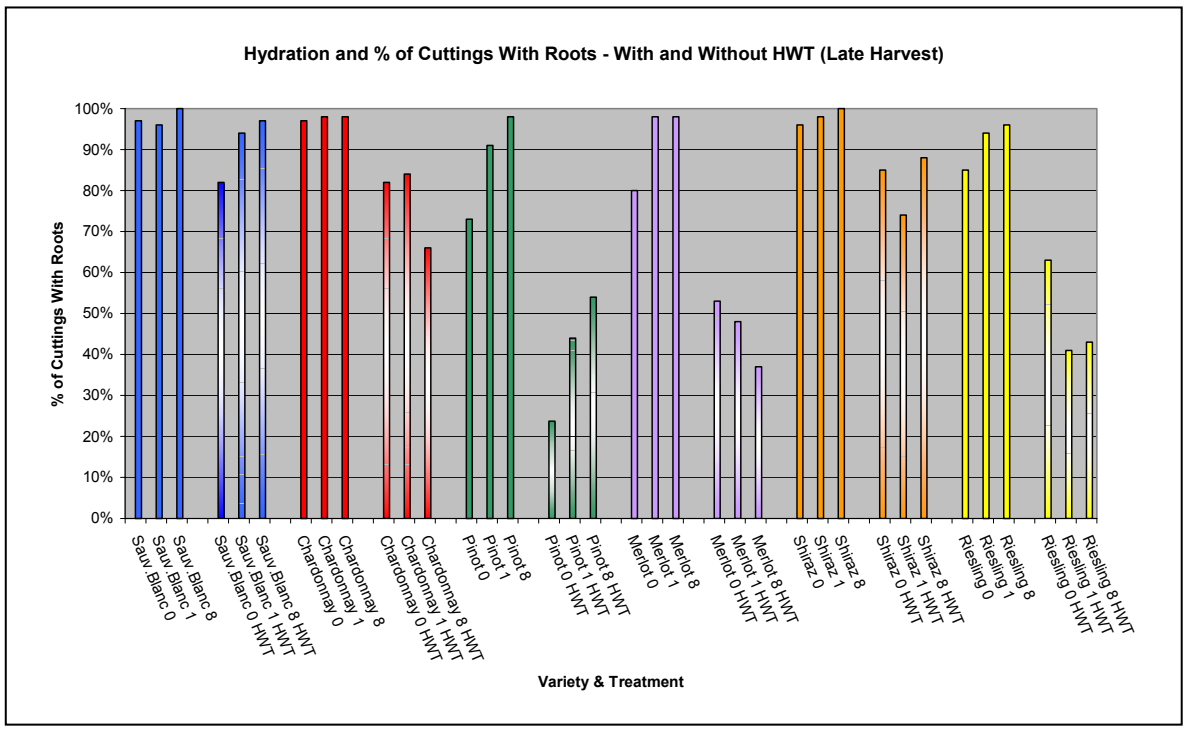
Graph 6; Percentage of cuttings with root initials, Late harvest date, no HWT

Influence of HWT

Analysis of root initiation data ± HWT indicated that late harvest cuttings with no exposure to HWT (all varieties and hydration durations) formed significantly more root initials than any other cuttings ($p<0.001$ at the 5% level). The same analysis revealed that there was no significant difference between the level of root initiation observed in Standard harvest cuttings –HWT and Late harvest cuttings +HWT. Standard harvest cuttings +HWT returned significantly fewer root initials than any other group ($p<0.001$ at the 5% level).



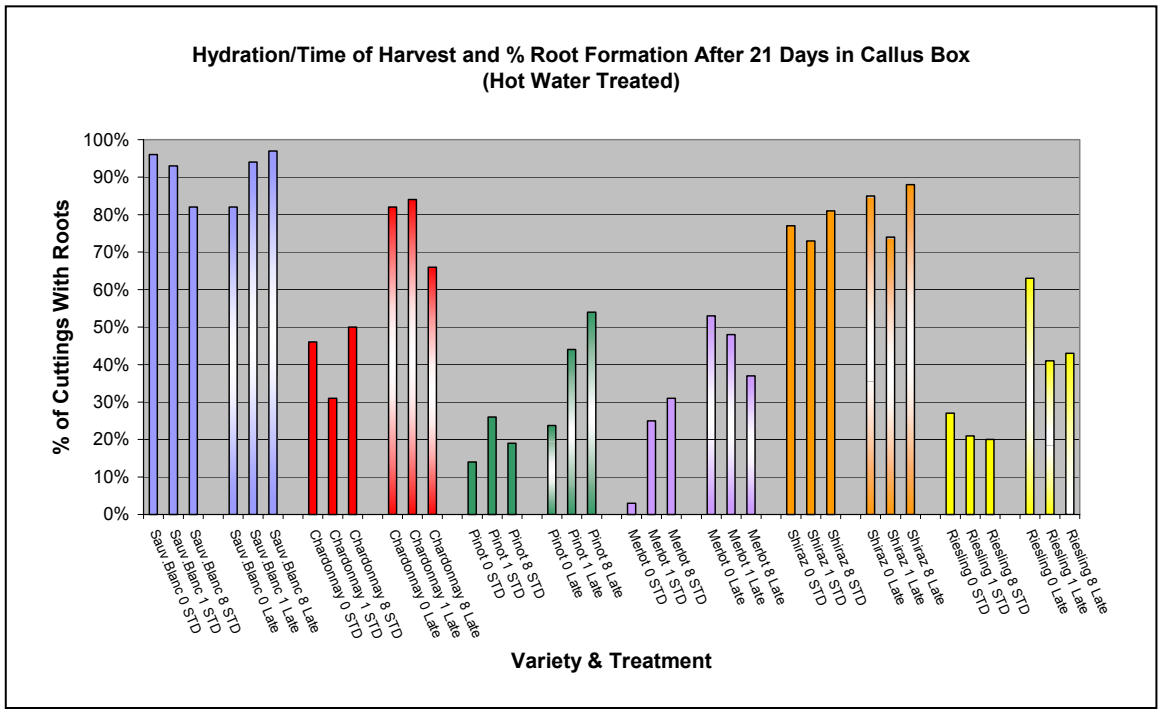
Graph 7; Hydration duration and % root formation, ±HWT, Standard harvest date



Graph 8; Hydration duration and % root formation, ±HWT, Late cutting harvest date
The data revealed a strong interaction between varieties, harvest times and the presence or absence of HWT ($p<0.001$ at the 5% level). For all hydration durations, Standard harvest date Pinot Noir, Merlot and Riesling produced significantly fewer root initials than other treatment combinations when exposed to HWT. Correspondingly, for all hydration durations, Standard harvest date Sauvignon Blanc and Late harvest date Sauvignon Blanc, Shiraz and Chardonnay produced significantly more root initials than other treatment combinations when not exposed to HWT. This observation reinforces the hypothesis that differing wine grape varieties respond differently to HWT exposure and sensitive varieties may have fewer root initials and will be at risk of poor performance if not managed appropriately flowing HWT.

Interaction of HWT and harvest date

For cuttings exposed to HWT, analysis of the root initiation data revealed a strong interaction between variety, number of root initials and harvest date ($p<0.001$ at the 5% level). For all hydration durations, Late harvested Sauvignon Blanc and Standard harvest date Sauvignon Blanc produced significantly more root initials than any other variety or treatment combination. This was closely followed by Late harvest date Shiraz and Chardonnay. Conversely, Standard harvest date Riesling, Pinot Noir and Merlot produced significantly fewer root initials than other varieties or treatment combinations. This is illustrated in Graph 9, below.

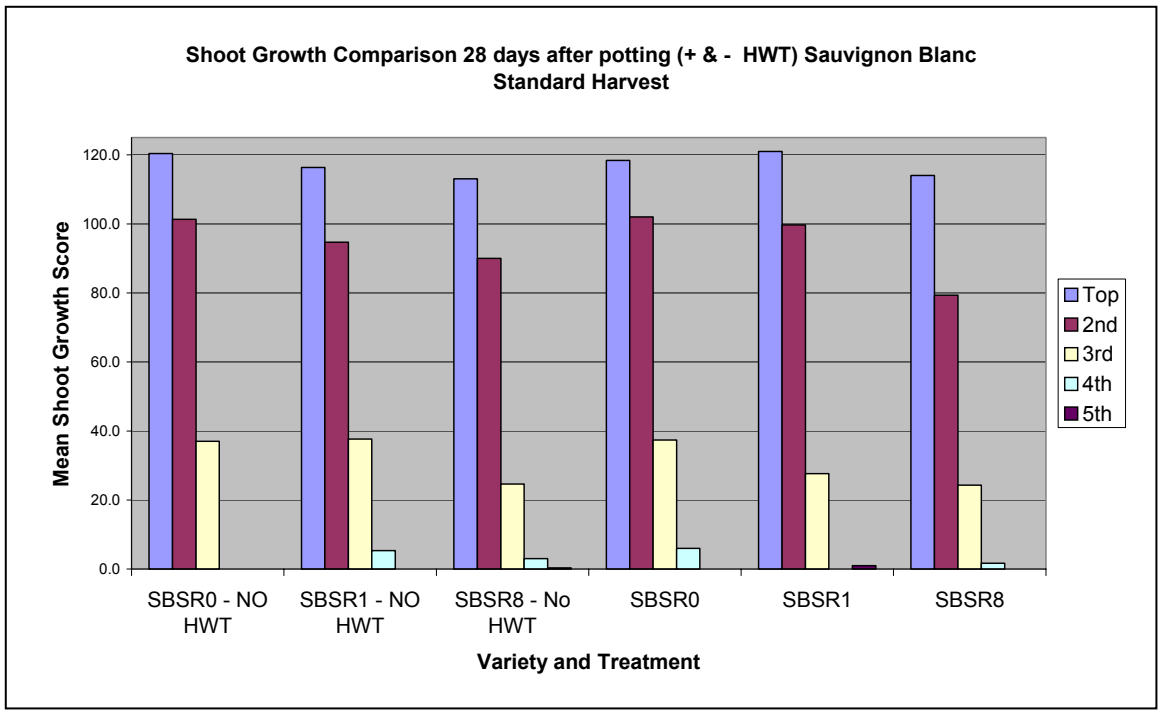


Graph 9; Hydration duration, harvest time and % root formation for HWT cuttings

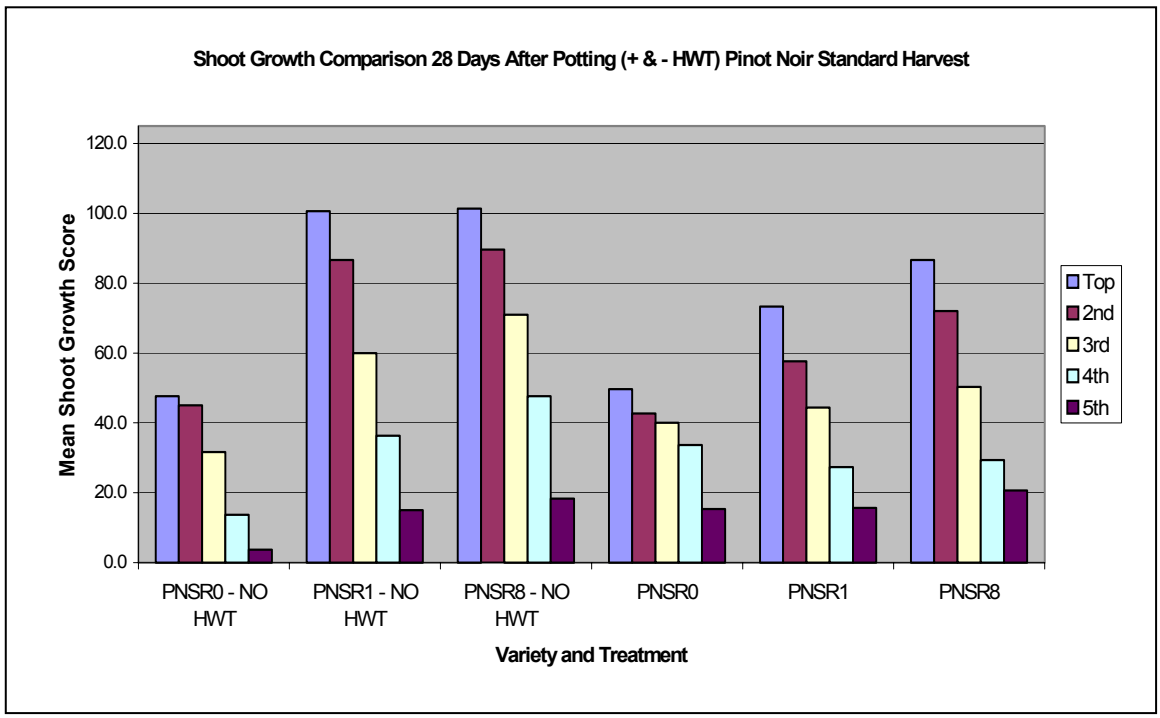
Further examination of the data indicated that Late harvest date Riesling, Merlot, Pinot Noir and (to a lesser extent) Chardonnay produced significantly more cuttings with root initials than their Standard harvest date counterparts ($p<0.001$ at the 5% level).

Shoot growth

Assessment of shoot growth characteristics 28 days after cuttings were potted into disposable plant bands revealed significant differences between the growth characteristics of tolerant and sensitive varieties ±HWT. This is illustrated in Graph 10 and Graph 11 (below) which contrast the mean shoot growth scores for the top 5 shoots of Standard harvest date, 8 hour hydrated Sauvignon Blanc (tolerant) and Pinot Noir (sensitive) in the presence and absence of HWT.



Graph 10; Mean shoot growth scores, Sauvignon Blanc ±HWT



Graph 11; Mean shoot growth scores, Pinot Noir ±HWT

28 days after potting, HWT tolerant varieties such as Sauvignon Blanc, Chardonnay and Shiraz characteristically display pronounced shoot extension in the first (top) and second buds, with little or no extension growth in the third or subsequent buds. There were no significant differences recorded between shoot growth characteristics ±HWT for Sauvignon Blanc.

HWT sensitive varieties such as Pinot Noir, Merlot and Riesling characteristically displayed comparatively less shoot extension in the first (top) and second buds, however, extension growth was also present in the third fourth and fifth buds. The amount of growth decreased with increasing distance from the apical bud. Sensitivity to HWT was generally expressed as a reduction in shoot extension observed in the first and second buds. The amount of shoot growth observed in third and subsequent buds remained relatively constant ±HWT for Pinot Noir.

Relative differences in shoot growth observed ±HWT is illustrated for Merlot in Photo 8, below…



Photo 8; Merlot shoot growth +HWT (right) and –HWT (left)

Discussion, Jacob's Creek Trial 2

Analysis of Trial 2 data reinforced many of the observations recorded in Trial 1 and presented several differences for consideration. In the absence of HWT, cuttings harvested earlier in the season (Standard harvest dates) produced significantly fewer root initials immediately after callusing than cuttings harvested towards the end of the season (Late harvest dates). This contrasted with the higher number of root initials observed for 'Standard' cuttings in Trial 1 and was thought to have been due to the influence of a relatively dry winter prior to harvest of the Standard cuttings in July 2001.

In the 4 weeks between the 18th of June and harvest of the last Standard cuttings on 18th of July 2001, only 20.4mm of rain was recorded at Langhorne Creek. Contrastingly, 70.2mm of rainfall was recorded in the 4 weeks between the 12th of August and harvest of the last Late cuttings on the 12th of September 2001. This result implied that low source area soil moisture could impact upon cutting performance in the nursery and should be investigated as a component of future research in Trial 3.

Trial 2 demonstrated that differing varieties again responded differently to HWT. When assessed immediately following callusing, Pinot Noir, Merlot and Riesling generally formed significantly fewer root initials than less sensitive varieties including Sauvignon Blanc, Chardonnay and Shiraz. This result reinforced the view that sensitive varieties have potentially fewer roots following HWT, predisposing them to the potential for poor performance when exposed to moisture stress in the shade house or field nursery.

Analysis of shoot growth observations highlighted the difference between varieties sensitive to and tolerant of HWT. Within 28 days of potting, sensitive varieties characteristically demonstrate retarded growth on apical, second and tertiary buds, with some level of growth demonstrated on at least the first 5 buds down from the tip of the potted cane. Tolerant varieties demonstrate pronounced growth on the apical and second buds, with little or no bud development or shoot extension on the third or subsequent buds. This trend was also observed in Trial 1 and won't be examined in detail during Trial 3.

Examination of rootling data following lifting from the field nursery and grading returned similar trends to Trial 1. HWT exposure did not significantly influence long-term cutting survival where nursery management practices were good and vines were not subjected to moisture stress in the shade house or field nursery.

Results – Jacob's Creek Trial 3, 2002/2003

Notes: (1) All 2002/2003 trial data was subjected to Analysis of variance using Statistix 7. (2) Reference to 'Standard' harvest date cuttings infers that cuttings were harvested on the 10th of July 2002 for all varieties except Cabernet Sauvignon, which was harvested on the 17th of July 2002. Reference to 'Late' harvest date cuttings infers that cuttings were harvested on the 21st of August 2002 for Chardonnay and Pinot Noir, the 28th of August for Riesling and the 11th of September for Cabernet Sauvignon. (3) Reference to 'root initials' describes the number of root primordia observed per cutting immediately after the cutting has been incubated in a callus room for 21 days at 26^{0}C.

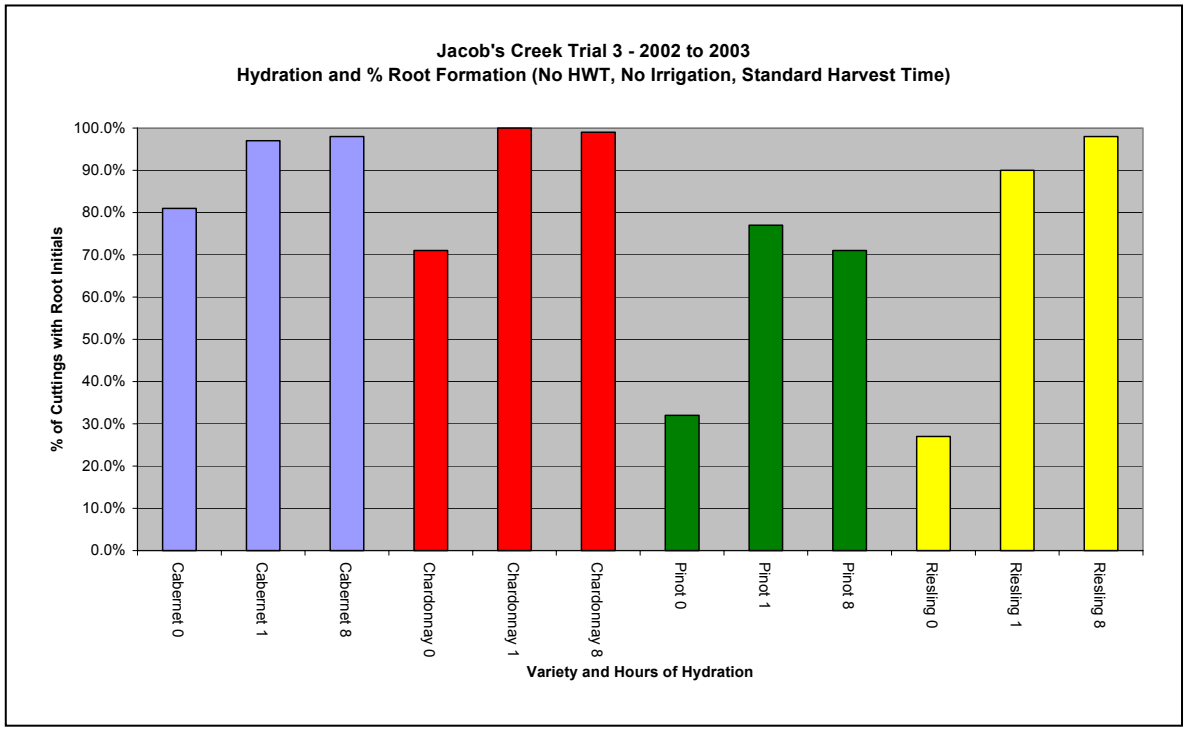
Data Capture

Trial 3 provided the first full test for the bar code driven data capture system for trial observations. All data associated with the assessment of root initials was recorded directly into a Denso BHT-5000 data capture unit through specifically tailored software and was downloaded into a PC without the need for manual transcription. This process generated 9600 lines of data by 4 data fields and saved approximately 3 weeks of data entry and verification time.

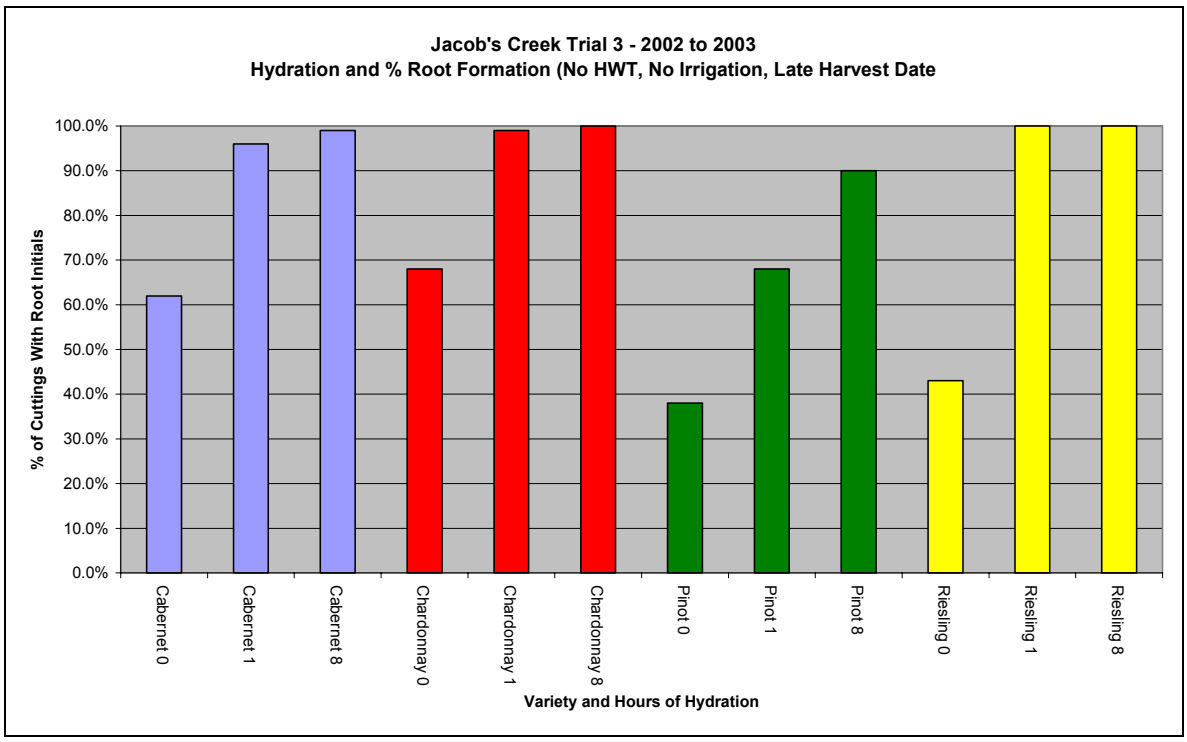
1. Harvest date, hydration and proportion of cuttings with root initials

When Trial 3 root initiation data was analysed in the absence of HWT and post vintage irrigation treatments, observations demonstrated that Standard harvest date cuttings of all varieties and hydration regimes returned cuttings with marginally fewer root initials than the equivalent group of Late harvest date cuttings, however, this difference was not statistically significant (p=0.6950 at 5%). Note Graphs 12 and 13 below.

Where all varieties and treatments are included, the Standard harvest date cuttings produced significantly fewer cuttings with root initials overall than the Late harvest date cuttings (p=0.0129 at the 5% level), although this result was marginal, involved mainly the '0 Hydration' treatments was and near the critical value for comparison of means.



Graph 12; Hydration, % Root Formation (Std. Harvest Time, No HWT, No Irrigation).



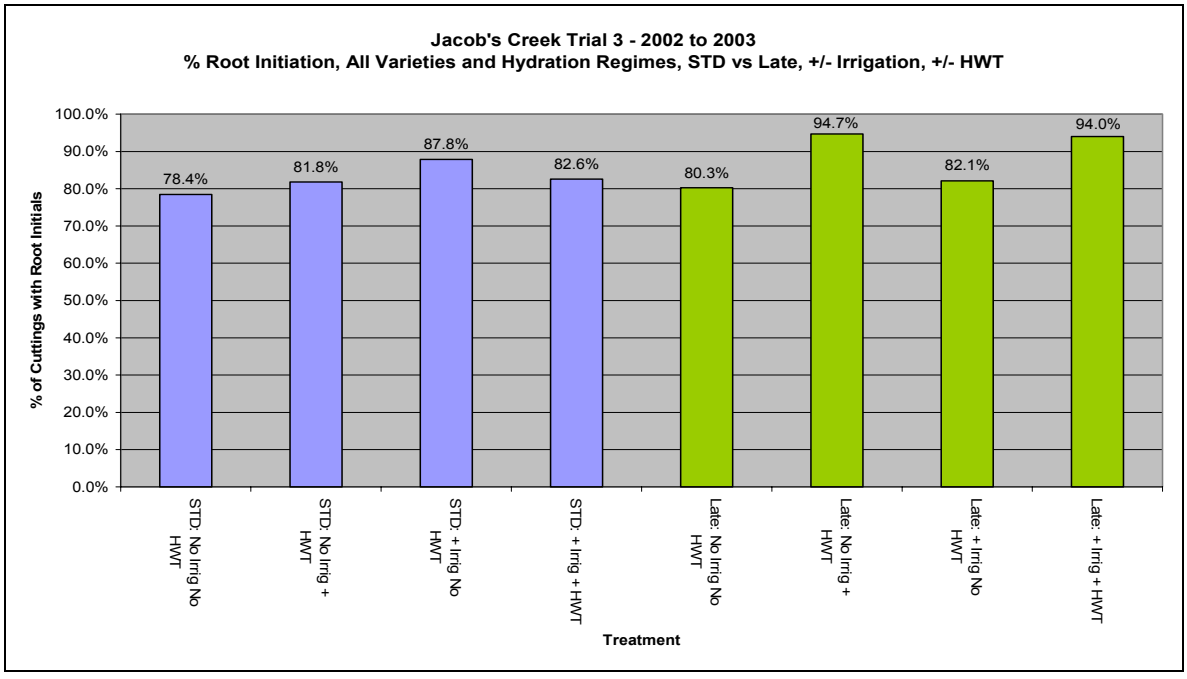
Graph 13; Hydration, % Root Formation Late Harvest Time, No HWT, No Irrigation).

In the absence of HWT, for all harvest date and supplementary irrigation regimes, Nil Hydration treatments consistently resulted in cuttings that produced fewer early root initials than cuttings receiving 1 or 8 hours of hydration ($p<0.001$ at the 5% level). 8 hour hydrated, non-HWT cuttings produced significantly more root initials than other treatment regimes. When HWT was applied, there was no significant difference overall between the number of root initials observed under 0, 1 or 8 hour hydration regimes.

2. Application of post-vintage, pre-cutting harvest source area irrigation

Initial analysis of post callusing root initiation data conducted in May 2003 grouped all varieties, hydration regimes and hot water treatments and indicated that source area plots receiving supplementary irrigation applications between vintage and cutting harvest returned significantly more cuttings with root initials overall than those not receiving additional irrigation ($p<0.001$ at the 5% level). Subsequent detailed analysis of the data later contradicted this assumption and proved that overall differences between irrigated and non-irrigated plots were not significant ($p=0.1668$ at the 5% level).

When the data was analysed for all varieties with separation of harvest date, irrigation and HWT regimes, late harvested HWT cuttings with and without supplementary irrigation produced significantly more cuttings with root initials than Standard harvest date cuttings with no supplementary irrigation and no HWT ($p<0.001$ at the 5% level). Contrastingly, Standard harvest date cuttings that received irrigation and were not HWT performed significantly better than their non-irrigated counterparts. Unfortunately, separation of treatment regimes supported the later analysis that supplementary irrigation effects were non-significant and did not reveal a consistent pattern with respect to root initial formation. Graph 14 (below) illustrates this result for all varieties and hydration regimes.



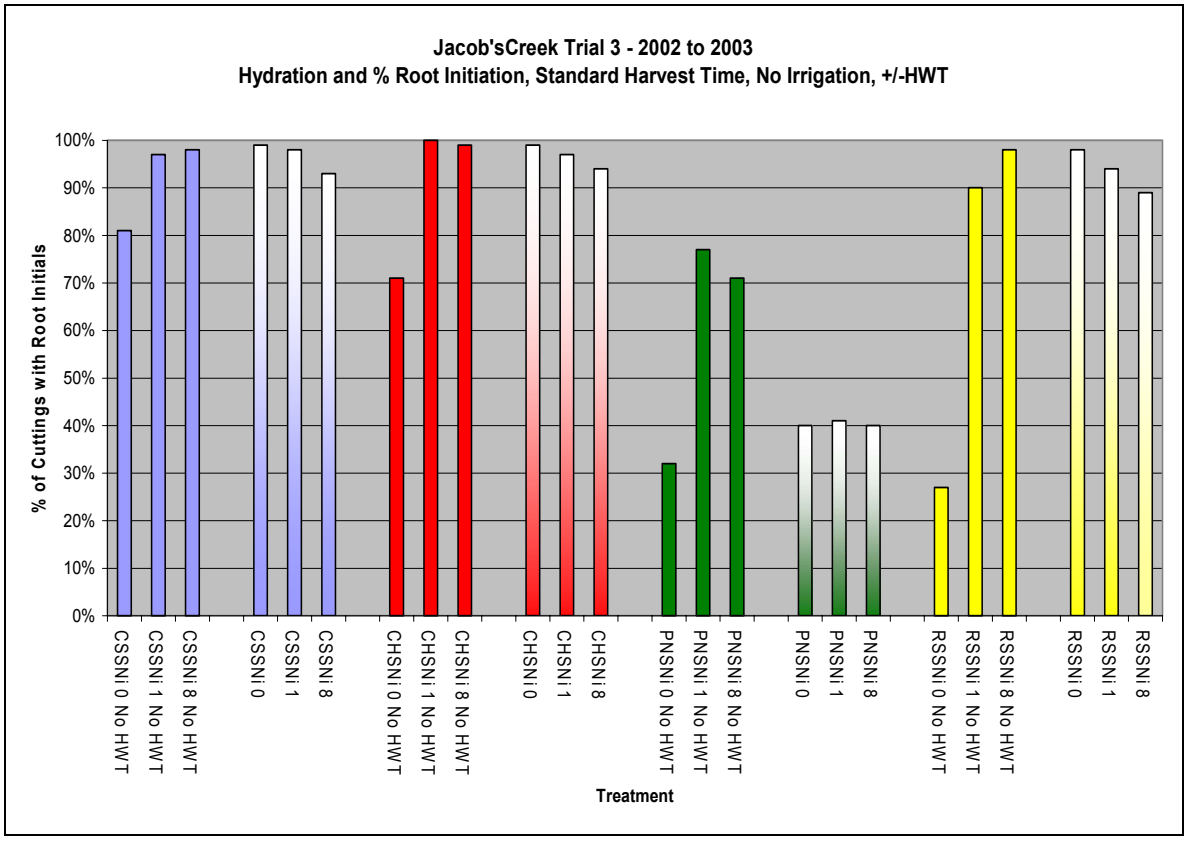
Graph 14; % Root initiation, all varieties and hydration regimes, +/- Irrigation & HWT

4. Influence of supplementary irrigation and HWT

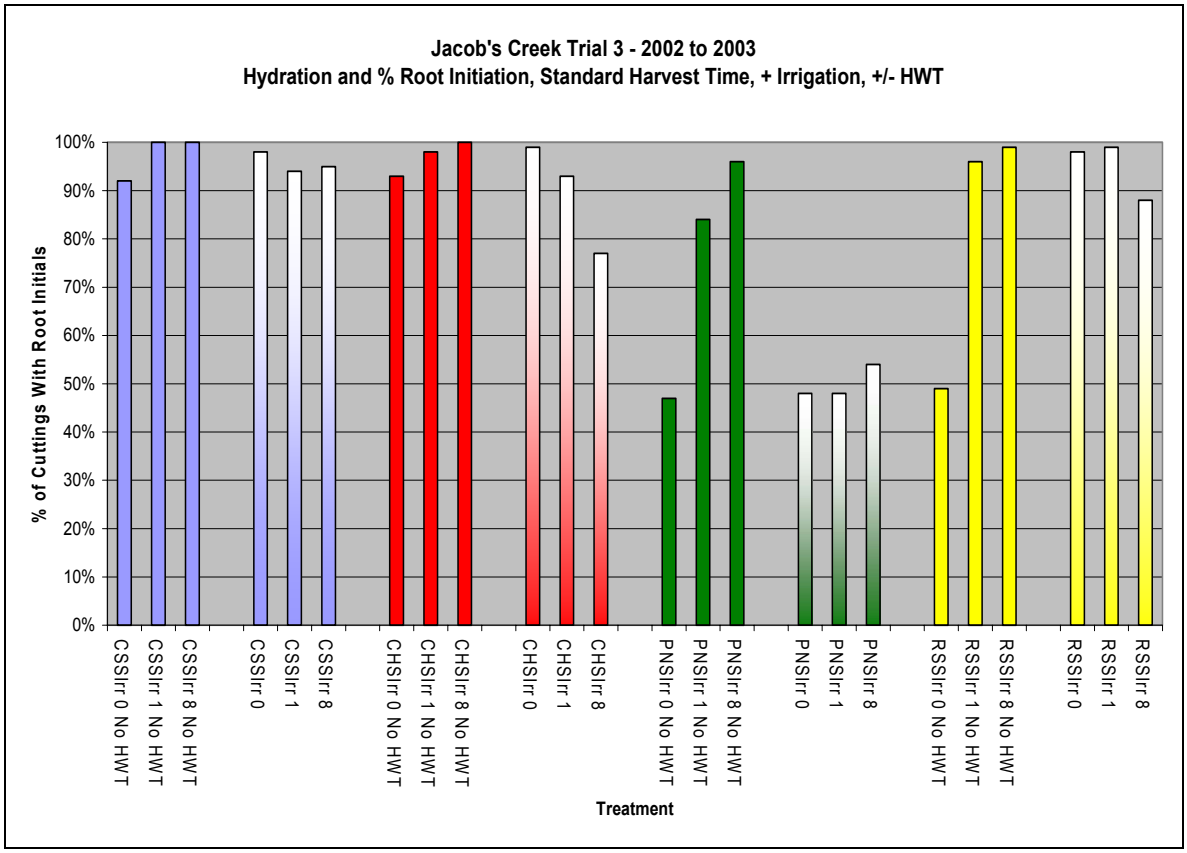
Graphs 15 to 18 illustrate the influence of HWT and hydration duration on the proportion of cuttings with root initials after callusing for all treatment combinations examined during Trial 3. Treatment means for Standard harvest date cuttings with and without irrigation are presented in Graphs 15 and 16 respectively. Treatment means for Late harvest date cuttings with and without irrigation are presented in Graphs 17 and 18 respectively. Treatment codes used in Graphs 15 to 18 have 5 elements and are constructed as follows:

CS[1] S[2] Ni[3] 1[4] No HWT[5]

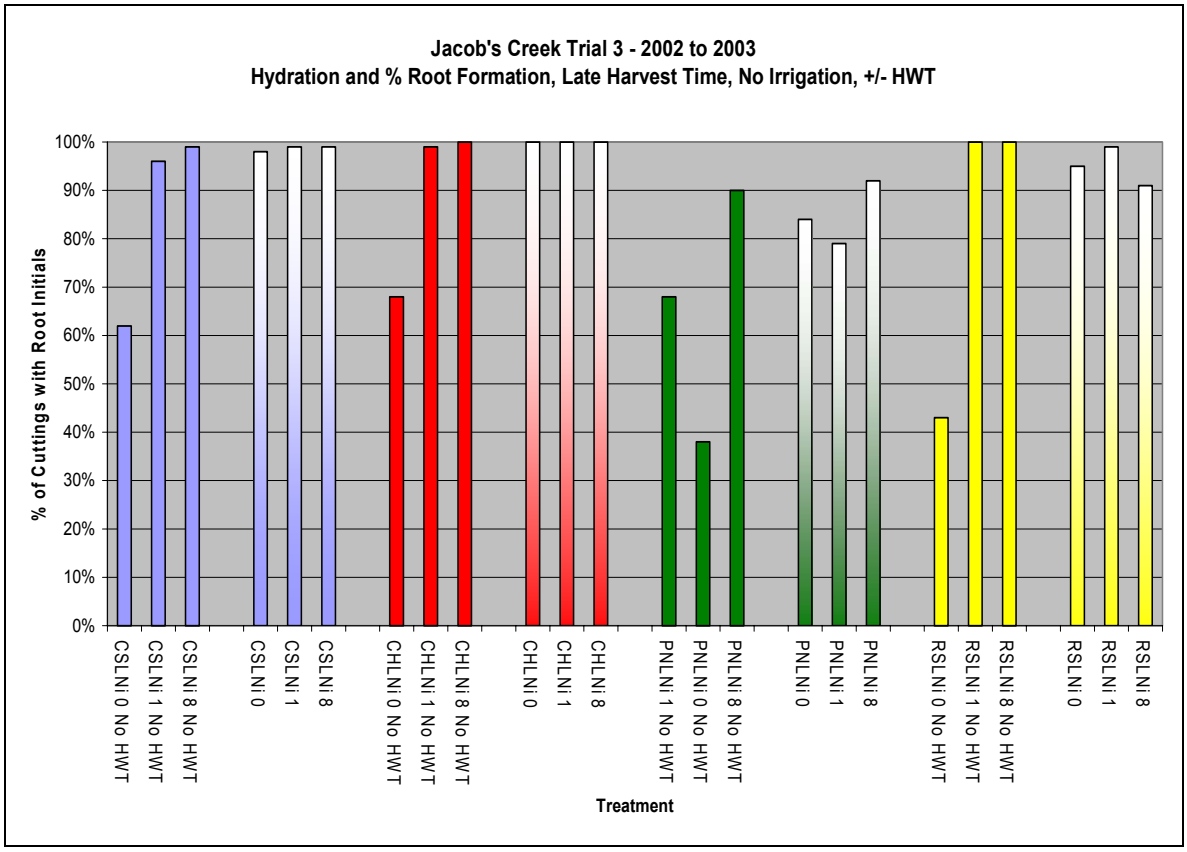
(1) Variety code, CS=Cabernet Sauvignon, CH=Chardonnay, PN= Pinot Noir, RS=Riesling (2) Harvest time, S=Standard, L=Late; (3) Irrigation code, Ni=not irrigated, Irr=irrigated (4) Hydration duration, 0=No Hydration, 1=1 hour, 8=8hour (5) HWT code, No HWT= No HWT applied, no reference in position 5 indicates that HWT was applied.



