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Ensuring optimal grape quality through management strategies for *Botrytis cinerea*



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ENSURING OPTIMAL QUALITY THROUGH MANAGEMENT STRATEGIES FOR *BOTRYTIS CINEREA*

A final report to the Grape and Wine Research Development Corporation

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Abstract

This study considered host nutrition as a management option in reducing the potential severity of Botrytis grey mould in vineyards. Levels of nitrogen (as ammonium nitrate), calcium (as calcium sulphate) and potassium (as potassium sulphate) were applied as either soil drenches or foliar sprays to Chardonnay (field) and Chardonnay and Cabernet sauvignon (glasshouse) vines. Applications were made at fruit set, early pre-bunch closure and late bunch closure.

Preliminary results suggest that managers of vineyards with high nitrogen in petioles should avoid further applications and wait for natural depletions to occur. Calcium/potassium additions resulted in less compact bunches although the bunch numbers were higher. Applications of nitrogen, calcium and potassium did not affect winemaking parameters such as pH, Brix or titratable acidity (TA).

In glasshouse trials, increased addition of calcium did correlate with reduced Botrytis leaf blight severity. Lower Botrytis incidence was reported from spur-pruned vines suggesting that light penetration to the bunch zone was greater in spur pruned than in cane-pruned vines.

The results reported relate to induced Botrytis expression through laboratory incubation and do not equate to natural Botrytis expression in the vineyard at harvest.

Executive summary

Botrytis cinerea, the causal agent in the disease commonly called Botrytis bunch rot or grey mould*,* is a ubiquitous fungal pathogen that is widespread in the vineyard environment. It requires warm, wet or cool, wet conditions, ie high humidity conditions, in which to sporulate and infect a host plant*. B. cinerea* is a weather-driven pathogen that infects most commonly at flowering under conducive weather conditions, after which the infection may remain latent until harvest Expression at harvest and potential resultant loss of, or reduction in quality, of the crop occurs only in the presence of wet weather conditions. Botrytis can infect at flowering or later in damaged tissue caused by environmental events such as hail or frost, or mechanical damage caused by vineyard management events.

Growers have identified environmentally sensitive management protocols as the major issue for disease management. The apparent less than adequate protection afforded by conventional chemicals results, in part, from the inability to deliver lethal doses of active ingredients to protect the crop.

Successful management of diseases requires a careful combination of vineyard management, vine nutrition and targeted chemical application. *Botrytis cinerea* is a fungal pathogen responsible for serious losses in vineyards in years of wet weather at critical stages in the season such as flowering and harvest.

The results of 2 year's work have been reported but climatic conditions (dry vintages) and the inability to have two consecutive years on the experimental site have affected the reporting of data. This is particularly important when looking at effects of nutrients applied to the soil. It is known that several seasons may be needed to the alter soil nutrient levels. However, at the time the site was chosen it was not known that the vineyard manager would rework the site so that it was no longer suitable for our trials. The second site had to be chosen quickly and was selected because of its history of susceptibility to *Botrytis* infection and, more importantly, expression at harvest, (ie the site has a high botrytis bunch rot risk and has a soil nitrogen level considered to be on the high side of adequate). Interestingly in the initial experiments at each site, neither recorded an increased uptake of N. An extension of this is to develop techniques in any future projects that encourage field expression even in seasons that do not have weather events that do not favour Botrytis development, so the effects of treatments on field expression can also be determined. Grapes which were not frozen but moist incubated show a level of infection in the bunches but does not equate with field expression.

This study considered host nutrition as a management decision in reducing the potential severity of Botrytis grey mould in vineyards. Levels of nitrogen (as ammonium nitrate), calcium (as calcium sulphate) and potassium (as potassium sulphate) were applied as either soil drenches or foliar sprays. Applications were made at fruit set, early pre-bunch closure and late bunch closure.

During the 2002/2003 season, ten-year old Chardonnay, trellised to Scott-Henry, in a commercial vineyard "Fernhill" at Coldstream, Yarra Valley, Victoria, on sandy loam soil, watered by drip-irrigation, were selected

for this study. This vineyard was not available in 2003/2004, so 6 year-old Chardonnay, trellised to Vertical shoot positioning, in a commercial vineyard "Meroo" at Yarra Glen, Yarra Valley, Victoria, on clay loam and drip irrigated, were used.

Glasshouse experiments were carried out at Department of Primary Industries, Knoxfield, Victoria, using Chardonnay and Cabernet Sauvignon. Nitrogen was applied using Hoagland's Solution as the base concentration with calcium and potassium levels adjusted accordingly to give the appropriate ratios of the 3 elements.

Botrytis spores were applied to the field and glasshouse vines at the rate of 1×10^4 spores per ml. Artificially inoculated bunches from the vineyard were harvested and incubated to initiate expression of Botrytis.

Shoot growth, light penetration, canopy density and bunch compactness were parameters measured to determine the effect of nutritional applications on infection by *B. cinerea*.

Irrigation, or careful use of water, assists with canopy management in minimising canopy density, a known factor in predisposing the fruit zone to increased disease pressure.

Initial studies in manipulating levels of calcium and nitrogen in grapevines in potted plants and in a commercial vineyard have shown that managers of vineyards with high nitrogen in petioles should avoid further applications and wait for natural depletions to occur. Calcium/potassium additions resulted in less compact bunches although the bunch numbers were higher. Applications of nitrogen, calcium and potassium did not affect winemaking parameters such as pH, Brix or titratable acidity (TA).

In glasshouse trials, increased addition of calcium did correlate with reduced Botrytis leaf blight severity. Lower Botrytis incidence was reported from spur-pruned vines suggesting that light penetration to the bunch zone was greater in spur pruned than in cane-pruned vines.

Results suggest that faster shoot extension prior to flowering and good light penetration into the canopy may reduce Botrytis incidence. Both these factors may be related to the increased size of internodal spaces at the basal end of the cane. Application of calcium and potassium were found to reduce bunch compactness.

It must be noted that the levels of Botrytis artificially expressed in the laboratory do not approximate the expression in the vineyard under normal growing conditions.

Background

Botrytis bunch rot caused by *Botrytis cinerea* Pers: Fr. is a severe disease at harvest if prevailing environmental conditions favour disease expression. This fungus is endemic in the natural environment with a wide host range and ability to infect numerous plant organs and reproduce rapidly in the field. In Australia, botrytis bunch rot is a particular problem in regions that have a cool, wet or a warm wet macroclimate that is favourable for disease development.

Damage to grape berries caused by botrytis bunch rot cost the industry an estimated $20 million in the 1999/2000 season. Such losses occur despite expenditures of $2-10 million annually on botryticides. In years of high disease pressure, the combination of chemical and other management practices does not provide sufficient control to ensure production of good quality wine. A recent CRCV survey (Aitken *et al*. 1999) revealed that most growers are dissatisfied with the efficacy of current management practices available for botrytis bunch rot control. Moreover, feedback at Research to Practice workshops indicates that growers are now very keen to reduce chemical inputs (David Braybrook, pers. comm. Vitisolutions). They are interested in taking a more holistic approach that incorporates non-chemical management options to suit their individual growing environment.

At the ASVO seminar series 'Managing Bunch Rots' held in Mildura in 2000, Dr Balasubramanian, Hortscience, New Zealand, emphasised that an understanding of the host-pathogen interaction with vineyard conditions is crucial to the development of 'whole vine' management strategies. At the recent (2001) Grapecheque 'Botrytis and Powdery Mildew' seminar held in the Yarra Valley, Prof. Wilcox, USA Department of Agriculture, suggested that among management practices, although still relatively poorly understood, grapevine nutrition (eg. nitrogen) plays a critical role in susceptibility to disease.

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An important outcome from the recent research carried out with GWRDC RITA and IDC funding shows that infection and expression are not necessarily directly linked (Cole *et al*, 2004). There is now clear evidence that *B. cinerea* infection is weather driven and that infection at flowering occurs only after periods of warm, wet or cool, wet weather conditions. If the flowering weather is dry, then flower infections are minimal. Infections may occur at other times during the season but these can be linked to weather events and damage to the vine or fruit. Infection at flowering or at any other time during the season does not lead directly to expression at harvest. *B. cinerea* must be actively growing in the vine or fruit to produce bunch rot at harvest and the associated problems such as laccase production. This event requires wetness. Latent infection that has not been activated will have no influence on the quality of the fruit at harvest and, therefore, the resultant wine. Management of the vine must be such that expression is minimised rather than infection prevented (Cole *et al,* 2004).

In this study we have used scientifically designed experiments to identify through both (1) association (ie. field-based practice and response correlations) and (2) cause and effect (ie. glasshouse-based treatment and response analysis) studies, management practices that alter the level of a vine's resistance to botrytis bunch rot. The complex interaction of the many host, pathogen, environment and management factors that influence disease incidence and severity makes this proposed research somewhat high risk. However, such work needs to be undertaken and associated with the results from both past and other ongoing *B. cinerea* research projects in order to gain a proper understanding of the grapevine-*B. cinerea* interaction. Resultant understanding will lead to more holistic vine management systems that successfully manage botrytis bunch rot and reduce reliance on chemical control.

The directions taken in this research proposal arise from recommendations made as outcomes of the May 1999 workshop (Aitken *et al*. 1999) for *B. cinerea* researchers in Australia. The directions also reflect recent growing international understanding on the interaction between plant condition (eg. nitrogen / calcium ratios) and disease. It also incorporated new experimental techniques developed in projects DAV95/1 and 95/2 (eg. improved inoculation protocols) and improved understanding of *B. cinerea* biology from projects CSU 99/3 and GVWIDC-Monash (eg. latency and strain variation).

With the potential for ongoing fungicide resistance problems, the increasing interest in ‘organic’ products, MRL limitations being set by overseas importers, and the move towards lower chemical inputs, non-chemical control options that are economically viable should be investigated. Alternative non-chemical approaches to control need to be validated under Australian conditions and incorporated into regional strategies. These strategies could include enhanced host resistance, stimulation of beneficial fungi populations and manipulation of the host-pathogen interaction.

In seasons conducive to disease expression, there is no effective chemical management for botrytis grey mould or bunch rot caused by *B. cinerea*. Resistant grape varieties are not available, and biological control methods are currently only at research and/or developmental stages. Development of integrated management programs is the most immediate and effective solution to the control of this pathogen.

Organic growers report generally lower levels of *B. cinerea* infection than neighbouring vineyards under traditional spray programs and do not consider botrytis bunch rot to be a significant problem in organic viticulture (D. Bruer, pers. comm. Temple Bruer Vineyard, Langhorm Creek, South Australia). Thus, fruit grown under organic protocols may be less susceptible to botrytis bunch rot. However, most of these reports are neither adequately documented nor scientifically validated. None-the-less, by way of example, losses due to botrytis bunch rot were high in the Mudgee area of NSW in 1999, but two organic vineyards in this region had lower levels of botrytis bunch rot without the use of botryticides. Characteristics of these vineyards were lower yields, pruning and vine management systems which resulted in smaller more open bunches and less inter-bunch congestion, organic soil amendments and minimal to no pesticide use. The growers considered that their vines were ‘in balance’, and therefore more resistant to *B. cinerea* infection.

A parallel project, supported by GWRDC RITA funds and funds from the Greater Victorian Wine Grapes Industry Development Committee, has been investigating different botrytis bunch rot management techniques during the past two seasons in high disease pressure vineyards in the Yarra Valley, Mornington Peninsula and Alpine Valleys. The outcomes from the project are a CD ROM containing a check list of possible options for growers to follow at the critical growth stages in the vineyard and a booklet describing the latest knowledge on *B. cinerea* distribution and epidemiology (Cole, M., Whiting J., & Braybrook D, 2004). Workshops were held in Beechworth, Knoxfield, Macedon and Bendigo, facilitated by the Victorian Department of Primary Industries Grapecheque program, where the growers were presented with scenarios for botrytis bunch rot management. It was apparent at these workshops that growers needed knowledge and confidence to implement this knowledge into their management practices. The major outcomes from the research were that *B. cinerea* is a weather driven fungus that is present in the total plant environment all of the year (Cole, M., Whiting, J., & Braybrook, D., 2004).

Effects of plant nutrition on grapevine health and susceptibility to *B. cinerea* are emerging as a very topical area of interest to both researchers (Balasubramanian *et. al*., 2000; Dubos, 2000) and growers (Erika Winter and Jane Fisher from DPI, Knoxfield pers. comm.). Encouraging results from research on other fruit crops, such as apple, peach and avocado, show that susceptibility to disease is influenced markedly by plant nutrition, particularly the balance between N and Ca. Manipulation of N and Ca tissue concentrations can be used to enhance resistance to disease (Conway *et al*. 1987; Sugar *et al*. 1992; Hofman *et al*. 1999). Management of pear trees for low N and high Ca content in the fruit is used to reduce the severity of postharvest fungal decay (Sugar *et al*. 1992). Such nutritional manipulations are highly compatible with other methods of disease control, such as chemical and biological measures.

How vineyard management practices such as plant nutrition impact flower and berry susceptibility to *B. cinerea* was the focus of this proposal. Using glasshouse-based experiment studies, we investigated the possibility of manipulating the balance between N and Ca tissue concentrations in order to increase the grapevines' natural resistance to *B. cinerea*. We investigated, in field-based correlation studies, the relationships between various management practices including canopy management and nitrogen, calcium and potassium fertilisation, and vine N and Ca levels and the incidence and severity of botrytis bunch rot. A PhD project is looking at the impact of N nutrition and irrigation on cell wall thickness, cuticular wax deposition and berry levels of resveratrol and other stilbenes implicated in botrytis resistance.

This holistic research strategy should provide a solid foundation for prescribing long-term and durable approaches to botrytis bunch rot management. Such approaches would reduce reliance on or even eliminate the need for chemical control, particularly in low risk regions and/or seasons.

Many factors make *B. cinerea* difficult to manage in grapevines. Even after decades of systematic agronomical and biochemical research (Dubbos 1999), *B. cinerea* and its interactions in the vineyard are not fully understood. Currently, key international and Australian research is focussing on pathways of infection, disease epidemiology, and biochemistry of the causal organism. Past research has focused on chemical control, epidemiology and the effects of environmental and biological factors, including canopy microclimate and insect herbivory on disease severity.

Studies in GWRDC project DAV 92/1 indicated that less botrytis bunch rot developed in small loose, well dispersed bunches on open vine canopies (Emmett *et al*., 1994). GWRDC project DAV 95/1 investigated the effects of spore level, environmental conditions and light brown apple moth activity on bunch rot development, the use of incidence monitoring to predict the need for fungicide treatments, and the efficacy of pre and post-infection fungicide treatments for bunch rot control. A bunch inoculation technique was also developed that can be used to evaluate chemical, biological and cultural treatments for *B. cinerea* control. Non-chemical control measures are extremely important in the management of botrytis bunch rot, but have not been sufficiently publicised (Dubos 1999). Previous work (GWRDC DAV 95/1) has shown that risk can be minimised by management practices such as pruning, air blasting, leaf plucking and control of light brown apple moth (LBAM).

When spray applications fail to give control it is often assumed that the pest has become resistant. Although this may sometimes be the case, recent research (DAV 98/2) has shown that some spray applications fail to deliver a lethal dose particularly in canopies where bunches are not easily accessible to sprays. Chemicals may still have a role in *B. cinerea* control particularly in crisis situations and high-risk regions. However, most growers have indicated that they would like to reduce their chemical inputs.

Reliance on natural infection and subsequent unfavourable seasons for botrytis bunch rot development has seen field trials fail in the past. As part of projects (DAV95/1) and (DAV 98/2) on-vine-inoculation and incubation techniques were developed which ensure disease development and improve the chances of obtaining useful trial information.

Modelling, using data collected over several seasons and across growing regions, showed that the level of bunch rot at harvest was not fully correlated or explained by the degree of early season *B. cinerea* infection, even when the length of season, amount of rainfall and number of heat days were taken into account (Mebalds *et al.* 1998). Incidence monitoring was not a reliable method for predicting the level of bunch rot at harvest. Current research has shown that there is no correlation between infection and expression (Cole, M. 2004) and that 100% infection incidence can result in 0% expression if weather conditions at harvest are dry. Vineyards showing high *Botrytis cinerea* incidence at all sampling times throughout the season, delivered exceptional quality bunches to the winery at harvest. Weather conditions at harvest were dry and hot. It is now apparent that it is actively growing *Botrytis cinerea* stimulated by wet weather that rots the bunches and produces laccase in the resultant wine.

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Latent Botrytis, even though known to be present by artificial expression through incubation, has no affect on wine quality.