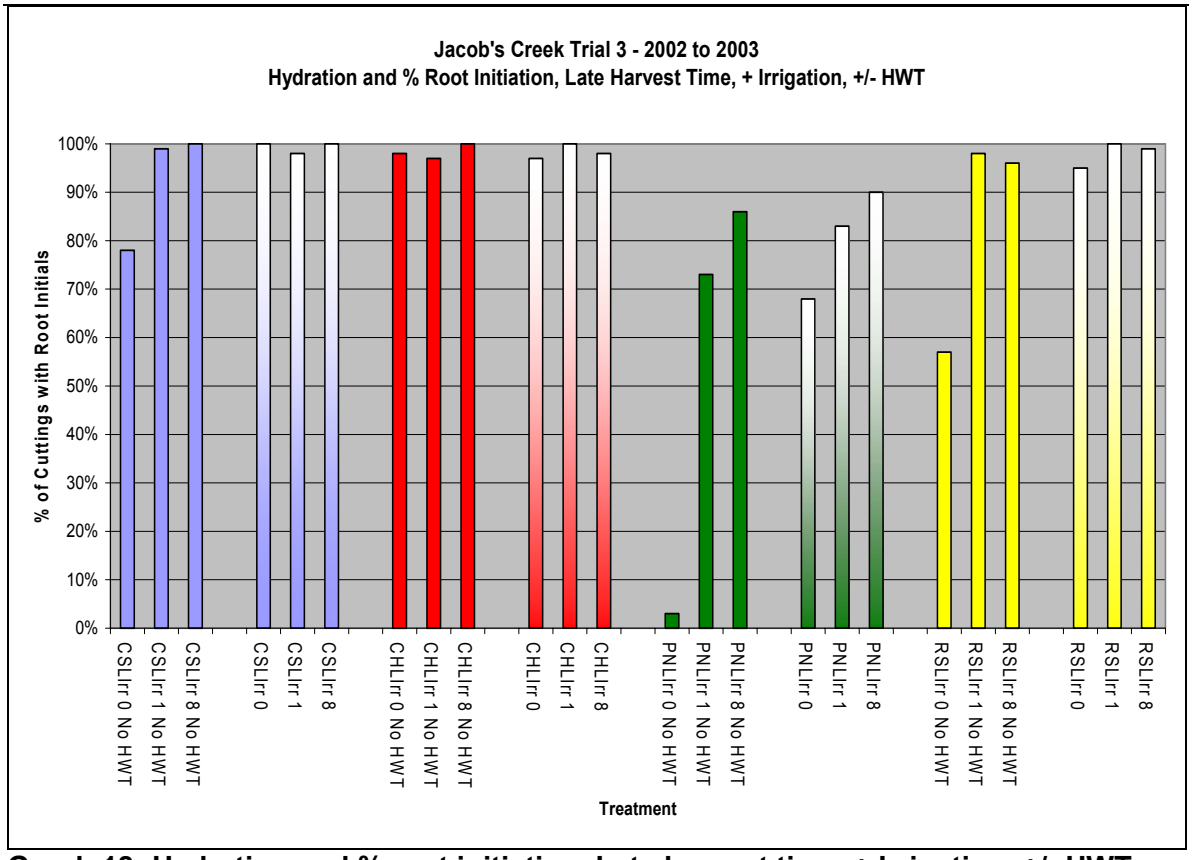
Graph 15; Hydration & % root initiation, Standard harvest time, 0 irrigation, +/- HWT



Graph 16; Hydration & % root initiation, Standard harvest time, + Irrigation, +/-HWT



Graph 17; Hydration and % root formation, Late harvest time, No Irrigation, +/- HWT



Graph 18; Hydration and % root initiation, Late harvest time, + Irrigation, +/- HWT

The greatest overall differences between the mean number of root initials observed for cuttings of each variety were recorded for Pinot Noir. All other varieties produced notable differences that imply the presence of an interaction between harvest date, supplementary irrigation and HWT, but on analysis, the differences recorded proved to be non-significant at the 5% level.

Analysis of variance for the Pinot Noir data revealed a number of significant differences between the mean number of root initials recorded for differing harvest date/irrigation and HWT regimes ($p<0.001$ at the 5% level), however there was no consistency or clear trend between the presence or absence of supplementary irrigation or HWT and the number of root initials recorded following callusing.

For example, Late harvest date, HWT Pinot cuttings not receiving supplementary irrigation returned significantly more root initials overall than the equivalent group of Standard harvest date cuttings (grouped means, all hydration levels 85%:40% respectively). Where supplementary irrigation was applied, Late harvest date, HWT Pinot cuttings also returned significantly more root initials overall than the equivalent group of Standard harvest date cuttings (grouped means, all hydration levels 80%: 50% respectively).

5. Harvest and grading of rootlings

Overall analysis of the final survival data for Trial 3 was confounded by the intermixing of several data sets at an unknown point between dispatch from Jacob's creek as potted vines and return to Jacob's Creek for grading as 1 year rootlings. The combination of missing data and variance within the remaining data sets raised doubts about overall data integrity, making reliance on observed trends unwise.

Cautious examination of the remaining intact data indicated that the effect of post-vintage supplementary source area irrigation could still be observed through changed mortality rates, particularly in the case of Riesling. Analysis of survival data for Standard harvest date, non-HWT rootlings of all varieties and hydration regimes indicated that non-irrigated Riesling had a significantly higher mortality rate of 25% when compared with the 6% mortality rate of similar irrigated Riesling rootlings ($p=0.3960$ at the 5% level). Survival rates for other Standard harvest date, non-HWT rootlings did not reveal significant differences between treatments.

While this outcome is disappointing, it still suggests that supplementary post-vintage source area irrigation may have a long-term effect on cutting performance. This bears further investigation in a drier environment where supplementary irrigation treatments are not confounded by rainfall and seasonal variation.

Discussion, Trial 3

Analysis of Trial 3 data served to further validate both the observations of Waite and May (1999) and the data returned by Trials 1 and 2. The overall outcome of this research leaves little doubt that differing vine varieties respond differently to the imposition of HWT and other cultural practices within the vine propagation process.

Trial 3 highlighted the early root initiation response of cuttings to differing harvest dates, with Standard harvest date cuttings performing marginally less well overall than the Late harvest date cuttings. This result was similar to that observed in Trial 2 and highlights the role of seasonal variation in cutting performance, particularly where post-vintage source area soil moisture levels may become deficient in dry seasons, or significant winter rainfall arrives later than mid-July. Contrastingly, with normal winter rainfall patterns experienced in Trial 1, Early and Standard harvest date cuttings performed better than their Late harvest date counterparts.

Trail 3 demonstrated that differing post-delivery cutting hydration durations produced variations in the early ability of cuttings to form root initials. In the absence of HWT, cuttings that were not hydrated recorded significantly fewer early root initials than those receiving 1 or 8 hours of hydration. When cuttings were subjected to HWT during Trial 3, this trend became less distinct with no significant differences then recorded between hydration regimes. This was possibly due to the combined influence of HWT on early root initiation and the total of 1 hour of hydration that cuttings inevitably receive during the HWT and cool-down process.

The role of post-vintage, pre-cutting harvest source area irrigation initially appeared to be statistically significant and beneficial to overall early root initiation performance, however, detailed analysis later disproved this association. While several statistically significant differences could be identified between treatment regimes, the presence or absence of supplementary source area irrigation did not return consistently beneficial root initiation trends overall. This result was disappointing and may have been be the product of interaction between rainfall and irrigation treatments during 2002.

The inconclusive outcome of Trial 3 with respect to supplementary irrigation treatments should not serve to discount the potential value of maintaining post-vintage source area soil moisture levels, particularly in drier regions or low rainfall seasons. The impact of post-vintage supplementary irrigation on cutting performance warrants further investigation in an environment where rainfall is less likely to impact on trial outcomes.

Conclusions – Jacob's Creek Trials 1, 2 and 3

Data from the 2000 to 2003 Jacob's Creek trial series clearly highlighted differing varietal sensitivities to HWT, the influence of varied propagation regimes and the influence of pre-cutting collection seasonal factors on the early development of vine propagules.

The comparative assessment of cutting root initiation immediately following 21 days of callusing at 26^0C provided a unique window of opportunity to capture measurable difference in the performance of cuttings to applied propagation regimes under controlled conditions. This technique consistently revealed varietal differences that would later become masked where cuttings were grown under optimal nursery management conditions.

Results obtained during the three Jacob's Creek trials are summarised under the following headings:

Cutting Harvest Date

Rainfall and seasonal variation significantly impacted upon the root initiation performance of cuttings harvested at dates ranging from early June (Early), to early July (Standard) and late August to early September (Late). During the relatively normal 2000 season, Standard harvest date cuttings recorded generally more root initiation than Early or Late harvest dates.

This contrasted with results obtained during 2001 and 2002, where winter rainfall was either low or late. Under these conditions, Standard harvest dates recorded comparatively low levels of root initiation when contrasted with the performance of cuttings following rainfall from the Late harvest dates.

Variations in early root development generally did not result in long-term impact on vine mortality and results suggest that cutting harvest dates between mid June and late July remain a good compromise in most cases. In dry seasons or seasons where rainfall is late, results suggest that delaying cutting harvest into August was not detrimental to cutting performance.

Cutting Handling Speed

The interval between cutting collection and cold storage offers the potential for cuttings to progressively dehydrate, particularly where ambient temperatures at the time of harvest are moderate, cuttings are exposed or they are subjected to the drying action of wind during transport over long distances.

Trial 1 examined the influence handling speed where cuttings were subjected to rapid handling (within 24 hours) versus delayed handling (5 days delay). In most cases, delayed handling did not result in significant reductions to root initiation performance under the relatively mild 2000 weather conditions. However, a notable exception was recorded where delayed handling in combination with HWT significantly reduced root initiation in Pinot Noir and only 6% of cuttings formed early root initials roots under this regime.

This observation was corroborated by anecdotal evidence, which cited an extreme case where Pinot Noir cuttings were harvested then stored under hot, dry conditions for 21 days prior to delivery to the nursery. The cuttings reportedly became so dehydrated that they required additional weight (bricks) to submerge them in a hydration tank and they performed dismally when propagated.

Results suggest that cutting dehydration following harvest plays a significant role in the suppression of root initiation. Rapid handling, delivery and cold-storage of cuttings with attention given to protection from dehydration is prudent as post-delivery hydration does not appear to fully compensate for cutting dehydration in transit.

Hydration Duration

Examination of hydration treatments applied to cuttings prior to cold storage consistently demonstrated that hydration durations beyond 8 hours did not necessarily result in improved early root initiation. Conversely, in the absence of HWT, cuttings receiving no hydration formed fewer root initials overall than those receiving hydration treatments of either 1 or 8 hours. When subjected to HWT, this relationship became less predictable as increasing hydration duration from 0 to 8 hours did not necessarily result in increased root initial formation.

Early root initiation responses observed due to interactions between HWT and hydration treatments were strongly influenced by varietal differences and seasonal factors during each of the three Jacob's Creek trials. When viewed in a generalised fashion, hydration durations of 1 or 8 hours appear to result in higher levels of early root initiation ±HWT, however there were notable exceptions in the case of sensitive varieties.

Varietal Responses

Throughout the Jacob's Creek Trial series, Cabernet Sauvignon, Sauvignon Blanc, Chardonnay and to a lesser extent Shiraz proved to be relatively tolerant of HWT and not particularly responsive to varying harvest date, handling or hydration regimes. This was evident through the production of significantly more root initials than more sensitive varieties and relatively little impact of HWT on early root initiation.

Pinot Noir, Merlot and to a lesser extent Riesling proved to be relatively sensitive to HWT and responded often significantly, to varying propagation protocols through a notable reduction in the formation of root initials.

At the conclusion of the Jacob's Creek trial series, it is apparent that previously unexplained cases of poor cutting performance following HWT may have been largely due to the combined effect of inherently different varietal responses to HWT and the manner in which treated cuttings were handled prior to delivery and managed in the nursery.

The impact of this interaction would have been particularly noticeable where the potential existed for young Pinot Noir, Merlot or Riesling vines to have fewer early root initials following HWT and were then exposed to prolonged moisture stress in the shadehouse or field nursery. Under these circumstances, susceptible varieties may have impaired access to soil moisture and would expire, resulting in batch failure, which was only in part due to the application of HWT.

Experience gained during the Jacob's Creek trials suggests that sensitive cutting varieties have the ability to recover from the impact of initially low root initiation following HWT if they are not subjected to soil moisture in the vine nursery or following transfer to a field nursery. Analysis of eventual mortality levels for rootlings treated during the Jacob's Creek trials suggested that there was little measurable long-term impact on vine health where good quality source material was used as cutting stock, vine nursery management practices are sound and field nursery management practices allowed sensitive varieties to recover from the influence of HWT.

Source Area Soil Moisture

When results obtained by Helen Waite (Program 3, pp 94-99) are viewed in conjunction with those obtained during Jacob's Creek Trials 1 and 2, the role of post-vintage source area soil moisture in cutting performance during propagation and HWT appears to be significant. Though results obtained during Trial 3 were inconclusive, it remains very likely that cutting performance will be improved in dry seasons or low rainfall regions by the provision of supplementary irrigation to source areas between vintage and cutting harvest. Further research is required to substantiate the benefit of supplementary irrigation, however, it is unlikely that appropriately scheduled supplementary irrigation would prove to be detrimental to cutting performance.

Recommendations.

Results gained during the Jacob's Creek trials have been integrated into existing HWT protocols to produce a revised HWT protocol document for distribution to VINA and AVIA members.

The revised protocol is an advisory document that recommends adherence to a HWT procedure that incorporates current best practice and the major outcomes achieved during the various program elements contained within SAR 99/4. The new protocol forms an integral component of VINA's 'Vine Industry Nursery Accreditation Scheme' and will serve as a new benchmark to assist vine nurseries in the successful execution of 'Effective, Efficient and Reliable Hot Water Treatments'.

The revised protocol document is provided in its entirety commencing on page 131 of this report.

References:

(See Appendix 3, pp. 146 to 149 for a compiled list of references)

5.2 - Program 2: Post - HWT Issues, Cold Storage

Department of Primary Industries, Victoria

P5.21

The Effect of Storage Treatments on the Viability of Grapevine Cuttings

Adam Wightwick[1], Sue McConnell[1], and Graeme Fletcher[1]

[1] Department of Primary Industries Victoria, Sunraysia Horticulture Centre, Box 905, Mildura, Victoria 3502.

Abstract

In 2001, Chardonnay grapevine cuttings received pre-storage treatment of hot water treatment or non hot water treatment as well as chlorination treatment (0, 10 or 100ppm). They were then placed in plastic bags with either venting at one end of the bag or no venting (sealed) and stored for 8 or 20 weeks. The cuttings in the bags with venting at one end were found to have less free moisture and a healthy earthy smell in comparison with the cuttings in the no vented bags. The cuttings from the vented bags also propagated better than the cuttings from the sealed bags. The chlorination treatment had no effect.

In 2002, Chardonnay grapevine cuttings received pre-storage treatment of hot water treatment or non hot water treatment. They were then placed in plastic bags with venting at one end of the bag, venting both ends of the bag or no venting (sealed) and stored for 10 or 20 weeks. Cuttings from the vented bags had a healthy earthy smell after both storage periods in comparison with the cuttings from the sealed bags, some of which had a wine smell. However propagation results were inconsistent. The cuttings in the bags with venting at one end propagated the best after 10 weeks storage however there were no significant differences between all treatments after 20 weeks storage.

Introduction

The mortality rate of grapevine cuttings following their treatment and storage continues to be a concern to the vine nursery industry (Waite and May, 1999). Often grapevine cuttings are kept in cool storage for long periods of time following treatment (eg hot water treatment). It is believed that these periods of storage are having a significant impact on the viability of the grapevine cuttings (Waite and May, 1999). Traditionally grapevine cuttings have been sealed in plastic bags and stored in a cool room at 0 – 2^{o}C. In the past this treatment has lead to significant amounts of post harvest decay syndrome and necrosis of the vine tissue leading to high mortality rates amongst the cuttings. It is believed that the amount of moisture within the bags has an influence over the decay and viability of the grapevine cuttings. Preliminary investigation has shown that the venting of bags during storage can reduce moisture levels and thus reduce the amounts of decay and necrosis.

The objectives of this two-year study were to assess the effect of storage bag ventilation on the viability of grapevine cuttings and then determine the optimum level of ventilation.

Materials and Methods

Pre-storage treatment of cuttings

Prior to being placed in cool storage grapevine cuttings were subjected to pre-storage treatments.

Year 1

14,800 Chardonnay grapevine cuttings were obtained from Victoria and Murray Valley Vine Improvement Association (VAMVVIA). Half of these cuttings received a one-hour hot water treatment. The remaining cuttings were placed in a control water hydration tank (non-hot water treated). Even numbers of cuttings were then placed in cool down tanks containing 0, 10, and 100 ppm of chlorine for 30 minutes.

Year 2

18,000 Chardonnay grapevine cuttings were obtained from VAMVVIA. Half of these cuttings received a one-hour hot water treatment. The remaining cuttings were placed in a control water hydration tank (non-hot water treated). Cuttings were then placed in cool down tanks for 30 minutes.

Ventilation treatments

After receiving the pre-storage treatments both the hot water treated and non-hot water treated grapevine cuttings were sealed in plastic bags.

Year 1

200 grapevine cuttings were sealed in each bag. A venting treatment was applied to half of the bags by punching two 1cm-diameter holes in one end of the bag. There were three bags for each pre-storage treatment (hot water treated (H), non-hot water treated (N), chlorination – 0, 10, and 100 ppm), ventilation treatment (sealed (S), vented (V)), and storage period (8 and 20 weeks) combination. The bags of grapevine cuttings were then placed in a cool room at 2^{o}C for 8 and 20 weeks.

Year 2

500 grapevine cuttings were sealed in each bag. Three different ventilation treatments were then applied being sealed (no ventilation), vented one end and vented two ends. Venting was achieved by punching 10 evenly spaced 1cm diameter holes along the sealed end/s of the bag. There were three bags for each pre-storage treatment (hot water treated (H), non-hot water treated (N)), ventilation treatment (sealed (S), vented one end (V1), vented two ends (V2)), and storage period combination. The bags of grapevine cuttings were then placed in a cool room at 2^{o}C for 10 and 20 weeks

Post-storage assessments

After both cool storage periods (Year 1 – 8 & 20 weeks, Year 2 – 10 & 20 weeks) half of the bags of grapevine cuttings were removed from the cool room. The bags of grapevine cuttings were then opened using a knife and assessed for:

Year 1

- Smell – an assessment of initial smell rated as earthy, wine or musty.
- Fungal growth – a visual assessment of fungal growth.
- Necrosis – buds from randomly selected grapevine cuttings in each bag were cut open and visually assessed for brown staining.

Year 2

- Smell – assessment of initial smell rated as earthy, wine, or musty.
- Fungal – a visual assessment of fungal growth (no growth or on less than 25% of the cuttings, growth on 25 – 80% of the cuttings, or growth on greater than 80% of the cuttings) and severity (weak, mild, or strong).
- Moisture – a visual assessment of moisture in the bag rated as dry, moist, or with free water.

Planting of grapevine cuttings

After the post storage assessments, bundles of grapevine cuttings were randomly planted in sand filled propagation beds containing heat mats set at 25^{o}C. The grapevine cuttings were planted so that approx half of the length of the cuttings were covered by sand. Grapevine cuttings were watered weekly to keep the sand moist and remained in the propagation beds until the average shoot length was approximately 10 cm.

Year 1

One bundle of 20 randomly selected cuttings was planted into the propagation beds from each post-storage treatment (hot water treated, non-hot water treated, chlorination treatment – 0, 10, and 100 ppm) and ventilation treatment (sealed, vented) combination.

Year 2

Four bundles of 10 randomly selected cuttings were randomly planted into the propagation beds from each post-storage treatment (hot water treated, non-hot water treated) and ventilation treatment (sealed, vented one end, vented two ends) combination.

Post-planting assessments

After the shoot lengths reached approximately 10cms the bundles of grapevine cuttings were removed from the propagation beds and assessed for:

- Budburst – visual assessment of the number of cuttings in each bundle that had reached bud burst.
- Root growth – visual assessment of the number of cuttings in each bundle with strong, medium, weak, and zero root growth. Also in Year 2 the roots from each bundle were removed and weighed.
- Shoot growth – the shoots from each bundle were removed and weighed.
- Callusing – visual assessment of the number of cuttings in each bundle with no roots but some callusing on the base of the cutting.

Statistical Analysis

The results were analysed in GenStat (Version 6.1) by Dr John Reynolds (Biometrician, Department of Primary Industries, Victoria, Australia) using the Analysis of Variance (ANOVA) model.

Results

Pre-planting assessments

Following their removal from cool storage, the bags of grapevine cuttings were cut open and assessed for the initial smell. A wine smell is an indicator of post harvest decay whilst an earthy smell is an indicator of healthy viable cuttings. In 2001 after 20 weeks of storage (Fig. 1) it was observed that a much higher proportion of the vented bags (94.44%) had a healthy earthy smell compared with the sealed bags (11.11%). This pattern was also observed in 2002 when the level of ventilation was increased. After both 10 and 20 weeks of storage (Fig. 2 and 3) 100% of the vented one end and vented two end bags produced an earthy smell.

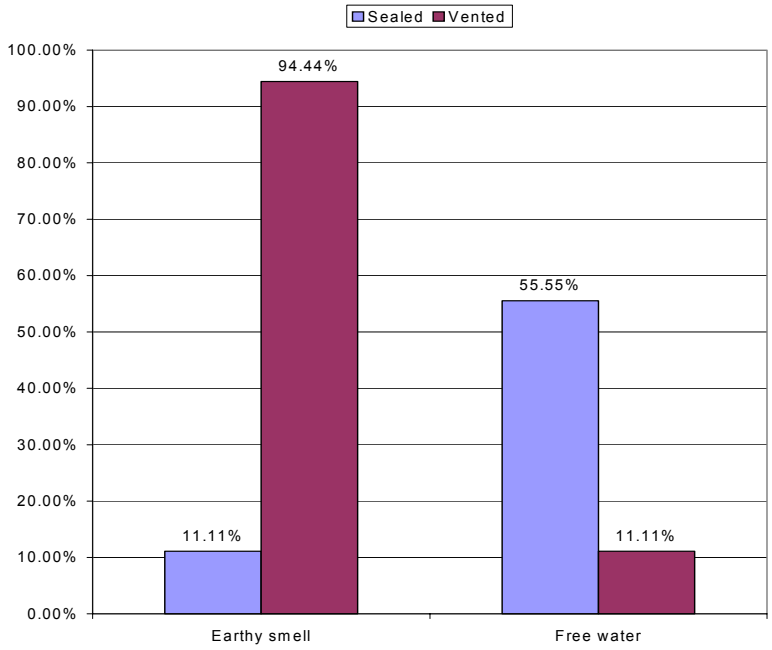


Figure 1 – 2001 Week 20 percentage of vented and sealed bags with an earthy smell and with free water

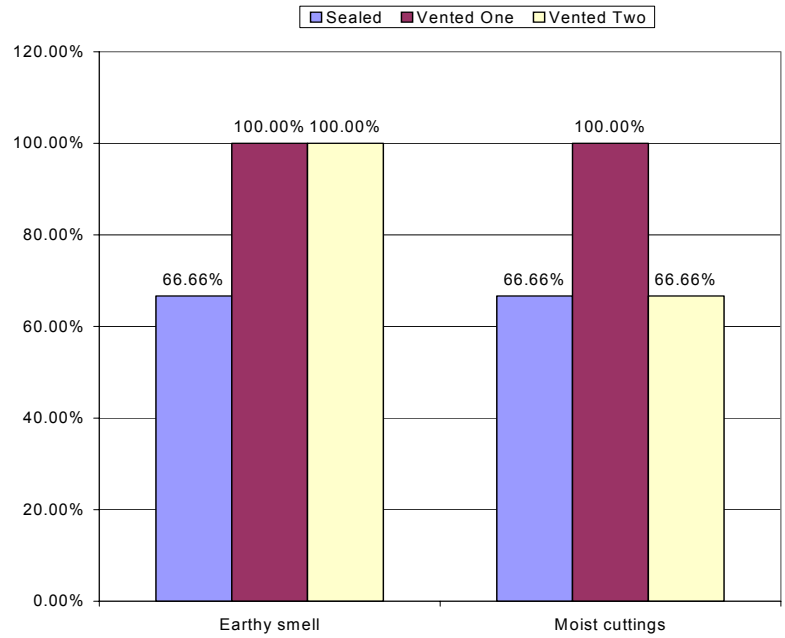


Figure 2 – 2002 Week 10 percentage of sealed, vented one end and vented two end bags with an earthy smell and with moist cuttings

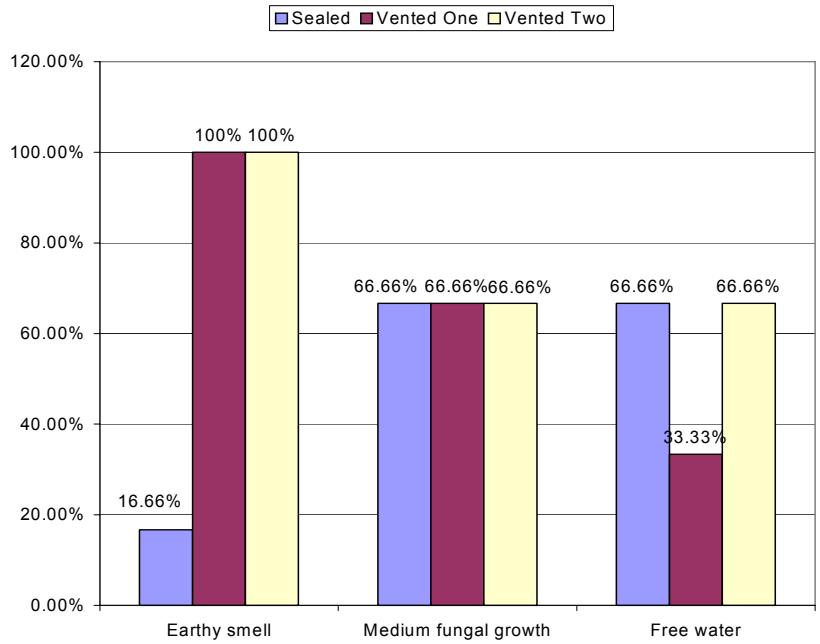


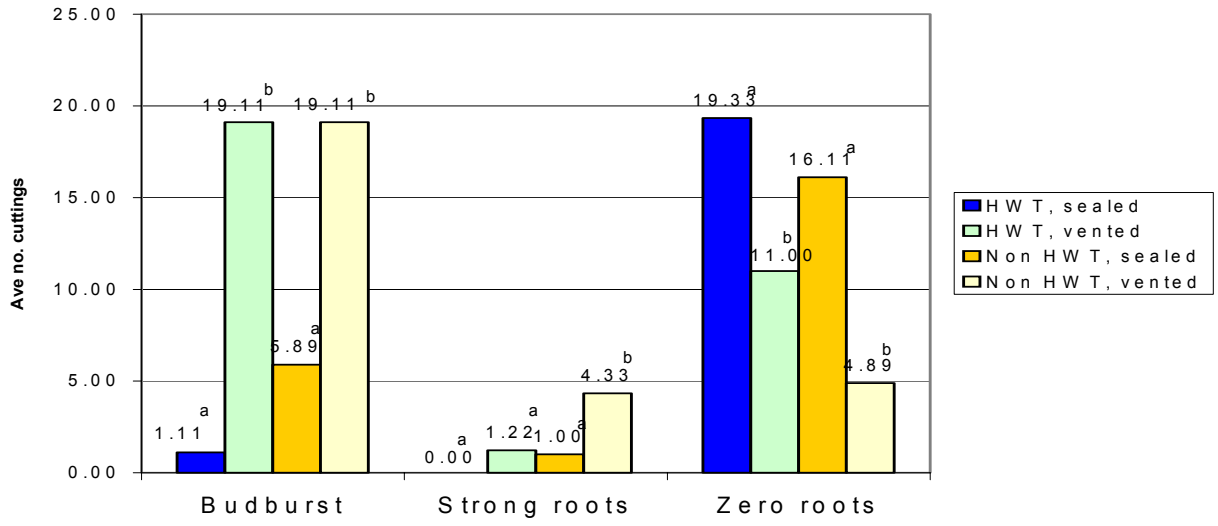
Figure 3 – 2002 Week 20 percentage of sealed, vented one end, and vented two ends bags with an earthy smell, medium fungal (growth on 25-80% of cuttings), and with free water

The moisture within the bags was also assessed. High moisture content within the bag is likely to lead to higher levels of decay. In 2001 (Fig. 1) the sealed bags had higher moisture levels than the vented bags with 55.55% of the sealed bags containing free water. After 20 weeks of storage in 2002 (Fig. 3) the vented one end bags (33.33%) had the lowest proportion containing free water, whilst both the sealed (66.66%) and vented two end bags (66.66%) had equal proportions of bags with free water.

In 2001 it was found that the chlorine treatments applied did not have any effect on the bags of cuttings. In both 2001 and 2002 fungal growth was evident on the cuttings however this growth did not affect propagation.

Post-planting assessments

After the cuttings were removed from the propagation beds their survival rate and strength of growth were assessed. In 2001 (Fig. 4 and 5) the cuttings from the vented bags performed better than those cuttings from the sealed bags. This is shown by the average number of cuttings per bundle that reached budburst (Fig. 4) where HV (19.11) and NV (19.11) were significantly ($P > 0.05$) greater than HS (1.11) and NS (5.89). This is further supported by looking at the proportion of cuttings per bundle with zero roots (Fig. 4) where HS (19.33) and NS (16.11) produced significantly ($P > 0.05$) greater proportions than HV (11.00) and NV (4.89).



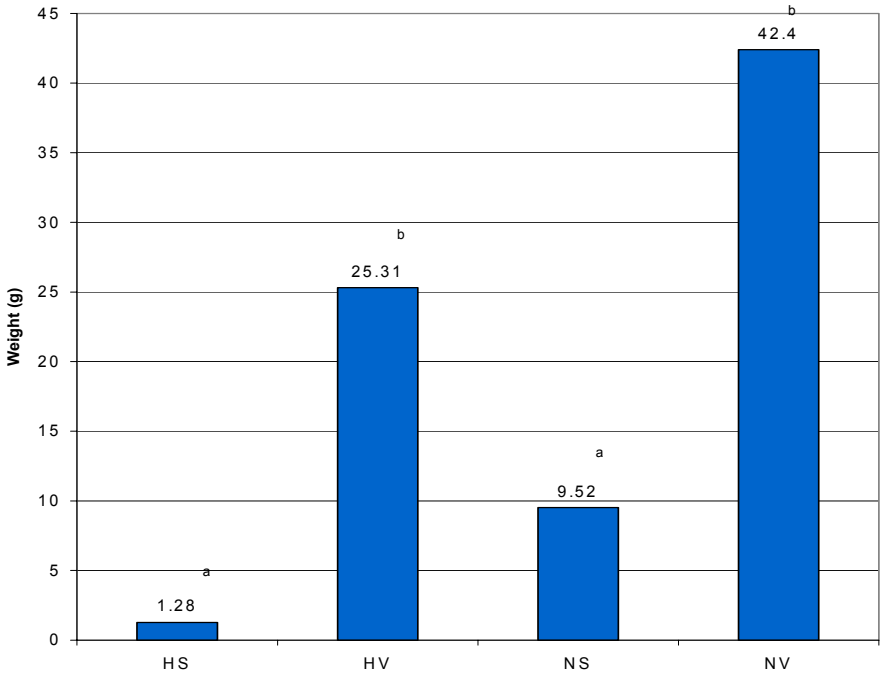


Figure 5 – 2001 Week 20 average shoot weights (g) from bundles of cuttings

In 2002, for the 10-week storage period (Fig. 6) the cuttings from the vented one end bags that had received hot water treatment performed the best. This is shown by the shoot weights (g) where HV1 (37.29) was significantly (P > 0.05) greater than NS (28.42), HV2 (27.59), HS (25.57), NV1 (16.58) and NV2 (15.06). HV1 (14.00) also produced root weights (g) significantly (P > 0.05) higher than HV2 (10.57), NS (9.51), HS (9.28), NV2 (5.02), and NV1 (4.80).

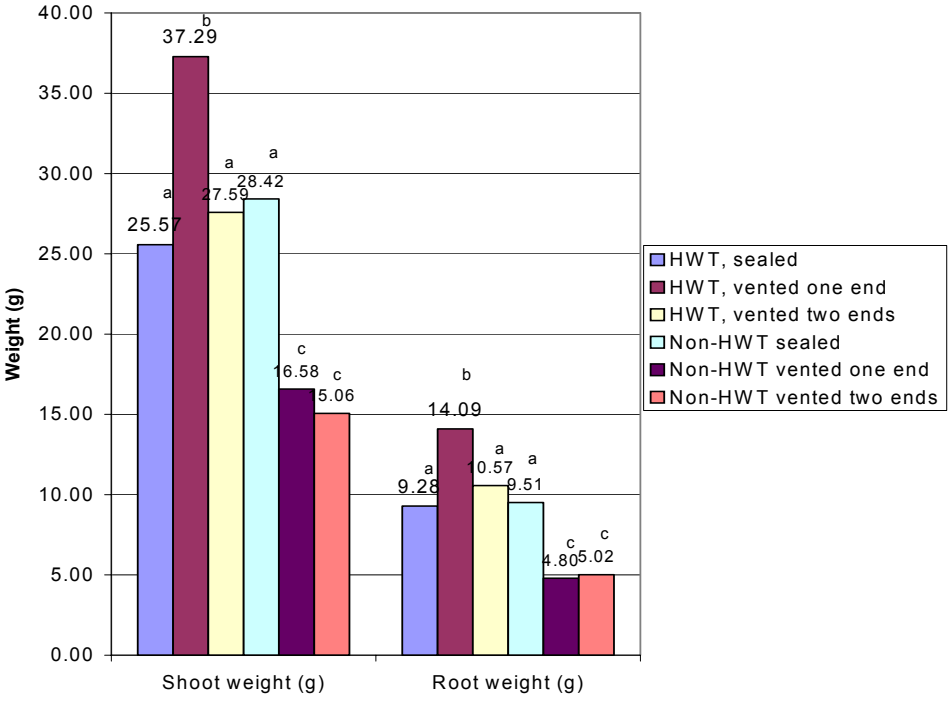


Figure 6 – 2002 Week 10 average shoot weights (g) and root weights (g) from bundles of cuttings

The 20-week storage period cuttings in 2002 had a low survival rate and no statistically significant differences were detected between treatments (Figure 7).

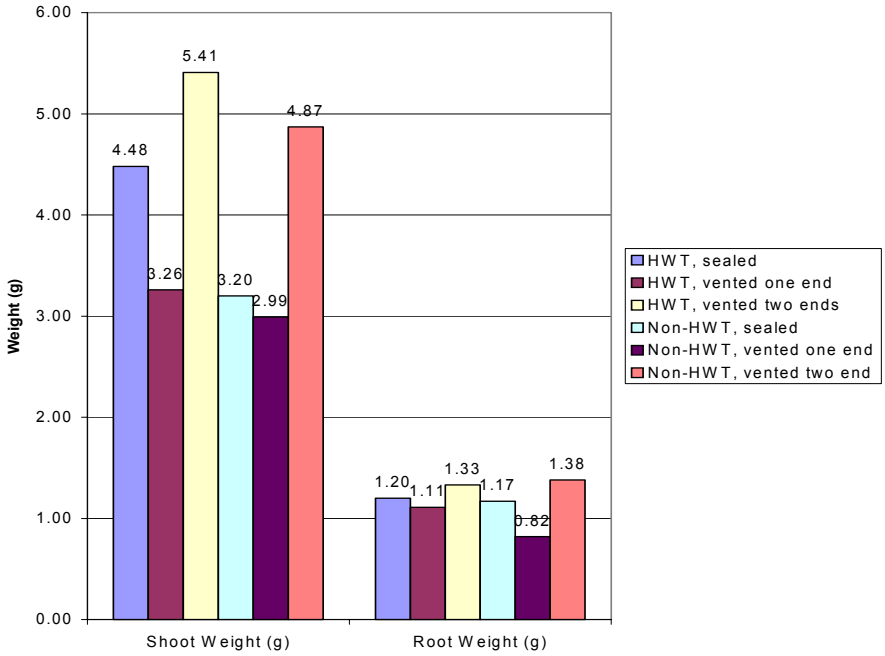


Figure 7 – 2002 Week 20 average shoot weights (g) and root weights (g) from bundles of cuttings

Discussion

In 2001 it was found that the chlorine treatments applied did not have any effect on the cuttings. For this reason, no chlorine treatments were applied in 2002.

In 2001 the pre-planting assessments showed that the vented bags typically produced a healthy earthy smell and contain little free water, this suggested that the cuttings from these bags would perform much better than those from the sealed bags. The post-planting results confirmed this with both the hot water treated and non hot water treated cuttings from the vented bags performing the best.

The results from the assessments of the bags suggest that venting can reduce both decay and moisture levels leading to the improved viability of grapevine cuttings. However the results from 2002 were inconsistent with some of the bags which were vented at both ends having problems with moisture. This may have been due to the position of this treatment in the bins during storage.

The pre-planting results for the 10-week storage period in 2002 showed that the bags for all of the ventilation treatments contained little moisture. This suggested that the cuttings would show a similar performance with the vented one end treatment performing the best. The post-planting results did not confirm this theory; the results were inconsistent however one of the vented one end treatments (HV1) did perform better than the rest. Some variability between bundles of grapevine cuttings for each treatment was observed so it is possible that not all of the bundles received the same heat conditions whilst in the propagation beds. This may be one explanation for the inconsistent shoot and root weight results.

The pre-planting results for the 20-week storage period in 2002 showed that the sealed and vented two end bags contained more moisture than the vented one end bags. This suggested that the cuttings for the vented one end bags would perform the best. This was not shown by the post-planting results.

The 20-week storage period cuttings in 2002 were planted late in the year (10th April). As a result the cuttings took much longer to shoot and the survival rate was a lot lower than with the 2001 and the 2002 10-week storage period cuttings. As a result of the low survival rates no statistically significant differences were detected.

Conclusions

- Compared with using sealed bags the venting of bags of grapevine cuttings during storage can:

    - Reduce the incidence of post harvest decay syndrome;
    - Reduce moisture levels within storage bags; and
    - Can lead to improved survival rates and growth when the grapevine cuttings are propagated.

- Further research is required to determine and validate the optimum level of venting for bags of grapevine cuttings during storage.

Acknowledgements

We wish to thank Ryan Baddock and Lance Hancock from VAMVVIA for their assistance in processing cuttings and Christina Porteous, DPI, for her technical assistance. Special thanks to Dr John Reynolds, DPI biometrician, for analysis of the results.

References:

(See Appendix 3, pp. 146 to 149 for a compiled list of references)

5.3 - Program 3: Nursery Practice & Cellular Level Effects of HWT

University of Melbourne

P3.1 Effects of pre-HWT nursery practices on dormant cuttings of *V. Vinifera*

P3.11:

The Effects Of Pre Hot Water Treatment Hydration and Order of Nursery Operations On Cuttings Of *Vitis vinifera* cvs. Cabernet Sauvignon and Chardonnay

Helen Waite[1] and Peter May[2]

[1]Helen Waite, Institute of land and Food Resources, University of Melbourne, Dookie College, Victoria, 3647, hwaite@unimelb.edu.au

[2]Peter May, Burnley College, 500 Yarra Boulevarde, Richmond Victoria 3121

Abstract

This study examined the effects of hot water treatment (HWT) at 50^0C for 30minutes, order of HWT and storage (store/HWT & HWT/store), and 3 hydration times (0, 4 & 15 hrs) on root and shoot development and final condition in dormant cuttings of Cabernet Sauvignon and Chardonnay. Although there were significant ($P<0.05$) treatment effects on callus development and root development in both varieties at callusing time, there were no differences between any of the treatments in either variety at final assessment when the vines were well established in cardboard plant bands and ready for sale. The benign conditions of the protected environment of the glasshouse and shade house in which they were grown may have enabled the cuttings to recover from the stresses imposed by the various treatments. Had the cuttings been grown on in a field nursery as is sometimes the practice, there may have been differences between treatments at final assessment.

Introduction

Some Australian nurseries report that grapevine propagating material that is HWT after cold storage is less likely to suffer a loss of quality than material that is HWT before cold storage (Wright pers. comm. 1997). However Wample (1997) stated that Cabernet Sauvignon cuttings that were stored at 3-4°C after HWT showed better root and shoot development than those treated before storage. Wample (1997) also suggested that the results of that trial held only for Cabernet Sauvignon grown in Washington State where winters are very cold and that other cultivars in other climates may perform differently.

In addition, it is generally accepted by the vine nursery industry in Australia that the standard practice of soaking propagating material overnight is beneficial and enhances the tolerance of cuttings to HWT (Nicholas, Chapman & Cirami 1992; Wright pers. comm. 1997). However there is indirect evidence that pre soaking plant material that is to be hot water treated may reduce tolerance to HWT (Baker 1962). Most other researchers investigating HWT (von Broembsen & Marais 1978; Orffer *et al.* 1979; Orffer & Goussard 1980; Burr *et al.* 1989; Burr et al. 1996; Wample 1997) do not mention periods of pre HWT soaking, although Wample *et al*. 1991 mention a 30 min. pre HWT soaking.

During the course of previous work (Waite & May 1999) it was observed that cuttings removed from the hydrating tank after overnight soaking had changed colour from a light, bright brown to a dull black and that the buds tended to be soft and mushy. On the basis of this evidence and the indirect evidence of Baker (1962) it was thought that soaking had the potential to damage grapevine cuttings. A small preliminary trial was designed to test the effects of pre HWT soaking on the quality of Semillon cuttings. In this preliminary trial 3 bundles of 10 Semillon cuttings were soaked for 0, 6 and 15 hours prior to HWT and callused in clean coarse sand. They showed suppression of rooting after 6 and 15 hours soaking compared with no soaking (Figure 1). These observations suggested that a more thorough examination of the effects of HWT, soaking and storage on the performance of grapevine cuttings was warranted.



Figure 4.1: Callused Semillon cuttings subjected to 0, 6 & 15 hours hydration prior to callusing.

Cabernet Sauvignon and Chardonnay were chosen as they had been used in the previous trial and, in the case of Cabernet Sauvignon, to use the same variety as Wample (1991) in order that some comparison might be drawn between that work and this study. A shortage of propagating material prevented the inclusion of Pinot Noir in this study.

Materials and Methods

A total of 900 cuttings, 450 each of the varieties Cabernet Sauvignon and Chardonnay were collected from the Victorian and Murray Valley Vine Improvement Association (VAMVVIA) mother vine source blocks at Irymple in north west Victoria on 21/6/1999. The cuttings were selected to conform to VAMVVIA standards; 7-12mm in diameter and approximately 350-400mm long, without obvious signs of disease or damage. The cuttings were allocated at random to 90 bundles of 10 cuttings, 45 bundles of each variety. Fifteen bundles (150 cuttings) of each variety were assigned at random to either no HWT, pre storage HWT or post storage HWT. Each group of 15 bundles was further divided into 3 groups of 5 bundles (50 cuttings) and subjected to 0, 4 or 15 hours hydration in the commercial hydration tanks at the VAMVVIA HWT facility. Clean potable water was used as per standard industry practice.

Following hydration, the 15 bundles of each variety that were to undergo pre storage HWT were treated at 50°C for 30 min. as part of a commercial lot at the VAMVVIA HWT facility at Irymple. On removal from the HWT tank the cuttings were immediately plunged into a cool down tank of clean potable water at ambient temperature for 30 minutes. The cuttings were then removed from the cool down tank and allowed to drain until there was no free moisture on the surface of the cuttings. All cuttings, including the group for post storage HWT and the group that did not receive any HWT, were then sealed in new plastic bags and stored in the VAMVVIA cool room at 1-2°C. All cuttings were remove from storage on 24/8/99 and left in the bags overnight to allow the material to stabilize to ambient temperature (approx. 10-12°C). The following day (25/8/99) the 15 bundles of each variety to be HWT post storage were HWT (50°C/30min.) at the VAMVVIA facility following the same protocols that were used for the pre storage HWT bundles.

All cuttings were then packed in moist vermiculite in new polystyrene vegetable boxes with the top cut out and incubated in the callusing room at Boulevarde Nurseries Pty Ltd, Irymple at 27°C (+/-0.5°C) and 95% humidity for 2 weeks as per standard industry practice. The cuttings were removed from the callusing room on 8-9/9/99, assessed and potted into cardboard plant bands (bottomless containers 50mm square) using a standard nursery potting mix and placed in a glasshouse with other potted grapevine cuttings and cared for in the same manner. Fungicidal sprays were applied at regular intervals and the shoot growth trimmed once with a brush cutter. By the time of final assessment on 6/10/99 the cuttings had become established in the plant bands and were hardened off ready for removal to the holding area for saleable plants.

Assessment criteria

The results of an earlier experiment (Waite & May 1999) demonstrated only weak relationships between callus and root development and root development and final condition in all three varieties that were evaluated. However callus development and root initiation are widely regarded by industry as indicators of cutting viability and development potential. Consequently it was decided to assess both callus and root development in this experiment. Callus development was assessed by estimating the percentage of callus formed around the basal end of each cutting. A complete ring of well-developed callus tissue was scored as 100%. For cuttings where the callus did not form a complete ring the score was calculated as a percentage to the nearest 5%. Root initials for each cutting were scored as 1=absent, no visible sign of root initiation; 2=present, root initials present but less than 3mm long; and 3=extended, root initials present and longer than 3mm. Means of callus and root development were calculated for each bundle of 10 cuttings.

Since the cuttings in this experiment were propagated in a commercial nursery specializing in container grown grapevine rootlings, final condition was assessed at the time the cuttings would normally be ready for sale as green potted vines for planting in the vineyard in late spring or early summer. The cuttings were assessed on 6/10/99 when they had been hardened off and were ready for planting. They were assessed as either “A” grade, with a well developed root system emerging from the pot and two or more well developed shoots; “B” grade, with a strong root system that had not yet emerged from the pot and at least one moderately well developed shoot; “C” grade, with poor root and shoot development, or dead. “A” grade cuttings were assigned a score of 3, “B” grade cuttings were scored as 2 and “C” grade or dead cuttings were scored as 1 since for commercial purposes, both groups are not saleable and are discarded.

Data Analysis

Data was analysed using the Minitab statistical package and Microsoft Excel 97. Details of the analyses used are given in the results section. Any Minitab printout will be indicated by the use of Courier font.

Results and Discussion

The results of a previous trial (Waite & May 1999) showed different responses to HWT between varieties, therefore the results for Chardonnay and Cabernet Sauvignon in this experiment are reported separately.

Chardonnay

Initially the data was analysed using a General Linear model ANOVA with the factors HWT and hydration. The levels of HWT were no HWT, HWT before storage and HWT after storage. The levels of hydration were no hydration, 4 hours hydration and 15 hours hydration.

Callus development

Although there was a significant main effect of HWT protocols on callus development in Chardonnay in this experiment (Table1), the interpretation of this effect is hampered by a significant interaction between HWT protocols and hydration (see interaction plot below). Discussion of these results is therefore based on a one-way ANOVA with 9 individual treatments covering all possible combinations of HWT protocols and hydration times (Table 2). Means separation was performed using Tukey's pairwise comparisons test ($P<0.05$) (Appendix 1).

Table 1: ANOVA table for the effect of HWT protocols and hydration time on callus development in Chardonnay

Source	DF	Seq SS	Adj SS	Adj MS	F	P
hwt2	2	1815.07	1815.07	907.53	9.98	0.000
soak2	2	359.95	359.95	179.97	1.98	0.153
hwt2*soak2	4	2131.55	2131.55	532.89	5.86	0.001
Error	36	3274.99	3274.99	90.97		
Total	44	7581.56				

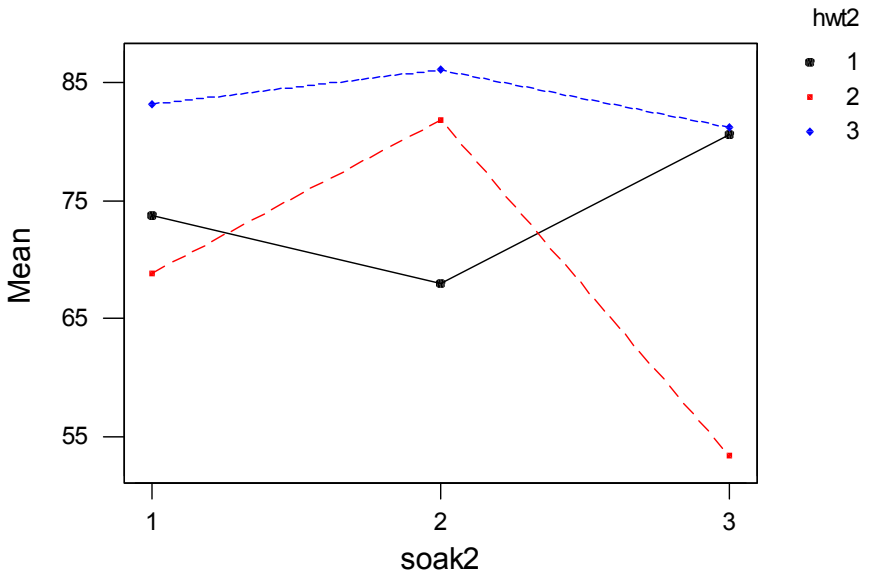


Figure 2: Interaction plot for main effects HWT protocols and hydration times on callus in Chardonnay cuttings. (Key: soak, 1=0hrs, 2=4hrs, 3=15hrs; HWT, 1=HWT/store, 2=store/HWT, 3=no HWT/store)

Table 2: One-way ANOVA table for effect of 9 treatment combinations of HWT protocols and hydration time on callus development in Chardonnay

Source	DF	SS	MS	F	P
treat_1	8	4306.6	538.3	5.92	0.000
Error	36	3275.0	91.0		
Total	44	7581.6			

The development of callus tissue in Chardonnay in this experiment was consistently, but not always significantly (P<0.05) greater in HWT cuttings that were stored before HWT than either the group that was not HWT or the group that was HWT before storage regardless of hydration time. In the post storage HWT group there was also less variation in callus development between hydration times than for the group that was HWT before storage or the group that was not HWT. There was a significant difference (P<0.05) in callus development between cuttings subjected to 4 and 15 hours hydration and HWT before storage. The cuttings that were hydrated for 4 hours showed the most advanced callus development (82%) compared to the cuttings that were hydrated for 15 hours (53%). However neither group was significantly different (P<0.05) to the group that received no hydration (69%) (Table 3).

Table 3: Chardonnay – means of callus development

Hydration time hrs	HWT/store	Store/HWT	Store/no HWT
0	69^{ab}	83^{a}	74^{a}
4	82^{a}	86^{a}	68^{ab}
15	53^{b}	81^{a}	81^{a}

Superscript applies to all means in table – figures with the same superscript letters are not significantly different (P<0.05).

In this experiment, 15 hours hydration applied to cuttings that were then HWT before storage suppressed or delayed callus development. The prolonged period in the relatively low oxygen environment of the hydrating tank (Gray 1999) may have resulted in intracellular flooding (Baker 1962b) and the activation of anaerobic respiration that may persist since the cuttings are saturated during hydration (Baker & Chandler 1957; Baker 1962b). Under these conditions respiratory efficiency might be compromised (Raven & Johnson 1992) resulting in delayed recovery from the stress of HWT and suppressed or delayed callus development.

The significantly greater ($P<0.05$) callus development in 15 hour hydrated cuttings that were stored before HWT compared to the cuttings that were stored after HWT, suggests that in storage the cuttings might have recovered from the effects of prolonged hydration before they were subjected to the additional stress of HWT. The relatively warm (27^0C) environment of the callusing box and the resulting increased respiratory rate might also enable quicker recovery from the stress of HWT in cuttings that are HWT after storage compared to those that are HWT before storage. It is interesting to note that unlike cuttings that were HWT before storage, callus development in cuttings that were not HWT or stored before HWT was not significantly affected ($P<0.05$) by variations in hydration times. However callus development in cuttings that were not HWT was more variable than in the group that were HWT post storage, indicating that the interaction between HWT and hydration is possibly affected by the order of storage and HWT.

Root development

In this experiment, root development in Chardonnay was significantly affected ($P<0.05$) by HWT protocols and hydration time at the time of assessment (Table 4). However interpretation of these effects is hampered by a significant interaction between HWT protocols and hydration time (Figure 3). Discussion of these results is therefore base on a one-way ANOVA with 9 individual treatments covering all combinations of the 2 factors (HWT protocols and hydration times) (Table 5). Means separation was performed using Tukey's pairwise comparisons (Appendix 2)

Table 4: ANOVA table for the effect of HWT protocols and hydration time on root development in Chardonnay

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Hwt	2	4.92578	4.92578	2.46289	46.28	0.000
Soak	2	0.64711	0.64711	0.32356	6.08	0.005
Hwt *soak	4	1.81422	1.81422	0.45356	8.52	0.000
Error	36	1.91600	1.91600	0.05322		
Total	44	9.30311				

Table 5: One-way ANOVA table for the effect of HWT protocols and hydration time on root development in Chardonnay

Source	DF	SS	MS	F	P
Treat	8	7.3871	0.9234	17.35	0.000
Error	36	1.9160	0.0532		
Total	44	9.3031			

Table 6: Chardonnay – means of root development

Hydration time hrs	HWT/store	Store/HWT	Store/no HWT
0	2[a]	1.9[a]	1[b]
4	1[b]	1[b]	1[b]
15	2[a]	1.8[a]	2[a]

Superscript applies to all means in table – figures with the same superscript letters are not significantly different (P<0.05).

The results of this experiment indicate that root development at the time of assessment was most advanced in material that was HWT but not hydrated, or hydrated for 15 hours. By contrast root development in material that was neither hydrated or HWT, or hydrated for 15 hours regardless of HWT protocols was significantly less advanced (P<0.05). It is interesting that 15 hours hydration appeared to stimulate root development, but 4 hours hydration apparently had no effect. It is also interesting that in this variety in this experiment, HWT apparently had no effect on root development in hydrated material but did affect root development in material that had not been hydrated.

The mean score of root development for material that was not hydrated or HWT was significantly (P<0.05) lower than for either group cuttings that were HWT regardless of order of storage. By contrast, in the experiment reported by Waite and May (1999) where all cuttings were hydrated overnight (15-16 hours) root development in Chardonnay cuttings collected in mid season at a similar time to the collection date in this experiment was less advanced than in cuttings that were not HWT. Since the cuttings used in the present experiment were collected from the same block of vines as those used by Waite and May (1999) it would appear that one or more unidentified factors not examined in these experiments may affect the physiological state of the cuttings. Such factors might include climatic variations such as temperature and rainfall, or variations in management practices such as irrigation scheduling and fertilizer applications.

Final Condition

There were no significant (P<0.05), or observable differences between any of the treatment groups when the Chardonnay cuttings, now green potted vines, were assessed for final condition on 6/10/99 (Table7). All the cuttings in each treatment group were alive and healthy when assessed and had well developed roots and shoots. Means of final condition of all combinations of HWT and hydration are shown in Table 8 below.

Table 7: ANOVA table for the effect of HWT protocols and hydration time on final condition in Chardonnay

Source	DF	Seq SS	Adj SS	Adj MS	F	P
hwt2	2	0.05824	0.05824	0.02912	1.57	0.222
soak2	2	0.01317	0.01317	0.00659	0.36	0.703
hwt2*soak2	4	0.06155	0.06155	0.01539	0.83	0.515
Error	36	0.66752	0.66752	0.01854		
Total	44	0.80048				

Table 8: Chardonnay – means of final condition

Hydration time hrs	HWT/store	Store/HWT	Store/no HWT
0	2.9	2.8	2.9
4	2.7	2.8	2.8
15	2.9	2.8	2.8

There are no significant differences (P<0.05) between any means in this table

Although there was a significant interaction effect of HWT protocols and hydration on callus development and root development in this variety, mean final condition scores for all treatment groups was relatively high (Table 8) indicating that the effects of hydration, HWT and order of HWT and cold storage on the cuttings at callusing were transient and did not affect the capacity of the cuttings to develop into sound rootlings.

At final assessment all the rootlings were sound and vigorous green potted vines and had they then been planted out in a permanent vineyard as would happen in normal commercial practice, is unlikely that there would have been any significant differences in vigour between treatment groups during the establishment phase.

Cabernet Sauvignon

Callus Development

In this experiment there were significant main effects of both HWT protocols and hydration on callus development when the results were analysed as a 2 way factorial. There was no interaction between the 2 factors (Table 9).

Table 9: ANOVA table for the effect of HWT protocols and hydration time on callus development in Cabernet Sauvignon

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Hwt	2	11088.4	11088.4	5544.2	74.93	0.000
Soak	2	1221.7	1221.7	610.9	8.26	0.001
hwt*soak	4	527.5	527.5	131.9	1.78	0.154
Error	36	2663.7	2663.7	74.0		
Total	44	15501.3				

To identify which levels of each factor were significantly different, a one-way ANOVA was run for each factor (Tables 10 &12). In this analysis, only HWT protocols had a significant effect ($P<0.05$) on callus development. Means separation was performed using Tukey's pairwise comparisons test (Appendix 3).

Table 10: One-way ANOVA Cabernet Sauvignon – means of callus development over 3 hydration times.

Source	DF	SS	MS	F	P
soak	2	1222	611	1.80	0.178
Error	42	14280	340		
Total	44	15501			

When the data were analysed as a one-way ANOVA for hydration time there was no significant effect of hydration time on callus development (Table 11).

Table 11: Cabernet Sauvignon – means of callus development over 3 hydration times.

0 hydration	4 hours hydration	15 hours hydration
60	72	69

There are no significant differences ($P<0.05$) between any means in this table

Table 12: One-way ANOVA table for the effect of HWT protocols on callus development in Cabernet Sauvignon

Source	DF	SS	MS	F	P
hwt	2	11088	5544	52.77	0.000
Error	42	4413	105		
Total	44	15501			

Callus development in cuttings that were not HWT was significantly less (P<0.05) than that in either of the groups that were HWT. Greatest callus development occurred in cuttings that were HWT after storage (Table 13).

Table 13: Cabernet Sauvignon – means of callus development over 3 HWT protocols.

HWT/store	Store/HWT	Store/no HWT
73[b]	83[a]	46[c]

Superscript applies to all means in table – figures with the same superscript letters are not significantly different (P<0.05).

Harvest date (21/6/99) for cuttings in this experiment was similar to that of the mid season collection date (17/6/97) for cuttings in the experiment reported by Waite and May. The results of that experiment also demonstrated a significant (P<0.05) stimulatory effect of HWT on callus development in Cabernet Sauvignon cuttings collected in mid season. In this variety HWT may provoke a wounding response in the tissue exposed by the basal cut that results in the stimulation of callus tissue. It is interesting that the pattern of callus development in Cabernet Sauvignon is broadly similar to that in Chardonnay in this experiment.

Root Development

In this experiment root development in Cabernet Sauvignon cuttings at callusing was significantly affected by variations in HWT protocols, but not by hydration. There was no interaction between the 2 factors (Table 14).

Table 14: ANOVA table for the effect of HWT protocols and hydration time on root development in Cabernet Sauvignon.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Hwt	2	2.24311	2.24311	1.12156	26.85	0.000
soak	2	0.09644	0.09644	0.04822	1.15	0.327
hwt*soak	4	0.09556	0.09556	0.02389	0.57	0.685
Error	36	1.50400	1.50400	0.04178		
Total	44	3.93911				

Discussion of these results is therefore based on a one-way ANOVA with 3 individual treatments covering all possible combinations of HWT protocols (Table15). Means separation was performed using Tukey's pairwise comparisons test (P<0.05) (Appendix 4).

Table 15: One-way ANOVA table for the effect of HWT protocols on root development in Cabernet Sauvignon.

Source	DF	SS	MS	F	P
Hwt	2	2.2431	1.1216	27.77	0.000
Error	42	1.6960	0.0404		
Total	44	3.9391			

Root development in cuttings that were not HWT was significantly more advanced than in cuttings that were HWT (Table 16).

Table 16: Cabernet Sauvignon – means of root development over 3 HWT protocols

HWT/store	Store/HWT	Store/no HWT
2.5[b]	2.4[b]	2.9[a]

Superscript applies to all means in table – figures with the same superscript letters are not significantly different (P<0.05).

The order of HWT and storage did not affect root development in the present experiment. Wample (1997) reported increased root numbers in Cabernet Sauvignon cuttings receiving pre storage HWT. However in this experiment root development rather than root numbers were assessed since root initials are very fragile and easily knocked off during handling in commercial situations making it difficult to measure root numbers accurately.

It is interesting that HWT suppressed root development but stimulated callus development. The results of a regression analysis (Figure 3) showed a strong negative relationship between callus and root development (r=-0.93) in Cabernet Sauvignon in this experiment. The results of this experiments contradict the perception in industry that well developed callus is a good indicator of potential root development.

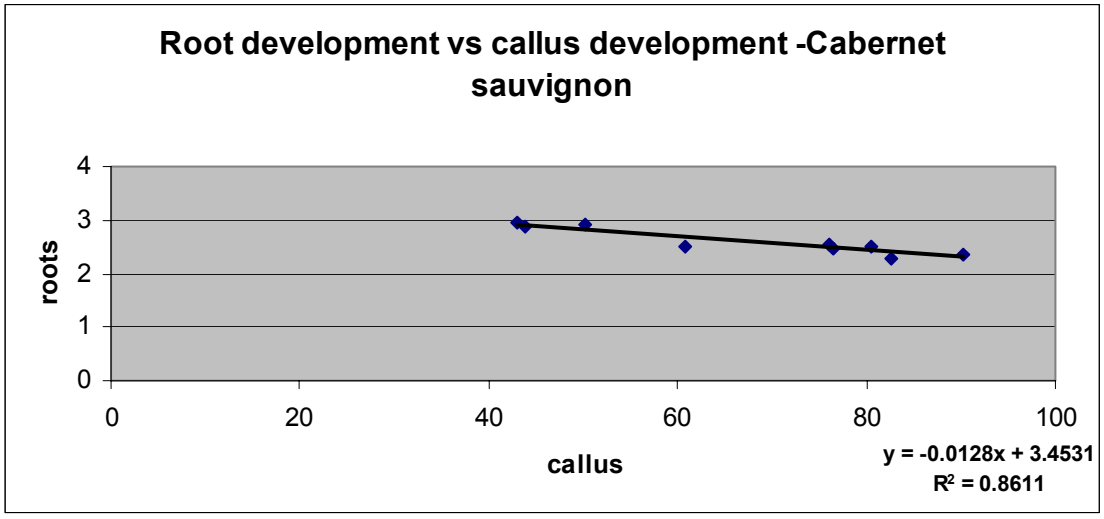


Figure 3: Cabernet Sauvignon regression plot – root development versus callus development.

Final Condition

There were no significant (P<0.05), or observable differences between any of the treatment groups when the cuttings, now green potted vines, were assessed for final condition on 6/10/99 (Table 17).

Table 17: ANOVA table for the effect of HWT protocols and hydration time on final condition in Cabernet Sauvignon.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Hwt	2	0.04133	0.04133	0.02067	1.09	0.348
Soak	2	0.02533	0.02533	0.01267	0.67	0.520
hwt*soak	4	0.04933	0.04933	0.01233	0.65	0.631
Error	36	0.68400	0.68400	0.01900		
Total	44	0.80000				

All the cuttings in each treatment group were alive and healthy when assessed and had well developed roots and shoots. Means of final condition of all combinations of HWT and hydration are shown in Table 18 below.

Table 18: Cabernet Sauvignon– means of final condition

Hydration time hrs	HWT/store	Store/HWT	Store/no HWT
0	3	2.9	2.8
4	3	3	3
15	2.9	3	2.9

There are no significant differences ($P<0.05$) between any means in this table

As for Chardonnay in this experiment, the high mean final condition score for all treatment groups indicates that the effects of HWT protocols on Cabernet Sauvignon cuttings at callusing were transient and did not affect the capacity of the cuttings to develop into sound rootlings. As was the case with the Chardonnay cutting in this experiment, all the rootlings were sound and vigorous at final assessment and it is unlikely that there would have been any significant differences in vigour between treatment groups once the rootlings were exposed to the harsher conditions in the vineyard.

Summary and Conclusion

The effects of variations in hydration times and HWT protocols on cuttings of Chardonnay and Cabernet Sauvignon at callusing were variable with different responses observed between varieties. In Chardonnay, callus development was consistently greater in cuttings that were HWT post storage than in cuttings that were HWT pre storage or stored, but not HWT. Callus development was suppressed in cuttings that were HWT pre storage and hydrated for 15 hours. In Cabernet Sauvignon callus development was greatest in cuttings that were hydrated for 4 hours and cuttings that were stored before HWT showed greater callus development than either cuttings that were HWT post storage or cuttings that received no HWT.

Root development in Chardonnay was significantly affected by both HWT protocols or hydration and an interaction between the two. Root development was most advanced in HWT Chardonnay cuttings that received no hydration and in all Chardonnay cuttings that were hydrated for 15 hours regardless of HWT. These results are very interesting in that HWT apparently had no effect on root development in cuttings that were hydrated, but appeared to stimulate root development in cuttings that were HWT but not hydrated. These results contrast with the results of the experiment reported by Waite and May (1999) where root development was apparently suppressed in HWT cuttings collected in mid season. These contrasting results suggest that other unidentified factors such as climate and mother vine management may affect the physiological state of Chardonnay cuttings and hence their response to propagation treatments.

In Cabernet Sauvignon cuttings root development was affected by HWT protocols, but not by hydration. Most advanced root development in this variety at the time of assessment was in the group that were not HWT indicating that HWT suppressed early root development in this variety.