Some growers are using regulated deficit irrigation (RDI) to produce smaller berries that do not touch each other. Researchers in New Zealand are experimenting with the use of deep-rooted inter-row plants such as chicory, that compete for nutrients and water in order to reduce vine vigour (Dr Deann Glenn, pers. comm.). At least one grower in the Yarra Valley is using summer grasses to remove excess soil moisture to control vine vigour (David Shearer, pers. comm.). Several practices such as mulching, use of cover crops, RDI, reduced N inputs, and pruning systems aimed at reducing vine vigour and susceptibility to *B. cinerea* were being trialed as part of the CRCV On-Farm Trials project. In our proposed study, the efficiency of this research was to be enhanced by utilising replicated trial sites established as part of the On-Farm Trials Project within the Sustainable Viticulture CRCV Program 5.3. Unfortunately, the On-Farm Trials project concluded in July 2003 and therefore these sites were no longer available.

Project Aims and Performance targets

1. To collate available information on the grapevine-*Botrytis cinerea* interaction, with particular emphasis on practices that may increase resistance to infection (preliminary study: Chapter 1-Literature Review).
2. To develop a botrytis bunch rot control project that builds upon information from preceding and on-going projects in order to improve understanding of grapevine, *Botrytis cinerea*, environment and management interactions (Completed end of year 1).
3. To investigate, in field studies, the enhancement of grapevine resistance to *Botrytis cinerea* by varying management practices (eg. canopy management, irrigation, nutrition, organic vs. conventional).
4. To investigate, in glasshouse studies, the enhancement of grapevine resistance to *Botrytis cinerea* by manipulating plant nitrogen and calcium nutrition.
5. To generate non-fungicide based recommendations for grapevine management that enhances natural resistance to botrytis bunch rot.
6. To communicate findings to growers and researches through publications and workshops.

Literature review

Preliminary and Previous Research

Many factors make *B. cinerea* difficult to manage in grapevines. Even after decades of systematic agronomic and biochemical research (Dubbos 1999), *B. cinerea* and its interactions with vineyard environment and management are not well understood. Currently, key international and Australian research is focussing on pathways of infection, disease epidemiology, and biochemistry of the causal organism (eg. Cole *et al* 1999, Holz, Pezet, Viret *et al* 2001, Viret *et al*. in press and Wiechel and Cole, 2001). Also, past research (eg. DAV 95/1) has focused on chemical control, epidemiology and the effects of environmental (eg. canopy microclimate) and biological (eg. insect herbivory) factors on *B. cinerea* disease severity. Studies in GWRDC project DAV 92/1 indicated that less botrytis bunch rot developed in small loose, well dispersed bunches on open vine canopies (Emmett *et al*., 1994). Project GWRDC DAV 95/1 investigated the use of incidence monitoring to predict the need for fungicide treatments, the effects of spore level, environmental conditions and light brown apple moth (LBAM) activity on bunch rot development, and, the efficacy of pre and post-infection fungicide treatments for botrytis bunch rot management. A bunch inoculation technique was also developed that can be used to evaluate chemical, biological and cultural treatments for botrytis bunch rot management.

Role of inoculum

Incidence monitoring of *B. cinerea* in unsprayed flower clusters and bunches undertaken in project DAV 95/1 showed that there was fluctuation in the level of *B. cinerea* within each season, between seasons and between regions. Modelling showed that the level of rot at harvest was not well correlated or fully explained by the degree of early season rot, even when the length of season, amount of rainfall and number of heat days were taken into account (Mebalds *et al.* 1998). Thus, incidence monitoring alone during bunch development was not a reliable method for predicting the level of bunch rot at harvest.

Recent research in France demonstrated that two sub-species of *B. cinerea* were active on the same vines, one in spring and the other after veraison (Jaeger, 1999), the first preferring green plant parts and the second being more prevalent on berries. Strain variability within vines and between seasons is in Australian vineyards is currently being examined in GWRDC project CSU 99/3. Both sub-species are present in Australian vineyards but are present all through out the season and are not quite as defined in their distribution as was found in France (Cole *et al,* 2004).The existence of two species could have major implications for development of management strategies.

In project (DAV95/1) *B. cinerea* isolated from several weeds and flowering plants commonly found in and around the vineyard, including windbreak Melaleuca flowers. It is now known that all strains collected to date from the vineyard environment are able to infect grapevines, therefore it would be appropriate to assume that inoculum from other hosts have the potential to contribute significantly to *B. cinerea* infections on vines (Cole *et al*, 2004). Project CSU99/3 has shown that *B. cinerea* isolates from different hosts infect grapevines and that they are sexually compatible.

Overall the removal of sources of inoculum (eg. canes with sclerotes and bunch residues) is advisable, but this is probably not a key factor in comparison with the phase of receptivity of the plant (DAV 95/1). Results from project DAV 95/1 demonstrated a positive relationship between the number of spores present at flowering and the severity (number of berries infected per bunch) of infection at harvest (Warren, Becker *et al.* 1999). Bulit *et al* (1970) found that epidemics of grey mould did not occur if concentrations of airborne conidia were below 40 m^{-3}, despite the occurrence on suitable temperature and relative humidity conditions. However, inoculum is probably always present in sufficient quantity to ensure some disease development.

Results from controlled on-vine inoculation in the field showed that 10^4 spores/mL applied to inflorescences were necessary for infection and disease expression at harvest (Warren, Becker *et al.* 1999). Latent infection could also be achieved on bunches at several growth stages (ie. late flowering, pre-bunch closure and veraison) when high numbers of spores were applied (Emmett and Rozario 1999; Warren, Becker *et al.* 1999). Several groups have shown that berry damage is required for spore germination and penetration (Cole *et al*. 1999, Holz 1999; Keller *et al*.2003). The cap scar at the pedicle is the initial damage point for infection at flowering. How long the cap scar remains infective is not yet known. Damage to berries can be caused by many factors such as rubbing within tight bunches and LBAM feeding.

Role of Damage

Even though there is evidence that damage is necessary to permit infection by *B. cinerea* on berries, the damage may be microscopic, eg micro-cracks in the wax cuticle on the berry surface that allow the fungus direct access to the epidermis. Damage to berries seems to be a primary factor in infection, and as such is a key issue for management.

LBAM

In some seasons and regions there was a relationship between incidence of LBAM and the incidence and severity of botrytis bunch rot. McMahon and Wicks (2000) showed that LBAM larvae are capable of transmitting viable *B. cinerea* spores (externally on mouthparts head and feet, and internally within the gut and released in faeces) between bunches and have the potential to spread further infection through grapevines.

Skin Damage

During the 1999/2000 harvest in Mudgee it was observed that berries from vineyards under traditional spray programs that were *B. cinerea* infected had a dark matt appearance as apposed to the natural glossiness still visible on berries from organic vineyards (Dr Rogiers pers comm). It has been hypothesised that certain agrochemicals and their added surfactants that make the tissue more permeable alter the structure or composition of the bloom and this facilitates infection (Dr Rogiers, GWDRC CSU02/01). Moreover, there is evidence from previous projects (Murphy and Warren, 2000; Riches et al., 2002) that application of sublethal chemical doses may actually increase the amount of *B. cinerea* at harvest compared with unsprayed bunches. This may be due to chemical sprays killing or inhibiting other microflora on the berry, which normally suppress or compete with *B. cinerea* or because of damage to the berry skin or a combination of factors. Dr Rogiers is currently investigating the effects of agrochemicals on the berry wax layer, susceptibility to *B. cinerea* and surface populations of other microorganisms. Scanning electron microscopy of berries grown in the vineyard without exposure to pesticide sprays showed that waxes on the surface are arranged in intricate upright platelets with a lacy fringe often lining the ends of these platelets (Rogiers *et al*. 2000). Exposure of the berry surface to fungicide alone slightly affected the lacy structure of the wax, while the wetters and spreaders generally had an even harsher effect on the wax structure, potentially creating more entry points infection by *B. cinerea*. Some adjuvants when applied alone (without the antifungal) facilitated *B. cinerea* growth on the berry (Rogiers *et al*. 2000). Surfactants and fungicides may not only have a direct effect on the waxes of the berry but also on the natural microflora on the berry. Furthermore a significant decrease in yeast population on berries that had received sprays was also reported and many of these yeast populations are known to be inhibitory to growth of *B. cinerea*. Microflora populations in washings taken from berries grown under organic and traditional vineyard practices are being compared in a parallel project undertaken by the principle investigator Dr Cole (GVGIDC-2001). This data is still to be assessed; however the fungal populations were similar in composition and B. cinerea was present in all vineyards, organic and conventional. It was the expression in the vineyard that was less or absent.

Bernard (1997) has shown that the most resistant grape varieties have a thicker skin, this being more difficult for *B. cinerea* to penetrate. Prudent (1994) and Chenet (1997) have shown that the quantity and quality of pectins, major components of cell walls, and the quantity of phenolic compounds, vary according to susceptibility of the variety to the disease. For instance, susceptible varieties also have a greater quantity of water-soluble pectins, which are more easily broken down by *B. cinerea* enzymes, as well as lower concentrations of phenolic compounds (Dubos, 2000).

Chemical control

Various product efficacy trials have shown that the registered botryticides generally do give management of botrytis bunch rot (reduced incidence and severity of berries infected) compared with unsprayed provided that a lethal dose of chemical actually contacts the flowers and bunches (McMahon and Wicks, 2000). The timing of spray applications during bunch development also has an effect on the efficacy of spray programs (McMahon and Wicks, 2000; Rozaria *et al*. 1998). McMahon and Wicks, (2000) reported that the best efficacy was achieved when chemicals were applied at flowering. On-vine inoculations undertaken in project DAV 98/1 showed that a single application of Scala^{R} at capfall significantly reduced the number of infected berries at harvest compared with no spray.

Many of these chemical efficacy trials apply sprays using a backpack sprayer resulting in higher chemical doses and better coverage than is often achieved by commercial spray equipment. Project DAV 98/1 used bioassays and dose-response curves to determine the lethal/effective dose of two commonly used botryticides, Rovral®

(iprodione) and Scala® (pyrimethanil) against *B. cinerea.* Lethal doses were then compared with the doses landed in a series of field trials using commercial equipment. In a typical field application using commercial Airshear sprayer onto Chardonnay on a VSP trellis at pre-bunch closure, 67% of bunches had residues lower than the dose required to kill 50% of *B. cinerea* infections. Failure to delivery a lethal chemical dose to flowers and bunches may explain why some growers fail to achieved adequate control of botrytis bunch rot despite following recommended spray programs (Warren and Riches 2001). Recent research (DAV 98/2) has shown that some spray applications fail to deliver a lethal dose; particularly in canopies where bunches are not easily accessible to sprays.

B. cinerea is a fungus with a high potential to develop resistance to chemicals. Resistance to both benzimidazoles and dicarboximides has been found in Australian vineyards. However, little is known about the nature of resistance or its practical effects on the efficacy of control programs. GWRDC project SPY/1 showed that doses of iprodione (Rovral®) deposited by applications after pre-bunch closure gave little to no control when bunches were infected with an iprodione resistant strain of *B. cinerea*.

Chemicals may still have a role in botrytis bunch rot control particularly in crisis situations and high-risk regions. It may be possible to reduce spray programs down to one well-timed and targeted application. However, most growers have indicated that they would like to reduce their chemical inputs.

Other non-chemical control options

With the potential for ongoing resistance problems, the increasing interest in 'organic' products and the MRL limitations being set by overseas importers, the need for non-chemical control options is a priority. Non-chemical control measures may be important in the management of botrytis bunch rot, but have not been sufficiently either researched or publicised (Dubos 2000). Developing IVM (integrated vine management) protocols utilising new soil amelioration products that improve soil and vine health need evaluation and integration into holistic management approach.

Anecdotal evidence suggests that mulching under vines may reduce the severity of botrytis bunch rot. However, this proposition needs to be tested experimentally. Researchers in New Zealand are experimenting with deep-rooted plants as inter-row plantings that compete for nutrients and water in order to reduce vine vigour and susceptibility to *Botrytis cinerea*. Previous work (GWRDC DAV 95/1) has shown that botrytis bunch rot can be minimised by canopy and insect management practices such as pruning, air blasting, leaf plucking and control of light brown apple moth (LBAM).

Host Considerations

Host resistance

The breeding of disease resistant varieties has become a more viable option with the availability of many genetic tools and the ability to undertake gene manipulation. However in existing vineyards replanting with resistant varieties may take many years.

Host modification

Many modifications may be made to the host to minimise the microclimate effect within the canopy that is conducive to disease. Some of these are already being practised, such as an open trellis type (eg Scott Henry), leaf plucking, and vine trimming to reduce vine vigour. Growers are already adopting practices that reduce vine vigour and result in open canopies, resulting in a microclimate less favourable to *B. cinerea* and development of botrytis bunch rot. Some of these practices have been shown to improve disease control and have the added advantage of improving spray penetration.

DAV 92/1 concluded that viticulturists should aim to produce small open bunches, no matter what the canopy system, in non-congested bunch zones distributed over the vine, and maximise the efficiency of chemical spray applications. Vines supporting small, loose bunches distributed in non-congested rather than in tightly packed bunch zones are the best option for successful botrytis bunch rot management (Emmett *et al*. 1994).

Most grape varieties have a tight bunch cluster that creates a microclimate favourable to *B. cinerea*. These have more contact points between berries leading to rubbing and it is often difficult for sprays to penetrate. Compact bunch structures with more tightly packed berries generally predispose bunches to increased damage from splitting, increased humidity within the bunch and in turn increased botrytis bunch rot. The non-contact surfaces

of grape berries tend to be more resistant to *B. cinerea* infection than the contact surfaces. The epicuticular wax of non-contact surfaces contains partially overlapping platelets while contact surfaces lack wax platelets and appears to have amorphous surface wax (Marois, Nelson *et al.* 1986). botrytis bunch rot is more prevalent in grape varieties that have tight compact bunches with high levels of contact surface area that results in flawed epicuticular wax and cuticle structure, eg. Riesling. However, other varieties with an open architecture but with large berries and low levels of cuticle that resulted in berry splitting were also prone to *B. cinerea* infection (Percival, Sullivan *et al.* 1993).

Natural clonal variation and artificial modification of bunches have been used to produce more open bunch architecture. Examples of these are the gamma ray-induced mutations of Barbera, and loose-berry clones of Mariafeld from Switzerland. Spraying gibberellic acid at flowering can cause elongation of berries and a percentage of fruit drop which can have beneficial effects for botrytis bunch rot management**.**

Current research by Dr Ian Dry and his team at CSIRO aims to develop genetically modified clones with open bunches to limit *B. cinerea* infections in vineyards. The success of this approach depends upon the genetic control of inflorescence structure being relatively simple, therefore allowing mapping and introduction of the desirable genes into selected cultivars an easier task. This is a long-term approach and it cannot be utilised on existing grapevine plantings.

Host defence mechanisms and vine health

Phytoalexins

Many plants, eg grapevines and berries, are stimulated to produce defence compounds in response to tissue damage from biotic and abiotic events. Stilbenes belong to one group of these secondary metabolites called phytoalexins, which are responsible for a level of resistance to *B. cinerea* infection. Phytoalexins are absent from healthy tissue but accumulate following an infection or damage, eg insect feeding, UV radiation, and kill cells in the immediate vicinity of the damage site. The major phytoalexin is resveratrol that decreases as berries ripen to maturity and is not present in sufficient quantities to avoid the development of botrytis bunch rot at harvest. Several reasons have been proposed for the reduction in resveratrol levels as berries ripen: the gene is not switched on, the stilbene synthase protein is inactive, or the chemical precursors for stilbene synthesis may not be available at that stage in berry development. If stilbene synthase gene expression could be induced more rapidly then protection of *B. cinerea* infection would be more effective at later stages of berry ripeness.

The role of stilbenes in flowers is not as clear: Inoculation of inflorescences with *B. cinerea* spores at full bloom led to the highest disease severity (Keller and Cole, 2002). Stilbene phytoalexins in the flowers were measured by HPLC and it was found that constitutive piceid levels did not change following inoculation. However, resveratrol did accumulate after pre-bloom and full bloom inoculation though not preventing infection while ∈-viniferin was found in necrotic tissues only. These results suggest that stilbenes are either not accumulated fast enough or to insufficient levels to prevent flower infection. There is a question as to why the vine would go to the trouble of synthesising metabolically costly stilbenes if they have no purpose. It has been hypothesised that *B. cinerea* (being a necrotrophic pathogen) elicits stilbene production to induce host cell death and enhance its infection rate (Cole and Keller). Thus the relationship between cause and effect is still not clear and requires further research.

Markus Keller and his team are trying to find manageable practices that get the vine to produce more stilbenes, which would make it less susceptible to *B. cinerea*.