It is interesting to note that although callus and root development were affected by the treatments in this experiment, the final condition of the cuttings of both varieties was not affected by any treatment applied in this experiment. No cuttings of either variety died and all the cuttings were of good commercial quality and considered saleable by the host nursery. The callused cuttings were potted into cardboard plant bands and grown on in the protected environments of a glasshouse and shade house until they were considered to be ready for planting in a vineyard. The favourable conditions of the glasshouse and shade house compared to the more rigorous conditions in a field nursery where cuttings are exposed to climatic fluctuations may have aided recovery from any stress induced by HWT, hydration, or storage. Had the callused cuttings been planted in the field nursery instead of the more protected nursery environment the final condition of the cuttings may have been different.

The results of this experiment indicate that although grapevine cuttings are affected by HWT and other nursery operations during the propagation phase, the effects observed at callusing are not a useful indicator of final condition. If, as previous work (Waite & May 1999) and nursery industry experience indicates, HWT has detrimental effects on grapevine cuttings, post-callusing handling is likely to play an important role in grapevine propagation. The high quality of the rootlings resulting from the experiment reported here indicates that the protected environments of glasshouses and shade houses reduces the stress on cuttings during the establishment phase, enabling recovery from the stress of HWT and other nursery processes and resulting in fewer losses.

References:

(See Appendix 3, pp. 146 to 149 for a compiled list of references)

P3.12:

Using 1-Methylcyclopropene To Preserve Grapevine Rootlings During Storage'

Adam Upton[1], Ian Wilkinson[1] and Helen Waite[1]

[1]Institute of land and Food Resources, University of Melbourne, Dookie College Victoria, 3647, hwaite@unimelb.edu.au

Abstract

Viticulturalists frequently store dormant grapevine rootlings in cool rooms with ethylene producing fruit such as apples. It is thought that exposure to atmospheric ethylene may have been a contributing factor in some of the cases of planting material failure attributed to HWT. This study examined the effects of 1-methylcyclopropene (1-MCP), a substance that blocks the effects of ethylene in plant tissue, on dormant Chardonnay rootlings held in an ethylene-contaminated (0.66ppm) cool room atmosphere for 12 weeks before planting.

Mortality rates at the end of storage were significantly (P<0.05) lower in the rootlings treated with 1-MCP compared to the untreated controls and bud burst and shoot growth were also significantly (P<0.05) more advanced in the treated group indicating that atmospheric ethylene has a negative effect on rootlings in cold storage and treatment of rootlings that are to be stored with ethylene generating produce with 1-MCP is likely to result in improved strike rates and earlier, more even bud burst.

Introduction

Vines need to be in full production by the third year after planting. Poor quality planting material does not perform to expectations, delaying production and affecting the quality of the harvested fruit (Crocker *et al*. 2001).

Many vineyards and grapevine nurseries face problems storing dormant grapevine cuttings, or rootlings for spring planting. They are frequently obliged to place the vines in coolrooms that are occupied by ethylene producing fruit (predominantly apples and pears).

Ethylene is thought of as the "ageing hormone in plants" (Treshow, 1970) and is known to cause abnormalities in growth and premature senescence of plant material including cut flowers and fruit (George 1993). Ethylene is known to have a wide range of effects on plants depending on the age and sensitivity of the plant. The effects of ethylene on plants include loss of chlorophyll, abortion of plant parts, stem shortening, abscission of plant parts, and epinasty (bending of stems). It has also been found to be damaging to dormant nursery stock (Blankenship, 2001). Ethylene can cause plants to die and may be produced when plants are injured, either mechanically or by disease. According to George, (1993) the presence of very low concentrations of even 0.1 ppm ethylene has been known to cause plants to grow abnormally.

The ideal conditions for storing cuttings should be below 0.05 ppm ethylene concentration at 1-2^{o}C and stored in vented bags with no free water. Conditions above 2^{o}C favour the growth of Botrytis and other potential pathogens. A chemical product, "EthylBloc", 1-Methylcyclopropene (1-MCP) has been developed to block the action of ethylene in plants. The mode of action of "EthylBloc" is via a preferential attachment to the ethylene receptor in the plant tissue, blocking the effects of ethylene from both endogenous and exogenous sources. This product has been tested in the flower and fruit industries in America and Australia and has been found to be effective at an extremely low dose (10-1000 ppb) (Warner H.L. & Kollman G.E. (n.d.)).

The aim of this experiment was to investigate the effects of 1-MCP applied prior to cold storage on post storage growth rates of Chardonnay rootlings that were exposed to normal levels of ethylene during cold storage for 3 months.

Materials and Methods

The 300 Chardonnay I10V1 rootlings used for this trial were sourced from Jacob's Creek Nursery in South Australia. The rootlings were hot water treated (50^{o}Cfor 30min) before being transported in moist sawdust. It is noted by Crocker *et al.* (2001) that hot water treatment (HWT) of grapevines at 50^{o} C for 30 minutes offers an effective control method of eliminating a wide range of both external and internal pests and diseases from planting material. HWT is used by many nurseries to satisfy local and interstate quarantine requirements.

The rootlings were divided into two even groups of 150 vines each. One group remained as the untreated control. The two groups were placed in separate plastic airtight waterlock treatment boxes at 20^{o}C. A vial of 1000 ppm 1-MCP was placed in the treatment box.

Water was injected into the vial by syringe through a membrane to initiate the volatilisation reaction of the 1-MCP. The rootlings were retained in the treatment box for 18 hours to allow the 1-MCP to attach to the ethylene receptor sites. Following treatment, both groups of rootlings were covered with perlite to retain moisture and stored in a plastic bag in a commercial orchard coolroom for 12 weeks. Apples and pears were present in the coolroom which was operated normally throughout the trial. Mean atmospheric ethylene concentration in the coolroom during the period of storage was 0.66 ppm (Figure 1).

On removal from the cool room all rootlings were trimmed to two buds before planting with the top bud being named 'Bud 1' and the lower 'Bud 2'. Both groups of rootlings were then planted 300mm apart in 2 parallel rows 1m apart in a uniform clay loam.

Drip irrigation was supplied and the rootlings were watered in. Shoot growth from the top 2 buds (bud 1 & bud 2) was measured weekly for 6 weeks after planting to determine if there was a difference in bud survival and bud length between the treated and the untreated rootlings.

Data was analysed with Microsoft Excel using a two tailed t-test.

Results

Cool Room Ethylene Concentration

The ethylene concentration of the coolroom was initially high (1.76 ppm) (Fig. 1) because the coolroom had not been opened for a two week period (the room was used by the owner for normal operations throughout the trial as would occur in normal commercial practice) and dipped to 0.22ppm in the 2 weeks before the end of the storage period before rising again (0.44ppm) by the end of the storage period. The mean concentration of the room over the 3 months of storage was 0.66 ppm.

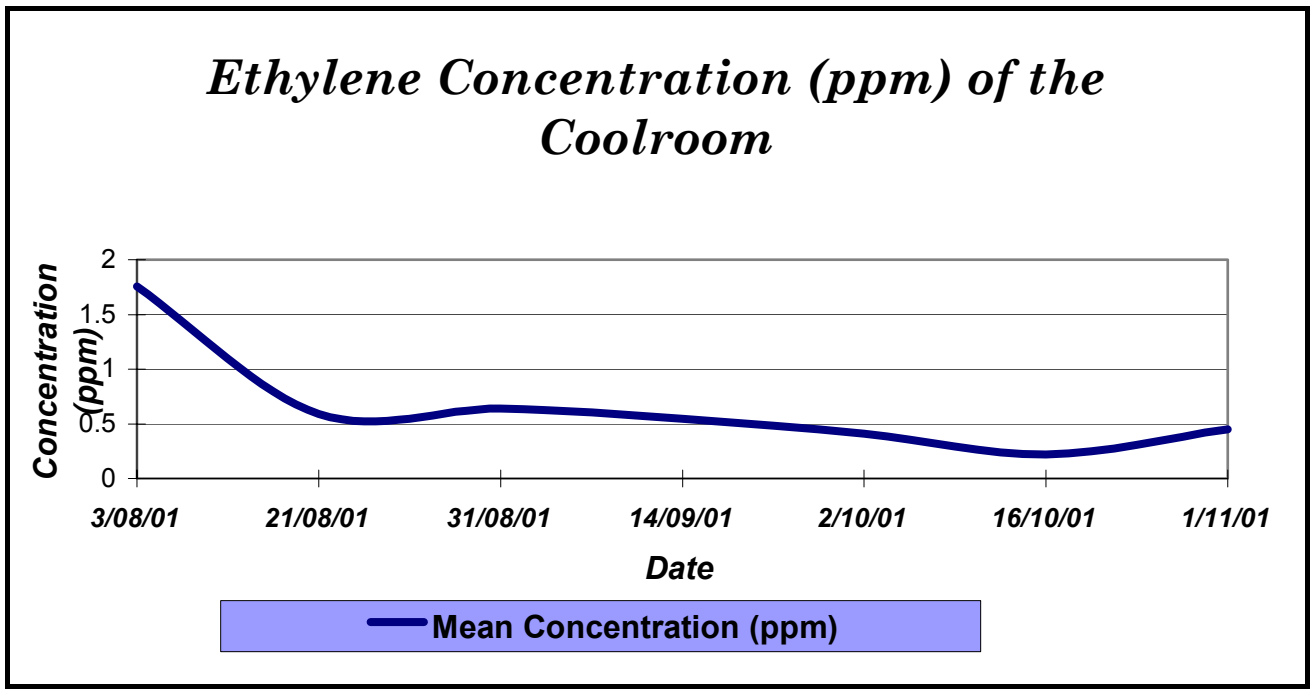


Figure 1 Ethylene Concentration of the Coolroom over the 3 months storage period

Bud Development and Shoot Growth

Shoot development in the rootlings treated with 1-MCP began earlier and was more advanced than in the untreated group throughout the trial. Bud burst in the untreated group of rootlings was generally delayed by one week compared to the rootlings that had been treated with 1 –MCP. Growth of both treated and untreated groups was slow through the initial 2 weeks of the trial (figure 2) because of an unusually cool spring/summer. According to the Bureau of Meteorology, November was generally cool with average minimum and maximum temperatures one degree lower than normal. Melbourne's mean maximum temperature for December (21.5 degrees) was the equal-fifth lowest on record.

By the end of the trial the treated group had on average 17 cm more growth over the six week period. The mean shoot growth for the untreated rootlings was 33 cm compared to 50 cm for the treated group.

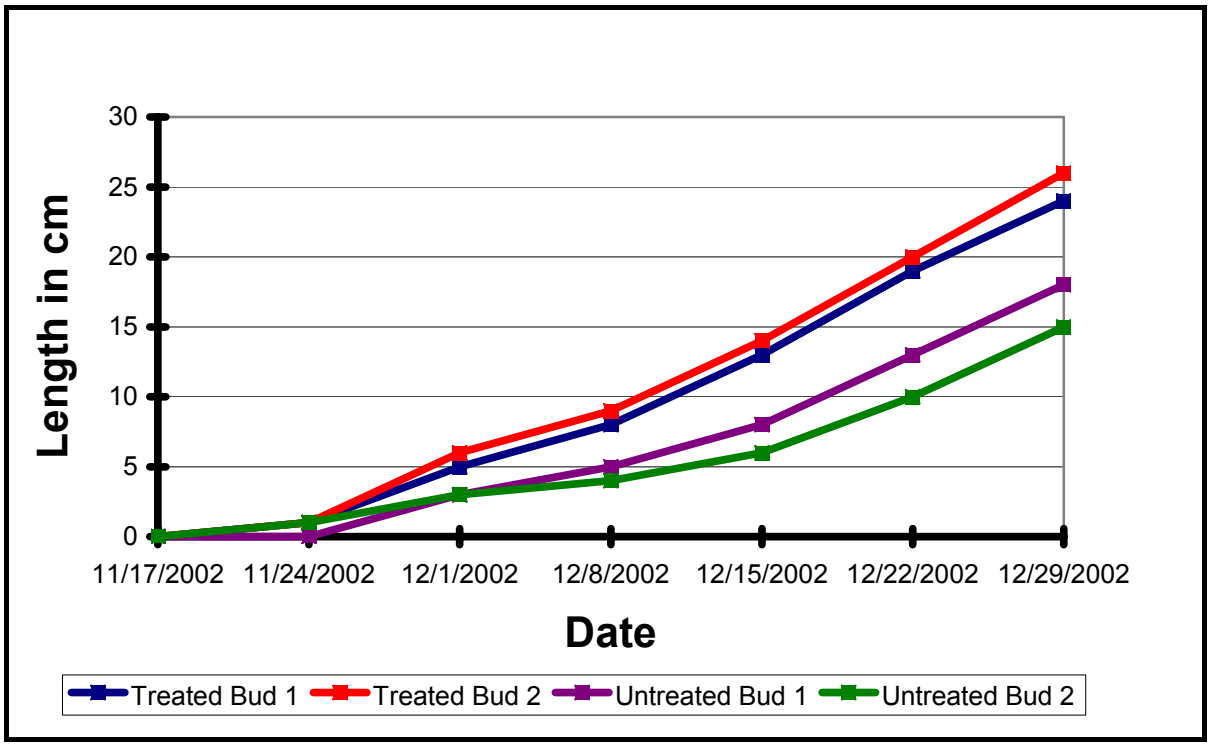


Figure 2 Cumulative Shoot Growth of vines over 7 weeks

Vine Mortality

In the untreated control group 12 vines failed to make any growth and were found to be dead, this equals an 8% mortality rate compared to a 0.6% mortality rate of the treated group where only 1 vine died (table 1).

Table 1 No of dead vines (no growth)

	Live Vines	Dead Vines
Treated Vines	149	1
Untreated Vines	138	12

Data Analysis

The trial was conducted on 300 grapevine rootlings, 150 treated and 150 untreated. A greater sample number may have shown higher accuracy of results and allowed different methods of Analysis of Variance, however due to the general shortage of planting material meant that large numbers of rootlings were not available.

It was noted that there was a great amount of variation in the data for the untreated group. Because there were many plants that had little or no growth, some plants that had less competition for water and nutrients which therefore grew disproportionably larger.

A t-test was used to calculate the level of significance between the two groups of data. Results indicated that there was statistical significance at both 1% and 5% confidence levels. Student's t distribution table values for 140 degrees freedom equals 1.656 at 5% and 2.353 at 1%. The calculated t value of 6.94 was greater than both table values, therefore it can be said that there is a significant difference between the untreated and the treated groups.

Discussion

Although this trial did not directly measure the effects of ethylene on the grapevine rootlings, the highly specific action of 1-MCP and the delayed and stunted shoot development observed in the untreated vines in this trial indicate that the untreated vines were most likely affected by the ethylene present in the cool room atmosphere. The poorer growth of the untreated vines conforms 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.

In the trial, bud burst was delayed on average by one week in the untreated group. This meant that the treated group effectively had an extra week of growth. It was also observed that the untreated rootlings appeared stunted compared to the growth recorded for the treated rootlings and it is thought that the influence of ethylene may have contributed to the inability of the untreated group to develop strong shoots. This is consistent with the findings of George (1993) who observed that ethylene inhibited the ability of some plant seedlings to produce shoots. Had the levels of ethylene been higher in the cool room the effects on the vines may have been more severe. However the objective of this trial was to determine the potential benefits of 1-MCP as a treatment for rootlings stored in a typical commercial environment and it was felt that the cool room used reflected the usual commercial situation.

The unusually cold spring/summer growing conditions (Bureau of Meteorology, 2002) were not ideal and may have delayed bud burst and slowed growth. However conditions were the same for both samples and it is thought that the impact of the cold weather did not affect the 2 groups differently.

It would have been preferable to monitor the growth of the rootlings until dormancy to determine if the effects of 1-MCP persisted beyond the initial stages of shoot growth, but time constraints did not allow extended monitoring.

Conclusion

This project has demonstrated that the use of 1-MCP as a treatment for rootlings that are to be stored in ethylene contaminated atmospheres is likely to result in earlier bud burst followed by faster and stronger growth in the first season in the vineyard and result in more even vineyards that are consequently easier and cheaper to manage.

Since vineyard managers require early returns from their vines, the improved vigour and strike rate in rootlings treated with 1-MCP indicates that the use of this substance on stored planting material is likely to be of economic benefit.

Further experiments will be required to find the most appropriate concentration of 1-MCP to be used and the period of time exposed to this chemical.

References:

(See Appendix 3, pp. 146 to 149 for a compiled list of references)

P3.13:

Dehydration Rates in Dormant Cuttings of *Vitis vinifera* cvs. and the Effects on Root Initiation in *Vitis vinifera* cv. Sauvignon Blanc'

Helen Waite[1]

[1]Helen Waite, Institute of land and Food Resources, University of Melbourne, Dookie College, Victoria, 3647, hwaite@unimelb.edu.au

Abstract

Grapevine cuttings are sometimes left exposed to the air in the time between cutting from the mother vine and processing in the nursery. This study investigated the rate of moisture loss in 4 *Vitis vinifera* cvs. over a period of 19 days at ambient temperatures and the effects of moisture loss on root initiation in *V. vinifera* Sauvignon Blanc. The moisture content of all 4 varieties (Sauvignon Blanc, Merlot, Shiraz and Cabernet Sauvignon) was close to 50% of fresh weight and the rate of dehydration was similar for all four varieties. Mean moisture loss after the first 24 hours was 4%, increasing to 6% after 48 hours and 36% after 19 days. Root initiation was significantly affected by dehydration with a decline in the number and length of root initials over the 19 day period. The mean number of roots initiated by cuttings not subjected to dehydration was 18 compared to 10 after 3 days dehydration, 6 after 11 days, and 1 after 19 days dehydration. Mean root length declined from 80mm prior to dehydration to 38mm after 11 days and 6mm after 19 days. The results indicate that even short periods of exposure of a day or 2 can affect the ability of cuttings to initiate roots and as a consequence likely to affect the quality of rootlings grown from the cuttings.

Introduction

In commercial grapevine propagation cuttings are often harvested from sites that are remote from the nursery. The large numbers of cuttings that are harvested and the necessity of transporting the cuttings over long distances is thought to result in levels of dehydration that may affect the performance of the cuttings in propagation.
In some circumstances cuttings may lie on the vineyard floor or be left exposed on shed benches for 12 hours or more before being transported to the nursery where processing may be delayed for several days when the nursery is busy. Although standard nursery practice has been to hydrate cuttings by submersing them in water for 12 or more hours prior to propagation to compensate for any moisture loss that may have occurred, the precise effects of dehydration at various levels on grapevine propagating material is unknown.

However the effect of dehydration on the quality of propagating material in other species is generally regarded as deleterious and likely to result in a reduced strike rate or poor quality plants (Hartmann, Kester & Davies 1990).

In order to determine the rate of dehydration in dormant *Vitis vinifera* cuttings the moisture content and rate of moisture loss in cuttings exposed to the air on a nursery bench was measured over 19 days in the 2000 and 2001 seasons (2000:Cabernet Sauvignon, Chardonnay & Pinot Noir. 2001:Cabernet Sauvignon, Shiraz, Merlot & Sauvignon Blanc). The effects of dehydration on root and bud development in dormant Sauvignon Blanc cuttings were also measured in the 2001 season.

Materials and methods

2000 season

One hundred dormant cuttings of each of 3 *V. vinifera* cvs. Cabernet Sauvignon, Pinot Noir and Chardonnay were divided into sub bundles of 10 cuttings, weighed, reformed into bundles of 100 cuttings and laid out on a nursery bench on 27/7/2000. The Cabernet Sauvignon cuttings were supplied by Dookie College vineyard and the Chardonnay and Pinot Noir cuttings were supplied by the Orlando Wyndham Group from source blocks in the Barossa Valley. The cuttings were weighed at 24 hour intervals over 19 days (25/7-12/8/2000) and then oven dried at 50^{o}C and weighed when weight loss had ceased. Total moisture was calculated as a percentage of total weight at the start of the experiment (25/7/2000). Daily moisture loss in each variety was calculated as a cumulative percentage of total moisture.

2001 season

One hundred dormant cuttings of each of 4 *V. vinifera* cvs. Cabernet Sauvignon, Shiraz, Merlot and Sauvignon Blanc were divided into sub bundles of 10 cuttings, weighed, reformed into bundles of 100 cuttings and laid out on a nursery bench on 29/8/20001. The cuttings were weighed at 24 hour intervals over 19 days (29/8-17/9/2001) and then oven dried at 50^{o}C and weighed when weight loss had ceased. Each bundle of cuttings was divided into the sub bundles for weighing and reformed after weighing. Total moisture was calculated as a percentage of total weight at the start of the experiment (29/8/2001). Daily moisture loss in each variety was calculated as a cumulative percentage of total moisture.

A further 6 bundles of 100 Sauvignon Blanc cuttings were also laid out on the nursery bench on 29/8/2001. One of these bundles of Sauvignon Blanc cuttings was removed and placed in a callusing box with clean moist coarse sand on each of days 1, 3, 7, 11, 15 and 19 and incubated at ambient temperatures. All cuttings were removed from the callusing box on 15/11/2001 and assessed for root number and length and bud development. Buds were scored as either 1, buds closed with no sign of loose scales or emerging leaves; 2, buds open with loose scales and emerging leaves or 3, buds extended with a clearly visible shoot (100-300mm) and leaves.

The Sauvignon Blanc and Shiraz cuttings were supplied by Dookie College vineyard and the Cabernet Sauvignon and Merlot cuttings were supplied by the Orlando Wyndham Group from source blocks in the Barossa Valley.

Results and discussion

Moisture content and dehydration rates

Of the 3 varieties examined in the 2000 season, Cabernet sauvignon had the lowest moisture content at 45% and Pinot Noir the highest moisture content at 48% with Chardonnay falling between with a moisture content of 46% of total weight (table 1). Moisture loss from exposure was also highest in Pinot Noir in the first 24 hours of exposure at 8.5% of total moisture compared to 5.5% of total moisture in Chardonnay and 3.5% in Cabernet Sauvignon. At the end of the 19day period cumulative moisture loss from dehydration was highest in Chardonnay at 38.5% and lowest in Cabernet Sauvignon at 27% (figure 1).

Table 1: Moisture content of *V. vinifera* cvs. Cabernet Sauvignon, Chardonnay and Pinot Noir 2000.

	Cabernet Sauvignon	Chardonnay	Pinot Noir
Total moisture	45%	46%	48%

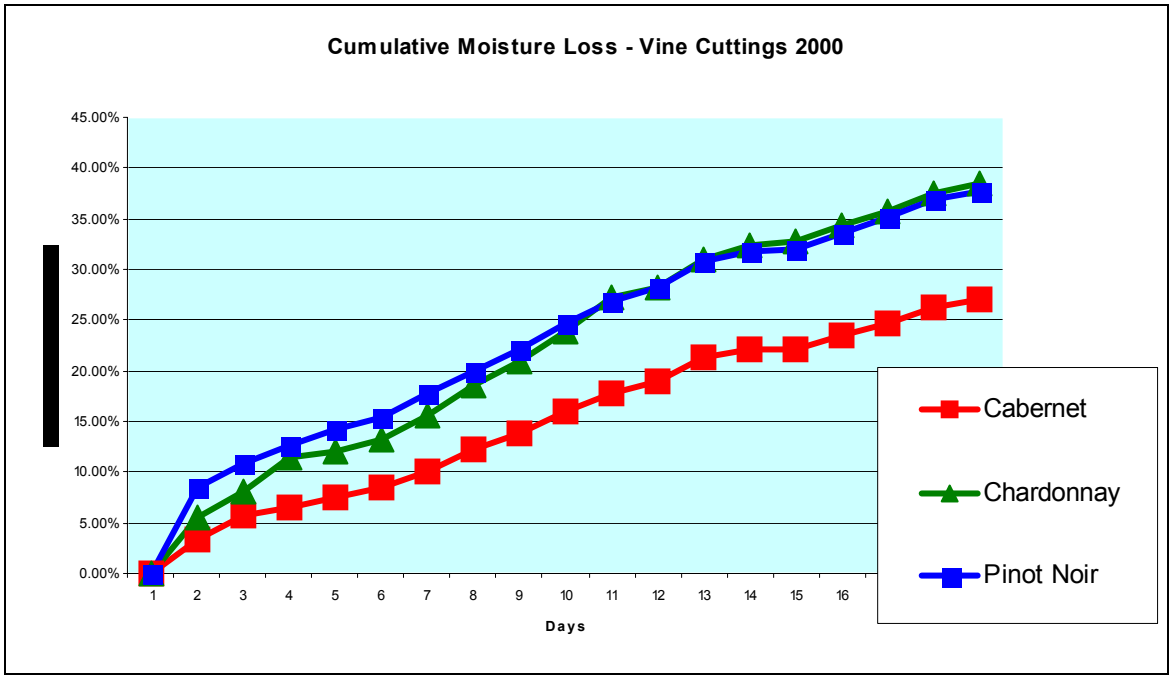


Figure 1. Cumulative moisture loss in Cabernet Sauvignon, Chardonnay and Pinot Noir over 19 days dehydration

By contrast cumulative moisture loss over 19 days in cuttings of Cabernet Sauvignon, Shiraz, Merlot and Sauvignon Blanc examined in the 2001 season was similar for all varieties and although total moisture content varied between varieties (table 2). The different moisture content of Cabernet Sauvignon cuttings observed over the 2 seasons may have reflected differences in climate and management practices between the 2 source areas, or different levels of respiratory activity since the cuttings used in the 2001 trials were collected at a later time and may have been emerging from dormancy.

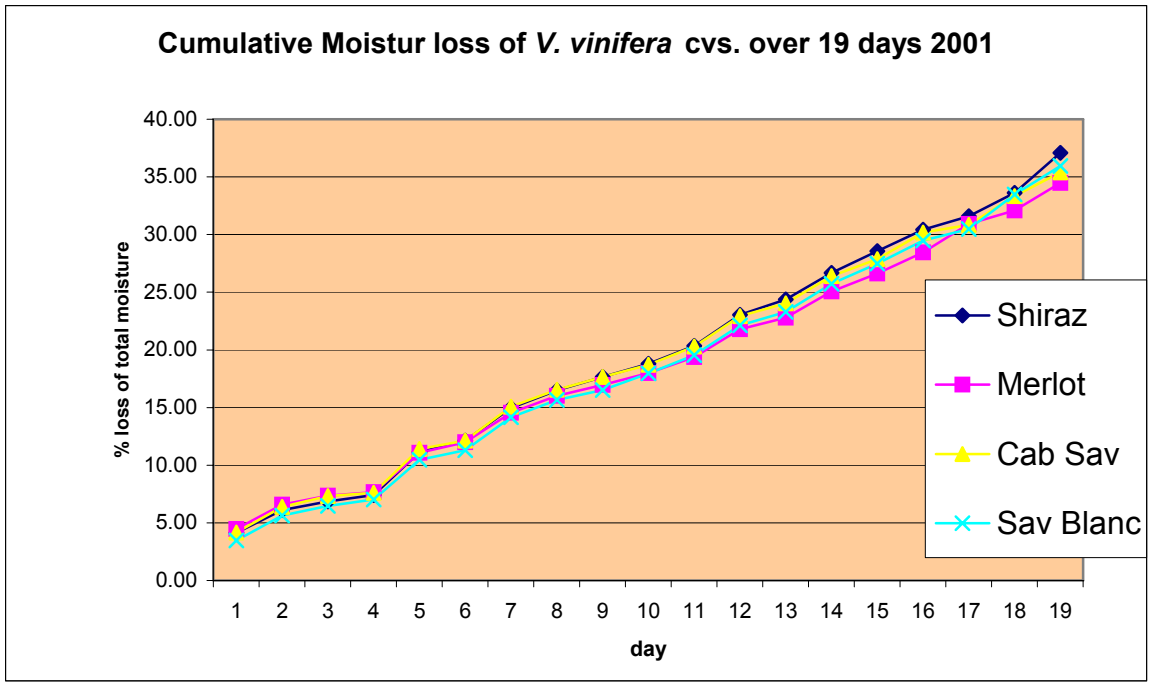


Figure 2: Cumulative moisture loss in Cabernet Sauvignon, Shiraz, Merlot and Sauvignon Blanc cuttings over 19 days dehydration.

Root development (Sauvignon Blanc)

Dehydration resulted in the formation of progressively fewer root initials at the base of Sauvignon Blanc cuttings as moisture loss increased (r^2=0.86). Cuttings that were not dehydrated developed an average of 16 root initials per cutting compared to less than 2 root initials per cutting in cuttings that had been exposed to dehydration for 19 days and lost 37% of their moisture content (fig.3).

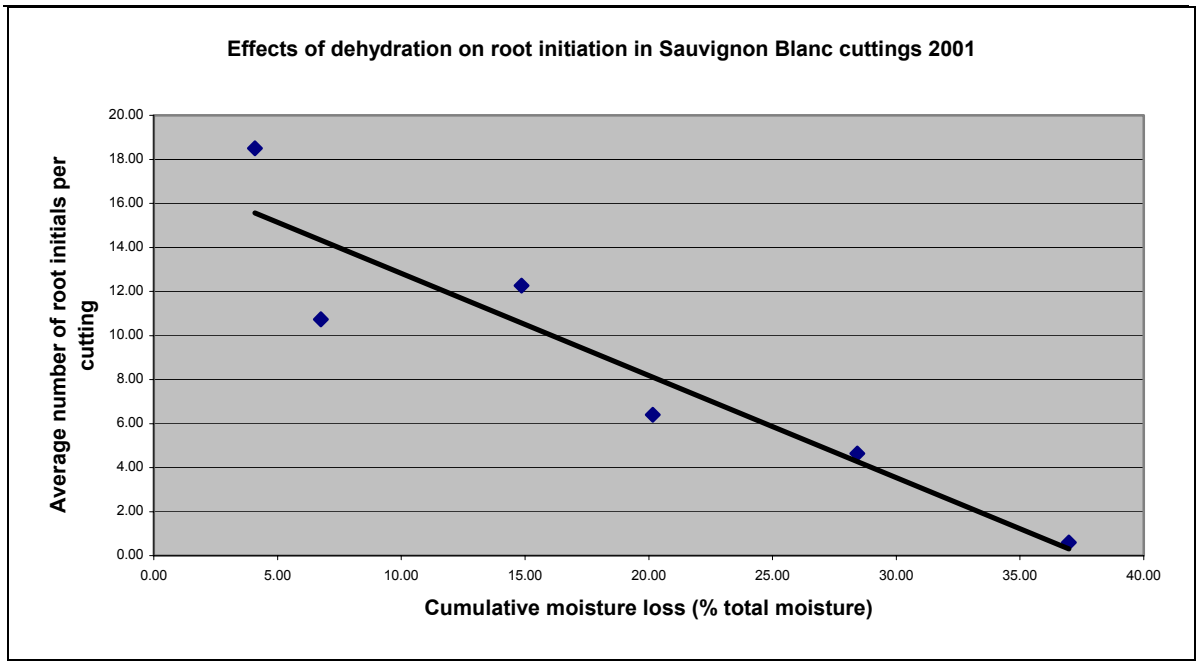


Figure 3: The effects of dehydration on the number of root initials forming on Sauvignon Blanc cuttings exposed to dehydration over 19 days.

Although root length was also measured in the Sauvignon Blanc cuttings, the reduced root length that was observed as moisture loss increased (fig. 4) may also be due to the variation in the duration of the incubation period. The first cuttings to be placed in the callusing box were incubated for 19 days longer than the cuttings that were placed in the callusing box last and although the incubation period was at ambient temperatures and prolonged for all cuttings (7-10 weeks) the effects of the variation in incubation times cannot be discounted. However the cooler ambient temperatures in the first 2-3 weeks of callusing are also likely to have delayed development in the earlier cuttings thus reducing the difference between cuttings placed in the callusing box at the beginning and end of the 19 day dehydration period.

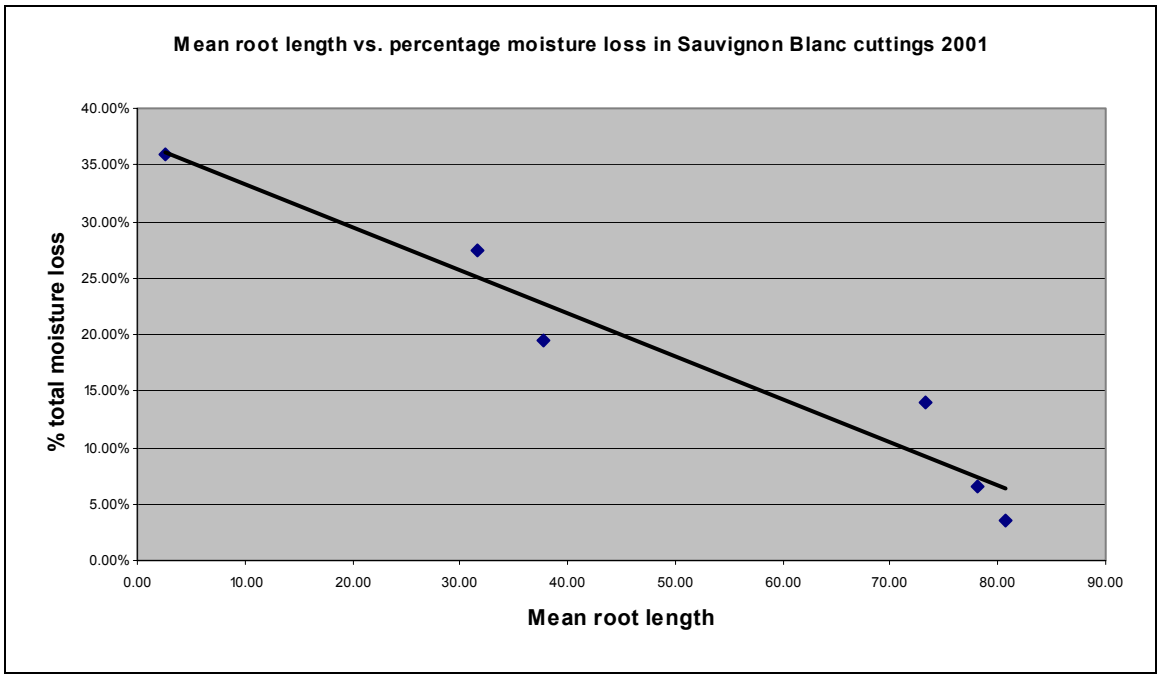


Figure 4: The effects of dehydration on root length in Sauvignon Blanc cuttings at callusing.

Bud development (Sauvignon Blanc)

Dehydration resulted in progressively inhibited bud development with increasing levels of dehydration in Sauvignon Blanc cuttings (fig. 5). Bud development was most advanced in cuttings that were not dehydrated and least advanced in cuttings that had been dehydrated for 19 days (r^2=0.87). Again, as with root length, the variation in callusing times may have affected bud development, however the extended period spent in the callusing box by all cuttings (7-10 weeks) was regarded as more than adequate for bud development particularly as grapevine bud development precedes root development by 3-4 weeks (Coombe 1988). The cooler ambient temperatures in the first 2-3 weeks of callusing are also likely to have delayed development in the earlier cuttings thus reducing the difference between cuttings placed in the callusing box at the beginning and end of the 19 day dehydration period.

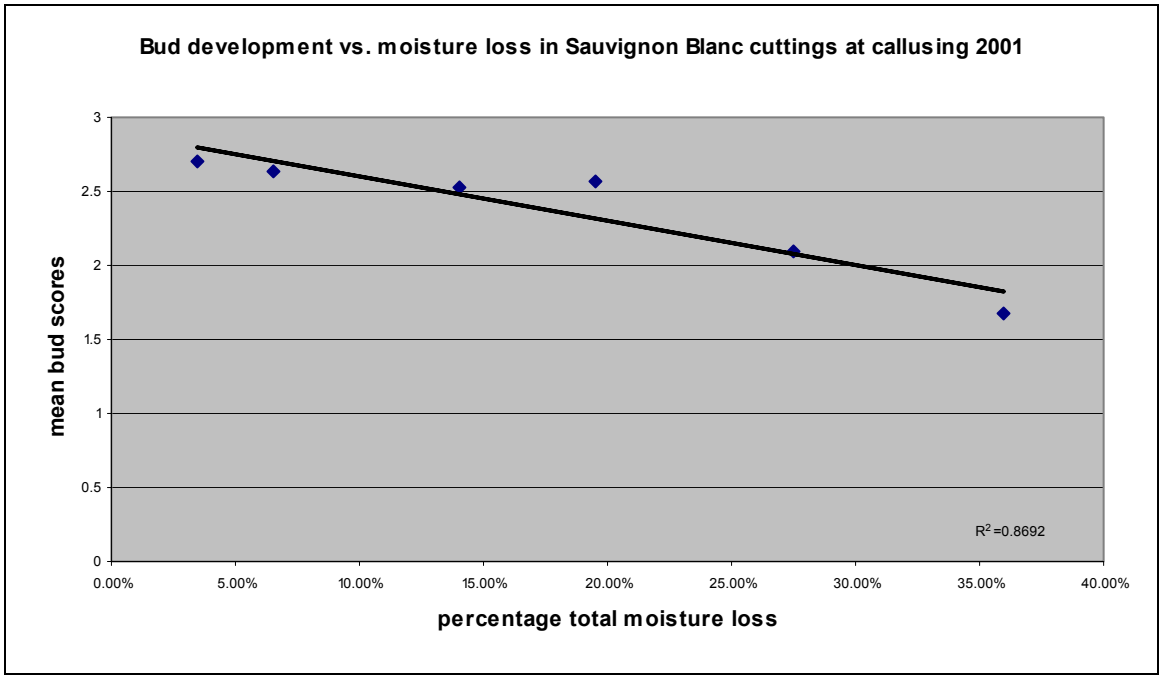


Figure 5: The effects of dehydration on bud development in Sauvignon Blanc cuttings at callusing, 2001.

Conclusion

The results of the 2000 and 2001 trials indicate that the moisture content of dormant *V. vinifera* cuttings is close to 50% and that when left exposed to the air on a nursery bench cuttings lose approximately 4-5% of their total moisture in the first 24 hours and up to 37% after 19 days. The strong relationship (r^2=0.86) between the number of root initials forming at the base of callused Sauvignon Blanc cuttings and the level of moisture loss through dehydration indicates that root development in cuttings that have been exposed to dehydration during collection and handling is progressively suppressed as dehydration levels increase. This may result in cuttings with poorly developed root systems that are more susceptible to environmental stress than cuttings that have not been exposed to dehydration. In addition, the suppressed root length and bud development associated with increasing levels of dehydration observed in this trial also indicates that dehydrated cuttings are likely to suffer delayed development that may result in poor quality plants or loss of the material.

The results of this trial indicate that rapid handling and processing of grapevine cuttings to avoid exposure and consequent dehydration is more likely to result in high quality plants than if cuttings are exposed to dehydration during the propagation process.

References:

(See Appendix 3, pp. 146 to 149 for a compiled list of references)

P3.14:

The Effects Of Hydration On Moisture Content and Root Initiation In *Vitis Vinifera* Cv. Sauvignon Blanc'.

Helen Waite[1] and Peter Steenholdt[1]

[1.]Institute of land and Food Resources, University of Melbourne
Dookie College, Victoria, 3647. E-mail, hwaite@unimelb.edu.au

Abstract.

This study examined the rate of moisture uptake in dormant cuttings of *V. Vinifera* cv. Sauvignon Blanc during hydration and the effects of hydration on root initiation. The percentage increase in the fresh weight of Sauvignon Blanc cuttings hydrated for 1,4,8 and 24 hours was 3.5%, 5%, 7% and 8.5% respectively. When assessed at callusing there was a significant effect ($P<0.05$) of hydration time on root number and root length with both increasing with increasing hydration time up to 8 hours beyond which there was no significant effect ($P<0.05$) of increasing hydration time.

Introduction

The rapid expansion of the Australian wine industry over the past decade has led to a greater demand for planting material and pressure on vine nurseries to supply large quantities of planting material. There are several factors affecting the production of healthy rootlings, one of these is thought to be hydration of dormant cuttings prior to callusing and the initiation of roots, a practice so common as to be almost universal in vine propagation and recommended by Nicholas *et al*. (1999). However hydration of propagating material is not common practice in the wider nursery industry and very little literature has been published on the subject.

Hydration, or pre-soaking of seed before hot water treatment, is referred to by Baker (1957), but there is no specific reference to *Vitis vinifera*. Baker (1957) recommends pre-soaking should be considered whenever plant material has trapped enough air to cause it to float.

Discussions with nurserymen and growers indicated that hydration is generally viewed as beneficial and hydration times for propagating material varied from one hour to six days, with no real rationale for the length of time cuttings are soaked. However it is thought that prolonged hydration of cuttings may lead to anoxia in the tissue (Baker 1962) and affect the capacity of the cuttings to initiate roots. It is also thought that the transmission of disease, particularly fungal infections, is likely to be increased with hydration of cuttings for periods longer than necessary for optimum root initiation.

Many physiological and environmental factors affect the ability of a cutting to develop roots. Physiological factors include stored carbohydrates; nitrogenous compounds, auxin levels and vitamins (Janick 1963). The environmental factors surrounding the cutting also play an important roll. These factors are temperature, light, humidity, oxygen and rooting media (Janick J. 1963). Humidity must be kept high to discourage stem desiccation. The water content of the stem helps translocate necessary auxin and stored carbohydrates to the callused area to promote root initials. Desiccated cuttings with insufficient water content will generally not root at all.

Even though very little water is used for plant nutrition itself, it is the essential carrier of nutrients through both the xylem and the phloem for plant function (Hudson 1981). The energy required for cell growth and the formation of callus is provided by carbohydrates and other nutrients transported through the phloem to the base of the cutting. Therefore certain levels of water are necessary within the cutting for root initiation as this requires cell nutrition. It is thought that cutting material taken from vines that have not received rain in the period from vintage until dormancy show poorer root initiation (Crocker *et al*. 2002)

It was because of the large variation in soaking times used by propagators and the lack of an industry standard that it was decided to undertake this work to determine if there was an optimum time for hydration to achieve maximum root numbers and cutting health. This study investigated the effect of hydration on root initiation and development in dormant cuttings of *V. vinifera* Cv. Sauvignon Blanc.

Materials and Methods

Prior to conducting the main research a preliminary trial using 30 cuttings of *Vitis vinifera* cv. Sauvignon Blanc was conducted. These cutting were soaked for 1 hour, 4 hours, 8 hours and 24 hours to determine if water uptake was continuing. The maximum percentage increase was obtained in the first hour (3.5%.) It took a further 3 hours to gain another 1.5% (a total of 4 hours of hydration.) After 8 hours of hydration there was only another half percentage increase in weight over the 4 hour group (5.5%). The results of 24 hours of soaking showed a total increase in weight of 8.5%.

Cuttings for the main trial were taken from the Dookie vineyard in mid June 2003 and selected from dormant healthy canes of 7-12mm diameter. Each cutting was 350 mm long. These cuttings were randomly divided into bundles of 10 canes each. Ten bundles of 10 canes formed each treatment group (100 cuttings) 0,1,4,8 and 24 hours of hydration. The water used for hydration was potable tap water with only the control receiving no hydration.

Following hydration the cuttings were kept in a cool dry cupboard under a moist hessian so as to retain their moisture and the next day placed in incubation boxes of moist clean granite sand. The cuttings were incubated there for twelve weeks to allow sufficient root development for assessment. The boxes were then opened and the cuttings assessed for root number, root length and root health. Statistical analysis for root number and length was by one-way ANOVA using Microsoft Excel. Analysis of root health was by Chi Square.

Results

Root Length

There was a significant difference ($P<0.05$) in root length between all groups except those hydrated for 8 hours and 24 hours (Table 1). Root length generally increased with increasing hydration time except in the groups treated for 8 and 24 hours, which were not significantly different ($P<0.05$) from each other.

Table 1: Mean root length following hydration for 0, 4, 8 and 24 hours.

Hydration time	0 hrs	1hr	4 hrs	8 hrs	24 hrs	LSD (P<0.05)
Mean root length mm	65.63^{a}	79.16^{b}	95.21^{c}	114.22^{d}	121.67^{d}	8.7

Means with the same superscripts are not significantly different.

Root Number

Root number was significantly ($P<0.05$) affected by hydration time. Root numbers generally increased with increasing hydration time except in the groups treated for 4 and 8 hours, which were not significantly different from each other (Table 2).

Table 2: Mean root number following hydration for 0, 4, 8 and 24 hours.

Hydration time	0 hrs	1hr	4 hrs	8 hrs	24 hrs	LSD (P<0.05)
Mean root number	3.86^{a}	6.65^{b}	8.42^{c}	9.18^{c}	10.65^{d}	1.35

Means with the same superscripts are not significantly different.

Root Health

A Chi squared test was applied to the subjective assessment of root health and showed a significant difference between all groups. Root health generally deteriorated with increasing hydration time.

Discussion

Root Length

These results indicate that the more water available to the cutting, the greater the length of the roots. The little extra water absorbed by the 8 hrs. & 24 hrs groups, had no significant effect as there was most likely adequate moisture already available in the cuttings to enable full transfer of nutrients to the roots for the continuance of growth.

Root Number

Root number increased with increased hydration time except with the 4 hour and the 8 hour group which were not significantly different. This is thought to be because the extra amount of water absorbed during that additional 4 hour period, approximately 2% as shown by the results of the preliminary trial, was not enough to increase the transfer of nutrients to the newly formed roots.
The extra 16 hours of hydration with the 24 hour group, an increase of a further 3.5% in moisture content, did increase root number. However root health was affected with increased hydration possibly a result of persistent anoxia in the tissue or the effects of pathogenic organisms.

Root Health

The results of the subjective assessment of root health when analysed by a Chi squared test showed a difference between the groups. The 24 hour group show the greatest difference in appearance; the roots being weak, withered and yellowed. This is possibly the result of anaerobic respiration brought about by the flooding of the tissue as a result of a relatively long period of hydration.

Conclusion and Recommendations

The results of this trial suggest that the optimum time for hydration is between four and eight hours to ensure the maximum number of healthy roots of a suitable length to enable the rootling to establish quickly. However it is possible that water borne pathogens could enter the wounds at the top and bottom of the cuttings during hydration. To avoid this consequence hydrating water should be treated to control potential pathogens that may be either in the water, or on the surfaces of cuttings.

References:

(See Appendix 3, pp. 146 to 149 for a compiled list of references)

P 3.2 Effects of HWT and Nursery Practices on Cell Physiology

P3.21:

The Effects Of Propagation Processes (Harvest Time, Hot Water Treatment, Dehydration, Hydration and Storage) on Tissue Condition of Hardwood Cuttings of *V. vinifera* Cv. Chardonnay.

Helen Waite[1] and Peter May[2]

[1]Institute of Land and Food Resources, The University of Melbourne, Dookie College, Victoria 3647, Australia.

[2]Institute of Land and Food Resources, The University of Melbourne, Burnley College, 500 Yarra Boulevarde, Richmond 3121, Australia

Abstract

This study examined the effects of dehydration (5 days on shed bench), hydration (0,1,8,16 & 40 hours), and HWT on the tissue of dormant Chardonnay cuttings collected in early, mid and late season by measuring the electrical impedance of the tissue at high (10.0kHz) and low (<1.0kHz) frequencies and calculating the ratio using a Plant Impedance Ratio Meter (PIRM model BP2B by Biosensors). In healthy tissue with intact cell membranes the resistance to the flow of current decreases with increasing frequency and the impedance ratio is greater than 1. In tissue with compromised membrane integrity the impedance ratio approaches 1.

The electrical impedance ratio of the tissue of Chardonnay cuttings generally, but not always significantly ($P<0.05$), decreased with each step in the propagation process leading up to callusing, suggesting that the integrity of the cell membranes was affected by the treatments.

Introduction

The work of Wample, Bary & Burr (1991) and Wample (1997) and the results of previous work by the authors (Waite & May 1999) indicate that harvest time, HWT, hydration and storage all affect callusing, root initiation and bud burst in cuttings of *V. vinifera* cultivars. However the effects of these factors are not always consistent and although the physiological state of the cuttings including depth of dormancy and the possible activation of anaerobic respiration by hydration treatments (Gray 1999) may affect the response of cuttings, there appears to be no obvious explanation for this variability (Wample 1997). The results of previous work by the authors (Waite & May 1999) also indicated that the post-callusing environment of the cuttings has different effects depending on pre-callusing treatment and the nursery conditions. The harsher conditions in the field nursery appear to have a greater impact on material than the protected conditions of the glasshouse suggesting that the effects of pre callusing nursery procedures persist into the establishment phase.

The purpose of this study was to determine the effects of dehydration, hydration and HWT on the tissue of Chardonnay cuttings by measuring the electrical impedance of the tissue in order to establish the time of onset of physiological changes in the tissue and the effects of consecutive treatments. Although to the best of the author's knowledge, electrical impedance has not previously been used to assess grapevine tissue or cuttings during the propagation cycle, it has been used successfully to determine the health of the tissue in a number of other woody perennials including conifers, deciduous trees (Glerum 1970; Glerum & Krenciglowa 1970) and *Eucalyptus obliqua* (Moore 1979). This non-destructive technique uses the Plant Impedance Ratio Meter (PIRM) to measure impedance of the tissue at two frequencies (1.0kHz & 10.0kHz) and the impedance ratio is then calculated (Anon. 1991).

Because this technique is non-destructive it can be used to follow the same material through several processes enabling a clear picture to be developed of the cumulative effects of the treatments that are applied to the material. Since the electrical impedance of plant tissue is affected by its water content, the use of the impedance ratio gives a more accurate result than the use of simple impedance measurements at a single frequency (Moore & Blazé 1980).

If the tissue being tested is healthy and the cell membranes are intact, the resistance to the flow of current decreases with increasing frequency (Hayden *et al.* 1969) and the impedance ratio is greater than 1. However, if the tissue is damaged, the resistance to low frequencies (<1.0kHz) is low and similar to the resistance to high frequencies (10.0kHz) and the impedance ratio is close to 1 (Glerum & Krenciglowa 1970). Glerum and Krenciglowa (1970) recorded a mean impedance ratio of 3.25 for live poplar stems and 1.5 for the same material after boiling. Moore (1979) recorded a mean impedance ratio of 1.786 for live stem tissue of *Eucalyptus obliqua* and 1.008 for dead stem tissue. Although Moore and Blazé (1980) acknowledge the shortcomings of electrical measurements in plant tissue assessment when used in isolation, they state that these techniques can be useful tools for detecting tissue stress or damage without destroying the sample.

Materials and Methods

The variety Chardonnay was chosen for this trial since anecdotal evidence and the results of previous work by the authors (Waite & May 1999; Waite unpub. 2002) indicated that this variety is less sensitive to nursery processes than Pinot Noir and more sensitive than Cabernet Sauvignon and would consequently provide a mid range response to nursery processes.

A total of 1,000 Chardonnay cuttings were collected from Orlando Wyndham Group source blocks at Lyndoch in the Barossa Valley, South Australia, on each of 3 occasions (3,000 cuttings in total), 5/6/2000, 3/7/2000 and 21/8/2000, representing early, mid and late season cutting collection times. The cuttings were selected to conform to VAMVVIA standards; 7-12mm in diameter and approximately 350-400mm long.

At each collection date the cuttings were randomly divided into 100 bundles of 10 cuttings each. In order to test the effects of dehydration on cutting tissue over the three collection dates the cutting bundles were randomly divided into two groups. Half the cuttings (50 bundles) from each collection time were subjected to 5 days post-collection exposure to the air in the processing shed and half (50 bundles) were processed promptly to minimize dehydration. Each lot of 50 bundles in both the dehydrated and non-dehydrated groups was then randomly allocated to 5 groups of 10 bundles (100 cuttings) and randomly assigned to 0, 1, 8, 16 or 40 hours hydration.

The cuttings that were to be hydrated were hydrated in clean potable water at the Orlando Wyndham nursery at Jacob's Creek, South Australia. The bundles were then randomly allocated to two plastic lined 1 tonne wooden apple bins and placed in the cold storage facility at the nursery and held at 1-2^{o}C. Before they were placed in storage cuttings that had been hydrated were allowed to drain to the point where there was no free water on the canes.

The same protocols were followed at each of the three collection dates. Treatment times were chosen to encompass the most common hydration times used in the nursery industry and to determine the effects of no hydration and the extreme of 40 hours hydration that is sometimes reported to occur in industry. All cuttings from all collection dates were removed from cold storage on 18/9/2000, HWT at 50^{o}C for 30 min. before being packed in clean, moist vermiculite for callusing.

High and low frequency electrical impedance readings were taken for each cutting immediately after cutting collection, dehydration, hydration, storage and HWT as appropriate according to treatments. The ratio of each pair of readings was then calculated for each cutting. The various combinations of treatment protocols gave 129 treatments for this experiment.

The electrical impedance of the cutting tissue was measured using a Plant Impedance Ratio Meter (PIRM model BP2B by Biosensors) (Moore & Blazé 1980). For each pair of readings the PIRM probe (2 parallel stainless steel needles 5mm apart) was inserted 2mm into the tissue of the internode just below the first bud up from the basal node of the cutting (approximately 80mm. from the base of the cutting) bringing the probe into contact with both the phloem and xylem tissue of the cutting. When taking subsequent readings care was taken to avoid the small holes made by the probe in the cutting tissue to ensure that the slight damaged caused by the probe did not affect later readings (De Plater & Greenham 1959).

When readings were taken following hydration of the cuttings, the bundles were allowed to drain and dry to the point where there was no free water on the surface of the cuttings that may have affected the impedance measurements. However although the water status of the tissues being tested affects impedance readings it does not affect the impedance ratio (Moore & Blazé1980).

Data analysis

For data analysis the mean impedance ratio was calculated for each bundle of 10 cuttings after each treatment. Each of these means became a replicate. Thus there were 10 replicates in each treatment group. Data was analysed using the Minitab statistical package and Microsoft Excel 97. Details of the analyses used are given in the results section. Any Minitab printout will be indicated by the use of 'Courier' font.

Results and Discussion

Because of the combinations of pre-treatments in this experiment it was not possible to use a factorial design. Analysis of these results is based on a one-way ANOVA with 129 individual treatments covering all the combinations of collection dates, dehydration and hydration times. Means separation was performed using Tukey's pairwise comparisons test The results showed a significant ($P<0.05$) treatment effect (see table 1).

Table1 One-way ANOVA for effect of collection date, dehydration, hydration, storage and HWT on the PIRM ratios of Chardonnay cuttings.

Source	DF	SS	MS	F	P
C1	128	4.60534	0.035979	60.23	0.000
Error	1160	0.692909	0.000597		
Total	1288	5.298249			

In this experiment the mean impedance ratios of healthy, freshly harvested Chardonnay cuttings were in the range of 1.29 to 1.46 (Table 2) and were consistent with ratios in other dormant grapevine cuttings tested by the author in the two preceding seasons (1998 & 1999). These readings agree with those found by the author of the PIRM manual (anon. 1991). Glerum (1970) found that impedance ratios for healthy plant tissue were generally in the range of 3.0 to 5.0 and for dead tissue in the range of 1.0 to 1.6, but states that impedance ratios are lower in dormant tissue than in active tissue.

Moore (1979) stated that the impedance ratios of healthy *Eucalyptus obliqua* tissue are somewhat lower (1.8) than those of the conifer species and maple tested by Glerum (1970), but that the impedance ratios of dead tissue were similar (1.0-1.1).

For the cuttings collected in early season, variation between the mean impedance ratios of each treatment group at collection (SD 0.03) was greater than the variation in mean impedance ratios between each treatment group in both mid (SD 0.02) and late season (SD 0.01) at collection. Least variability in the mean impedance ratios between replicates was in the material collected in late season at which time the cuttings showed active sap flow and the loosening of bud scales that precedes bud burst. Mean impedance ratios after HWT followed a similar pattern. Variability in mean impedance ratios was greater in material collected in early season (SD 0.04), less variable in cuttings collected in mid season (SD 0.02) and less variable again in cuttings collected in late season (SD 0.01).

These results indicate that the impedance of the tissue and consequently the state of the tissue was more variable at the beginning of the dormant season with variability decreasing as the season progressed. (see table 2).

It is interesting that the impedance ratios for cuttings collected in late season when dormancy was breaking were still considerably lower than the ratios Glerum (1970) found for active conifer and maple tissue (3.0-5.0) and slightly lower than the ratio Moore (1979) found for active *E. obliqua* tissue (1.8).

Table 2 PIRM ratio means[1] of all treatment combinations at each stage of treatment[2].

Collection time	Hydration time	Collection	Post dehydration	Post hydration	Post storage	Post HWT
Early	0	1.38^{c-f}			$1.28^{o-z,A-E}$	1.33^{f-m}
	1hr	1.36^{c-i}		1.30^{j-v}	$1.24^{yz,A-T}$	1.22^{K-X}
	8hrs	1.37^{c-h}		$1.27^{r-z,A-K}$	$1.26^{w-z,A-Q}$	1.22^{N-X}
	16hrs	1.38^{b-e}		$1.27^{q-z,A-K}$	$1.25^{x-z,A-R}$	1.20^{S-X}
	40hrs	1.29^{l-x}		$1.26^{u-z,A-P}$	1.23^{E-V}	1.20^{R-X}
	0	1.46^{a}	1.42^{ab}		$1.28^{n-z,A-C}$	1.19^{VWX}
	1hr	1.37^{c-g}	1.41^{bc}	1.29^{m-y}	$1.26^{t-z,A-P}$	1.23^{l-W}
	8hrs	1.32^{i-q}	1.39^{bcd}	$1.26^{t-z,A-O}$	$1.26^{s-z,A-N}$	$1.24^{z,A-T}$
	16hrs	1.37^{c-h}	1.39^{bcd}	$1.38^{n-z,A-D}$	1.19^{T-X}	1.22^{L-X}
	40hrs	1.37^{c-g}	1.46^{a}	$1.28^{o-z,A-I}$	1.21^{O-X}	1.18^{X}
Mid	0	1.36^{c-i}			1.31^{j-u}	1.23^{H-W}
	1hr	1.33^{f-m}		$1.29^{m-z,AB}$	$1.28^{n-z,A-E}$	1.24^{C-U}
	8hrs	1.35^{d-j}		$1.26^{v-z,A-Q}$	$1.25^{w-z,A-R}$	1.24^{D-V}
	16hrs	1.35^{d-k}		$1.25^{w-z,A-R}$	$1.27^{r-z,A-M}$	1.22^{K-X}
	40hrs	1.34^{e-l}		1.21^{R-X}	1.23^{H-W}	$1.24^{x-z,A-S}$
	0	1.33^{g-n}	1.31^{j-u}		$1.25^{x-z,A-R}$	$1.25^{w-z,A-R}$
	1hr	1.38^{b-f}	1.32^{g-n}	1.22^{M-X}	1.29^{m-z}	$1.25^{x-z,A-S}$
	8hrs	1.38^{b-e}	1.38^{b-f}	1.18^{WX}	$1.24^{x-z,A-S}$	$1.25^{w-z,A-R}$
	16hrs	1.38^{c-f}	1.30^{l-w}	$1.26^{r-z,A-M}$	$1.25^{x-z,A-S}$	1.19^{U-X}
	40hrs	1.38^{c-f}	1.32^{i-p}	$1.28^{o-z,A-F}$	1.24^{A-T}	1.23^{H-W}
Late	0	1.32^{g-o}			$1.28^{o-z,A-H}$	1.24^{D-V}
	1hr	1.31^{j-s}		$1.24^{yz,A-T}$	$1.27^{r-z,A-L}$	1.24^{B-T}
	8hrs	1.31^{j-t}		1.21^{P-X}	$1.28^{o-z,A-F}$	1.22^{K-X}
	16hrs	1.32^{h-p}		1.21^{N-X}	$1.28^{o-z,A-G}$	1.23^{F-V}
	40hrs	1.31^{j-v}		1.23^{J-W}	$1.24^{yz,A-T}$	1.23^{F-V}
	0	1.30^{l-v}	1.29^{m-z}		$1.26^{t-z,A-O}$	1.21^{Q-X}
	1hr	1.29^{l-x}	$1.29^{m-z,A}$	1.22^{J-X}	$1.27^{p-z,AJ}$	1.22^{K-X}
	8hrs	1.31^{j-r}	1.33^{f-m}	$1.27^{p-z,A-J}$	$1.26^{u-z,A-P}$	1.23^{G-V}
	16hrs	1.30^{j-v}	1.30^{j-v}	1.21^{Q-X}	$1.24^{yz,A-T}$	1.22^{K-X}
	40hrs	1.30^{j-v}	$1.29^{m-z,A}$	$1.24^{z,A-T}$	$1.25^{x-z,A-R}$	$1.24^{z,A-T}$

[1]Means followed by different superscript letters are significantly different from each other ($P<0.05$). Because of the large number of superscripts required to differentiate between all 129 means, both upper and lower case superscripts have been used and condensed, for example; a-d, A-D =abcdABCD.

[2]Blank cells occur where that factor was not part of the treatment cycle for that group

The effects of dehydration on the impedance of grapevine tissue

The effects of dehydration on the mean impedance ratios of the cuttings was variable in cuttings collected in early and mid season. In 3 of the 5 early season treatment groups the mean impedance ratio was not significantly affected by dehydration, but in 2 treatment groups the mean impedance ratio was significantly greater ($P<0.05$) following dehydration. By contrast, mean impedance ratios of cuttings collected in mid season was significantly lower ($P<0.05$) after dehydration in 3 of the 5 treatment groups, but not significantly different to the mean impedance ratios measured at collection in 2 treatment groups.

In the cuttings collected in late season there were no significant differences between mean impedance ratios in any of the 5 treatment groups of at collection and post dehydration. The increase in impedance ratios in 3 of the 5 treatment groups of cuttings collected in early season is consistent with reports of de Plater and Greenham (1959) and Moore and Blazé (1980) who state that tissue damaged by dehydration has a high electric resistance at all frequencies. However if this is the case, it is difficult to explain the decreased mean impedance ratios in dehydrated cuttings collected in mid season, unless that material was less susceptible to dehydration and damage to the tissue was not as severe, resulting in a decrease in the impedance ratios rather than an increase.

The effects of hydration on the impedance of grapevine tissue

In all except 2 groups, the post hydration mean impedance ratios of cuttings harvested on all 3 collection dates were significantly lower ($P<0.05$) than the mean impedance ratios of the groups at harvest, regardless of hydration time. The mean impedance ratios of group collected in early season and hydrated for 40 hours and the group collected in mid season and hydrated for 1 hour were not significantly different ($P<0.05$) to the mean impedance ratios of those groups at collection.

The reduction in mean impedance ratios following hydration in the majority of cuttings in this experiment indicates that hydration may have affected the integrity of the cell membranes of the cuttings. Although hydration may have compromised the integrity of cell membranes in this case, the damage was not fatal. When inspected at the end of the treatment cycle after storage and HWT all the cuttings were alive. These results are in concert with the results reported previously (Waite & May 1999) that showed hydration had no significant effect ($P<0.05$) on the final quality of the Chardonnay cuttings (in that experiment), suggesting that any damage caused by hydration was transient and did not result in permanent damage to the cuttings.

The effects of cold storage on the impedance of grapevine tissue

There was no significant effect ($P<0.05$) of cold storage on the mean impedance ratios of 16 of the 30 treatment groups, in 9 of the 30 treatments mean impedance ratios decreased and in 5 treatment groups the mean impedance ratio increased. In cuttings that were not hydrated or dehydrated mean impedance ratios were significantly lower ($P<0.05$) after storage regardless of collection time. In dehydrated cuttings collected in early and mid season, mean impedance ratios were significantly lower ($P<0.05$) after storage than the mean impedance ratios after dehydration. There was no consistent pattern to changes in mean impedance ratios after storage across the treatments although the significant increases in mean impedance ratios occurred only in cuttings collected in mid and late season.

The effects of hot water treatment on the impedance of grapevine tissue

Except for the group that was collected early in the season and not hydrated or dehydrated, the mean impedance ratio after HWT in all treatment groups (regardless of hydration and dehydration), was significantly lower ($P<0.05$) than the mean impedance rates at collection, indicating that the impedance of the tissue had altered during the propagation process and that the integrity of the cell membranes may have been affected.

For the group that was collected in early season and not hydrated or dehydrated there was no significant difference ($P<0.05$) between mean impedance ratios at collection and after HWT indicating that in this case tissue condition was not affected. However when post HWT mean impedance is compared with post storage mean impedance in this group the picture is less clear.

It is interesting that the mean post storage impedance ratio for this group is significantly lower than either mean impedance ratio at collection or after HWT. There is no obvious explanation for this result. In all other groups the mean impedance ratio after HWT is either lower than, or not significantly different to the mean impedance ratio after storage.

Conclusion

The electrical impedance of the tissue in Chardonnay cuttings generally, but not always significantly, decreased during the steps in the propagation process leading up to callusing, suggesting that the integrity of the cell membranes was affected during the process. However both the mean impedance ratios after HWT (>1.1) and visual inspection indicated that the tissue was not killed by any of the processes to which the material was subjected. This evidence supports the results of the experiments reported by Waite and May (1999), in which all cuttings were clearly alive after callusing and able to initiate roots.

The variability in initial mean impedance ratios between early, mid and late season collection dates indicates that the physiological state of the tissue of Chardonnay cuttings may vary during the dormant season.

The differences in variability in mean impedance readings over the 3 collection times found in this experiment may be due to a number of factors including technique and instrument function, variations in moisture content of the material and local variations within the vineyard from which the cuttings were taken. However Wample (1997) reported variations in root initiation over 4 different cutting collection times indicating that the physiological state of cuttings does vary over the dormant season and the differences in variability in mean impedance ratios between cutting times observed in this experiment may reflect the different physiological states of the material over the cutting season.

The variability in mean impedance ratios after dehydration is interesting and again appears to indicate differences in the physiological state of the cutting tissue over the dormant season. Although mean impedance ratios after dehydration were generally not significantly different ($P<0.05$), or lower than mean impedance ratios at cutting collection, they were significantly higher ($P<0.05$) in 2 groups of cuttings collected in early season. By contrast, in cuttings collected in late season, mean impedance ratios after dehydration were not significantly different ($P<0.05$) to mean impedance ratios at collection. This indicates that the internal condition of cuttings was less affected by dehydration in cuttings collected in late season than in cuttings collected in early or mid season.

This may be due to variations in moisture content of cuttings collected at different times, or to variations in the physiological state of the cuttings. It is interesting that the cuttings collected in late season showed signs of sap flow and loosening of bud scales. Both these are indicators of increased physiological activity and potentially increase the susceptibility of the cuttings to dehydration, rather than reducing susceptibility to dehydration as the results suggest.

Mean impedance ratios of all cutting groups after hydration were significantly lower ($P<0.05$) than post collection or post dehydration mean impedance ratios in 21 of the 24 treatment groups that were hydrated and lower in the remaining 3 groups, but not significantly so ($P<0.05$). It might be argued that impedance in tissue with increased moisture levels is lower and that these results merely reflect a change in moisture status in the tissue. However the use of the impedance ratio rather than direct impedance measurements is reported to minimize the effects of changes in moisture levels in tissue (Evert 1973; Moore 1979). If this is the case these results indicate that hydration begins to affect the condition of Chardonnay cutting tissue early in the hydration period since the results do not indicate that different hydration times have different effects on the mean impedance ratios of the tissue of Chardonnay cuttings.

Although electrical methods of assessing plant tissue have been in use for more than 40 years (de Plater & Greenham 1959), this technology does not appear to have been used previously to determine the effects of nursery processes on plant tissue or on the tissue of grapevines either as cuttings or *in situ*. However the similarity of the results of this experiment and the experiments reported by the authors (Waite & May 1999) that also found differences in the response of grapevine cuttings to HWT and hydration support the assertion of Moore and Blazé (1980) that impedance ratio measurements of plant tissue can be a useful tool for the assessment of tissue condition in plants, particularly when used in concert with other assessments. Further studies are required to determine the usefulness of this technique for assessing grapevine propagating material since the results of this experiment may have been affected by factors other than the condition of the tissue, including operating technique and moisture content of the tissue.

Although the results of the experiment reported here may indicate that the processes of HWT, hydration, dehydration and cold storage can affect cell membrane integrity in the tissue of Chardonnay cuttings, there may be other cellular level effects of these processes that have not been measured, but have an equal or greater effect on the normal functioning of cells and tissues. Such effects may include the disruption of enzyme systems (Baker 1962), or damage to organelles such as mitochondria (Dangeard 1951).

It would appear from the results of this experiment and the experiments reported by others (Orffer 1980; Wample Bary & Burr 1991; Wample 1993 & 1997) that the damage caused to grapevine cuttings in the production phase leading up to callusing is not lethal, but that the effects may persist into the establishment phase and result in an increased susceptibility of the cuttings to environmental stress.

References:

(See Appendix 3, pp. 146 to 149 for a compiled list of references)

P3.22:

The effects of Hot Water Treatment on Cell Ultrastructure of Dormant *Vitis Vinifera* cv. Pinot Noir tissue.

Helen Waite[1] and Gunta Jaudzems[2]

[1]Institute of Land and Food Resources, The University of Melbourne, Dookie College, Victoria 3647

[2]Monash Microimaging, Monash University, Clayton, Victoria, 3800

Abstract

Samples of internode tissue from dormant Pinot Noir cuttings were embedded in Spurr's resin under vacuum and examined with a light microscope and a transmission electron microscope (TEM). Light microscope examination of ray tissue (x100) revealed cells with secondary thickening that were packed with amyloplasts. Some of the amyloplasts in the ray tissue of HWT material appeared to be fractured.

The apparent fracturing of the starch grains may be a result of disruption of the protein membrane around the amyloplast and/or expansion of the starch grains caused by water absorption during gelatinisation that occurs at 50^{o}C. TEM examination of ray tissue showed the cytoplasm of HWT cells to be much more electron dense than in cells that were not HWT.