Transgenics

Ian Dry and colleagues at CSIRO Plant Industry are investigating transgenic strategies for *B. cinerea* management. The gene for stilbene synthase has been isolated and incorporated using rDNA techniques into tobacco, wheat, rice and barley. Some of these transgenic plants have demonstrated an increased resistance to *B. cinerea*. A difficulty with this approach is that resistance to *B. cinerea* appears to be polygenic (ie more than one gene involved) whereas currently they only have the capability of working with 1-2 genes at a time. Furthermore, this technology cannot be utilised on existing grapevines.

Induced resistance

Studies with other crops suggest that by using certain natural compounds, plant extracts and other chemicals know as elicitors, it is possible to activate natural defence mechanisms (eg phytoalexin and PR-protein accumulation) in plants against pathogens. The use of elicitors to induce the grapevines natural defence responses offers novel opportunities for disease management. Recent overseas research by Jeandet *et al*. (2000) has shown that a metallic salt, hexahydrated aluminium chloride ($AlCl_3$), can act as an inducer of phytoalexin synthesis in grapevine leaves resulting in increased resistance to *B. cinerea*. Furthermore, $ALCL_3$ also enhanced the efficiency of iprodione (Rovral®) when they were used in combination (Jeandet *et al*., 2000).

Nutritional management

Improving vine health through improved soil health may activate the vine's own defence mechanism, providing added protection from disease. This is a new and exciting area of research.

Many environmental factors affect vine vigour and, in turn, the microclimate of the berries, how much water the plant absorbs, yield, how the grapes are disposed, the size of the berries and state of the skin, early ripening, and, efficacy of spraying (Dubos, 2000). It has long been suspected that excessive nitrogen (N) fertilisation promotes development of botrytis bunch rot. However, according to Dubos (2000), there are no definitive data to support this assertion. None-the-less, a good correlation between botrytis bunch rot levels and the amount of nitrogen in musts has been reported (Dubos, 2000). Overseas studies have shown that high nitrogen levels can increase bunch stem necrosis (Keller and Koblet, 1995; Christensen and Boggero, 1985), which increases the susceptibility of vines to *B. cinerea* infection through damaged tissues. High fruit N has been associated with increased susceptibility to fungal decay in apple fruit (Sugar *et al*. 1992). It has also long been suspected that calcium nutrient status also affects the susceptibility of the vine to damage from pathogens, as it does in other fruit crops. High fruit Ca concentration has been correlated with low levels of postharvest disease in apple (Conway and Sams, 1987) peach (Conway *et al*. 1987) and avocado (Hofman *et al*. 1999). It is thought that Ca reduces the post harvest development of disease by strengthening cell walls and maintaining membrane selective permeability and integrity (DeMarty *et al*. 1984). Calcium strengthens cell walls by cross-linking the pectic polymers in the cell wall and middle lamella (Carpita and Gilbeaut 1993), thereby making them less accessible to attack by fungal pectolytic enzymes (Bateman and Lumsden 1965 and Conway *et al*. 1992). Chardonnet and Doneche (1995) studied the Ca contents of the cell walls of grapevine berries at different developmental stages and on different varieties. They found Ca supply in both disease susceptible and resistant cultivars. High Ca concentrations in the epidermis resulted in a lower incidence of infection in both susceptible and resistant cultivars. Management of pear trees for low N and high Ca content in the fruit has been used to reduce the severity of postharvest fungal decay (Sugar *et al*. 1992). Such nutritional manipulations are compatible with other methods of decay control.

Studies on avocado in South Africa have shown that tree vigour can have a significant impact on fruit mineral accumulation (Cutting and Bower, 1990; Witney *et al.* 1990). In mango, an increase in tree vigour, measured as an increase in the leaf to fruit ratio, has been shown to cause a significant reduction in fruit Ca accumulation and an increase in postharvest disease susceptibility (Simmons *et al*. 1998). Greater plant vigour tends to lead to more vegetative shoots that may out-compete fruit for Ca (Kirkby and Pilbeam 1984; Ho *et al*. 1993). 'Conventional theory' suggests that host resistance to pathogen and pest attack comes at a price to the growth of the plant, since it may divert resources away from growth. However, recent research on soybeans and insect attack found that by manipulating the proportion of several macronutrients it was possible to increase resistance of the host to insect attack while maintaining good plant health and development (Busch and Phelan, 1999).

The observations and research reported above indicate an interaction between plant vigour and susceptibility to disease, and that tissue N and Ca concentrations are important factors in this interaction. In the proposed research, we plan to investigate, by correlation with variable management practices in the field, relationships between plant vigour and grapevine susceptibility to botrytis bunch rot. An emphasis on determining the importance of N and Ca tissue status and the N:Ca ratio will be underpinned by glasshouse grapevine nutrition experiments. A better understanding of the role of these variables in grapevine botrytis disease will assist in devising management recommendations to enhance grapevine disease natural resistance.

The anticipated whole plant health-based strategy should provide a long-term management approach to botrytis bunch rot management that would reduce reliance on or even eliminate the need for chemical control, particularly in low risk seasons and low risk regions.

Nutrition Trials

Introduction

Plant diseases are the result of a combination of host, pathogen and environmental factors over time. A severely nutrient stressed plant is frequently more vulnerable to disease than one at a nutritional optimum and a plant receiving large excess of a mineral element may also be more susceptible to disease (Huber 1981). A disease is seldom eliminated by the application of a mineral element but specific levels of nutrients do reduce the severity of many diseases. Mineral nutrients can either increase or decrease the resistance of plants to pathogens by effecting changes in growth pattern, the histological or morphological properties of its tissues and chemical composition (Huber 1981). A common generality is that nitrogen tends to increase disease while calcium and potassium tend to reduce disease.

Nitrogen

Nitrogen has been extensively studied in relation to host nutrition and disease severity for many years. Nitrogen is an essential requirement for plant growth but its availability in soil is usually limited and it has to be applied to soils as a fertiliser to sustain crop growth. Nitrogen influences host plant resistance by reducing the frequency of successful pathogen penetration or by retarding pathogenesis after penetration. Nitrogen is the main mineral element changing the amount of cellulose and thereby affecting the mechanical strength of the cell wall.

Nitrogen is available to plants as biological mineralisation of complex soil organic matter, microbial fixation of atmospheric nitrogen and fertilisers. Mineralisation and fixation result in the ammoniacal form of nitrogen. The NH_4 - N is then oxidised to NO_3-N. Plants will assimilate primarily NO_3 that is internally reduced to NH_2 prior to utilisation. However, most plants can use either form of nitrogen. The levels of amine, amide and protein in the plant are higher when they utilise NH_4 rather than NO_3 (Huber 1981).

Diseases caused by obligate and facultative pathogens respond differently to mineral nutrition. An increasing nitrogen concentration increases the severity of disease by obligate pathogens but may have the opposite effect on disease caused by facultative pathogens. These differences in response are based on the nutritional requirements of the two types of pathogens where obligate pathogens utilise assimilates supplied by living cells and facultative pathogens prefer senescing tissue. All factors which support the metabolic and synthetic activities of host cells and which delay senescence of the host plant increase resistance to facultative pathogens.

Botrytis cinerea is a facultative parasite or semi saprophyte and therefore, is unable to grow in the absence of dead cells. In some cases, optimal nitrogen leads to increased resistance because young, dark tissue is more resistant than mature or somewhat senescent leaves. High nitrogenous fertilisers inhibit tissue necrosis in the vigorous and young plant organs (Kiraly 1976).

In grapevines, it has long been speculated that excessive nitrogen fertilisation promotes the development of botrytis bunch rot but there is no definitive data to support this view (Dubos 2000). However, a good correlation between botrytis bunch rot levels and the amount of nitrogen in wine musts has been reported (Dubos 2000). High nitrogen fertilisation can increase bunch stem necrosis that inturn can predispose vines to botrytis infection through damaged tissues.

In grapevines, (Bavaresco and Eibach 1987) found that increasing nitrogen decreases the resistance of the plant to two obligate pathogens, powdery and downy mildew, and high rates of nitrogen decrease the synthesis of the plant defence compounds, stilbenes and this effect is variety specific.

In other plant systems when nitrogen is in excess the activities of some key enzymes of phenol metabolism are depressed and as a result host susceptibility is increased (Kiraly 1964).

Potassium

Potassium does not become a structural component of the plant but is involved in all cellular functions as a mobile regulator of enzyme activity. A balanced level of potassium induces thicker cell walls, accumulation of arginine and other amino acids and the production of new tissues (Huber 1981). Increasing potassium fertilisation reduces disease severity of both obligate and facultative pathogens (Marschner 1986).

In grapevines, 49% of the seasonal requirements of potassium are taken up from the end of bloom to veraison. At the 10 cm shoot growth stage potassium is remobilised from the root to the shoot. From veraison to harvest

potassium is remobilised from shoots and leaves to bunches. After harvest, potassium increases in all organs of the vine (Conradie 1981).

In Hungarian grapevine trials, potassium fertilisation reduced the susceptibility of grapevine cultivars to infection by *B. cinerea*. Infection is predisposed by wounding of grape tissues prior to harvest. High potassium nutrition may reduce wound injury and accelerate wound healing resulting in reduced susceptibility to infection (Kiraly 1976).

Calcium

Calcium is a structural component of the middle lamellae of plant cell walls and is involved in cell division, cell development and the neutralisation of metabolic acids (Huber 1981). Calcium is a less mobile nutrient and is not redistributed from older tissue therefore, plant requirements must be continually obtained from external sources.

The calcium content of plant tissues can affect the severity of disease in two ways. Firstly, calcium is essential for the integrity of biological membranes so when calcium levels are suboptimal low molecular weight compounds move from the cytoplasm into the apoplast. Second, calcium polygalacturonates provide cell wall stability by crosslinking the middle lamella. Most fungi invade plant tissue by secreting extracellular pectolytic enzymes that dissolve the middle lamella and calcium drastically inhibits the activity of these enzymes. Tissues of deficient plants are poorly organised and have thin cell walls, large intercellular spaces, and poorly defined middle lamella (Huber 1981). Calcium and cell walls resistant to enzymatic degradation are important to resist pathogens which cause soft and dry rots (Kiraly 1976).

In grapevines, half of the seasonal requirements of calcium are absorbed from the end of bloom to veraison. Calcium accumulates predominantly in the leaves from bloom onwards and leaf fall results in the vine losing 54% of its calcium. Grape bunches contain very low levels of calcium (Conradie 1981)

Calcium can have a direct effect on *B. cinerea* during infection as calcium is released from the host cell wall and the concentration increases in the free space between cells during fungal attack, which can limit the infection process. (Chardonnet, Sams *et al.* 1999) demonstrated that applying $CaCl_2$ causes a thickening of the fungal cell wall, a consequence of the high osmotic pressure developed. This could retard the elongation phase of the hyphae and result in a loss of cell wall elasticity.

Calcium chloride vacuum-infiltration of harvested grape berries did not have a significant effect on infection rate. However, it resulted in an increase in calcium content of the berry with the majority of calcium being found in the cell wall fraction of the skin (Chardonnet, L'Hyvernay *et al.* 1997). Externally applied calcium accumulates in the outer layers of cells in the skin of the grape berry. The cation promotes the stability of the cell wall by chelating the free carboxylic groups of galacturic units. The fungus hydrolyses the methylated carboxylic groups of the cell wall pectins with a pectin methyl esterase. In the presence of sufficient amounts of calcium, these carboxylic groups immediately chelate.

In wine grapes, Wuxal® calcium applied four times from fruit set to veraison increased the calcium content of berries and reduced the incidence and severity of bunch rot (Winter and Nicol 1998).

Calcium and potassium balance

Increasing potassium does not necessarily lead to an increase in disease as long as the calcium content of the plant is high. However, calcium reduced the severity of post harvest *B. cinerea* in naturally infected rose flowers but increasing potassium negated the ability of calcium to reduce disease probably due to competition for cation uptake (Volpin and Elad 1991).

Aim

A detailed knowledge of the means by which mineral nutrients increase or decrease disease must be gained in order to complement disease and pest control measures. Mineral nutrition is an environmental factor that can potentially be more easily manipulated. The aim of experiments described below was to determine the effect of N, K and Ca application on the nutrient status of petioles and grape bunches and the relationship of these parameters to *B. cinerea* resistance and grape composition.

Materials and Methods

Field trial

Season 2002/03

Vines were 10 year-old Chardonnay grown in a commercial vineyard "Fernhill" at Coldstream, Yarra Valley and spaced four vines per panel (vine spacing 1.5 m and row spacing 2.5 m). The trellis system was Scott-Henry and the yield was 7 t / ha in the previous year. Soil type was sandy loam and the vines were watered by drip irrigation. In previous years, the vineyard received a full spray program. In the experimental season, the vineyard received a full spray program with the exception of botryticides. However, a botryticide (Switch®) was unintentionally applied to the trial site at pre bunch closure.

Experimental design

The trial was a randomised block design with 8 treatments (Table 1). There were 5 replicates of each treatment (Figure 1). The treatment plots consisted of a panel of four vines of which the two centre vines were sampled.

	Rep 1		Rep 2		Rep 3		Rep 4		Rep 5	
	Panel 1	Panel 2	Panel 3	Panel 4	Panel 5	Panel 6	Panel 7	Panel 8	Panel 9	Panel 10
Row 1	1	2	8	7	5	6	3	4	1	2
Row 2	3	4	5	6	3	4	1	2	3	4
Row 3	5	6	3	4	1	2	4	8	5	6
Row 4	7	8	1	2	7	8	5	6	7	8

Figure 1 Trial site Fernhill vineyard 2002/03.

Nitrogen treatment was 60 kg / ha of nitrogen applied as NH_4NO_3; the potassium treatment was 120 kg / ha of potassium applied as K_2SO_4; the calcium treatment was applied as $CaSO_4$ at rates of 0.5% (1st application), 1.0% (2nd app) and 1.5% (3rd app). The N and K were dissolved in 5 L of water and applied to one side of the row. The Ca was dissolved in water and applied to bunch by a hand atomiser until runoff. Nutrient treatments were applied three times: at fruit set (> pea size)[11/12/02], early pre bunch closure (PBC) [9/1/03] and late bunch closure (LBC) [21/1/03].

Season 2003/04

Vines were 6 year old Chardonnay grown in a commercial vineyard "Meroo" at Yarra Glen and spaced five vines per panel (vine spacing 1.5 m and row spacing 2.5 m). The trellis system was vertical shoot positioned single (VSP) cordon and the yield was 12 t / ha in the previous year. The vines were spur pruned to approximately 55 nodes per vine using finger and thumb. The soil was clay loam and the vines were watered by drip irrigation. The vineyard and trial site received a full spray program during the experimental season including botryticides. Because this was such a high-risk site, the manager was not prepared to risk losing the crop by withholding botryticides.

Experimental design

The trial was a randomised block design with 8 treatments (Table 1). There were 5 replicates of each treatment (Figure 2). The treatment plots consisted of a panel of five vines of which the three centre vines were selected as data vines. Two of these vines were used for disease assessments and one for growth rate.

	Panel 1	Panel 2	Panel 3	Panel 4	Panel 5	Panel 6	Panel 7	Panel 8
Row 1	8	3	5	1	7	2	4	6
Mid row								
Row 2	4	2	7	5	6	3	1	8
Row 3	3	7	1	6	8	4	5	2
Row 4	7	2	8	6	5	4	3	1
Row 5	2	7	5	3	1	6	8	4

Figure 2 Trial site Meroo vineyard 2003/04.

Nitrogen treatment was 300 kg/ha of nitrogen applied as $NH_4 NO_3$; the potassium treatment was 300 kg/ha of potassium applied as K_2SO_4; the calcium treatment was 2.0 % and applied as $CaCl_2$. The N and K treatments were dissolved in 5 L of water and applied to the area directly under the vines (area - 2.7 m^2). The treatments were applied 4 times: 5 weeks after budburst (10 cm shoot length) (24/10/03), fruit set (2 weeks post flowering), veraison and directly after harvest prior to leaf fall. The Ca was dissolved in water and applied to bunches by a hand atomiser until runoff at fruit set (17/12/03), early PBC (14/1/04) and veraison (5/3/04). Botryticides were applied at 80% capfall (8/12/03) Teldor, pea-size (28/12/03) Switch, PBC (19/1/04) Captan and LBC (4/2/04) Fortress.

Table 1 Mineral nutrition field trial treatments for Fernhill vineyard 2002/03.

Treatment number	Nitrogen	Potassium	Calcium
1			
2			0.5%, 1.0%, 1.5%
3		120 kg/ha	
4		120 kg/ha	0.5%, 1.0%, 1.5%
5	60 kg/ha		
6	60 kg/ha		0.5%, 1.0%, 1.5%
7	60 kg/ha	120 kg/ha	
8	60 kg/ha	120 kg/ha	0.5%, 1.0%, 1.5%

Shaded areas are treatments applied.

Table 2 Mineral nutrition field trial treatments for Meroo vineyard 2003/04.