The increased density in the HWT cytoplasm appears to be in large part, a result of numerous ribosomes apparently clustered around endoplasmic reticulum indicating that the HWT triggered the production of large amounts of protein, most likely heat shock proteins that protect cells against the effects of heat. However the specimens had been in cold storage for several weeks prior to processing as is the normal practice in industry and the changes seen in the HWT may be due to the activation of other metabolic processes, possibly emergence from dormancy or repair to damaged cell ultrastructure. Further investigation using both microscopy and biochemical techniques are required to clarify this issue.

Introduction

Hot water treatment imposes a serious stress on grapevine cuttings and rootlings. Delayed and stunted development in the early stages of the propagation cycle has often been observed in material that have been subjected to hot water treatment (HWT) at 50^{o}C for 30 minutes (Waite 1998). It is thought that this slow and stunted growth may be a result of damage to cell ultrastructure that affects physiological processes and the capacity of the cutting to utilize the energy reserves in the tissue. In grapevines, energy is mainly stored as starch in the amyloplasts that are found in large numbers in the ray tissue of canes, but also in other tissues of the wood (Mullins, Bouquet & Williams 1992). The effects of higher than normal temperatures on plant tissue include temporary or permanent coagulation of enzymes and other proteins, the production of toxic metabolic by-products (Levitt 1980), and liquefaction of lipids (Giese 1962).

Previous work (Waite unpub. 2002) investigating the electrical impedance of grapevine internode tissue subjected to HWT indicated that HWT increased cell membrane permeability, possibly as a result of damage caused by coagulation of membrane proteins or liquefaction of lipids (Giese 1962, Levitt 1980).

In addition to the temporary or permanent disruption of metabolic processes, exposure to high temperatures also triggers the production of heat shock proteins that are synthesised in preference to normal cell proteins (Brodl 1990) and appear to provide some protection against damage from further exposure to high temperatures (Lin, Roberts & Key 1984; Morrell & Wample 1995; Woolf & Lay-Yee 1997). Gelatinisation of starch also occurs at 50^{o}C (Aurand, Woods & Wells 1987) and it is thought that HWT may cause swelling and gelatinisation in the amyloplasts and damage to the enclosing membranes preventing the utilization of the starch.

Although the early growth of HWT material is frequently slow and stunted, the capacity of cuttings and rootlings to apparently recover and make normal growth as the season progresses suggests that there is sufficient energy available within the cutting or rootling to maintain life and enable budburst, but not enough to fuel normal growth rates. Normal growth is observed to begin about 6 weeks after bud burst, when there is sufficient energy being produced by the leaves to meet demand (Mullins, Bouquet & Williams 1992).

The work reported here used both light and transmission electron microscopy (TEM) to investigate the effects of HWT (50^{o}C/30min.) on the cell ultrastructure of internodal tissue of dormant 1 year old canes of *Vitis vinifera* cv. Pinot Noir.

Materials and Methods

Twenty, dormant 2 node cuttings of *V. vinifera* cv. Pinot Noir were collected from Hanging Rock Vineyard near Macedon in Victoria on 29th July 2002 and randomly divided into 2 groups of 10 cuttings. On the day following collection one group of 10 cuttings was subjected to HWT at 50^{o}C for 30min. and immediately plunged into cold water at ambient temperature (15^{o}C) for 30min. to cool. On removal from the cooling bath the treated cuttings were allowed to drain overnight on the laboratory bench. The following day both the treated and untreated cuttings were placed in zip lock plastic bags and stored at 2^{o}C until mid October. A cutting was then selected at random from each group and small segments of internode were dissected out, killed and fixed in Karnovsky's fixative and embedded in Spurr's resin under vacuum. Sections were prepared and examined under a light microscope and by transmission electron microscope (TEM). Considerable difficulty was encountered in the processing of the tissue as the secondary thickening in the cell walls of the material impeded infiltration of the fixative and the resin.

Results and Discussion

Light Microscopy

Large numbers of amyloplasts were clearly visible in the cells of the ray tissue when viewed at 100x. In HWT tissue some of the amyloplasts appeared to be fractured, a feature not observed in the untreated tissue and although this may be an artefact of the embedding and sectioning techniques it indicates that there were differences between amyloplasts in HTW tissue and untreated tissue (fig. 1).

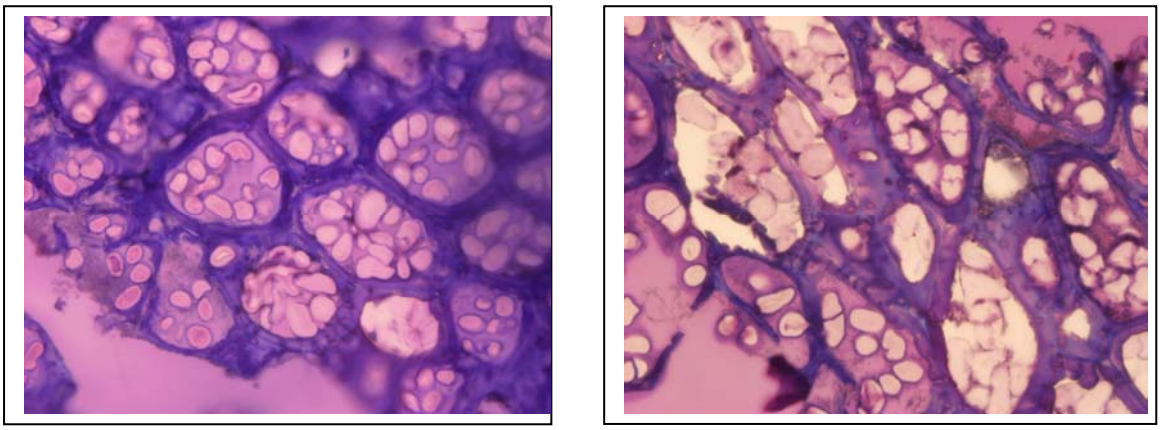


Figure 1. Light micrographs of ray tissue of *V. vinifera* cv. Pinot Noir (x100). HWT tissue on the right showing some apparently fractured amyloplasts compared to intact amyloplasts in untreated tissue on the left.

Transmission Electron Microscopy

TEM examination of ray tissue showed the cytoplasm of HWT cells to be much more electron dense than in cells that were not HWT (fig. 2). The increased density in the HWT cytoplasm appears to be in part, a result of numerous ribosomes apparently clustered around endoplasmic reticulum (fig. 3), indicating that the HWT triggered the production of large amounts of protein, most likely heat shock proteins that protect cells against the effects of heat and aid in repair of heat damage (Brodl 1990). However irreparable coagulation of cytoplasmic proteins including enzymes, may have also occurred inhibiting normal cell function.

Cell and organelle membranes were also more electron dense in the HWT treated tissue, possibly as a result of coagulation of constituent proteins. If membrane proteins and enzymes were coagulated either temporarily or permanently, the ability of cells to regulate the movements of substances across the cell membrane and in and out of organelles may have been affected, inhibiting the capacity for normal growth and development.

Other differences between HWT and untreated cells were also observed. Objects that appear to be mitochondria are visible in the cytoplasm of HWT cells but not in the cytoplasm of untreated cells. This is possibly a result of mitochondrial replication in response to increased energy requirements for the synthesis of heat shock proteins and repair activities.

Unlike other electron micrographs of mitochondria (Robards 1970) the cristae are not clearly visible, possibly a consequence of the difficulties experienced in the embedding process or the recent replication of the mitochondria.

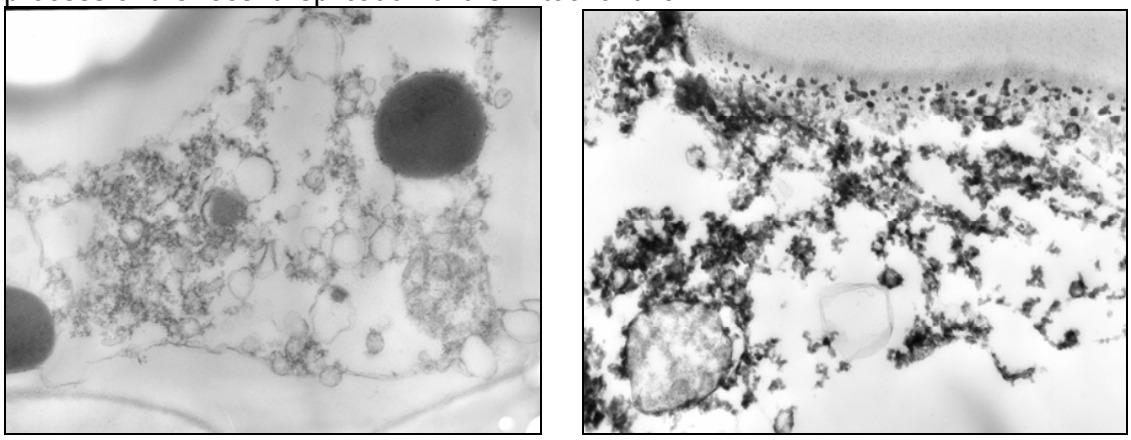


Figure 2. Electron micrographs of ray tissue of *V. vinifera* cv. Pinot Noir (x20,000). HWT tissue on the right showing increased electron density in the cytoplasm and cell membrane. Note the large number of vesicles in the cytoplasm of the untreated cell on the left and the mitochondrion in the lower right hand corner and the dark globules in the cell membrane at the top of the picture of the HWT cell on the right.

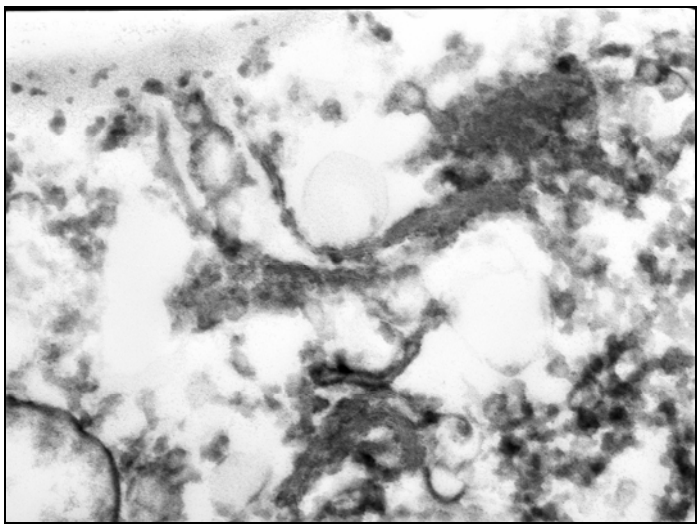


Figure 3. Electron micrograph of cytoplasm in HWT ray tissue of *V. vinifera* cv. Pinot Noir (x41,000) showing endoplasmic reticulum with ribosomes.

Differences between the plasmodesmata in HWT and non-HWT cells of ray tissue were also observed in tissue examined with TEM. Unlike the cell membranes and cytoplasm the plasmodesmata were more distinct in non-HWT cells than in HWT cells. In the HWT cells the cell membranes appear to have shrunk back from the cell walls and the plasmodesmata, possibly straining or stretching the desmotubules causing them to a appear less distinct in the electron micrograph (fig. 4).

Damage to the plasmodesmata during HWT may impede communication between cells and in the case of ray tissue might impede the movement of stored carbohydrates from the ray tissue to meristematic tissue where the demand for energy is high.

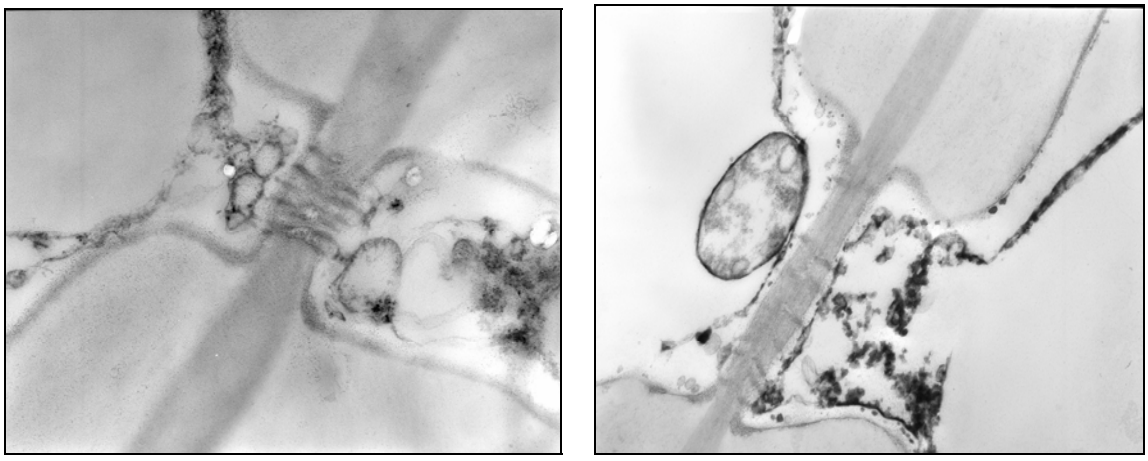


Figure 4. Electron micrographs of distinct plasmodesmata in non-HWT ray tissue of *V. vinifera* cv. Pinot Noir (x27,000) in micrograph on the left and indistinct plasmodesmata in HWT ray tissue of *V. vinifera* cv. Pinot Noir (x20,000) in micrograph on the right.

Conclusion and Recommendations

The changes in cell cytoplasm and membranes that have been observed in this work and the developmental delays observed in HWT cuttings and rootlings reported in previous work indicate that HWT has a profound effect on the physiology of treated grapevine tissue that persists while the material is held in storage and for some time after removal from storage.

The changes in the physiology of the treated material that diverts cellular activities to the production of heat shock proteins and repair activities in favour of normal cell processes indicate that the increased levels of losses that sometimes occur in HWT propagating material may be a result of an inability of the material to function normally in the early part of the season and withstand environmental stresses that sometimes occur during propagation.

In order to reduce unacceptable losses in propagation and rootling establishment during the time it takes the material to recover from HWT it is advisable to ensure that the material is not exposed to further stresses such as dehydration and poor drainage and physical damage from careless handling.

References:

(See Appendix 3, pp. 146 to 149 for a compiled list of references)

P3.23:

The Effect Of HWT And Cold Storage On The Respiration Rates Of Dormant *Vitis Vinifera* Cuttings Collected In Early, Mid And Late Season.

Helen Waite[1],

[1] Institute of Land and Food Resources, University of Melbourne Dookie College, Victoria, 3647; hwaite@unimelb.edu.au

Abstract

This study examined the effects of HWT and cold storage on the respiration rates of cuttings of Chardonnay, Cabernet Sauvignon and Pinot Noir collected in early, mid and late season by measuring the rate of CO_2 emission pre HWT, 1 hour after HWT, 24 hours after HWT and 24 hours after removal from a period of 14 weeks cold storage. Respiration rates in all 3 varieties varied with the time of collection. The respiration rate of Chardonnay was highest in early season, significantly lower ($P<0.05$), in mid season, and had begun to increase by late season, but not significantly ($P<0.05$) compared to mid season. A similar pattern was observed in Cabernet Sauvignon, but there were no significant differences ($P<0.05$) in respiration rates over the season. By contrast, the respiration rate in Pinot Noir cuttings was lowest in cuttings collected in early season, increased in mid season, but not significantly so ($P<0.05$), and was significantly higher in late season. The respiration rates of Cabernet Sauvignon cuttings collected early, mid and late season and of Pinot Noir cuttings collected in early and mid season were significantly lower than ($P<0.05$) the respiration rates of Chardonnay collected early, mid and late season apparently supporting anecdotal observations that dormancy is deepest and most prolonged in Cabernet Sauvignon and quite brief and shallow in Chardonnay.

When measured an hour after HWT, there was a significant rise ($P<0.05$) in the respiration rates of all cuttings regardless of collection time except for Chardonnay collected in early season. Although the respiration rate of the early season Chardonnay cuttings rose after HWT, the rise was not significant ($P<0.05$). When measured again 24 hours later the respiration rates of cuttings of all varieties from all collection times, including early season Chardonnay, had fallen significantly ($P<0.05$). In early season Cabernet Sauvignon, mid and late season Chardonnay and late season Pinot Noir the respiration rate was not significantly different ($P<0.05$) to the pre HWT rate. In early season Pinot Noir and in mid and late season Cabernet Sauvignon the respiration rate 24 hours after HWT remained significantly higher than the pre HWT rate. In early season Chardonnay the respiration rate was significantly lower ($P<0.05$) than the pre HWT rate.

Following removal from cold storage, the respiration rates of both HWT and non-HWT Pinot Noir cuttings, regardless of collection time, were significantly higher ($P<0.05$) than the rates at collection. There was a similar trend in Cabernet Sauvignon, although respiration rate following cold storage in both HWT and non-HWT cuttings collected in early season and non-HWT late season cuttings was not significantly different ($P<0.05$) to the respiration rate at collection. In Chardonnay the trend was somewhat different. The respiration rates of both HWT and non-HWT early season cuttings and late season HWT cuttings was lower after cold storage than at collection. However respiration rates after cold storage in both HWT and non-HWT mid season Chardonnay cuttings were significantly higher ($P<0.05$) than at collection. Late season non-HWT cuttings were the only group where there was no significant ($P<0.05$) change in the respiration rate between collection and removal from cold storage.

The generally large peak in the respiration rates of all three varieties of cuttings immediately after HWT, followed by a significant decrease over 24 hours is indicative of a wounding response as a result of exposure to the near lethal temperature of HWT. The high post HWT respiration rates also indicate a high oxygen demand during the first 24 hours following HWT. Consequently the practice of enclosing cuttings in plastic bags and placing them in cold storage shortly after HWT may impede recovery from HWT and result in oxygen deprivation during storage.

Introduction

Although there is now a considerable body of research on the effects of hot water treatment (HWT) on the development of grapevine cuttings, little is know of the effects of HWT on cutting physiology. However it is known that cuttings vary in their response to HWT depending on variety, time of cutting collection during the dormant season (Wample 1997, Waite unpub. 2002) and mother vine management. Previous work investigating dormancy in grapevine cuttings has demonstrated measurable differences in respiration rates between varieties and across the dormant season (Crocker *et al*. 2002). And it was though that this may, in part, explain the variable responses of cuttings to HWT that have been observed, but it continues to be difficult to predict the response of cuttings to HWT in all circumstances. In order to better understand the impact of HWT on cuttings of different varieties at various stages of dormancy respiration rates in *Vitis vinifera* cvs. Chardonnay, Pinot Noir and Cabernet Sauvignon cuttings collected in early, mid and late season were measured at collection, 1 and 24 hours after HWT and after 14 weeks in cold storage at 2^{o}C to determine the short and long term effects of HWT on cutting physiology. Anecdotal evidence from the vine nursery industry and previous research (Crocker *et al*. 2002, Waite unpub. 2002) indicates that these 3 varieties represent a range of responses to HWT. Cabernet Sauvignon is relatively resilient to HWT, Chardonnay somewhat sensitive and Pinot Noir quite sensitive. Respiration rates in plant material are normally measured by measuring the rate of CO_2 emission rather than oxygen consumption since oxygen is not only consumed during respiration, but enzymatic processes also use oxygen and this can result in artificially high readings for oxygen consumption and distortion of rates (Taiz & Zeiger 1998).

Materials and Methods

Cutting Material

Healthy and undamaged dormant 1 year-old canes of Chardonnay and Pinot Noir were harvested from the Hanging Rock vineyard in southern Victoria on 11/6/2002, 29/7/2002 and 26/8/2002. Dormant 1 year-old canes of Cabernet Sauvignon were also collected from the University of Melbourne vineyard at Dookie College in North East Victoria on the same dates (these cutting dates will be referred to as early, mid and late season). The canes were then cut into 2 node segments approximately 100mm long and 7-12mm in diameter. The 2 node cuttings from each collection time were then randomly allocated to 50 bundles of 5 cuttings of each variety. The cuttings were left covered, but not sealed, on the laboratory bench overnight to allow recovery from the wounding response caused by the cutting process. Each group of 50 bundles was then randomly divided into 2 groups of 25 bundles. One group of 25 bundles of each variety at each collection time was kept as the untreated control and the remaining 25 bundles subjected to HWT at 50^{o}C for 30min.

Measurement of CO_2 Emission

On the day following cutting collection, the rate of CO_2 emission from each bundle of cuttings was measured using a LI-6200 Portable Photosynthesis System (LI-COR Inc.) calibrated according to the instructions described in the accompanying manual. To determine the rate of CO_2 the change in CO_2 concentration (ppm) in the chamber of the LI-6200 over a period of 35 seconds was measured 3 times for each bundle of cuttings and the mean change calculated. Similar measurements of CO_2 emissions were also made 1 and 24 hours after HWT for the HWT groups. Both the HWT and control groups from each collection date were then enclosed in plastic bags with a small ventilation hole and placed in cold storage at 1-2^oC. All cuttings were held in cold storage for 14 weeks as per standard industry practice and removed on 23/9/2002, 4/11/2002 and 2/12/2002 depending on whether they were collected early, mid or late season.
After removal from cold storage the cuttings were left in their bags on the laboratory bench for 24 hours to enable them to equilibrate to ambient temperature when the rate of CO_2 emission of each bundle was measured again. Following the final CO_2 emission measurement the cuttings were oven dried at 50^oC and weighed when their weight had stabilized.

Calculation of Respiration Rates

The respiratory rates of the cuttings were calculated ppm/gm/second using the following formula:

$R = \Delta C/(m \times t)$ Where: ΔC = change in CO_2 concentration (ppm), m = mass (g) and t = time in seconds.

Data Analysis

Data were analysed by one-way ANOVA using the Minitab statistical package.

Results

Respiration rates of Cabernet Sauvignon, Chardonnay and Pinot Noir at cutting collection

The respiration rates of cuttings of the three varieties at the time of collection were generally variable depending on variety and collection date (table 1). In Chardonnay, cuttings collected in early season had a significantly ($P<0.05$) higher respiration rate than those collected in mid and late season. The respiration rates of Chardonnay cuttings collected in mid and late season were not significantly ($P<0.05$) different from one another, however the respiration rate of cuttings was lowest at mid season indicating that for this variety dormancy may not have been fully developed in early season and by late season respiration rates were beginning to increase and the dormant period was drawing to a close. A similar, but much more subtle and not statistically significant ($P<0.05$) trend was also apparent in the Cabernet Sauvignon cuttings. In these cuttings mean respiration rates at early (0.0068) and late season (0.0061) were very similar while the respiration rate at mid season (0.0039) was slightly, but not significantly ($P<0.05$) lower. It is interesting to note that although the respiration rates for these 2 varieties follow similar patterns, the respiration rates for Cabernet Sauvignon were significantly ($P<0.05$) lower than the respiration rates for Chardonnay, regardless of collection date. The lower respiration rate for Cabernet Sauvignon compared to Chardonnay recorded in this trial appears to support anecdotal observations from industry that dormancy in Cabernet Sauvignon is deeper and more prolonged than in Chardonnay, which is observed to have a very brief and shallow dormancy period.

In Pinot Noir respiration rates were lowest in cuttings collected in early season, slightly, but not significantly (P<0.05) higher at mid season and significantly higher (P<0.05) in late season, indicating that full dormancy in Pinot Noir is achieved earlier in the season than in Chardonnay or Cabernet Sauvignon. Although the respiration rate of early season Pinot Noir cuttings was not significantly (P<0.05) different to that of early season Cabernet Sauvignon cuttings, by mid season the respiration rate of Cabernet Sauvignon had decreased slightly and Pinot Noir had increased slightly to the point where the respiration rates of the 2 varieties were significantly (P<0.05) different. By late season this difference was significant (P<0.05). It is interesting to note that the respiration rate of late season Pinot Noir was not significantly (P<0.05) different to that of Chardonnay collected in early season.

Table 1. Respiration rates in Cabernet Sauvignon, Chardonnay, and Pinot Noir cuttings collected in early, mid and late season.

	Early season	Mid season	Late season
Cabernet Sauvignon	0.0068^{ef}	0.0039^{f}	0.0061^{ef}
Chardonnay	0.0217^{a}	0.0153^{bc}	0.0176^{b}
Pinot Noir	0.0092^{de}	0.0119^{cd}	0.0221^{a}

Note: means with the same superscripts are not significantly different (P<0.05).

Effects of Hot Water Treatment and Cold Storage on Respiration Rates of Cabernet Sauvignon, Chardonnay and Pinot Noir.

Cabernet Sauvignon

Regardless of cutting collection time, the respiration rate of Cabernet Sauvignon cuttings increased significantly (P<0.05) in the hour immediately following HWT and then decreased significantly (P<0.05) over the next 24 hours (Table 2). However, 24 hours after HWT, the respiration rate of cuttings collected in early season had fallen back to the pre HWT rate while the respiration rate of cuttings collected in mid and late season remained significantly (P<0.05) higher than the rate at collection.

Following cold storage the respiration rates of both HWT and non-HWT Cabernet Sauvignon cuttings collected in early season remained at the pre storage levels (Table 2). In HWT cuttings collected in mid season the post cold storage respiration rate was significantly higher (P<0.05) than the rate at collection, but not significantly different (P<0.05) to the rate 24 hours after HWT. The respiration rate of mid season non-HWT cuttings was significantly higher (P<0.05) following cold storage than the respiration rate at collection and significantly higher (P<0.05) than that of the HWT cuttings collected at the same time indicating that, in this case, HWT resulted in a depressed respiration rate following cold storage.

The post cold storage respiration rate of HWT Cabernet Sauvignon cuttings collected in late season was significantly higher (P<0.05) than the rate at collection and 24 hours after HWT, but not significantly higher (P<0.05) than the rate immediately following HWT or, interestingly, the rate of non-HWT cuttings collected in mid season. The post cold storage respiration rate of non-HWT Cabernet Sauvignon cuttings collected in late season was not significantly different to the rate at collection.

Table 2. Respiration rates in Cabernet Sauvignon collected in early, mid and late season +/-HWT.

Season	Treatment	Collection	HWT +1hr	HWT +24hrs	Post storage
Early season	-HWT	0.0068^{ghi}			0.0085^{fgh}
	+HWT	0.0088^{efgh}	0.0278^{a}	0.0088^{efgh}	0.0107^{ef}
Mid season	-HWT	0.0039^{ijk}			0.0214^{b}
	+HWT	0.0029^{k}	0.0193^{d}	0.0091^{efg}	0.011^{e}
Late season	-HWT	0.0061^{hij}			0.0062^{hij}
	+HWT	0.0039^{ijk}	0.0197^{c}	0.0103^{ef}	0.0208^{c}

Note: means with the same superscripts are not significantly different (P<0.05). Blank cells indicate where treatments were not applied.

Chardonnay

The respiration rate of Chardonnay cuttings collected in mid and late season increased significantly (P<0.05) in the hour immediately following HWT and then decreased significantly (P<0.05) over the next 24 hours. However there was no significant effect (P<0.05) of HWT on the respiration rate of cuttings collected in early season. The respiration rate of HWT early season cuttings 1 hour after HWT (Table 3) was not significantly different (P<0.05) to the pre HWT rate. It is interesting to note however, 24 hours after HWT, the respiration rate of mid and late season HWT cuttings was not significantly different (P<0.05) to the respiration rate of non-HWT cuttings, while the respiration rate of early season HWT cuttings 24 hours following HWT was significantly lower (P<0.05) than the respiration rate at collection time.

Following cold storage the respiration rates of HWT cuttings collected in early and mid season were not significantly different to the respiration rates of non-HWT cuttings, but the respiration rates of HWT cuttings collected in late season were significantly lower (P<0.05) than non-HWT cuttings following cold storage. These results indicate that Chardonnay cuttings collected in early season are least affected by HWT.

Table 3. Respiration rates in Chardonnay collected in early, mid and late season +/-HWT.

Season	Treatment	Collection	HWT +1hr	HWT +24hrs	Post storage
Early season	-HWT	0.0217^{cd}			0.0151^{e}
	+HWT	0.0249^{bc}	0.0282^{ab}	0.0175^{de}	0.0151^{e}
Mid season	-HWT	0.0153^{e}			0.0322^{a}
	+HWT	0.0164^{e}	0.0258^{bc}	0.0145^{ef}	0.0321^{a}
Late season	-HWT	0.0176^{de}			0.0173^{de}
	+HWT	0.0163^{e}	0.0225^{c}	0.0157^{e}	0.0102^{f}

Note: means with the same superscripts are not significantly different (P<0.05). Blank cells indicate where treatments were not applied.

Pinot Noir

Regardless of collection time the respiration rates of all HWT Pinot Noir cuttings increased significantly (P<0.05) in the hour following treatment and had fallen significantly (P<0.05) again 24 hours after HWT. However, the late season cuttings were the only group where respiration returned to the pre treatment rate. In both the early and mid season groups the respiration rate 24 hours after HWT was significantly higher (P<0.05) than the pre HWT rate.

Following cold storage, the respiration rate of early and late season HWT cuttings was significantly higher than the non-HWT early late season cuttings respectively, while the post cold storage respiration rate of the mid season HWT cuttings was not significantly different (P<0.05) to that of the mid season non-HWT cuttings.

Table 4. Respiration rates in Pinot Noir collected in early, mid and late season +/- HWT.

		Pre HWT	HWT +1hr	HWT +24hrs	Post storage
Early season	-HWT	0.0092^{i}			0.0189^{f}
	+HWT	0.0087^{i}	0.0287^{b}	0.0123^{h}	0.0152^{g}
Mid season	-HWT	0.0119^{h}			0.0152^{g}
	+HWT	0.0101^{hi}	0.0273^{bc}	0.0191^{ef}	0.0167^{fg}
Late season	-HWT	0.0221^{d}			0.0319^{a}
	+HWT	0.0208^{de}	0.025^{c}	0.0186^{f}	0.0271^{bc}

Note means with the same superscripts are not significantly different (P<0.05). Blank cells indicate where treatments were not applied.

Discussion

The differences in respiration rates between varieties at collection and the variable responses to the treatments are most likely attributable to interactions between the different genotypes and the environmental conditions, although it is possible that differences between Cabernet Sauvignon and the other 2 varieties might have been a result of different climatic conditions in the 2 vineyards. However there is a considerable body of anecdotal evidence in industry and the work of McIntyre, Lider and Ferrari (1982) that supports the much later date for bud break in Cabernet Sauvignon than either Pinot Noir or Chardonnay.

The significant increase in respiration rates in Cabernet Sauvignon and Pinot Noir and in mid and late season Chardonnay cuttings immediately following HWT are likely to be a result of stimulation of respiration rates caused by elevated temperatures and/or a wounding response to the unnaturally high temperature of HWT and the consequent increased activity involved in the production of heat shock proteins (Brodl 1990, Vierling 1991, Morrell *et al*. 1997). However it is interesting that although the respiration rate of early season Chardonnay cuttings increased, the response to HWT was not significant (P<0.05). It is difficult to explain this different response except to conclude that the Chardonnay mother vines, which were not yet fully dormant and still respiring at a relatively high rate when the cuttings were collected, may have produced heat shock proteins in response to high summer temperatures that were, at least in part, retained in the tissue until the vines were fully dormant. However this hypothesis is purely speculative and further investigation of the heat shock response in Chardonnay cuttings is required to explain this result.

The significant decline in respiration rates, in some cases to the pre HWT respiration rate, in the 24 hours following HWT indicates a degree of recovery from the immediate stress of the treatment. Some European nurseries report improved performance in cuttings that have been allowed to recover from HWT for at least 24 hours following HWT before consignment to cold storage (Waite 1999). This might be explained by the relatively high oxygen demand of cuttings with high respiration rates in the immediate post HWT period and the restricted oxygen supply inside packaging further exacerbating the stress of HWT. Consequently it may be beneficial to delay cold storage of HWT grapevine cuttings in commercial nurseries for at least 24 hours following HWT instead of the usual practice of storing cuttings within 24 hours of HWT.

The lack of a consistent pattern between pre and post storage respiration rates in cuttings collected in early, mid and late season and +/-HWT is further evidence of innate variability in cuttings at collection affecting the response to natural or imposed changes in the environment. As indicated above, the differences in response are likely to be a result of interactions between genotype and environment, including factors such as the completeness of dormancy and the relative activity of metabolic processes (Lavee & May 1997), management and health status of mother vines (Crocker *et al*. 2002) and climatic factors such as drought and extremes of temperature.

Conclusion and Recommendations

The variability in respiration rates in dormant grapevine cuttings pre and post cold storage, collected early, mid and late season and +/-HWT, reported in this work add substance to the findings of earlier studies that have demonstrated a variable response to HWT by cuttings of the same cultivars as used in this work collected early, mid and late season. Although it has been shown that respiration rates vary according to the nature of the material and the processes to which they are subjected, the exact nature of the observed variability is currently unknown. Further work to investigate cutting physiology during dormancy and the interaction between cuttings and natural and imposed environmental factors is required in order to elucidate these responses.

The elevated respiration rates observed in cuttings during the period immediately following HWT are likely to cause a commensurate increase in oxygen demand. If cuttings are placed in bags in cold storage within 24 hours of HWT there may be insufficient oxygen to meet requirements further stressing the cuttings. These observations and the anecdotal evidence from European nurseries indicate that it is likely to be beneficial to delay cold storage of HWT grapevine cuttings for at least 24 hours following HWT instead of the usual practice in nurseries of storing cuttings within 24 hours of HWT.

References:

(See Appendix 3, pp. 146 to 149 for a compiled list of references)

P 3.24:

The effects of source area and hot water treatment on the development of *Vitis vinifera* cv. Pinot Noir cuttings

Helen Waite[1]

1. Institute of Land and Food Resources, University of Melbourne Dookie College, Victoria, 3647; hwaite@unimelb.edu.au

Abstract

This study investigated the effects of source area and HWT on the development of *Vitis vinifera* cv. Pinot Noir. Cuttings were sourced from the Barossa Valley and Riverland of South Australia, Margaret River (WA) and Kyneton (Vic) representing a range of climates from hot and dry (Riverland) to cold and moist (Kyneton). Half the cuttings from each area (500) were HWT and the other half remained untreated. Cuttings were assessed at callusing for callus, root and bud development and at lifting for shoot and root development and mortality. Callus development was not affected by source or HWT.

The number of root initials forming at the base of cuttings varied between source areas, but not between treatments except in the cuttings sourced from Western Australia where cuttings that had been subjected to HWT had significantly ($P<0.05$) fewer roots than those that were not HWT. The mean length of roots formed at the base of the cuttings at callusing also varied between source areas and treatments. Longest roots were on non-HWT cuttings sourced from Western Australia followed by cuttings sourced from the Riverland, Barossa Valley and Kyneton in descending order. However HWT had no significant effect ($P<0.05$) on root length except in the groups sourced from WA. When root number and length are considered together cuttings sourced from the Riverland appears to develop a stronger root system in the early stages of the propagation cycle than cuttings from the other sources. Cuttings sourced from Western Australia and Kyneton and not HWT had the highest number of extended apical buds at callusing although cuttings sourced from Kyneton were not significantly different ($P<0.05$) from cuttings sourced from all other areas regardless of treatment. When the there was no significant difference ($P<0.05$) in the number of live vines recovered from each original bundle of 100 cuttings except in the HWT group sourced from Kyneton where mortality was significantly higher than in all other groups except HWT cuttings sourced from the Barossa Valley. The variations in performance observed between cuttings from different source areas in this trial may be the result of a number of factors including climatic differences, irrigation, vine nutrition and crop load or interactions between all these factors. On the basis of these results, growers may prefer to obtain cuttings from the Riverland or Barossa Valley.

Introduction

During a series of workshops held in December 2000 to communicate the results of recent hot water treatment (HWT) research to the vine nursery industry and vine improvement associations, the issue of the effects of source area on cutting development and response to HWT was raised. Anecdotal reports from members of industry indicated that the performance of cuttings during propagation varies between source areas. Cuttings from some areas were reported to develop into superior quality rootlings with fewer losses during the propagation cycle than cuttings from other areas and that differences were more pronounced in cuttings that were HWT. The view of nursery personnel was that the observed differences were the result of variations in growing conditions in the source vineyards and were possibly related to variations in climate or management practices, particularly irrigation regimes.

In response to these industry concerns a trial was established in 2001 to examine the effects of HWT on root, bud and shoot development and final condition in Pinot Noir cuttings sourced from different climatic areas. Cuttings were obtained from the Margaret River region of Western Australia, the Barossa Valley, the South Australian Riverland and the Kyneton district in Victoria. It was felt that these areas represented a variety of climates ranging from hot and dry (Riverland) to cold and wet (Kyneton). Difficulty of supply meant that cuttings could not be obtained from other areas. The variety Pinot Noir was chosen since earlier work in the Grape and Wine Research and Development Corporation (GWRDC) HWT project SAR 99/4 has identified Pinot Noir as a variety that is particularly sensitive to propagation practices including HWT.

Materials and methods

A total of 1,000 Pinot Noir cuttings were obtained from each of four source areas, Margaret River (D5V12), the Barossa Valley (D5V12), the Riverland (D5V12) and Kyneton (NV6). Cuttings from each source area were randomly divided into 10 bundles of 100 cuttings each to reflect standard industry practice. The cuttings were packed in clean plastic bags with two small holes for ventilation and stored at 1-2^{o}C until 15/10/2001.

Prior to storage all cuttings were hydrated in clean potable water for 1 hour. This hydration time was chosen since the results of trials conducted in 2000 as part of the GWRDC HWT project SAR99/4 indicated that a hydration period of 1 hour prior to storage was beneficial and results were better than no hydration, or hydration for periods greater than 1 hour.

Upon removal from storage 5 bundles of 100 cuttings from each source area were randomly allocated to one of two treatment groups. One group of 5 bundles from each source area was HWT at 50^{o}C for 30min. and the remaining group of 5 bundles from each source area was kept as an untreated control. All cuttings were then packed with clean moist vermiculite in clean wooden crates that were lined with new plastic bags. A covering layer of dry perlite was applied to a depth of 5mm over the surface of the packed cuttings as per industry best practice. The cuttings were then incubated at 27^{o}C in the callusing room at Adro Grafted Vines, Griffith, NSW. The callusing room was managed to ensure that no hot or cold spots developed during the incubation period.

All cuttings were removed from the callusing room on 6/11/2001 and assessed for root and bud development. Twenty cuttings were selected at random from each bundle of 100 cuttings for detailed assessment. Callus tissue at the base of each of the 20 cuttings randomly selected from each bundle of 100 cuttings was assessed as present or absent and root numbers and length were measured. The development of the apical bud on each cutting was also assessed by ranking buds as closed, with no sign of bud movement or loosening of bud scales; open, with loose bud scales and emerging leaves; and extended, with shoot and leaf development clearly visible.

Following assessment, all cuttings were planted 150mm apart in the field nursery at Adro Grafted Vines and grown on as standard commercial cuttings under flood irrigation. The soil in the field nursery is an even well drained sandy loam and was laser levelled to ensure even irrigation (Fig 1).

The cuttings were inspected in the field on 3/1/2001 and assessed for shoot development. The length of the top 3 buds on 20 randomly selected cuttings from each bundle of 100 cuttings was measured. At the conclusion of the trial in July 2002 when the rootlings were dug from the nursery beds shoot and shoot numbers were measured in 20 randomly selected cuttings from each group (formerly bundles) of 100 cuttings.

Data Analysis

Data analysis was carried out using the Minitab statistical package.

Results

Callus development

In this trial callus development was not affected by either source of cuttings or HWT. The majority of the cuttings were callused (table1) with the callus forming a complete ring at the base of the cutting.

Table 1: Mean number of cuttings per group of 20 with developed callus tissue.

	WA	Riverland	Barossa	Kyneton
No HWT	19.8	20	19.8	20
HWT	20	20	20	19.2

Root development at callusing

In this trial the number of root initials forming at the base of cuttings varied between source areas, but not between treatments except in the cuttings sourced from Western Australia where cuttings that had been subjected to HWT had significantly (P<0.05) fewer roots than those that were not HWT. Cuttings sourced from the Riverland developed the largest number of root initials followed by the Barossa Valley, Western Australia and Kyneton in descending order.
Root initiation was not significantly (P<0.05) suppressed by HWT in cuttings from the Barossa Valley, the Riverland and Kyneton. However there was a significant effect (P<0.05) of HWT on root initiation in cuttings sourced from WA (Table 2).

Table 2: Mean number of root initials per cutting from different source areas and +/- HWT

	WA	Riverland	Barossa	Kyneton
No HWT	3.61 [b]	5.45 [a]	3.83 [b]	1.08 [c]
HWT	1.05 [c]	4.42 [ab]	4.03 [b]	0.53 [c]

Superscript applies to all means in table – figures with the same superscript are not significantly different (P<0.05)

In this trial the mean length of roots formed at the base of the cuttings at callusing also varied between source areas and treatments. Longest roots were on non-HWT cuttings sourced from Western Australia followed by cuttings sourced from the Riverland, Barossa Valley and Kyneton in descending order. However HWT had no significant effect (P<0.05) on root length except in the groups sourced from WA. (Table. 3).
When root number and length are considered together cuttings sourced from the Riverland appears to develop a stronger root system in the early stages of the propagation cycle than cuttings from the other sources.

Table 3: Mean length of roots forming during callusing in Pinot Noir cuttings from different source areas and +/-HWT

	WA	Riverland	Barossa	Kyneton
No HWT	47.83 [a]	39.85 [ab]	31.88 [b]	13.38 [cd]
HWT	22.8 [bc]	42.8 [a]	25.88 [b]	6.98 [d]

Superscript applies to all means in table – figures with the same superscript are not significantly different (P<0.05)

Bud development

In this trial the development of apical buds at callusing varied between source areas and treatments. Cuttings sourced from Western Australia and Kyneton and not HWT had the highest number of extended apical buds at callusing although cuttings sourced from Kyneton were not significantly different (P<0.05) from cuttings sourced from all other areas regardless of treatment.

Table 4: Apical bud development at callusing in Pinot Noir cuttings from different source areas +/-HWT.

	WA	Riverland	Barossa	Kyneton
No HWT	2.54 [a]	2 [b]	2.03 [b]	2.26 [ab]
HWT	2.01 [b]	1.97 [b]	2.1 [b]	2.14 [b]

Superscript applies to all means in table – figures with the same superscript are not significantly different (P<0.05)

At the mid season assessment (3/1/2002) shoot development from the apical bud was suppressed in HWT cuttings from all source areas, but not always significantly so (P<0.05) (Table 5). The relatively advanced apical bud development observed at callusing in non-HWT cuttings sourced from Western Australia was carried through into the apical shoot development at mid season.
Figure 5: Length of mid season shoot growth from first 3 buds in Pinot Noir cuttings sourced from different areas and +/-HWT

Table 5: Apical bud development at mid season in Pinot Noir cuttings from different source areas +/-HWT.

	WA	Riverland	Barossa	Kyneton
No HWT	116.9[a]	84.4 [ab]	128.42 [a]	82.3 [ab]
HWT	74.53[b]	72.4[b]	90.11 [ab]	34[b]

Superscript applies to all means in table – figures with the same superscript are not significantly different (P<0.05)



Figure 1: Pinot Noir cuttings in the field nursery at Griffith in early spring. HWT cuttings on right of photograph and non-HWT cuttings on left of photograph. Note the delayed development in the HWT cuttings.

Final condition

When the cuttings, now fully developed rootlings, were lifted from the field and counted at the end of the growing season there was no significant difference (P<0.05) in the number of live vines recovered from each original bundle of 100 cuttings except in the HWT group sourced from Kyneton where mortality was significantly higher than in all other groups except HWT cuttings sourced from the Barossa Valley (Table 6).

Table 6: Mean number of live vines recovered from each bundle of 100 cuttings at end of season in Pinot Noir from different source areas +/-HWT.

	WA	Riverland	Barossa	Kyneton
No HWT	94.4[a]	95.6[a]	85.2[a]	87[a]
HWT	88.4[a]	95.4[a]	82.2[ab]	58.8[b]

Superscript applies to all means in table – figures with the same superscript are not significantly different (P<0.05)

The number of roots per live was not affected by source area or HWT (table 6), but shoot development was significantly affected (P<0.05). Shoot development from the apical bud varied with source areas and HWT.

Table 7: Mean root numbers at end of season in Pinot Noir cuttings from different source areas +/-HWT.

	WA	Riverland	Barossa	Kyneton
No HWT	9.54	8.9	9.96	7.76
HWT	8.36	8.01	8.73	8.43

HWT suppressed shoot development in all source areas except Kyneton and longest shoot were recorded on vines sourced from Western Australia and the Barossa Valley (table 8)

Table 8: Apical bud (shoot) development (mm) at end of season in Pinot Noir cuttings from different source areas +/-HWT.

	WA	Riverland	Barossa	Kyneton
No HWT	237.8[a]	146.1[b]	194.3[a]	105.6[bcd]
HWT	105.6[bcd]	85.9[d]	133[bc]	94.6[cd]

Superscript applies to all means in table – figures with the same superscript are not significantly different (P<0.05)

Discussion

Callus development in cuttings is often used in the nursery industry as a guide to cutting viability and potential vigour of the resulting rootling. Callus development in the cuttings in the trial reported here was not affected by either source of cuttings or HWT and the majority of cuttings had a complete ring of well-developed callus tissue when examined. Previous work (Waite 2002) indicated that although callus development in Pinot Noir is affected by HWT, time of cutting collection and the position of the cutting on the cane, the relationship between callus and root development is weak (R^2=0.1865) suggesting that callus development indicates that a cutting is alive, but is not a reliable indicator of the capacity of the cutting to form roots. In the trial reported here the uniform callus development, but variable root development between groups and treatments supports the previous findings that the presence of callus signifies the cutting is alive, but that callus development is not a useful indicator of cutting performance.

The reduction in root numbers and length observed at callusing in HWT cuttings sourced from Western Australia and Kyneton parallels the results of Orffer (1977) and Orffer *et al.* (1979) who found that HWT initially suppressed root development in grapevine cuttings. The response of cuttings collected from the Barossa Valley and the Riverland is somewhat different. Unlike the material collected from the other source areas, root numbers at callusing in the material collected from the Barossa Valley were not suppressed by HWT

however root length was suppressed. It is interesting that the reverse of this pattern occurred in the cuttings collected in the Riverland.

Other authors (Wample, Bary & Burr 1991; Wample 1997) report a variety of responses at a range of temperatures up to 60^{o}C. Wample (1997) discusses the variation in response to HWT in cuttings collected at different times during the dormant period and speculates that cuttings of different cultivars or cuttings of the same cultivar sourced from different climatic areas may respond differently to HWT.
The results reported here appear to add weight to this conclusion. The results of previous work (Waite 2002) demonstrated that although HWT affects root development at callusing, the relationship between root development and the final condition of cuttings is quite weak (R^2=0.0633).
Although root development at callusing was affected by source area and HWT the number of roots on surviving vines at the end of the season was not significantly affected ($P<0.05$) by source area or treatment.

The results of a study by Wample (1993) examining the development of Cabernet Sauvignon buds after various time and temperature combinations showed that HWT progressively inhibits bud development with increasing treatment temperature and duration (52^{o}C-60^{o}C and 10-30min duration). However Orffer and Goussard (1980) reported that HWT (50^{o}C for 30min) stimulated bud development in Ramsey and Jacquez cuttings. In the present trial apical bud development in HWT cuttings at callusing was generally less advanced than in non-HWT cuttings and apical bud development at callusing was generally reflected in apical shoot length at mid season indicating that the effects of HWT and source area persisted into the growing season. The more advanced bud development combined with the slower root development in cuttings sourced from Kyneton may have resulted in moisture stress and contributed to the higher mortality in the HWT cuttings.

The stunted early season growth in HWT cuttings of Pinot Noir observed in previous trials (Waite 2002) was also observed in the HWT cuttings in the present trial. It was also observed in both this trial and the previous trial (Waite 2002) that the early season stunting did not persist and the HWT cuttings grew normally from mid season onwards. The persistence of the effects of HWT into the early part of the growing season possibly increases the susceptibility of HWT cuttings to adverse environmental conditions in the field nursery early in the season and may result in losses or poorer quality rootlings.

Cuttings sourced from Western Australia were generally more advanced in their root and shoot development than cuttings sourced from other areas. Anecdotal evidence from industry sources in Western Australia indicates that dormancy for many *V. vinifera* cultivars is brief in that state and bud burst occurs earlier than in the Eastern states. This may explain the relatively advanced development of non-HWT cuttings from that area. Cuttings from Western Australia were also quite sensitive to HWT and both root and shoot development were suppressed. Cuttings sourced from the Riverland and Barossa Valley were generally less advanced than cuttings from Western Australia, but were less affected by HWT indicating that cuttings from these sources may be the preferred choice for propagation purposes. The generally poorer performance of cuttings sourced from Kyneton in this trial may be attributable to the particularly harsh growing conditions in the source vineyard or the characteristics of the clone (MV6 compared to D5V12 from the other source areas). The vineyard soil is a shallow granitic sandy clay loam and the climate in the Kyneton district is cold with snow falling in most winters. Drought conditions also prevailed in 2001. The warm summer temperatures experienced in the other districts may have also stimulated the development of heat shock proteins in the tissue which may have persisted for long enough to provide some protection from the stress of HWT.

Conclusion

The results from this trial indicate that cutting performance is affected by both source area and HWT and possibly by clonal variation. On the basis of the results reported here, growers may prefer to obtain cuttings from the Riverland or Barossa Valley. However it should be remembered that these results cover one season only and different results might be obtained in different seasons (Vierling 1991; Morrell & Wample 1995).

The variations in performance observed between cuttings from different source areas in this trial may be the result of a number of factors including climatic differences, irrigation, vine nutrition and crop load or interactions between all these factors. The effects of irrigation practices on cutting performance will be examined in a trial commencing in the 2002 season.

References: (See Appendix 3, pp. 146 to 149 for a compiled list of references)

5.4 Program 4: QA, Industry Adoption & Technology Transfer

P4.1 Extension of Research Results

1) HWT Research Update Seminars

Results obtained during SAR 99/4 were presented to Australian vine nursery operators in a series of interactive workshops convened in conjunction with VINA throughout southern Australia. The workshops provided a means of rapidly transferring research results to HWT practitioners in a setting that encouraged discussion by nurserymen and sought their input on future research directions.

The workshops were well attended by VINA, AVIA and vine improvement delegates and were convened as follows:

November to December 2000

1. Busselton WA, 29th Nov 2000
2. Griffith NSW, 5th Dec 2000
3. Dixon's Creek Vic, 7th Dec 2000
4. Lenswood SA, 11th Dec 2000
5. Loxton SA, 13th Dec 2000
6. Irymple Vic, 14th Dec 2000

January 2002

1. Busselton WA, 18th Jan 02
2. Lenswood SA, 21st Jan 02
3. Loxton SA, 22nd Jan 02
4. Irymple Vic, 23rd Jan 02
5. Griffith NSW, 25th Jan 02

Details associated with each workshop and their outcomes were submitted to GWRDC as two individual workshop tour reports dated 29th June 2001 and 26th March 2002. Copies of each report can be obtained from the GWRDC website at www.gwrdc.com.au

2) Poster Presentations, Workshops and Conference Attendance.

The following poster, workshop and conference presentations served to further disseminate results obtained during SAR 99/4, both within Australian and internationally…

- Australian Wine Industry Association – Wagga NSW. Guest presentation at national conference July 2000
- 11th Australian Wine Industry Technical Conference – Adelaide SA. Poster presentation 'Hot Water Treatment – Implications of hot water treatment and other nursery practices in the production of quality grapevine planting material', October 2001
- 2nd ICGTD Workshop on 'Esca and Grapevine Declines', Lisbon Portugal, presentation of paper titled 'Implications of hot water treatment and other nursery practices in the production of quality grapevine planting material', September 2001
- Assistance with delivery of dormancy and HWT protocols modules to the Hot Water Treatment Operators Course, Nuriootpa SA May 2002
- Presentation on dormancy measurement and HWT research progress, SAVI Annual General Meeting 27th May 2002
- 14th ICVG Conference, Locorotondo, Italy, Presentation of a paper titled 'Australian advances in hot water treatment research', September 2003.

3) Published papers.

The following papers were published between 2000 and 2003…

Crocker, J.A., (2001) 'Development of Effective, Efficient and Reliable Hot Water Treatments, Workshop Tour Report'. Delivered to the Grape and Wine Research and Development Corporation, 29th June 2001, 26 pages.

Crocker, J., Fletcher, G., Waite, H., Wright, P., (2001) 'Australian Advances in Hot Water Treatment Research'*, The Australian Grapegrower and Winemaker, April 2001*.

Crocker, J.A., (2002) 'Development of Effective, Efficient and Reliable Hot Water Treatments, Workshop Tour Report'. Delivered to the Grape and Wine Research and Development Corporation, 26th March 2002, 25 pages.

Crocker, J., Waite, H., Wright, P. & Fletcher, G. (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*, No. 461a 2002, pp 33-37.

Crocker J., Wright P., Deverell P., and Waite H. (2003), 'Australian Advances in Hot Water Treatment Research'. Extended Abstracts, 14th Meeting of the International Council for the Study of Virus and Virus Like Diseases of the Grapevine., pp 71-72.

Crocker J., (2003), ' Report on an International Vine Nursery Study Tour and Summary of the 14th ICVG Conference, Locorotondo - Italy'. Conference and Tour Report to the Grape and Wine Research and Development Corporation. 96 pages.

Waite, H., Crocker, J., Fletcher, G., Wright, P., and deLaine, A. (2001) 'Implications of Hot Water Treatment and Other Nursery Practices in the Production of Quality Grapevine Planting Material'. *Phytopathologica Mediterranea, Vol. 40, Supplement 2001.*

Waite, H. (2002), The effects of hot-water treatment on grapevine propagating and planting material, Masters of Applied Science Thesis, Burnley College, the University of Melbourne.

P4.2 Improved AVIA and VINA HWT Protocols

The following publication integrates results obtained during SAR 99/4 to produce a revised HWT protocol document for distribution to AVIA and VINA members. This document revises known procedural thinking on HWT within Australian vine nurseries and was produced as a major outcome associated with the completion of SAR 99/4.

The following document forms the HWT module associated with VINA's Vine Industry Nursery Accreditation Scheme. It provides both a benchmark protocol and an advisory document for consideration by anyone contemplating HWT for vine cuttings, rootlings and scion wood.

Hot Water Treatment for Grapevine Cuttings, Rootstock Cuttings, Rootlings, and Scion Wood.

Version 1.0, November 2003.

John Crocker[1], Paul Wright[2] and Helen Waite[3]

[1] Senior Research Officer – Viticulture; SARDI - Lenswood Centre, Swamp Road, Lenswood South Australia 5240. Ph (08) 8389 8802, crocker.john@saugov.sa.gov.au

[2] Chairman – Vine Industry Nursery Association, PO Box 180, Mt Pleasant, SA 5235 Ph (08) 8568 2383. E-mail plwright@senet.com.au

[3] Lecturer, Horticulture and Viticulture; Institute of Land and Food Resources, University of Melbourne, Dookie College, Victoria 3647. Ph (03) 5833 9285, hwaite@unimelb.edu.au

1.0 Purpose, Responsibilities, and Disclaimer

1.1 Purpose of HWT

Hot water treatment is the process of immersing dormant vine cuttings or rootlings in clean hot water at defined temperatures and for defined time periods to significantly reduce the probability of transferring specific insect pests or specific bacterial and fungal pathogens to new vineyards through propagation material.

1.1 Responsibility

The authors recommend that only suitably trained and accredited hot water treatment operators are employed to carry out this process.

1.2 Disclaimer

Please note that the protocols discussed in this document are provided in good faith and are considered to be accurate at the date of publication. The following protocols are compiled from the collective commercial experience of the nursery industry, information available in the public domain, published scientific literature and the results of GWRDC sponsored research by Australian scientists.

Please Note:
Persons using the information contained in this document do so at their own risk

Any individual using the information presented in this document must do so in the knowledge that the authors cannot be held liable for any loss incurred by the individual or associated commercial entities. Control cannot be exerted by the authors over the activities of individuals using this protocol or the range of biological, engineering and procedural issues critical to the successful execution of individual hot water treatment batches.

2.0 Definition of Hot Water Treatment

Australian vine nurseries routinely use two main types of hot water treatment, namely:

2.1 Short Duration Hot Water Treatment

'The immersion of good quality, dormant vine cuttings or rootlings in clean hot water at *54^0C for 5 minutes with a temperature variation no more than +/-1^0C* during the treatment period.'

Short duration hot water treatment is the minimum requirement specified by Government Authorities for the movement of propagation material between regions in Australia and is an effective surface treatment for use against *Phylloxera*, nematodes and a discrete range of plant pathogens.

2.2 Long Duration Hot Water Treatment (referred to as 'HWT' in this document)

'The immersion of good quality, dormant cuttings or rootlings in clean hot water at *50^0C for 30 minutes with a temperature variation no more than +/-1^0C* during the treatment period.'

Long duration HWT is a surface and penetrating treatment that satisfies quarantine regulations for the movement of propagation material between regions in Australia and provides control of *Phylloxera* and nematodes, initial suppression of fungi including *Phytophthora* and *Phomopsis* and suppression of bacteria including Crown Gall.

Please note: *Hot water treatment is not suitable for the removal of viral infections from cuttings or rootlings and will not improve the performance of poor quality propagating material.*

3.0 Selection and storage of cuttings or rootlings suitable for Hot Water Treatment

Not all grapevine cuttings or rootlings will respond in the same manner to HWT. Research has shown that cuttings of some varieties (for example Pinot Noir, Merlot, Riesling and to a variable extent, Chardonnay) show an initial delay in their ability to form roots when subjected to long duration HWT. Cuttings generally recover from this through good management in the shadehouse and avoidance of moisture and heat stress if planted directly into field nurseries. Poor post-HWT nursery and management practices may result in varying degrees of mortality in sensitive varieties.

3.1 Notes on cutting collection, processing and pre-HWT storage

Grapevine cuttings have been shown to perform well following HWT where the cuttings were collected from well managed, source blocks that have received routine vine health inspections and comply with the following general criteria:

- The cutting source vines have been well managed
- Have received adequate nutrition
- Have not received excessive nitrogen
- Have not been overcropped
- Are free from disease
- Have known, traceable parentage
- The source vines have not been subjected to severe frost
- The canes should not be bruised, scarred or broken
- The canes must be fully lignified, not green
- The cutting source vines have not been subjected to excessive moisture stress during the cropping season and have received either post harvest irrigation or regular post harvest rainfall prior to cutting collection
- The source vines are fully dormant - Best results are generally observed from cuttings that are collected 4 weeks after leaf fall and 4 weeks before bud burst with no visible sign of sap flow when collected and no sign of budswell. (*Research into vine dormancy has shown that vines at Lenswood (SA) show internal signs of increased activity as early as 5 weeks before budswell)*.

Once cuttings have been harvested they perform well if handled carefully and are processed without delay – the same general principles apply for rootlings. Research has shown that good cutting moisture status and the protection of cuttings against moisture loss following collection have a significant, positive impact on root initiation and performance following HWT. As cuttings dehydrate they progressively lose their viability. Attention to the following critical points has the potential to significantly improve cutting performance following HWT:

- Cuttings collected and bundled in the field should not be contaminated by soil, should be shaded following collection and should be protected from dehydration

- All cuttings should be labelled at the point of collection. The label should clearly identify at least the following…1) cutting variety and clone, 2) source area location and 3) date of cutting collection

- Cuttings should be delivered as rapidly as possible (for example within 8 hours) to the nursery or packing shed and should be protected from wind, sunlight and dehydration during transportation and should not be stored in association with fuel or pesticides during transportation

- If desired, cuttings may be hydrated by immersing in clean, sanitised water (e.g. between 1 and 8 hours) as soon as possible after delivery to the nursery or processing shed. At this point, some nurseries chose to treat cuttings with fungicides prior to cold storage.

- Research has demonstrated that hydration, and particularly long duration hydration (8 hours +), is not necessarily beneficial and may initiate fermentative breakdown of cuttings during storage

- Hydration water should be clean, changed daily and should be sanitised through chlorination. (e.g. chlorinated up to 10ppm free chlorine using stabilised pool chlorine or similar product. Commercial chlorination rates vary from 5ppm to 50ppm).

- Note that cuttings and rootlings absorb chlorine from water during hydration and chlorine levels should be tested and adjusted after every batch.

A poorly managed hydration tank is a rapid method of spreading disease to successive cutting batches – care and frequent change of chlorinated hydration water is needed here!

- Following hydration (or fungicide treatment), cuttings should be packed into plastic bags or plastic lined bulk storage bins. The cuttings should be packed with no free surface moisture and one or two 5mm vent holes per bag. Alternatively, bulk bin liners can be loosely closed to provide ventilation for the cuttings.

- Cuttings should be stored at 2^0C +/-1^0C and high humidity. Good air circulation and temperature uniformity within the cool room is advised. A regular electronic or manual log of cool room temperature should be maintained (see point 6.0 below).

- Avoid frequent opening of the cool room as this causes room temperature to fluctuate allowing botrytis and other undesirable organisms to germinate.

Note – poor performance has been recorded where cuttings are cold stored for extended periods without storage bag ventilation…particularly following HWT. Dormant rootlings have been known to perform poorly if exposed to HWT and are returned to the cool room.

Poor performance has been recorded where cuttings are stored in association with fruit and vegetables or are exposed to any other ethylene sources (eg…combustion by products from LPG heaters and forklifts).

Good performance is regularly observed if cuttings are removed from the cool room and allowed to return to room temperature before HWT and propagation/ grafting (12 to 24 hours).

Similarly, few problems are experienced with rootlings if they are removed from the cold room and are allowed to equilibrate before HWT *immediately prior to dispatch to the customer.*

Research has demonstrated that cutting respiration rates increase significantly in the period immediately following HWT and gradually decline over the next 24 hours. Storing HWT cuttings in sealed bags immediately after HWT may not be beneficial as the cuttings have a significantly higher oxygen requirement for at least 24 hours

4.0 HWT tank design, engineering, equipment and operating considerations

4.1 HWT Tank Design

HWT tanks and equipment should be located to avoid contamination by wind blown dust, debris or pesticide overspray.

A great variety of HWT tank designs are currently in use by commercial nurseries. There is little standardisation between designs, however, all successful HWT tanks have the following elements in common:

- HWT tanks are commonly constructed from biologically inert, corrosion resistant materials including galvanised steel, stainless steel and reinforced plastics.

- The tank may be insulated to improve operating efficiency and may be constructed with a lid to exclude foreign matter and prevent heat loss between batches or overnight.

- Tank volume should be sufficient to allow for approximately 0.5 litres of water per cutting to be treated or 1.0 litres of water per rootling. Total tank volume will be determined by the batch size individual nurseries wish to process. HWT tanks capable of processing in excess of 5000 cuttings per batch are not uncommon.

- HWT tank water can be heated by a variety of techniques ranging from steam injection, gas fired heat exchangers and gas burners to electric and oil fired heat sources. The only limitations are that the heat must be applied to the HWT tank water indirectly, uniformly and at a sufficient distance away from the treated material to avoid localised hot spots within the tank.

- HWT tank water must be agitated to circulate hot water through the treated material and make the tank water and treated material achieve the uniform target temperature through-out the entire load mass. This is generally achieved through the use of a pump with sufficient water circulation capacity.

Note: Cuttings should be packed in treatment baskets so that the cuttings do not obstruct water flow (i.e. the cuttings should be packed parallel to the direction of water flow in the tank). Similarly, baskets should not be tightly packed as this will also impede water flow and result in uneven treatment temperatures

- When cuttings are immersed in the HWT tank, the combination of tank heating source and agitation employed in each individual tank design must be capable of allowing the tank to rapidly recover to target temperatures (this should take approximately 2 minutes) at which point the treatment time commences

Please note that a tank of similar or larger volume filled with clean chlorinated cool water (20^0C or less) will be required to immediately submerge and cool down HWT material following the required duration in the HWT tank. The cool down tank must be of similar or larger dimensions to the HWT tank to allow use of the treatment basket as outlined below.

4.2 Treatment basket or dipping cage design

To facilitate ease of handling and allow appropriate control over the duration of HWT, a basket or cage must be constructed to contain bundled cuttings or rootlings. The basket should be constructed with the following key design considerations:

- The basket should be composed of corrosion resistant, biologically inert material

- It should be constructed of mesh or a similar open design to permit unimpeded water circulation through the treatment batch and may need a mesh lid to prevent cuttings from floating during HWT

- The basket should be designed to fit within the HWT tank while allowing 300mm to 350mm clearance on all sides to permit water circulation and allow easy entry and extraction from the HWT tank

- The basket should be sufficiently robust to safely carry the batch size to be treated

- The basket must be fitted with lifting points and approved lifting tackle rated to carry the wet weight measured from the maximum load of bundled cuttings possible within the basket's dimensions

4.3 HWT Tank commissioning and certification

Prior to treating cuttings or rootlings in a new or modified HWT tank, the unit must be calibrated and certified by an appropriate, accredited certifying agency to ensure that the HWT tank is heating uniformly when fully loaded and is performing to the specifications detailed in AS2853.

In some regions within Australia, appropriate hot water vessel compliance testing will need to be completed by State authorities. VINA and AVIA can provide contact details for a number of providers of these services.

Following analysis of temperature and performance data by the certifying agency, the HWT tank will either be certified as operationally satisfactory or the certifying agency will indicate areas for tank design improvement to rectify hot or cold points and raise tank performance before HWT tank certification is approved.

4.4 Temperature control and record keeping

An accredited nursery must accurately monitor and record HWT tank temperature for every cutting batch processed. This information must be stored either electronically or manually and be easily retrieved as required. Ongoing control of HWT tank operating temperature and batch record keeping are critical.

Batch temperature monitoring can be carried out with a three-channel data logger connected to three temperature probes. Each temperature probe must be accurate to within 0.5^0C.

Temperature probes should record ***at least*** the following…(1) Cutting load temperature (core or 'centre' of the cutting load), (2) HWT tank water temperature (near the recirculation inlets) and (3) HWT recirculation water outlet temperature.

For complete cutting batch temperature monitoring, an additional two probes could be used within the treatment basket with one additional probe located centrally within the cutting basket -100mm into the top of the cutting load and one probe located 100mm from the bottom of the cutting batch.

NOTE

Automatic or thermostatically controlled temperature regulation can be unreliable. An accredited HWT operator should be employed to monitor HWT treatment progress as indicated by the tank's data logger/temperature probe array. The operator should be capable of manually adjusting HWT tank temperature if it begins to vary from the acceptable standard.

The data logger should be configured to provide the following <u>minimum</u> information for every treatment batch:

- 3 probe temperature measurement
- Temperature logging every 5 minutes, all probes
- Data printing or manual data recording, all probes
- Electronically stored data saved to disk daily

Remember that this is the minimum information requirement for each treatment type. A higher rate of data recording is possible with most equipment and is encouraged by the Authors.

Regardless of the treatment duration, the following minimum information must be recorded and stored for every treatment batch:

- Batch reference number
- Identification of the operator
- Treatment date
- The maximum and minimum temperatures recorded during the treatment period

4.5 Temperature probe testing and test record keeping

All temperature measuring equipment must be tested at least annually (before the start of the treatment season). A Certified Thermometer must be used as a reference point and calibration tool. Frequent testing will ensure that temperature probe calibration does not vary with time.

Testing by an approved instrument calibration testing service is preferred.

Self Testing Procedure for Temperature Probes

Where nurseries wish to self-test their temperature probes, the following procedure may be used and must be fully documented:

- Place the temperature probes and Certified Thermometer together in a suitable water bath. Ensure that the probes and thermometer are located at the same depth, do not touch the bottom of the water bath and are not in contact with each other.
- Start the data logger and gradually heat the water to a target temperature of 50^0C either directly by heating on a stove, or indirectly by pouring in very hot (not boiling) water and gently stirring the water to mix and stabilize the water temperature.
- The operator must read and record the temperature of each of the probes against the certified thermometer when 50^0C is reached on the certified thermometer
- Probes returning different temperatures to the certified thermometer must be marked and clearly identified
- Where testing indicates that probes need adjustment and the measuring equipment has the facility to do so, each temperature probe can be adjusted to read precisely the same value as the certified thermometer.
- Repeat this process for a 55^0C target temperature

Further advice on this calibration method can be obtained from the Authors.

Where your measuring equipment has no adjustment facility, temperature probes that do not read the same temperatures as the certified thermometer must be replaced or clearly marked with the temperature differential for continued use.

Each time you perform a calibration test, the following information must be recorded and stored:

- The date the test was performed
- The name of the operator performing the test

- The identification number or serial number of the Certified Thermometer
- The identification numbers or serial numbers of the temperature probes tested
- A record of the temperatures reported by each probe against the temperatures shown on the certified thermometer
- Signature or initials of the operator confirming that each temperature probe is accurate

5.0 Hot Water Treatment Procedure

- Pre-season - Thoroughly clean the HWT tank and cool down tank to remove any plant matter, soil residue or other foreign matter.

1) Fill the HWT tank with clean water free of sediment or foreign matter
2) Fill the cool down tank with clean water free of sediment or foreign matter. Chlorinate cool down water (e.g. to 10ppm free chlorine using stabilised pool chlorine or similar product)
3) Raise the water temperature of the HWT tank to between 1^0C and 2^0C above the target temperature. This will allow the load to heat up to the target temperature rapidly while the bath temperature recovers to approximately the target temperature
4) Load a treatment basket with cuttings and position temperature probes.
5) Lower the treatment basket into the HWT tank until the cuttings or rootlings are completely submerged and at least 100mm under the water surface
6) Allow the tank temperature to recover
7) Begin to log treatment temperatures, record treatment batch details on record sheet. Adjust tank temperature during treatment if necessary
8) At the required duration (5 or 30 minutes), lift the treatment basket from the HWT tank and immediately lower the treatment basket into the cool down tank
9) Keep the treatment basket in the cool down tank for an equivalent period of time as the HWT
10) Unload the treatment basket and avoid contamination of the cuttings. Clearly label the batch as 'hot water treated' to avoid inadvertent re-treatment or contamination by untreated cuttings
11) Check chlorine levels in the cool down tank and re-chlorinate as required (see note 5.2 below).