Treatment number	Nitrogen	Potassium	Calcium
1			
2			2 %, 2%, 2%
3		300 kg/ha	
4		300 kg/ha	2 %, 2%, 2%
5	300 kg/ha		
6	300 kg/ha		2 %, 2%, 2%
7	300 kg/ha	300 kg/ha	
8	300 kg/ha	300 kg/ha	2 %, 2%, 2%

Shaded areas are treatments applied.

Botrytis inoculation

Season 2002/03

Botrytis cinerea cultures were grown on PDA for seven days till sporulating. Spores were collected in distilled water with 0.001% Tween and, using a haemocytometer, adjusted to 1 x 10^4 spores per mL (Warren, Becker *et*

al. 1999). In the vineyard, 10 inflorescences were randomly chosen across 2 vines per replicate. Of these 5 inflorescences were inoculated with 5 mL of the spore suspension and 5 were left for natural infection. All 10 inflorescences were moist incubated in a zip lock bag for 24 h.

Season 2003/04

Fungal inoculum was produced as in the previous season. In each replicate, one vine was randomly designated the "flowering" vine and one as the "harvest" vine. At flowering, four inflorescences were inoculated with spores on each of these vines and four chosen for natural infection. All inflorescences were moist incubated for 24 h in a ziplock bag.

Disease assessment

Season 2002/03

At harvest, bunches were assessed for botrytis bunch rot in the field and samples taken back to the laboratory to undergo surface sterilisation for 3 minutes in 1% bleach and moist incubation for 10 days. Disease severity was measured as the percentage of berries showing sporulating *B. cinerea*.

Season 2003/04

One week after inoculation, the inflorescences from the "flowering vine" were removed, surface sterilised in 1% bleach and moist incubated. Severity of *B. cinerea* infection was measured after 10 days. At harvest, bunches from the "harvest vine" were removed for assessment as described at flowering.

Plant tissue nutrient analysis

Petiole

Season 2002/03

Leaves positioned directly opposite bunches were sampled from two vines per replicate at harvest. The petioles (20 g fresh weight) were separated from leaf blades and washed and dried at 65ºC for two days, then ground to a powder with a plant tissue grinder and stored at room temperature in tubes until analysed.

Season 2003/04

Leaves positioned directly opposite bunches were sampled from one vine per replicate at flowering. The petioles (20 g fresh weight) were separated from leaf blades and washed and dried at 65ºC for two days, then ground to a powder with a plant tissue grinder and stored at room temperature in tubes until analysed.

Grape

Season 2002/03

Ten randomly selected bunches were collected at harvest from across two vines in each replicate and pooled. The bunches were stored at –20ºC until analysis for quality parameters.

Season 2003/04

Ten randomly selected bunches were collected at harvest from two vines in each replicate. The bunches were stored at –20ºC until analysis for quality parameters.

In both season 2002/03 and season 2003/04 two bunches per replicate were homogenised to give approximately 250 mL of macerated tissue. The homogenate was dried at 65ºC for seven days until all the moisture was removed.

Nutrient Analysis

The petiole and homogenised grape tissue from treatment 1 (no nutrient applied) and treatment 8 (N, Ca, K applied) was analysed for total N, nitrate N, K and Ca via ICP suite. State Chemistry Laboratory Victoria performed these analyses.

Soil collection and nutrient analysis

In season 2002/03, one soil core (30 cm depth) was taken from each replicate and combined for treatments 1 and 8. In season 2003/04, three soil cores (30 cm depth) were taken from each replicate for treatments 1 and 8.

A soil analysis for total nitrogen, nitrate and ICP suite was performed on soils collected from treatment 1 (no added nutrients) and treatment 8 (N, K, Ca). State Chemistry Laboratory Victoria performed these analyses.

Vine growth and canopy density

Season 2002/03

These measurements were not taken in this season.

Season 2003/04

One shoot from the centre vine was tagged for measurements at weekly intervals and shoot length (cm), node and bunch number per shoot were recorded. The tagged shoots were measured on (29/10/03) E-L stage 12, (5/11/03) E-L stage 14, (12/11/03) E-L stage 15, (19/11/03), E-L stage 17 (26/11/03) and E-L stage 18 (3/12/03). The growth rate was calculated based on shoot extension over time.

The canopy density was determined at 80% capfall and veraison in the bunch zone using a light meter to determine the level of light penetration into the canopy. The percentage light penetration was based on the average of 3 readings at each panel and was proportioned against the average of the light intensity outside the rows prior to assessment and after assessment. A low lux index value indicated low light penetration.

The lux index is

$$\frac{\text{lux in the bunch zone} \times 100}{\text{average lux outside the canopy}}$$

Bunch compactness

Season 2002/03

These measurements were not taken in this season.

Season 2003/04

At flowering and harvest the collected bunches were weighed and the bunch length (cm) recorded. A bunch compactness index was calculated as a ratio of bunch weight / length (Emmett, 1995).

Fruit yield and quality parameters

Fruit weight per vine was recorded at harvest. Total fresh fruit weight was calculated as the mean of the fruit yield of two vines per replicate. The average berry weight was determined from 1 bunch per replicate for each treatment. Total soluble solids (TSS) in fruit juice were measured using a hand refractometer. A single bunch from each replicate was used to assess TSS. The titratable acidity of juice (TA) was determined from the juice of one bunch per replicate which was measured twice (Iland).

Statistical analysis

Analysis of variance was performed on all data using Genstat (Laws Agricultural Trust). All data were tested for normality and the disease severity, light penetration at flowering and veraison data was angular transformed to satisfy assumptions of constant variance. Estimated means and LSI boundaries were back transformed and graphed. The plant nutrient analysis data for total calcium was square root transformed, total nitrogen was inverse square root transformed and total potassium was not transformed.

Glasshouse trial

Potted vine establishment

The glasshouse experiment was started in September 2002 at DPI Knoxfield, using the varieties Chardonnay (clone I10V1) and Cabernet Sauvignon (clone SA125). Unrooted, dormant cane cuttings 35 to 38 cm in length were planted to a depth of approximately 10 cm in individual pots containing a medium of acid-washed, coarse sand. For each variety, seventy-two potted plants were established in a glasshouse with temperature maintained near an average 22^{o}C and humidity unregulated.

Watering & nutrient solution application

Following planting, pots were initially irrigated with tap water. When 50% of the plants were at the second leaf stage (growth stage 9), near the end of September, the watering regime for each plant was changed to include deionised water on Mondays, Wednesdays, Fridays Saturdays and Sundays, and one of 18 different nutrient solutions on Tuesdays and Thursdays. On nutrient days, each potted vine was hand-watered with 167 ml of solution. On deionised water days, deionised water was administered three times a day (9 am, 2 pm and 5 pm) for two minutes by automated irrigation. At each watering time around 600 ml (enough for flushing and run through) was delivered through sprinkler heads which gave a wetting radius of between 3 and 5 cm near the base of each cane.

The base solution was a modified, half-strength Hoagland's solution. In the first year (2002/03) the nitrogen levels used were - N2, N1 (modified, half-strength Hoagland's), N0.5, N0.25, N0.125, N0.0625. Each of these N levels was used in combination with calcium at levels - Ca2, Ca1 and Ca0. A further six treatments with K2Ca2, K2Ca1, K2Ca0, K0.125Ca2, K0.125Ca1 and K0.125Ca0 (Table 3). Nitrogen sources were ammonium nitrate and/or potassium nitrate and/or calcium nitrate 4-hydrate. Potassium sources were potassium nitrate and/or potassium dihydrogen orthophosphate and/or potassium chloride. Calcium sources were calcium chloride and/or calcium nitrate 4-hydrate. It was considered that some of the low nutrient treatments (especially T13 through to T18) had left the vines in a poor state of vigour.

In the second year (2003/04) the experimental design was a resolvable row column design consisting of 3 replicates of 6 rows by 6 columns. The calcium levels were Ca2, Ca1 and Ca0, and 6 K-N combinations N2: K1, N1: K1, N0.5: K1, N0.25: K1, N1: K2, N1: K0.125). There were a total number of 108 vines. The N1, K1 and Ca1 were equivalent to half-strength Hoagland's (Table 4).

After the end of March, automatic applications of deionised water continued at three per day but only on Saturdays and Sundays, Tuesdays and Thursdays. Nutrient solutions were thereafter applied on Mondays, Wednesdays and Fridays. It was considered that some of the low nutrient treatments (especially T13 through to T18) had left the vines in a poor state of vigour and that additional nutrients were required to enhance health prior to winter dormancy. All vines received either nutrient solution 4, 5 or 6, depending on which of these matched the Ca level administered during the trial.

In the middle of May, all potted vines were removed from the glasshouse and placed outdoors for over-wintering.

Table 3 Nutrient treatments 1st year 2002/03

SOLUTION/ TREATMENT	N	P	K	Ca
1 (N2Ca2)	15	0.5	3	5
2 (N2Ca1)	15	0.5	3	2.5
3 (N2Ca0)	15	0.5	3	0
4 (N1Ca2)	7.5	0.5	3	5
5 (N1Ca1)	7.5	0.5	3	2.5
6 (N1Ca0)	7.5	0.5	3	0
7 (N0.5Ca2)	3.75	0.5	3	5
8 (N0.5Ca1)	3.75	0.5	3	2.5
9 (N0.5Ca0)	3.75	0.5	3	0
10 N0.25Ca2	1.88	0.5	2.5	4.99
11 N0.25Ca1	1.88	0.5	3	2.49
12 N0.25Ca0	1.88	0.5	3	0
13 N0.125Ca2	0.94	0.5	2.5	4.97
14 N0.125Ca1	0.94	0.5	3	2.5
15 N0.125Ca0	0.94	0.5	3.04	0
16 N.0625Ca2	0.48	0.5	2.5	5
17 N.0625Ca1	0.48	0.5	3	2.54
18 N.0625Ca0	0.47	0.5	3	0
19 (K2Ca2)	7.5	0.5	6	5
20 (K2Ca1)	7.5	0.5	6	2.5
21 (K2Ca0)	7.5	0.5	6	0
22 K0.125Ca2	7.5	0.5	0.375	5
23 K0.125Ca1	7.5	0.5	0.375	2.5
24 K0.125Ca0	7.5	0.5	0.375	0

2 = full strength Hoagland's
1 = half strength Hoagland's

Table 4 Nutrient treatments 2nd year 2003/04

SOLUTION/ TREATMENT	N	K	Ca
1 (N2Ca2)	15	3	5
2 (N2Ca1)	15	3	2.5
3 (N2Ca0)	15	3	0
4 (N1Ca2)	7.5	3	5
5 (N1Ca1)	7.5	3	2.5
6 (N1Ca0)	7.5	3	0
7 (N0.5Ca2)	3.75	3	5
8 (N0.5Ca1)	3.75	3	2.5
9 (N0.5Ca0)	3.75	3	0
10 N0.25Ca2	1.88	3	4.99
11 N0.25Ca1	1.88	3	2.49
12 N0.25Ca0	1.88	3	0
19 (K2Ca2)	7.5	6	5
20 (K2Ca1)	7.5	6	2.5
21 (K2Ca0)	7.5	6	0
22 K0.125Ca2	7.5	0.375	5
23 K0.125Ca1	7.5	0.375	2.5
24 K0.125Ca0	7.5	0.375	0

2 = full strength Hoagland's
1 = half strength Hoagland's

Botrytis inoculation

Leaf bioassays were used to assess the effects of nutrition on *B. cinerea* infection, since the one year old rootlings did not produce adequate numbers of flowers in the first or second year of the project and therefore bunch bioassays were not possible. Flowers would be expected after 3-4 seasons.

In March, the first fully expanded leaf was removed from each treatment. The leaves were surface sterilised in 1% bleach for 3 min and rinsed twice in sterile deionised water. The leaves were inoculated with a plug of *Botrytis cinerea* culture (B33) growing on PDA with mycelium side down. The leaves were incubated for 7 days at room temperature in a tray sealed with plastic wrap also containing with moistened paper towel and dishcloth.

Botrytis assessment

In the first year (2002/03), the disease severity was scored on a scale from 0-3.
0 – no lesion, 1 – small lesion, 2 – medium lesion (1/2 leaf) and 3 – whole leaf diseased.

In the second year (2003/04), the leaves were photographed under glass and the percentage disease severity determined using Sigma Scan software. The disease area was recorded, as was the total area of the leaf. If the lesions were difficult to distinguish the leaves were cleared in absolute ethanol to remove chlorophyll for 2 days and then photographed and the percent disease severity determined.

Sampling to establish glasshouse leaf nutrient levels

At the end of March 2003, whole leaves (blades and petioles) were taken from glasshouse plants. Starting near the base, the original aim was to remove every second leaf. However, because leaves were scarce on the plants treated with low nutrient solutions, fewer than half the leaves were taken to ensure that plant health was not compromised. Collected leaves were weighed (fresh) and then oven dried in paper bags at 65^{o}C for 48 hours.

At the end of March 2004, whole leaves (blades and petioles) were taken from glasshouse plants. Starting near the base, the original aim was to remove every second leaf. Collected leaves were weighed (fresh), washed in deionised water to remove dust and spray residue and then oven dried in paper bags at 65^{o}C for 48 hours.

Winter pruning

In June of the first year, the vines were cut back to 2 nodes of previous years growth. The cane prunings were collected and the weights, lengths and the number of nodes recorded.

Statistical analysis

The percentage of disease area was modelled using the REML directive in Genstat (Lawes Agricultural Trust). The square root transformation was required to satisfy assumptions. Estimated means and LSI boundaries were back transformed and graphed.

Results

Field trial

Season 2002/03

The experiment was on 10 year old Chardonnay vines at "Fernhill' during the 2002/03 season.

Disease Assessment

In the vineyard at harvest, there was a small amount of botrytis bunch rot within the trial area but this was only on bunches around posts and not on the experimental vines. Inoculation of tagged bunches at flowering resulted in significantly higher levels of botrytis bunch rot at harvest ($P<0.05$) following surface sterilisation and moist incubation in the laboratory compared to uninoculated bunches (Figure 3). As such they were treated separately for statistical purposes. However, there was no significant difference between treatments in the inoculated bunches or the uninoculated bunches ($P>0.05$).

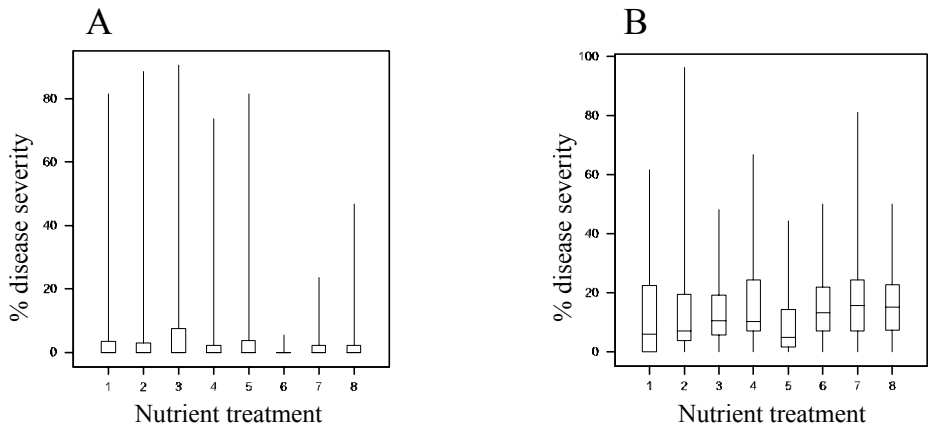


Figure 3 The effect of N, K and Ca treatments on botrytis disease severity of A) naturally infected and B) inoculated bunches at harvest following surface sterilisation and moist incubation. Disease severity is the number of berries infected per bunch of 5 bunches per replicate. 1 – no nutrient applied, 2 – Ca only, 3 – K only, 4 – Ca and K, 5 – N only, 6 – N and Ca, 7 – N and K and 8 – N, K, Ca. Box and whisker plots, median is marked as a line within each box. The whiskers indicate the 10th and 90th percentiles.

Fruit yield and quality parameters

The nutrient treatments had no effect on TSS, pH and TA (*P>0.05*) (Table 5). The Ca only treatment tended to increase yield, while the Ca and N, N only and N, K and Ca tended to reduce the yield compared to the control, however, this was not significant (*P>0.05*). All treatments with the exception of Ca and N resulted in an average berry weight greater than the control that was not significant (*P>0.05*).

Table 5 Effect of mineral nutrition on grape quality parameters Fernhill 2002/03

Treatment	Yield (kg) [a]	Average Berry weight (g) [b]	TSS [b] ºBrix	pH [b]	Total acidity [c]
1 Control	2.92	0.89	22.3	3.77	4.64
2 Ca	3.21	1.05	21.3	3.76	4.49
3 K	2.87	1.03	21.8	3.81	4.84
4 Ca K	2.72	0.95	21.2	3.71	4.96
5 N	2.42	1.08	21.1	3.82	5.15
6 N Ca	2.27	0.91	21.8	3.79	4.68
7 N K	2.75	1.03	21.4	3.83	4.56
8 N K Ca	2.45	1.03	21.3	3.82	5.21
Significance	ns	ns	ns	ns	ns

[a] Mean for two vines per treatment for 5 replicates
[b] Average of 1 bunch per treatment for 5 replicates
[c] Mean of 1 bunch per treatment for 5 replicates measured twice

Soil nutrient analysis

The three soil applications of nitrogen to vines did not significantly increase the total N present (0.18) compared to the control (0.19). The three soil applications of potassium did not significantly increase the total K present (0.22) compared to the control (0.14).

Plant tissue nutrient analysis

The addition of nitrogen to the soil did not significantly increase the level of total N or total NO_3 detected in the petioles or bunches at harvest ($P>0.05$) (Figure 4, Figure 5). The NO_3 levels in petioles taken from Fernhill at harvest were deficient (<10 mg/kg) according to Reuter and Robinson (1986). The addition of calcium to the bunches did not significantly increase the level of calcium present in the petioles or bunches at harvest. The calcium levels in petioles taken at harvest were adequate. The addition of potassium to the soil did significantly increase the level of potassium detected in the grapevine petioles and bunches at harvest ($P<0.05$). The potassium levels in petioles taken at harvest were deficient (<1 %w/w).

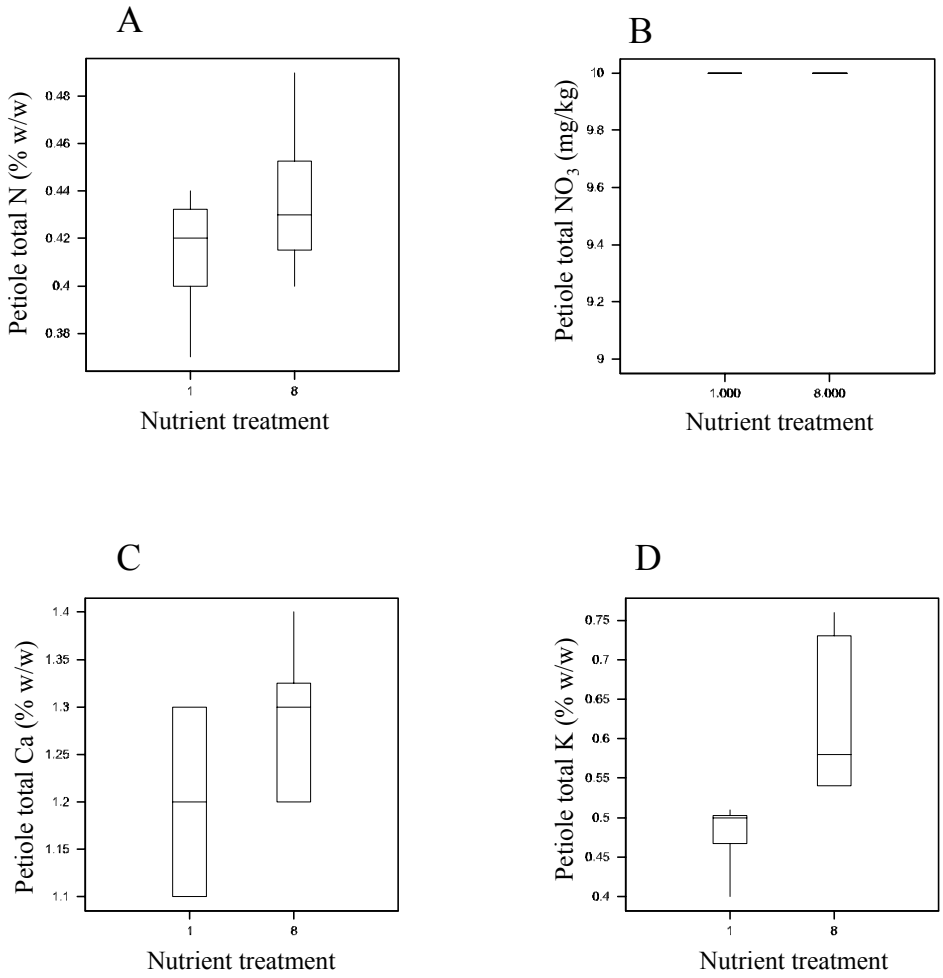


Figure 4 The effect of mineral nutrition on grapevine nutrient status of the petiole at Fernhill 2002/03. A) total N, B) total NO_3, C) total Ca and D) total K. 1- control no applied nutrition and 8 – applied nitrogen, potassium and calcium. Samples collected at harvest.

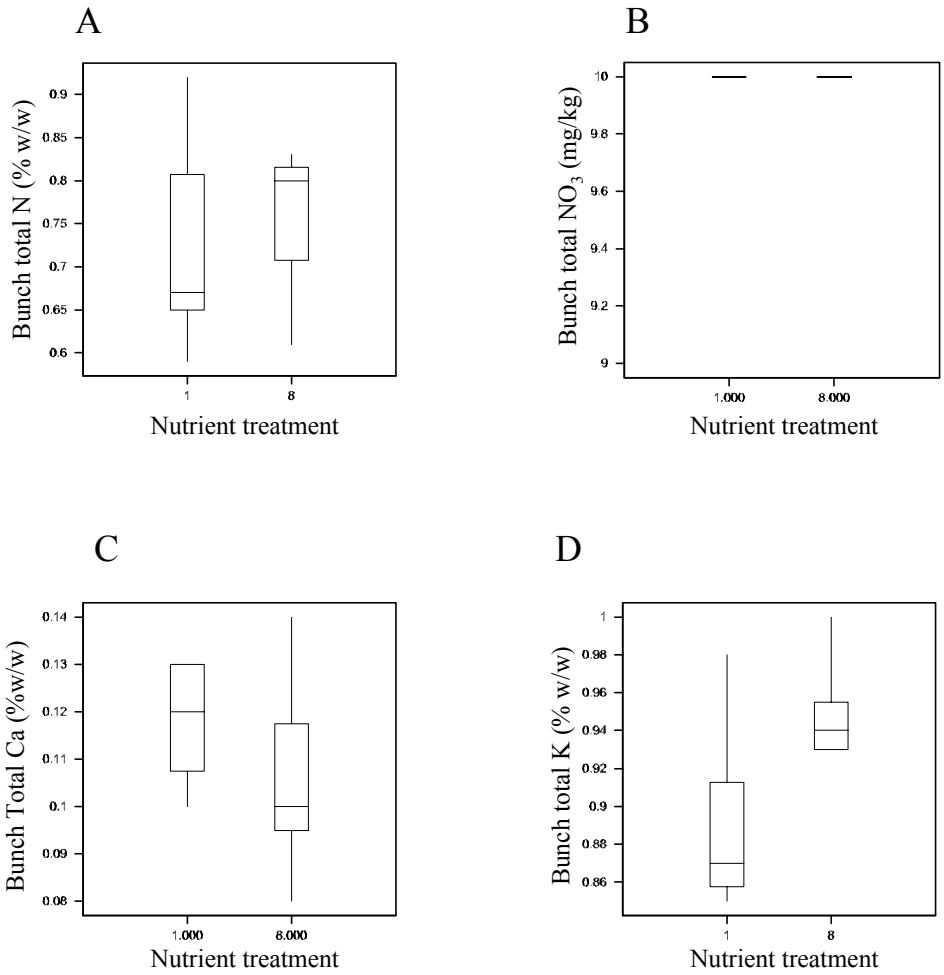


Figure 5 The effect of mineral nutrition on grapevine nutrient status of the grape bunch at Fernhill 2002/03. Average of one bunch per treatment for five replicates. A) total N, B) total NO_3, C) total Ca and D) total K. 1- control no applied nutrition and 8 – applied nitrogen, potassium and calcium. Samples collected at commercial harvest.

Season 2003/04

The experiment was on 6 year old Chardonnay vines at "Meroo" vineyard.

Disease Assessment

Botrytis bunch rot at harvest was prevalent in the trial site due to rainfall for three consecutive days leading up to harvest (Figure 6) but only two tagged bunches showed sporulating *B. cinerea* prior to moist incubation. At the trial site natural levels of infection were high and artificial inoculation of bunches at flowering did not significantly increase disease expression observed on moist incubated bunches after harvest ($P>0.05$) (Figure 7).

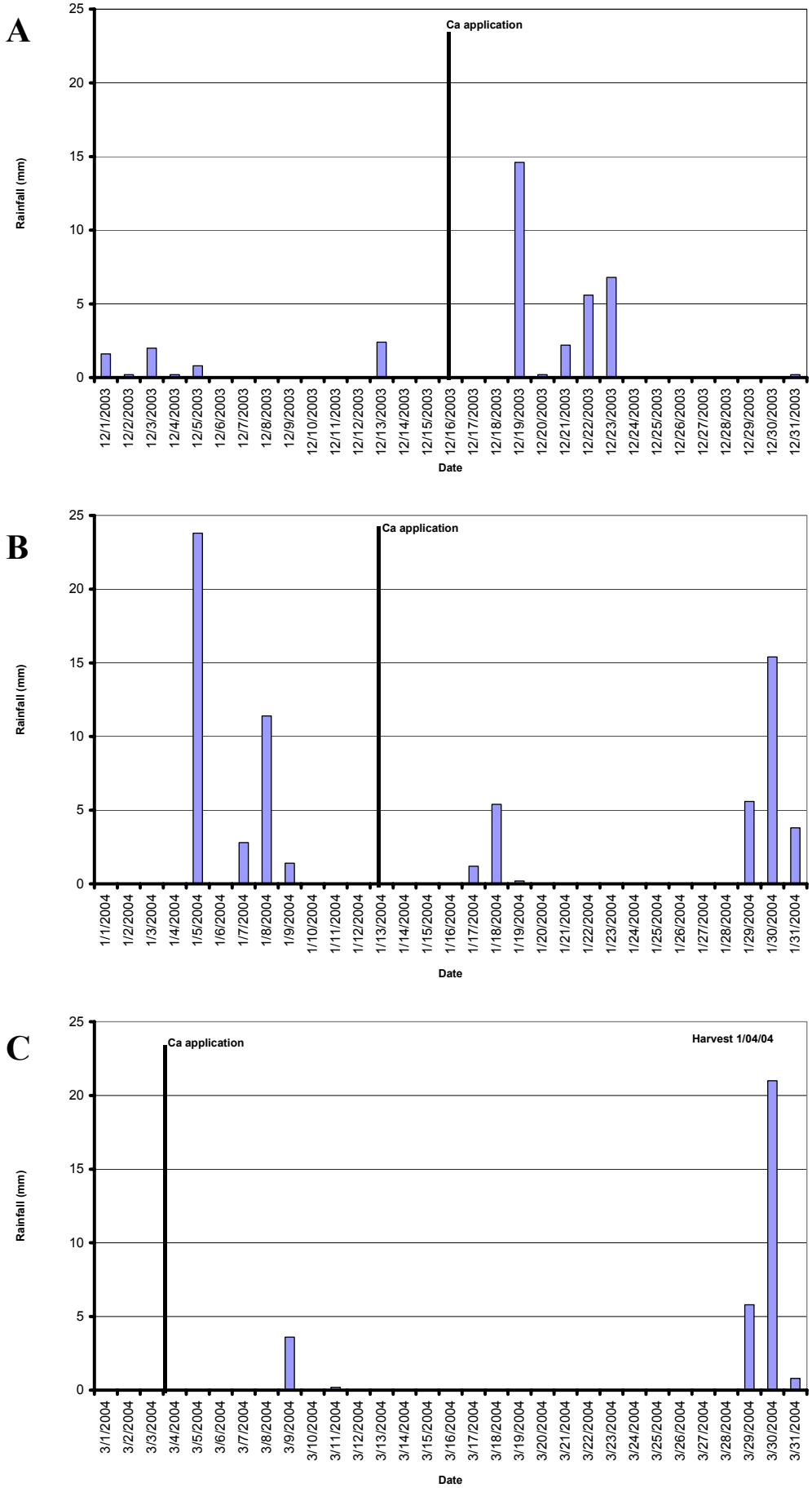


Figure 6 Rainfall during season 2003/2004. A) December, B) January and C) March.

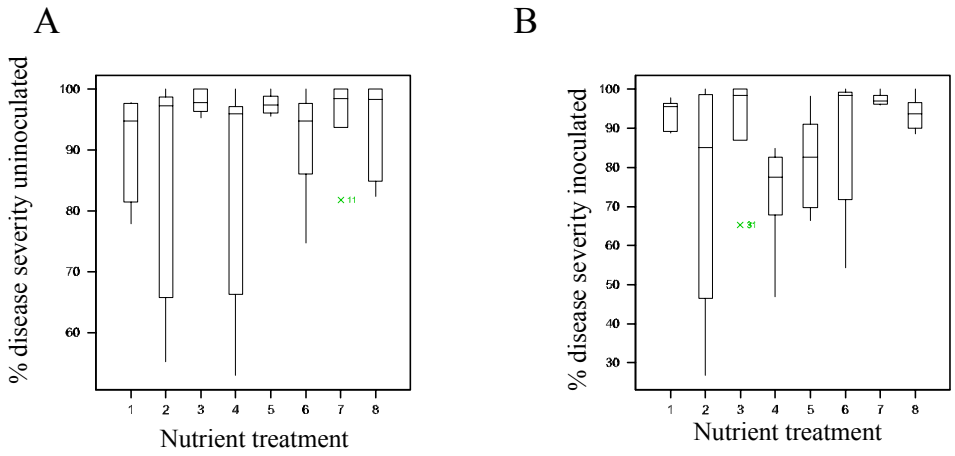


Figure 7 The effect of mineral nutrition on the percent botrytis bunch rot severity of A) naturally infected and B) artificially inoculated bunches from Meroo at harvest following moist incubation at 2003/04. 1 – no nutrient applied, 2 – Ca only, 3 – K only, 4 – Ca and K, 5 – N only, 6 – N and Ca, 7 – N and K and 8 – N, K, Ca.

Vine growth and canopy density

After one application of soil applied nitrogen and potassium the shoot growth rate, measured over five weeks up to flowering was unaffected by the treatments except for N and K which was greater (Figure 8). At flowering, the light penetration into the bunch zone was reduced in all the treatments except Ca compared to the control (Figure 9). At veraison, all treatments resulted in less light penetration than the control. The higher the lux indexes the greater the light penetration into the bunch zone.

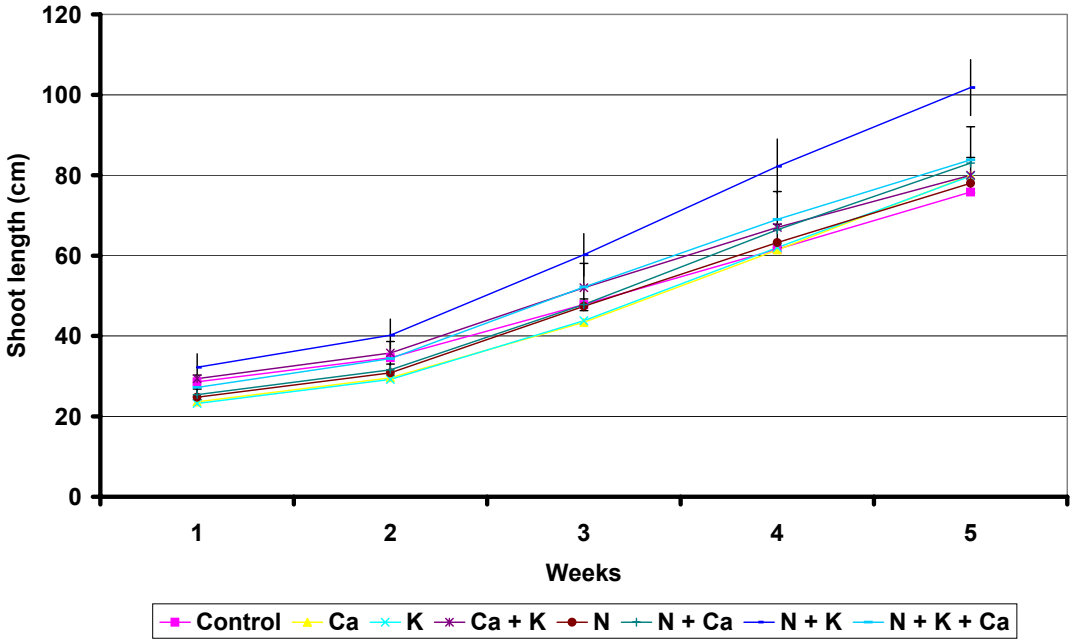


Figure 8 The effect of mineral nutrition on the actual growth rate of Chardonnay over 5 weeks up to flowering at Meroo. Data represent mean of one shoot per treatment of 5 replicates. Bar represents SE.