5.1 Note – Hot Water Treatment Tank Maintenance

The maintenance water quality in the hot water treatment tank is critical to the success of this process and to the health of treated cuttings or rootlings.

- Use only the best quality water available to fill the hot water tank.
- Ensure the water is free from sediment, silt or foreign matter.
- During continuous operation, empty and clean the tank at the end of each day.

5.2 Note – Cool Down Tank Maintenance

As the cool down tank is the last stage in the HWT process it is essential to keep the water sanitised. Your attention to cool down tank hygiene is absolutely critical to the health of all material that you treat. You must maintain chlorine levels of at least 10ppm in your cool down tank between batches. You must also empty and clean the tank daily (at least), using only the best quality water available to re-fill the tank.

Note: Rainwater contains a wide range of microorganisms. It may be palatable for drinking, but it is not a sterile source of water and must be chlorinated prior to use.

If the cool down tank becomes visibly contaminated with soil, sediment or foreign matter at any stage during the day, the tank should be emptied, cleaned and refilled with clean chlorinated water before proceeding.

6.0 Cool store monitoring and record keeping

Cuttings should be stored at 2^0C +/-1^0C and high humidity. Good air circulation and temperature uniformity within the cool room is advised. A regular electronic or manual log of cool room temperature should be maintained. A continuous log of cool room temperature is preferable with temperatures sampled and recorded hourly.

Cool room temperature logs should be printed continuously and filed daily. Data loggers should be downloaded to disk daily.

Cool rooms can be fitted with audible alarms to indicate that the room temperature has varied either over or under the set point. Where the cool room is located out of hearing range or the supervisor is not on-site, the cool room could have the facility to alert the storage supervisor of problems 24 hours per day, 7 days per week. This may be through an automatic messaging facility linked to a phone or pager. Freezing damage can rapidly kill stored cuttings and temperatures above 5^0C will progressively reduce storage life.

P4.3 HWT Quality Assurance

Facilitated Development of a Nursery Bar Code System

Paul Wright[1], Stephen Peacock[2] and John Crocker[3]

[1]Chairman – Vine Industry Nursery Association, PO Box 180, Mount Pleasant, SA 5235 Ph (08) 8568 2383. E-mail plwright@senet.com.au

[2]Technical Consultant - Tracking Labels and Consulting, PO Box 4103, Seaton SA 5023 PH (08) 8244 1608, E-mail spjm@chariot.net.au

[3]Senior Research Officer – Viticulture; SARDI - Lenswood Centre, Swamp Road, Lenswood South Australia 5240. Ph (08) 8389 8802, crocker.john@saugov.sa.gov.au

Aim

To facilitate the development of an electronic vine tracking, quality assurance and trace-back system for the Australian vine nursery industry and create a research tool to allow individual cuttings to be tracked through commercial vine propagation processes.

Background

Vine propagation protocols used by Australian vine nurseries vary considerably between enterprises, but contain many elements that are universally necessary for the successful production of healthy vines. From the point of cutting receipt by the nursery to dispatch of a finished product, propagation protocols contain numerous critical control points where the success or failure of a propagation batch can be easily decided by the actions of nursery staff.

Most vine nurseries employ varying paper based record keeping systems to log critical data associated with individual vine batches during the propagation process. Such paper based systems form the mainstay of VINA and AVIA nursery accreditation schemes and must stand independent audit if individual nurseries are to attain accreditation under either scheme.

In the event that a vine nursery delivers a propagation batch to a customer and the batch subsequently dies or fails to perform in the vineyard, litigation may ensue and the nursery must rely on propagation records to defend against punitive action by the customer. With paper-based systems, the search for relevant batch records can be time consuming, particularly in the case of nurseries with high production outputs or where records are sought several years after product delivery.

Bar code product identification and database systems are widely used in the food and manufacturing sectors to track componentry through production lines, store information associated with the manufacture of products and track finished products to the point of supply. Scope existed for the adaptation of commonly used bar code technologies to create a viable means of identifying individual cuttings or cutting batches and electronically store and collate data at critical propagation control points.

Project Initiation and Collaborators.

Expressions of interest were sought from a number of potential collaborators who routinely develop and service commercial bar code driven stock and quality control systems. Of the companies contacted, only Comware Pty Ltd was initially prepared speculate on involvement in this proposal.

Issues of Intellectual Property were forgone on the basis that the collaborator accepted sole responsibility for all costs and any risks associated with software development, product development, marketing and eventual product support. Assistance with concept development and initial testing was offered without encumbrance by Paul Wright who provided technical input on propagation procedures and ensured that the evolving system remained industry relevant at all times.

A project team consisting of Comware's Stephen Peacock, Paul Wright and John Crocker, met to define the scope and functionality of the proposed bar code system and commence drafting the database structure and information flow requirements. Comware Pty Ltd eventually withdrew support after 6 months for commercial reasons when Stephen Peacock resigned. Further development of the bar code system was suspended for over 12 months and was not progressed further by Comware.

In 2001, Stephen Peacock founded 'Tracking Labels and Consulting Pty Ltd' (TLC) and re-established contact with the bar code project team with view of reinitiating development of the nursery bar code system. The project team agreed to assist Stephen and recommenced system development from first principles.

System Structure

The project team developed entirely new data capture and database programming code for production the prototype bar code system. Detailed discussion with Paul Wright facilitated the construction of a propagation process flow chart that was inclusive of most elements common to Australian vine nurseries. The flow chart presented in Fig. 1, formed the basis for initial database architecture used in the prototype system.

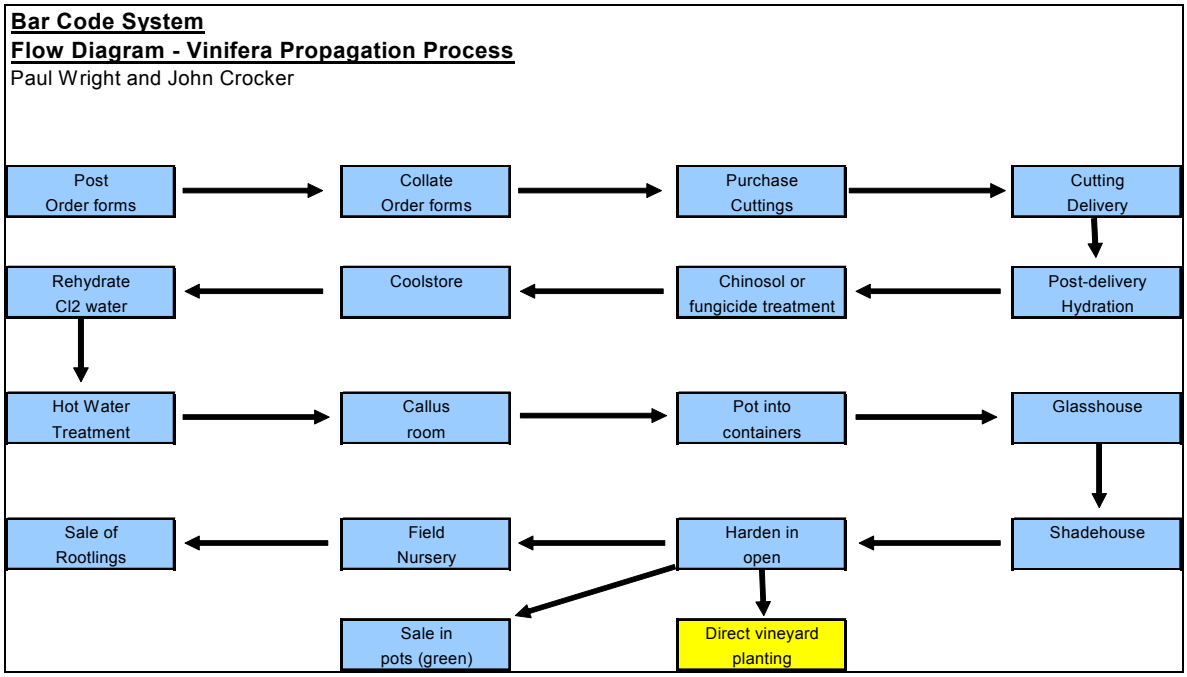


Figure 1; Propagation flow chart.

Each segment of the propagation flow chart indicates a critical control point in the generalised vine cutting propagation process and additional critical control points were required for propagation systems involving grafted vines. In this case, data associated with both scion wood and rootstock were merged in the grafting control point to form a new composite individual encompassing both data sets.

Data capture and reporting modules were also written as separate research tools, allowing the hand-held bar code data capture unit to be used for trial assessment at the post-callusing root initiation stage, shoot growth stage and final rootling grades stage. Automated trial assessment data capture cut labour requirements by weeks and removed the need for manual data entry prior to statistical analysis. The data capture software included auto checking to ensure the correct number of observations were taken at each step and auto verification to ensure that assessment codes were entered correctly. Figure 2, provides and example of the pre-programming data entry flow chart required to carry out rootling grading assessments.

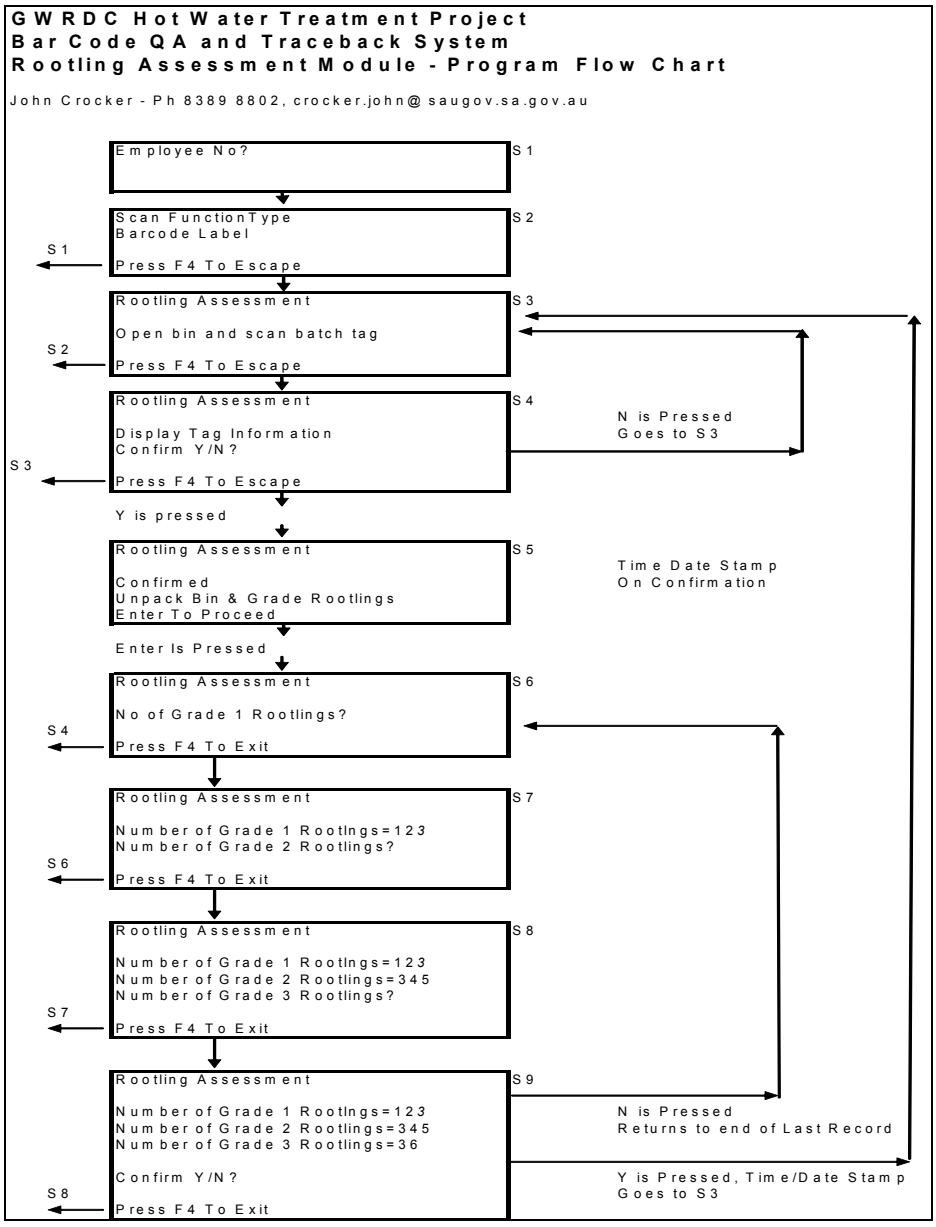


Figure 2; Rootling grading assessment – example of programming flow chart

The final prototype version was also modified to include modules for the assimilation of cutting production data to accommodate nurseries that produce rootstock cuttings, scion wood and *V vinifera* cuttings for sale and internal propagation requirements.

Database reporting is provided through a series of paper and on-screen reports formatted to comply with QA audit and record keeping specifications associated with the VINA and AVIA vine nursery accreditation schemes.

System testing and modification

The initial prototype system versions were used to label and track individual vine cuttings through Jacob's Creek Trials 1 and 2 (2000 to 2002), Photo 2, below. A modified version including trial assessment and data collation modules was used to track cuttings through Jacob's Creek Trail 3 from 2002 to 2003. System functionality and relevance issues were continuously adjusted from a vine nursery context through consultation with Paul Wright to arrive at final system architecture.



Photo 1; Bar code data handset



Photo 2; Bar coded vines, Jacob's Creek

A pre-commercial 'beta release' version of the full database and QA system is under evaluation during 2004 at the Jacob's Creek Vine Nursery through collaboration between Stephen Peacock, Philip Deverell (Nursery Manager) and Tim Scheiner (Nursery Supervisor).

While hand-held data capture units proved useful for single station data entry (Photo 1), hardware advances since 2000 will enable the final commercial version to operate in real-time through radio frequency data links to a central PC. This will facilitate simultaneous data capture and reporting from multiple bar code handsets dispersed at key operational stations through the entire Jacob's Creek Nursery operation.

Commercial release

TLC plans completion of the final commercial release database version prior to commencement of the 2005 propagation season. After the investment of significant capital and time resources into system development by TLC over the past 4 years, several commercial nurseries have expressed interest in the system and are likely to purchase hardware and software from TLC towards the end of 2004. Marketing issues including system naming product cost and support packages are yet to be resolved by TLC, however, adoption of the product name 'UniTrack' is likely.

6. Outcome/ Conclusion

SAR 99/4 performed well against all planned outputs and performance targets. Final project report delivery was delayed due to structural and duty changes within collaborating organisations impacting on time available for data collation and analysis.

All objectives detailed in the original application were achieved, with the exception of those listed in Section 4.2 'Notes and Amendments to Original Project Objectives' p.11. The refinement of an automated data collection system and inputs from our project collaborators resulted in significant cost savings to GWRDC during the passage of SAR 99/4. All unspent funds were returned to GWRDC before the end of the 2003/2004 financial year.

SAR 99/4 improved the vine nursery industry's understanding of long duration hot water treatment and the range of interacting propagation practices that potentially impinge on the performance of vine cuttings following HWT. Delivery of research results directly to vine nurseries through interactive HWT workshops facilitated the rapid uptake of research outcomes and ongoing modification of nursery propagation protocols to accommodate new information.

The identification of varietal sensitivities to HWT enabled nurseries to be aware of potential reductions in early root initiation and the potential for poor performance in some varieties if they are exposed to water stress following HWT. Together with an improved understanding of the detrimental role of cutting dehydration, over-hydration and poor nursery management practices, SAR 99/4 will assist nurseries to reduce the probability of poor cutting performance following HWT.

The amalgamation of the major research results obtained during SAR 99/4 into an improved HWT protocol document will allow Australian vine nurseries to readily integrate new information into their routine nursery practices. GWRDC's own cost-benefit analysis for the research conducted during SAR 99/4 quotes an NPV for this research at an estimated $19.4m with a BCR of 21.5 : 1. The break-even risk for GWRDC's R&D outlay is a 2% to 3% reduction in vine failure, which will be easily achieved given good industry adoption rates.

7. Recommendations

The research needs of vine nursery operators were prioritised with the aid of a project reference group and were compiled into a new project titled 'Advances in Hot Water Treatment for Rootstocks, Rootlings and Grafted Vines'. The following list represents Industry priorities for R&D provided by vine nurseries, vine improvement and the PGIBSA in 2003;

1. ***Alternatives to Chinosol***: Development of safe, effective and environmentally sustainable alternatives to Chinosol for the treatment of cuttings prior to long term cold storage. Recommendation of industry standards for pre-cold storage treatments following the de-listing of Chinosol and halt of production by Aventis in June 2002.

2. ***Cutting Moisture Status:*** Development of a routine test for rootstock, scion and *Vitis vinifera* cutting moisture status at the point of delivery to vine nurseries using readily available equipment and procedures. Recommendations for time to optimum re-hydration for given cutting moisture contents. Recommendation of a standard for minimum acceptable moisture content and inclusion of the standard into nursery protocols.

3. ***HWT of Rootstocks, Rootlings & Scions:*** Assessment of techniques for the handling, HWT and storage of rootstock cuttings, grafted rootlings, own-rooted rootlings and scion wood to maximise storage life while satisfying quarantine regulations where appropriate. Recommendation of industry protocols to extend the period over which grafting can be successfully carried out and grafted rootlings successfully stored.

4. ***Bio-indicators for Cutting Dormancy State:*** Use of cutting respiratory measurements to correlate changes in rootstock and *V. vinifera* cutting dormancy state with budburst and flowering in other deciduous species. Development of recommendations for flowering indicator species suitable for planting in association with source areas to provide a visual indication of changed dormancy state in rootstocks and *V. vinifera* scions.

5. ***Virus/Disease Status & HWT:*** Assessment of the impact of virus status and disease load as a further untested factor influencing the viability and survival of a range of rootstocks and scions following HWT. Recommendation of protocols for management/rejection/acceptance of cuttings with known viral loads and endogenous diseases.

6. .***Extension of R&D and Industry Consultation:*** Formation of an industry based Project Reference Group to oversee and steer project outcomes. Delivery of regular research summary workshops in each state to extend research results and seek direct industry input on future research priorities. Delivery of technical papers and posters at relevant national and international conferences. Annual publication of R&D results in relevant industry journals and industry newsletters.

GWRDC supported a new R&D program with a proposed commencement date of 01July 2003. The program was reliant on co-funding from voluntary contributions (VC's) by VINA and accompanied matching contributions by HAL. Unfortunately, HAL experienced a VC funding shortfall in 2003 and was unable to contribute to the new project, resulting in postponement of commencement. The details relating to VINA's VC scheme for R&D funding is under negotiation, but is expected to commence during the 2004/2005 financial year.

Recommendation: That the Vine Nursery Industry project reference group revisit the R&D priorities listed above before resubmission of a project proposal to GWRDC, HAL and VINA members.

8. Appendix 1: Communication:

Please refer to Section 5.4 (Program 4): 'HWT QA, Industry Adoption & Technology Transfer', pp. 129-143 for detailed explanation of the communication strategies employed during SAR 99/4.

9. Appendix 2: Intellectual Property:

No IP with commercial value to GWRDC, SARDI, University of Melbourne, DPI-Victoria or any of the other collaborators involved in SAR 99/4 was generated. All IP was generated as the result of collaborative effort and remains in the public domain. The 'Unitrack' bar code system was developed through facilitated input by Paul Wright and John Crocker in conjunction with Stephen Peacock of TLC Pty Ltd. TLC fully funded the development of 'Unitrack' and retains all IP rights to this product.

10. Appendix 3: References

A compiled list of all references cited within SAR 99/4 is as follows:

Antcliff, A.J. and May, P. (1961) Dormancy and bud burst in Sultana vines. *Vitis* 3, pp1-14.

Aurand, L. W., Woods, A.E. & Wells M.R. (1987), *Food composition and analysis*, Van Nostrand Reinhold, New York, USA.

Baker, K.F. (1957), 'Damping off and related diseases', in *The UC system for producing healthy container-grown plants,* ed. K.F. Baker. University of California, Division of Agricultural Sciences, Section 3, pp. 38.

Baker, K.F. (1962), 'Principles of heat treatment of soil and planting material', The Journal of the Australian Institute of Agricultural Science, June 1962, pp. 118-126.

Baker, K.F. (1962), 'Thermotherapy of planting material', *Phytopathology*, vol 52, pp. 1244-1255.

Bidwell, R.G.S. (1979) Plant Physiology 2nd ed., Macmillan Publishing Co., Inc., New York, pp. 529-546.

Blankenship, S. (2001), *Ethylene: The ripening Hormone*, Washington State University Database, Tree Fruit Research and Extension Centre, 16th Annual Postharvest Conference, Yakima, WA, USA, (hppt://postharvest.tfrec.wsu.edu)

Brodl, M. R. (1990), 'Biochemistry of heat shock responses in plants', in *Environmental Injury to Plants*, ed. F. Katterman, Academic Press, Chapter 6.

Bureau of Meteorology (2002), Media Release, *Climate - Victoria*, Australia. (http://www.bom.gov.au/announcements/media_release/vic/20011101.shtml)

Burr, T.J., Ophel, K., Katz, B.H. & Kerr, A. (1989), 'Effect of hot water treatment on systemic *Agrobacterium tumefaciens* Biovar 3 in dormant grape cuttings', *Plant Diseases*, vol. 73, No. 3, pp. 242-245.

Burr, T.J., Reid, C.L., Splittstoesser, D.F., & Yoshimura, M. (1996), 'Effect of heat treatments on grape bud mortality and survival of *Agrobacterium vitis in vitro* and in dormant grape cuttings'. *Am. J. Enol. Vitic.* 47, pp. 119-123.

Coombe, B.G. (1988), 'Grape phenology' In *Viticulture volume 1 resources*, Eds. B.G. Coombe & P.R. Dry, Winetitles, Adelaide, Chapter 7**.**

Crocker, J., Fletcher, G., Waite, H., Wright, P., (2001) Australian Advances in Hot Water Treatment Research*, The Australian Grapegrower and Winemaker, April 2001*.

Crocker, J., Waite, H., Wright, P. & Fletcher, G. (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*, No. 461a 2002, pp 33-37.

Dangeard, P. (1951), 'Observations sur la destruction du chondriome par le chaleur, Compt. Rend., Vol. 232, 1274-1276.

De Plater, C.V. & Greenham, C. G. (1959), 'A wide-range bridge for determining injury and death', *Plant Physiology,* 34, pp. 661-667.

Evert, D.R. (1973), 'Factors affecting electrical impedance of internodal stem sections', *Plant Physiology,* 51, pp.478-480.

Geise, A.C., (1962), *Cell physiology*, 2rd edn, W.B. Saunders Company, Pennsylvania, USA.

George, E.F. (1993), *Plant Propagation by Tissue Culture,* Part 1, The Technology, Exegetics

Hamilton, R.P. (1997) Hot Water treatment of grapevine propagation material. *Australian Grapegrower and Winemaker*, April 1997.

Hartmann, H.T., Kester, D.E. & Davies, F.T.Jr. (1990), *Plant Propagation Principles and Practices.* (5th ed), Prentice - Hall International.

Janick J. (1963). *Horticultural Science*, W.H. Freeman and Co., Prudue University Press, Indiana, U.S.A.

Lavee, S. & May, P. (1997), 'Dormancy of grapevine buds – facts and speculation', *Australian Journal of Grape and Wine Research*, vol. 3, pp. 31-46.

Levitt, J. (1980) Responses of Plants to Environmental Stresses Volume 1, Academic Press Inc., New York, USA.

Lin, C-Y., Roberts, J.K. & Key, J.L. (1984), 'Acquisition of thermotolerance in soybean seedlings', *Plant Physiology*, 74, p.152-160.

McIntyre, G.N., Lider, L.A. & Ferrari, N. L. (1982), 'The chronological classification of grapevine phenology', *American Journal of Enology and Viticulture*, Vol. 33, No.2, pp. 80-85.

Moore, G. & Blaze, K. (1980), *The electrical assessment of plant health and injury*, Proceedings, From green fingers to electronic sensors, Burnley Horticultural College, Richmond, Victoria.

Moore, G. M. (1979), 'Electrical assessment of injury and mortality of eucalyptus Obliqua L'Herit. Seedlings after heat stress', *Australian journal of Plant Physiology* 6, pp. 317-22.

Morrell, A.M. & Wample, R. L. (1995), 'Thermotolerance of dormant and actively growing Cabernet Sauvignon is improved by heat shock', *Am. J. Enol. Vitic.,* vol. 46, no. 2, 1995.

Morrell, A.M., Wample, R. L., Mink, G.I. & Ku, M.S.B. (1997), 'Expression of heat shock proteins in dormant buds of grape (*Vitis vinifera* L.)', *Am. J. Enol. Vitic.,* vol. 48, no. 4, 1997.

Mullins, M.G., Bouquet, A. & Williams, L. E. (1992), *Biology of the Grapevine*, Cambridge University Press, Great Britain.

Myking. T. (1998) Interrelations between respiration and dormancy in buds of three hardwood species with different chilling requirements for dormancy release. Trees – Structure and Function, Springer-Verlag Heidelberg pp 224-229.

Nicholas, P.R., Chapman, A.P. & Cirami, R.M., (1999), 'Grapevine Propagation', in *Viticulture vol 2 Practices*, Eds. B.G. Coombe & P.R. Dry, Winetitles , Adelaide, Chapter 1.

Nicholas, P.R., Chapman, A.P. and Cirami, R.M. (1992) 'Grapevine propagation', In: Coombe B.G. and Dry P.R. Eds. *Viticulture volume 2 practices*, Winetitles Adelaide, Chapter 1.

Orffer, C.J. & Goussard, P.G. (1980), 'Effect of hot-water treatments on budburst and rooting of grapevine cuttings', *Vitis* 19, pp. 1-3.

Orffer, C.J. (1977), 'Hot-water treatment of vine propagating material- urgently necessary in RSA', *The Deciduous Fruit Grower*, July 1977, 224-231.

Orffer, C.J. Goussard, P.G. Bosman, D.C. Morkel, D.C. and Wiese, J.J. (1979), 'The effect of hot-water treatment and growth regulators on budburst and rooting of cuttings of the grapevine rootstock cultivar USVIT 2-1', *The Deciduous Fruit Grower,* October 1979, 340-344.

Pouget, R. (1963) Recherches physiologiques sur le repos vegitatif de la vigne (*Vitis vinifera* L.): la dormance et le mechanism de sa disparition. Annales d Amelioration des Plantes 13, no hors serie 1.

Taiz, L. & Zeiger, E. (1998), *Plant Physiology*, 2nd edn, Sinauer Associates, Inc.

Treshow, M (1970) Environment and Plant Response, McGraw-Hill Book Company, NY.

Vegis, A. (1965) Ruhezustände bei höeren Pflanzen. Induktion, Verlauf und Beendigung: Übersicht, Terminologie, allgemeine Probleme. In 'Encyclopedia of Plant Physiology Vol XV/2'. Ed. A. Lang (Springer Verlag: Berlin, Heidelberg, New York) pp. 499-533.

Vierling, E. (1991), 'The roles of heat shock proteins in plants', *Annu. Rev. Plant Physiology Plant Mol. Biol.* 42, pp.579-620.

Von Broembsen, S. & Marais, P. G. (1978), 'Eradication of *Phytophthora cinnamomi* from grapevine by hot water treatment', *Phytophylactica*, vol. 10, no. 1, pp. 25-27.

Waite, H. & May, P. (1999), *The effects of hot water treatment on the mortality of grapevine propagules, final report*, Institute of Land and Food resources, the University of Melbourne, Dookie College & Burnley College.

Waite, H. & May, P. (1999), The effects of Hot Water Treatment, hydration and order of nursery operations on cuttings of *Vitis vinifera* cvs., unpub.

Waite, H. (1999), '*To investigate the grapevine nursery industry – USA, France, Italy'*, Report to the Winston Churchill Memorial Trust of Australia.

Waite, H. (2002), The effects of hot-water treatment on grapevine propagating and planting material, Masters of Applied Science Thesis, Burnley College, the University of Melbourne.

Waite. H, May. P. (1999). 'The effects of hot water treatment on the mortality of grapevine propagules - Final Report to AVIA'.

Wample, R., Bary A. & Burr T.J. (1991), 'Heat tolerance of dormant *Vitis vinifera* cuttings', *Am. J. Enol. Vitic.,* vol. 42 no. 1 pp. 67-72.

Wample, R., Spayd, S.E., Evans, R.G. & Stevens, R.G. (1991), *Nitrogen fertilization and factors influencing grapevine cold hardiness,* International Symposium on Nitrogen in Grapes and Wine, pp. 120-125.

Wample, R. (1993), 'Influence of pre- and post-treatment storage on bud break of hot water treated cuttings of cabernet sauvignon', *Am. J. Enol. Vitic.,* vol. 44, no. 2, 1993.

Wample, R. (1997), 'Influence of pre- and post-treatment storage on rooting of hot-water-treated cuttings of cabernet sauvignon', *Am. J. Enol. Vitic.,* vol. 48, no. 2, 1997.
of cabernet sauvignon', *Am. J. Enol. Vitic.,* vol. 48, no. 2, 1997.

Warner, H.L. and Kollman, G.E. (no date.) *EthylBloc,* Rohm and Hass Company Research Laboratories, Spring House, USA.

Woolf, A.B. & Lay-Yee, M. (1997), 'Pre-treatment at 38°C of 'Hass" avocado confer thermotolerance to 50° C hot water treatments' *HortScience* vol.32 no.4, pp.705-708.

Wright, P. (1997 & 2001), Manager, Jacob's Creek Nursery, Orlando Wyndham Group Pty Ltd, PO Box 943, Rowland Flat 5352, South Australia.

11. Appendix 4: Staff

1. Project Team.

Dr Michael McCarthy[1]
Organisation: South Australian Research and Development Institute
Roles: Project Supervisor
Postal Address: SARDI, Nuriootpa Research Centre, PO Box 245 Nuriootpa SA 5355
E-mail: mccarthy.michael@saugov.sa.gov.au
Phone: +61 (08) 8568 6409, Fax +61 (08) 8568 6449

John Crocker[1]
Organisation: South Australian Research and Development Institute
Roles: Principal Investigator and Project Coordinator
Research Responsibilities: Pre -HWT Issues 'The Jacob's Creek Trials' and Measurement of Vine Dormancy
Postal Address: SARDI, Lenswood Research Centre, Swamp Rd Lenswood SA 5240
E-mail: crocker.john@saugov.sa.gov.au
Phone: +61 (08) 8389 8802, Fax +61 (08) 8389 8899

Helen Waite[2]
Organisation: University of Melbourne
Research Responsibilities: Nursery Practice & Cellular Level Effect of HWT
Postal Address: Institute of Land and Food Resources, University of Melbourne, Dookie College, Victoria 3647.
E-mail: hwaite@unimelb.edu.au
Phone: +61 (03) 5833 9285, Fax +61 (03) 5833 9201

Graeme Fletcher[3]
Organisation: Department of Primary Industries, Victoria
Research Responsibilities: Post – HWT Issues, Cold Storage
Postal Address: c/o Agriculture Victoria, Sunraysia Horticulture Centre, Box 905, Mildura, Victoria 3502.

(*Note…Graeme retired due to ill health and Sue McConnell coordinated the completion of Graeme's research*).

Sue McConnell[4] (*for Graeme Fletcher*)
Organisation: Department of Primary Industries, Victoria
Research Responsibilities: Post – HWT Issues, Cold Storage
Postal Address: Agriculture Victoria, Sunraysia Horticulture Centre, Box 905, Mildura, Victoria 3502.
E-mail: Sue.McConnell@dpi.vic.gov.au
Phone: +61 (03) 5051 4500, Fax +61 (03) 5051 4523

Principal Collaborators

Paul Wright[5]
Organisation: *Formerly*, Manager, Jacob's Creek Vine Nursery, Orlando-Wyndham Group
Research Responsibilities: Collaborator, Pre -HWT Issues 'The Jacob's Creek Trials' and Co-developer - Bar Code QA System
Postal Address: c/o 'Vinewright' PO Box 180, Mt Pleasant SA 5235
Email: plwright@senet.com.au
Phone: Ph +61 (08) 8568 2385 Fax +61 (08) 8568 2345

Philip Deverell[6]
Organisation: Orlando-Wyndham Group, Jacob's Creek Vine Nursery
Research Responsibilities: Collaborator, Pre -HWT Issues 'The Jacob's Creek Trials'
Postal Address: Orlando Wyndham Group, Jacob's Creek Vine Nursery, PO Box 943, Rowland Flat SA 5352
E-mail: Philip.Deverell@orlando-wyndham.com
Phone: +61 (08) 8521 3050, Fax +61 (08) 8563 3395

Stephen Peacock[7]
Organisation: Tracking Labels and Consulting Pty Ltd (TLC)
Research Responsibilities: Co-developer - Bar Code QA System
Postal Address: 5 Andrew Street, Hendon SA 5014
E-mail: spjm@chariot.net.au
Phone: +61 (08) 8244 1608, Fax +61 (08) 8244 1607

12. Appendix 5:

No additional reference material was submitted by the authors.

13. Appendix 6: Budget reconciliation

APPENDIX I
FORM B

GRAPE & WINE RESEARCH & DEVELOPMENT CORPORATION
Statement of Receipts and Expenditure
as at 31 January 2004

Trust Fund :	**RESEARCH TRUST FUND**	**FUNDING**	$
GWRDC			
Project No :	SAR 99/4	Salaries	0.00
Provider :	John Crocker	Travel	0.00
Title of Project :	Development of Effective Hot Water Treatments	Operating	0.00
		Capital	0.00
		Total Funding	0.00

	Salaries	**Travel**	**Operating**	**Capital**	**Total**
	$	$	$	$	$
A Uncommitted (c/f 1 July)	35,915.16	0.00	213.91	0.00	36,129.07
B Outstanding Commitments (c/f 1 July)	0.00	0.00	860.00	0.00	860.00
C Refunds of funding	35,915.16		213.91		36,129.07
D Cash Received From Trust Fund	0.00	0.00	0.00	0.00	0.00
E Approved transfers (from Form C)					0.00
F Cash available (A+B-C+D±E)	0.00	0.00	860.00	0.00	860.00
G Expenditure	0.00	0.00	860.00	0.00	860.00
H Outstanding Commitments (30 June)	0.00	0.00	0.00	0.00	0.00
I Total funds Committed (G+H)	0.00	0.00	860.00	0.00	860.00
J Uncommitted (30 June) (F-I)	0.00	0.00	0.00	0.00	0.00
K Other income (Paid to Trust Funds)					0.00

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Note 1 **Row B should be the same as Row H from the previous year and Row A the same as Row J from the previous year**

Note 2 **The above reconciliation should be inclusive of any GST commitments**

I hereby certify that this statement of expenditure is correct.		
Signature	Printed Name	Date