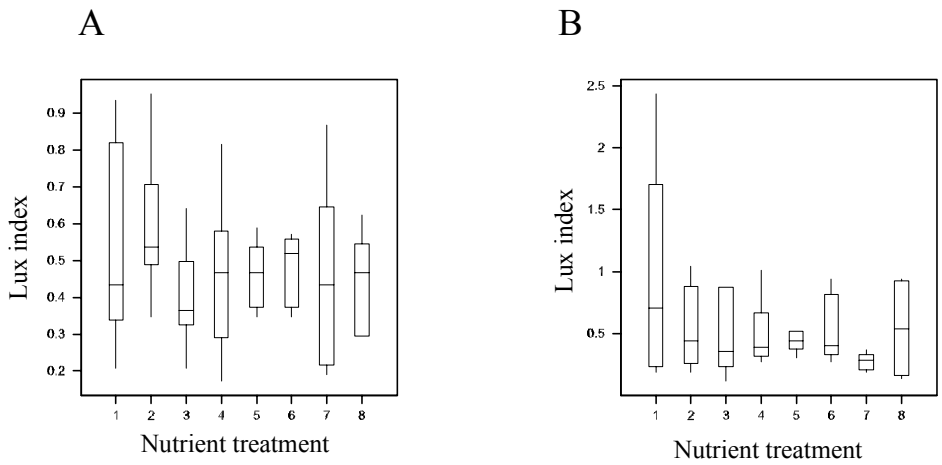


Figure 9 The effect of mineral nutrition on canopy density at A) flowering and B) veraison measured with light penetration index. 1 – no nutrient applied, 2 – Ca only, 3 – K only, 4 – Ca and K, 5 – N only, 6 – N and Ca, 7 – N and K and 8 – N, K, Ca.

Bunch compactness

The Ca and K treatment produced significantly less compact bunches with loosely packed berries than the other treatments ($P<0.05$) (Figure 10). This treatment also produced the smallest bunches with the average bunch weight of 124.4 g (Table 6).

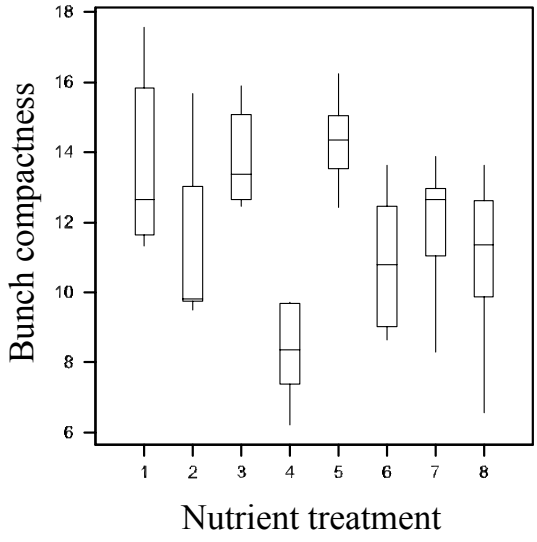


Figure 10 The effect of mineral nutrition on bunch compactness at harvest. The larger the number the tighter the bunch (greater compactness). 1 – no nutrient applied, 2 – Ca only, 3 – K only, 4 – Ca and K, 5 – N only, 6 – N and Ca, 7 – N and K and 8 – N, K, Ca.

Fruit yield and quality parameters

The Ca and N treatment produced a smaller yield in comparison with the other treatments (Table 6). The Ca and N treatment produced a lower bunch number per vine than the other treatments. The Ca and K treatment produced bunches with a lower bunch weight while the other treatments were similar to the control. The mineral nutrition did not effect the total soluble solutes (TSS) of the grape juice. The TA of the K and Ca treatments when applied alone increased but the K and Ca applied together decreased the TA of the juice. The K and Ca only treatments did not affect the pH of the juice but when applied together the juice pH was increased although not significantly.

Table 6 Effect of mineral nutrition on grape quality parameters at Meroo 2003/04.

Treatment	Yield (g) [a]	Bunch number [a]	Average Bunch weight (g) [b]	TSS [b] °Brix	pH [b]	Total acidity [c]
1 Control	7.49	52.3	143.6	20.56	3.73	6.77
2 Ca	8.77	63.2	142.2	21.45	3.725	7.77
3 K	7.98	57.6	137.8	21.43	3.765	8.415
4 Ca K	7.965	64	124.4	20.99	3.895	6.66
5 N	8.305	59.5	141.6	21.55	3.67	7.065
6 N Ca	6.855	48.5	141.4	20.75	3.805	6.795
7 N K	7.95	53.3	153.8	21.31	3.745	7.275
8 N K Ca	8.03	59.6	134.8	21.36	3.815	6.86
Significance	ns	ns	ns	ns	ns	ns

[a] Mean for two vines per treatment for 5 replicates
[b] Average of 1 bunch per treatment for 5 replicates
[c] Mean of 1 bunch per treatment for 5 replicates measured twice

Soil nutrient analysis

The four soil applications of nitrogen to vines did not significantly increase the total N present (0.22) compared to the control (0.22). The four soil applications of potassium did not significantly increase the total N present (0.48) compared to the control (0.56).

Plant tissue nutrient analysis

After one application of nitrogen up to flowering when petioles were collected, the petiole total nitrogen was greater in the untreated control than the treated vines (Figure 11). Three applications of nitrogen to the soil did not significantly increase the level of total nitrogen in the bunches at harvest compared to the no nitrogen control treatment (Figure 12). One application of potassium up to flowering when petioles were collected did not significantly increase the level of potassium in the petiole tissue compared with the control. Three applications of potassium to the soil did not significantly increase the level of potassium in the bunches at harvest compared to the no potassium control treatment.

In general, the NO_3 levels in petioles taken at flowering were excessive (>1200 mg/ kg) according to Reuter and Robinson (1986). The calcium levels were deficient (<1.2 %w/w) and the potassium levels were adequate (>1.5 %w/w).

The calcium was applied after flowering and so the petiole levels of calcium at flowering were similar between the non-treated and calcium treated vines. Three applications of calcium to bunches did not significantly increase the level of calcium detected in the bunch tissue compared to the no calcium control treatment.

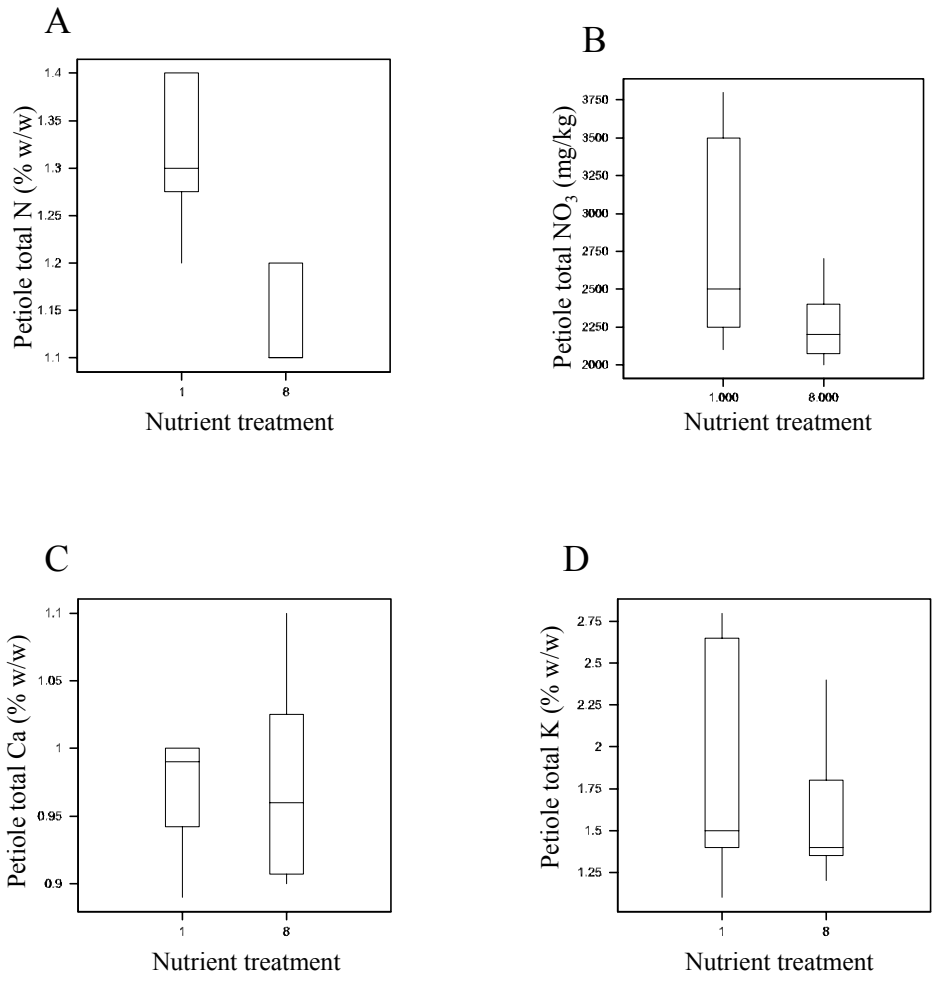


Figure 11 The effect of mineral nutrition on grapevine nutrient status of the petiole at Meroo 2003/04. A) total N, B) total NO_3, C) total Ca and D) total K. 1- control no applied nutrition and 8 – applied nitrogen, potassium and calcium. Samples collected at flowering.

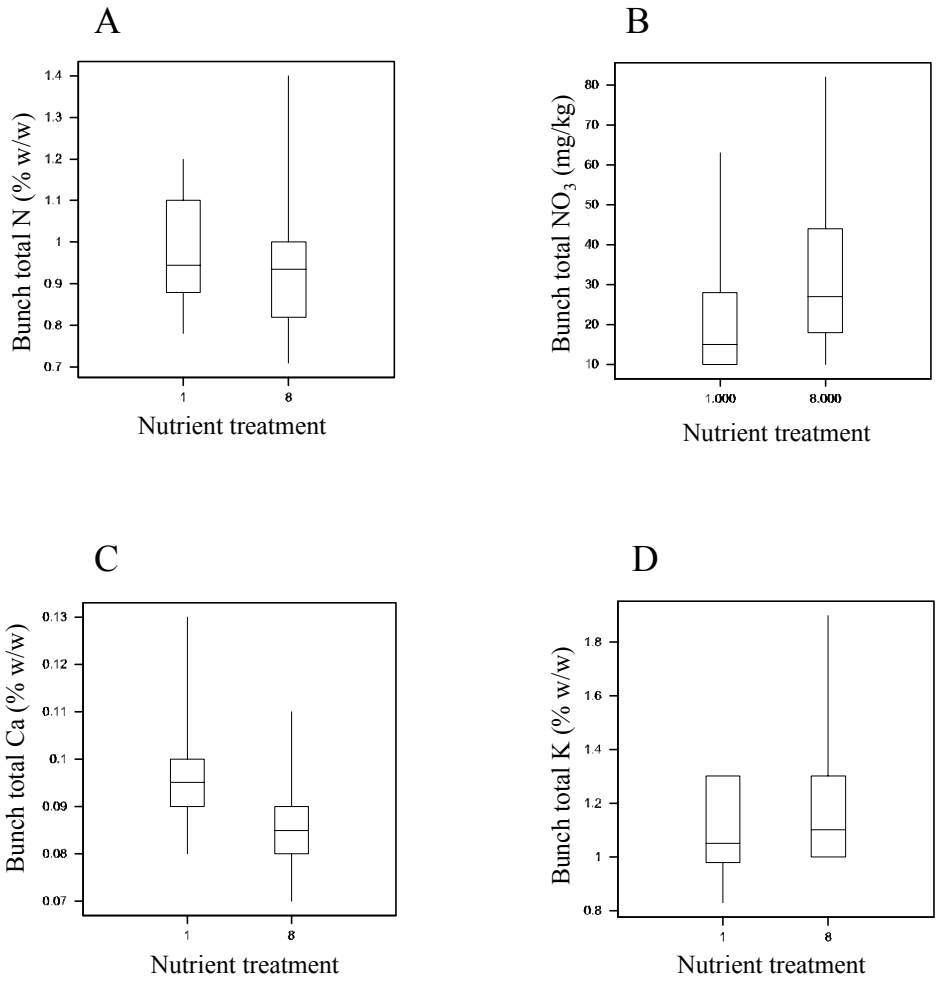


Figure 12 The effect of mineral nutrition on grapevine nutrient status of the grape bunch at Meroo 2003/04. Average of one bunch from two vines per treatment for five replicates. A) total N, B) total NO_3, C) total Ca and D) total K. 1- control no applied nutrition and 8 – applied nitrogen, potassium and calcium. Samples collected at commercial harvest

Glasshouse trial

First year

The experiment consisted of 24 nutrient treatments containing various levels of nitrogen, potassium and calcium were applied to 1 year old potted canes of Chardonnay and Cabernet Sauvignon in the glasshouse.

Botrytis assessment

There was a significant ($P<0.05$) difference between varieties, with Cabernet Sauvignon overall having more leaf area infected by *B. cinerea*. The nutrient treatments had no significant effect on disease severity seen in the leaf bioassay of potted Chardonnay and Cabernet Sauvignon (Figure 13).

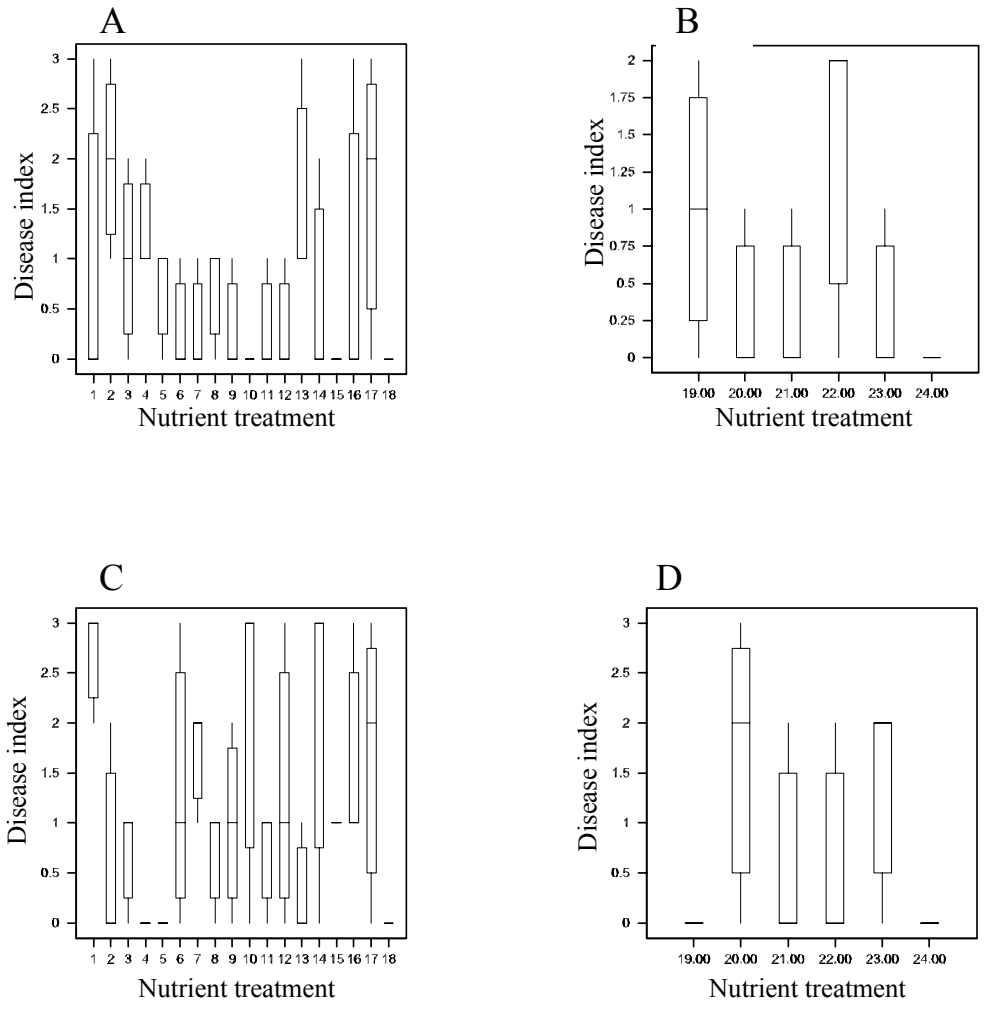


Figure 13 Effect of mineral nutrition on percentage botrytis severity of potted A) Chardonnay vines N x Ca, B) Chardonnay vines K x Ca, C) Cabernet Sauvignon N x Ca, D) Cabernet Sauvignon K x Ca (2002/03).

Vine growth parameters

Both variety and nutrient regime had a significant effect on the number of nodes on cane prunings produced by potted vines in the first year (P<0.001) (Figure 14). In Chardonnay, the node number per vine increased with increasing nitrogen concentration. N2Ca2 produced the highest number of nodes (24) while treatment with N0.125Ca2 resulted in an average of only 1.7 nodes per vine prunings (Figure 14). The Chardonnay vines produced significantly more nodes than CS (P<0.001).

Total cane length (cm) of prunings was significantly (*P*<0.001) affected by the nutrient treatments and increased with increasing N (Figure 15). Variety also had an effect on cane length with Chardonnay producing significantly longer canes than CS. However, there was not a significant interaction between variety and nutrient solution.

Nutrient treatments had a significant effect on cane pruning weight with weight increasing in response to additional N in both Chardonnay and Cabernet Sauvignon (Figure 16). However, for cane pruning weight there was no significant effect between varieties, or interaction between nutrient treatment and variety (*P*>0.05).

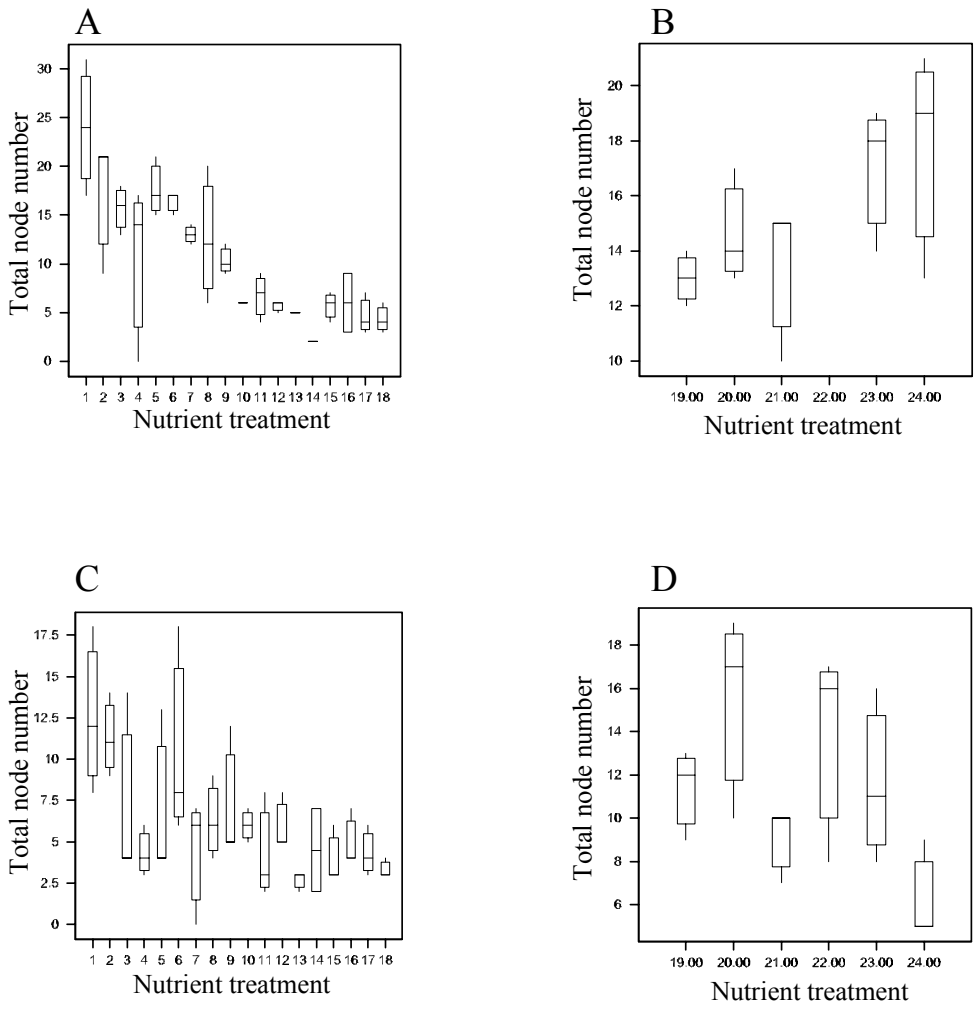


Figure 14 The effect of mineral nutrition on total node number of A) Chardonnay vines N x Ca, B) Chardonnay vines K x Ca, C) Cabernet Sauvignon N x Ca, D) Cabernet Sauvignon K x Ca (2002/03).

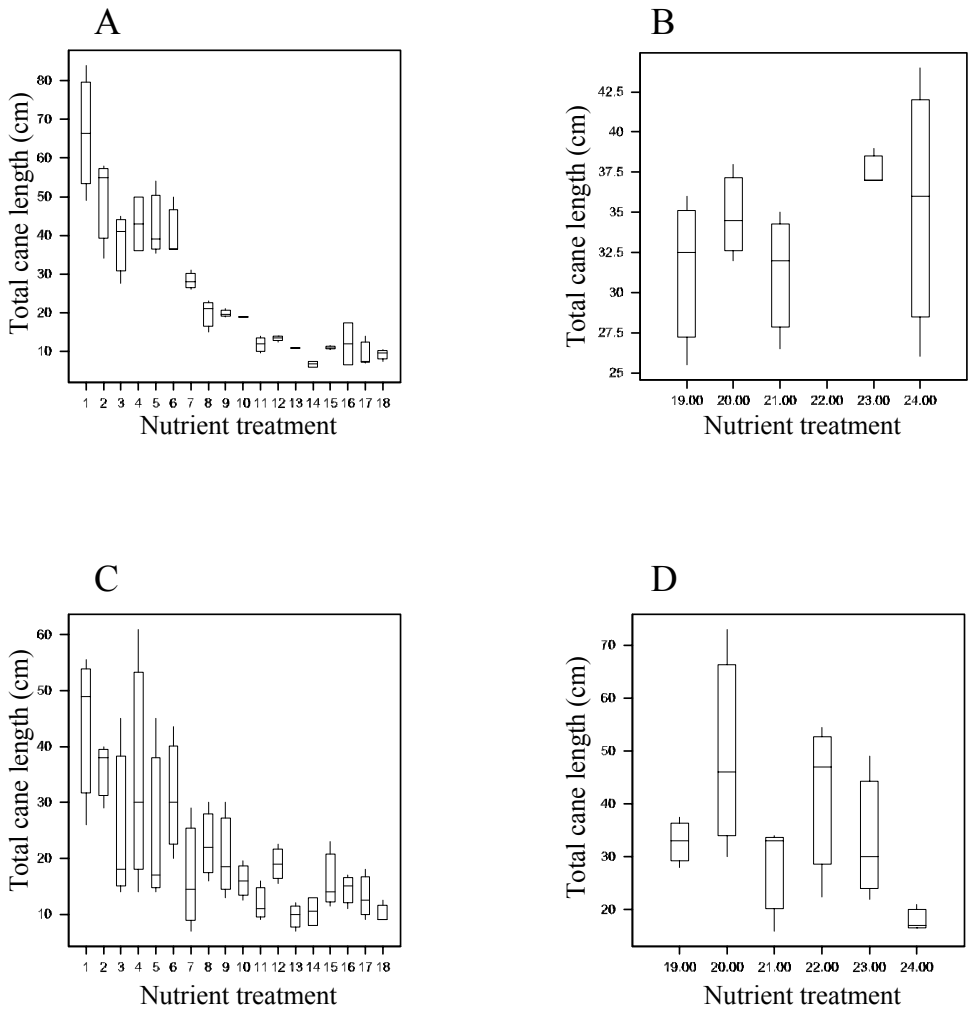


Figure 15 The effect of mineral nutrition on total cane length (cm) of A) Chardonnay vines N x Ca, B) Chardonnay vines K x Ca, C) Cabernet Sauvignon N x Ca, D) Cabernet Sauvignon K x Ca (2002/03).

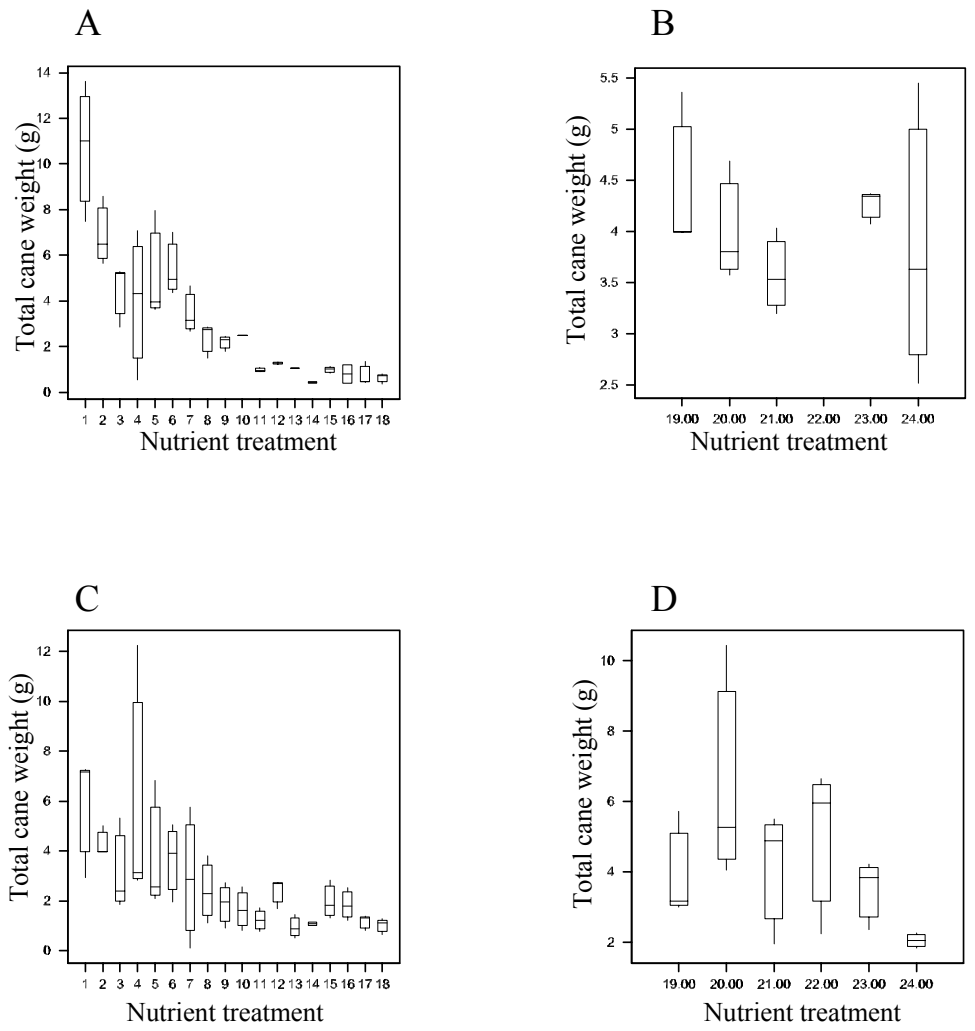


Figure 16 The effect of mineral nutrition on total pruning weight (g) of A) Chardonnay vines N x Ca, B) Chardonnay vines K x Ca, C) Cabernet Sauvignon N x Ca, D) Cabernet Sauvignon K x Ca (2002/03).

Second year

The experiment consisted of 18 nutrient treatments with various levels of nitrogen, potassium and calcium applied to potted Chardonnay and Cabernet Sauvignon vines for two seasons.

Botrytis assessment

In the second season, there was no significant difference between the disease severity of the varieties and the data was pooled (Figure 17). High calcium nutrition reduced the severity of botrytis leaf blight on potted vines (Figure 18, Figure 19). The severity of botrytis leaf blight on potted vines tended to be increased by a N:K ratio of 1:2.

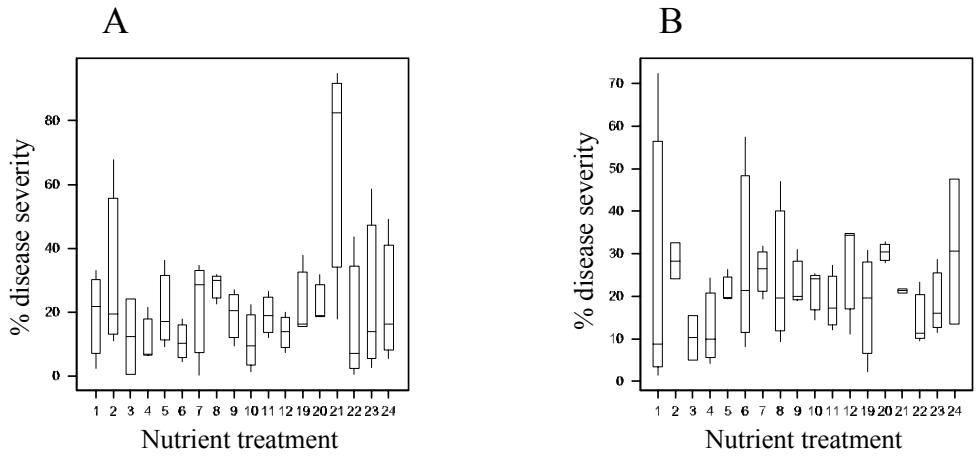


Figure 17 The effect of mineral nutrition on percentage disease severity of potted A) Chardonnay and B) Cabernet Sauvignon vines (2003/04).

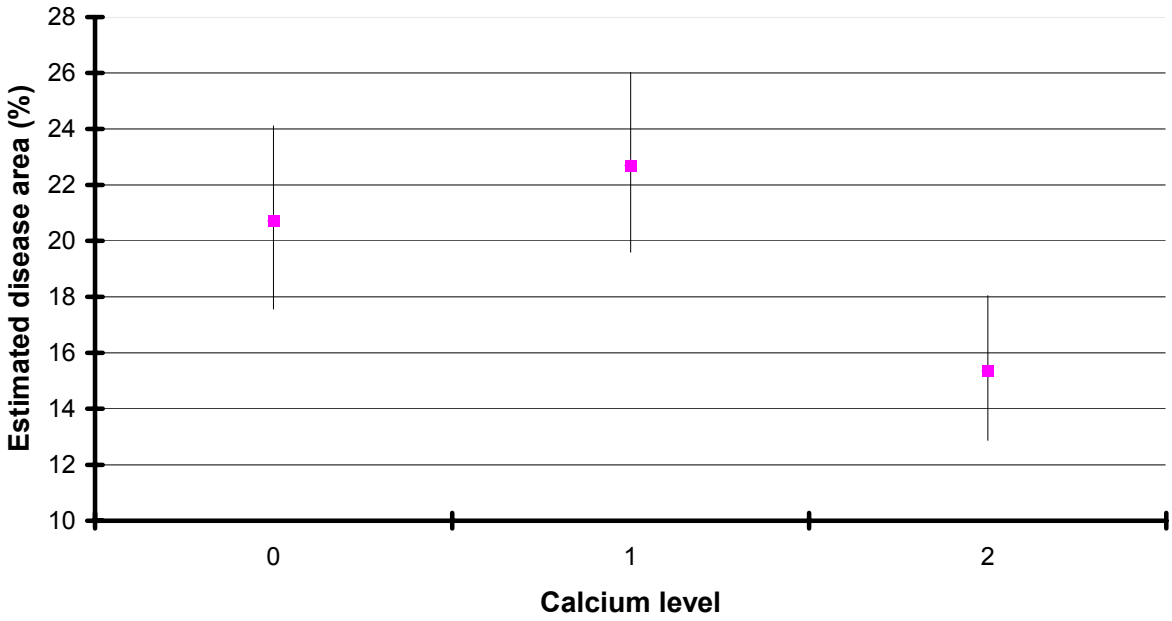


Figure 18 Effect of calcium nutrition on botrytis leaf blight severity of potted vines. In cases where tails do not overlap treatments are significantly different at P<0.05.

A



B



C



Figure 19 Effect of calcium nutrition on botrytis leaf blight severity of Chardonnay potted vines.

A) calcium 0 B) calcium 1 and C) calcium 2.

Plant nutrient analysis

There was a significant difference between the varieties with Cabernet Sauvignon having greater nitrogen content in leaves than Chardonnay ($P<0.001$). There was also a significant difference between the nutrient treatments ($P<0.001$). In Chardonnay potted vines, the addition of N2 increased the level of total nitrogen in leaf blades at harvest compared with N1 and N0.25 (Figure 20). The addition of N2 and N1 to Cabernet Sauvignon vines gave similar levels of total N in leaf blades at harvest (Figure 21), while the addition of N0.25 significantly reduced the total N in the leaf blades compared with N2 and N1 ($P<0.01$).

There was a significant difference between the varieties with Cabernet Sauvignon vines having greater levels of calcium in leaf blades than Chardonnay ($P<0.02$). The addition of calcium significantly increased the level of calcium present in the leaf blades of both Chardonnay and Cabernet Sauvignon potted vines ($P<0.001$). The addition of various levels of potassium did not significantly effect the levels of total K in the leaf blades.

There was a significant difference between potassium treatments ($P<0.02$) but the variety was only significant when assessing specific treatments. The addition of K1 and K2 significantly increased the levels of total K in leaf blades of Cabernet Sauvignon potted vines at harvest compared with K0.0125 ($P<0.01$).

In both Chardonnay and Cabernet Sauvignon potted vines assessed for nutrient levels in leaf blades all vines were deficient in nitrogen and calcium, however, potassium was optimal to excess according to Gartel (1994).

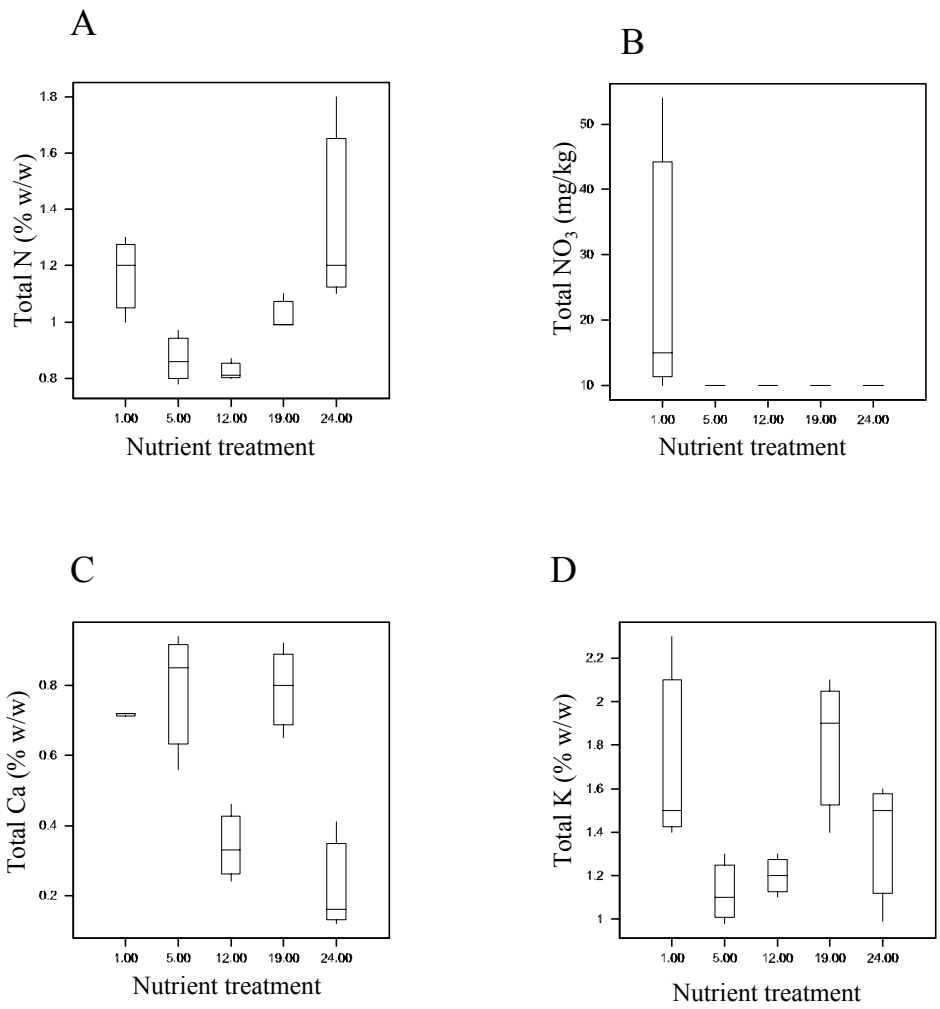


Figure 20 The effect of mineral nutrition on grapevine nutrient status of leaves from potted Chardonnay vines. A) total N, B) total NO_3, C) total Ca and D) total K. 1 – N2 K1 Ca2, 5 – N1 K1 Ca1, 12 – N0.25 K1 Ca0, 19 – N1 K2 Ca2, and 24 – N1 K0.125 Ca0. Samples taken at harvest.

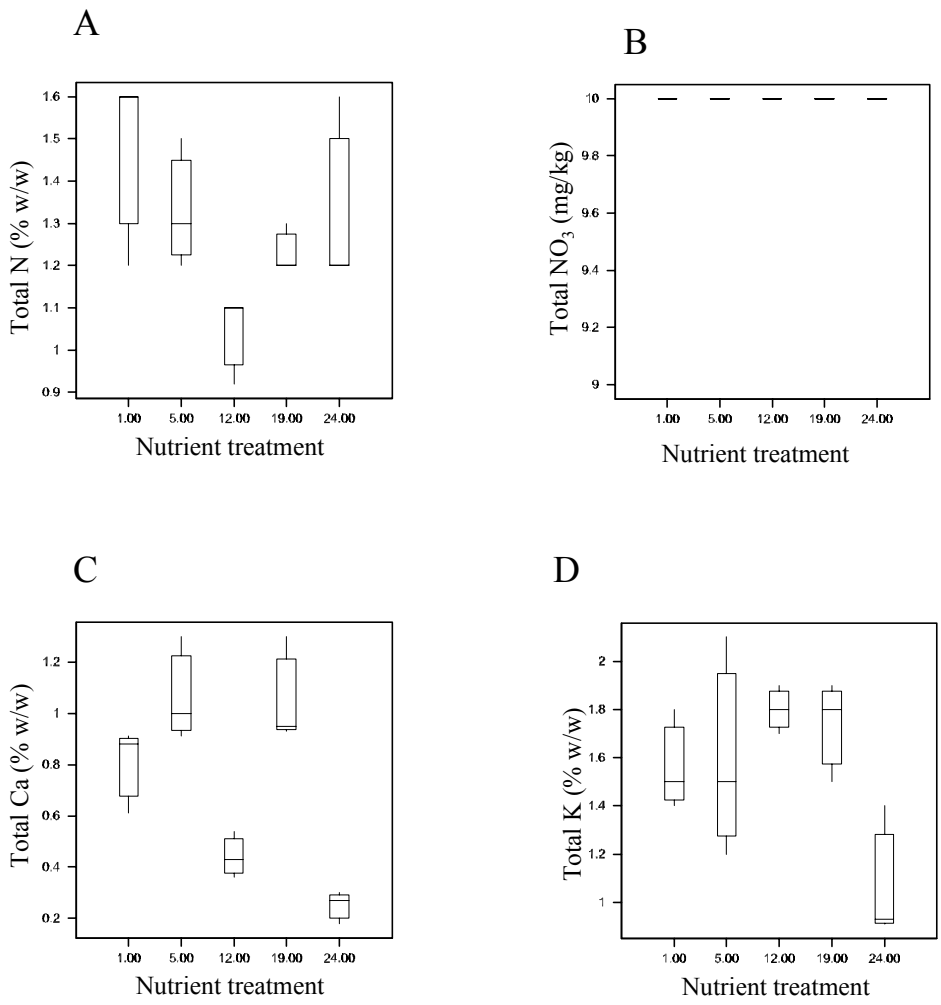


Figure 21 The effect of mineral nutrition on grapevine nutrient status of leaves from potted Cabernet Sauvignon vines. A) total N, B) total NO_3, C) total Ca and D) total K. 1 – N2 K1 Ca2, 5 – N1 K1 Ca1, 12 – N0.25 K1 Ca0, 19 – N1 K2 Ca2, and 24 – N1 K0.125 Ca0. Samples taken at harvest.

Discussion

The results from two years of field trials conducted at two different sites are reported here. The original intent was to conduct nutrient trials at the same site (Fernhill) over three consecutive years, which was considered to be particularly important when looking at effects of nutrients applied to the soil. It is known that several seasons may be needed to alter soil nutrient levels. However, at the time the site was chosen it was not known that the vines would be reworked, rendering the site unsuitable for continuation of the trial. The second site (Meroo) was selected because of its history of susceptibility to *B. cinerea* infection and, more importantly, expression at harvest (ie the site has a high botrytis risk). The soil nitrogen level at Meroo was considered to be on the high side of adequate and therefore it was unlikely that the control plot would represent a low nitrogen treatment. The levels of soil nitrogen at Fernhill were considered deficient. Interestingly, this study showed that a single season of applications of additional nitrogen did not significantly increase the level of total N or total NO_3 detected in the soil, petioles or bunches at harvest at either of the site.

Soil nitrogen is not a good indicator of nitrogen concentration in tissues, as uptake by the vine is dependent on many factors (eg amount of organic matter present). Measurements from petiole tissues are usually considered a better measure of the nitrogen nutrient status of grapevines.

Weather conditions at harvest (2002/003) were not conducive to disease expression in the field at Fernhill. Field expression was observed at Meroo during harvest in 2003-2004, but not on experimental bunches. If there was field expression, then the weather must have been conducive, and weather data collected from the site showed that there had been rainfall in the three days leading up to harvest. It is not clearly understood why the experimental bunches did not develop bunch rot under these conditions, although it is known that berry damage is an important factor.

Surface sterilisation and moist incubation of bunches collected from the field at harvest was used to determine the levels of latent infection present to provide an indication of the potential level of field expression if all conditions at harvest had been favourable. In the light of results from other research (Cole et al, 2004), 10 days of incubation does not give an indication of field expression so this in future research needs to be reconsidered with 72 hours of incubation being the maximum to mimic field expression because fruit would not be harvested after being exposed to that period of rain. Therefore, future projects should incorporate techniques that encourage field expression independently of natural weather events so the effects of treatments on field expression can be determined.

In 2002/03 at Fernhill, artificial inoculation at 80% capfall significantly increased the potential severity of disease at harvest, as measured by moist incubation in the laboratory. Disease levels in uninoculated bunches were too low for comparisons between treatments to be made. However, the application of nitrogen, potassium and calcium did not significantly alter the severity of botrytis bunch rot at harvest on the artificially infected bunches.

In 2003/04 at Meroo, there was no difference in the botrytis bunch rot severity between the naturally infected bunches and the artificially infected bunches. Since inoculum was applied to inflorescences at flowering, there was sufficient natural inoculum in the vineyard to have the same affect on severity as the application of 10^4 spores per mL of artificial inoculum. Inoculation studies using artificial inoculations (Warren et al 2000) indicate that bunch rot development is not only influenced by weather conditions but also by the number of *B. cinerea* spores in bunches.

As reported for Fernhill, the application of nitrogen, potassium and calcium at Meroo did not significantly alter the severity of botrytis bunch rot at harvest on either the naturally infected bunches or the artificially infected bunches. However, nutritional studies in other perennial horticultural crops have found that several years of nutrient application is required to enhance the mineral nutrition of the plant and reduce disease severity.

Due to budget constraints, nutrient analysis was performed only on tissues harvested from the control and treatment 8 (N, Ca, and K). The rationale for this was that N, Ca and K levels in treatment 8 would provide an estimate of levels of each of the nutrients in the respective treatments. This may not be a true reflection, however, as K and Ca are both cations and may compete for uptake by vine tissues. Research in other fruit crops such as apples and cherries shows that when K levels in tissues are high, uptake of Ca can be restricted.

Nutrient applications at Fernhill had no effect on levels of nitrogen and calcium but significantly increased potassium in the grapevine petiole and bunch tissue at harvest. *Botrytis cinerea* requires wounding to infect tissues and since potassium is thought to accelerate wound repair in tissues (Kiraly 1976), higher levels of potassium in the vine tissues may reduce susceptibility to *B. cinerea* infection and expression. In this present study, however, no decrease in the potential severity of botrytis bunch rot (as measured by moist incubation)

following applications of potassium was observed. It is noteworthy that the levels of potassium taken up by the vines, as determined by petiole analysis, could be considered as deficient (<1 %w/w), according to Reuter and Robinson (1986). Although the nutrient requirements information is not readily available for bunch tissue perhaps the potassium levels in bunches were also deficient and a threshold level of potassium is required before significant effects on disease severity can be observed. In Hungarian grapevine trials, K fertilisation reduced the susceptibility of vines to infection by botrytis (Kiraly 1976).

Because many factors may affect disease expression, with weather being one of the most important factors, direct comparisons cannot be made across sites and seasons. However, the difference in nutrient status between Fernhill and Meroo provide some opportunity to compare low nitrogen site with one considered having high nitrogen. At Meroo the soil nitrogen levels were considered to be high and this was reflected in the levels of petiole nitrogen. Managers of vineyards recording high petiole nitrogen would refrain from applying further nitrogen to the soil until natural depletion had occurred. How long this would take is not known and would probably vary greatly from site to site. Therefore, manipulation of calcium and potassium may be more promising as a shorter term botrytis bunch rot management strategy.

A PhD project, currently in its second year, will further investigate the relationship between soil nitrogen and susceptibility to botrytis bunch rot. If a relationship between high nitrogen and increased susceptibility is confirmed then practical management strategies need to be developed. As it may not be practical in the short term to reduce levels of nitrogen in the soil, alternative shorter-term measures are required.

Nutrient applications at Meroo did not significantly increase the levels of nitrogen, potassium or calcium in the grapevine petiole at flowering or the bunch tissue at harvest. These vines were very high in nitrogen, low in calcium and adequate for potassium (according to Reuter and Robinson 1986), and very susceptible to *B. cinerea* infection and expression with a potential disease severity of 90% infected berries infected (as determined by moist incubation of bunches in the laboratory).

Grape bunches naturally contain very low levels of calcium (Conradie 1981). Application of calcium by spraying directly onto bunches would therefore appear to be the most efficient way of increasing the calcium content of bunch cells. However, application of three calcium spray treatments (at flowering, pre-bunch closure and veraison) did not result in significantly less potential botrytis expression at harvest as determined by moist incubation in the laboratory at either site. In addition, the amount of calcium detected in berry tissues was not significantly increased by the calcium treatments at either site. Petiole calcium levels were determined to be adequate at Fernhill and low at Meroo, suggesting that little, if any, of the applied Ca was taken up by the vine cells. Further more, it also appeared that the applied calcium was removed from bunch and leaf surfaces. In these trials, calcium was applied as $CaCl_2$ or $CaNO_4$, dissolved in water and applied by hand-held sprayer until runoff. At Meroo, 5 mm rainfall was recorded 4 days after each of the three sprays was applied (at flowering, pre-bunch closure and veraison) (Figure 6), so it is possible that the applied calcium was washed off before it could be incorporated into berry cells. Manufacturers of commercially available calcium products claim that their product is chelated and therefore taken up more quickly by plant cells. Such formulations may also contain stickers for improved rainfastness. In wine grapes, Wuxal® calcium applied four times from fruit set to veraison increased the calcium content of berries and reduced the incidence and severity of bunch rot (Winter and Nicol 1998). Trials should be undertaken with commercially available products to determined calcium uptake and efficacy and bunch compactness.

At Meroo, the Ca K treated vines had significantly less compact bunches and the average bunch weight was less than the other treatments, but the yield was not significantly different because the bunch number was higher at harvest than all other treatments. Overall, a trend towards less compact bunches was observed when calcium treatments were applied. Less compact bunches are considered a highly desirable characteristic for reducing bunch susceptibility to Botrytis and is recommend by best practice (Cole, Whiting and Braybrook, 2004). Open bunches allow better airflow through bunches and faster drying times so that conditions within the bunch are less conducive to disease development. In addition, berries in more open bunches are less damage-prone. (Ellison, Ash *et al.* 1998)) demonstrated a good correlation between bunch tightness and susceptibility ratings. Berries of tight clustered cultivars were more susceptible to bunch rot due to more berry-to-berry contact sites, where the cuticle is poorly developed (Marois, et al, 1986), and a higher proportion of internal berries, which are prone to splitting (Vail and Marois, 1991). The potential for manipulating calcium and potassium nutrition to produce less compact bunches warrants further research.

At Meroo, the application of nitrogen and potassium at 5 weeks after bud burst increased the shoot length prior to flowering. The level of light penetration into the canopy at flowering was not significantly different for the nutrient treatments. However, at veraison all the nutrient treatments had a more shaded canopy than the control treatment.

The applications of nitrogen, potassium and calcium had no adverse effects on grape quality parameters including pH, Brix, and TA, which fell within the specifications for winemaking, according to the vineyard manager.

When this project was developed, it was anticipated that it might be difficult to significantly alter the nutrient status of soil and grapevines under field conditions within only one or two seasons. Therefore, a glasshouse trial was also established at Knoxfield so that nutrient levels could be more easily manipulated. In the first year of the glasshouse trial, there was a significant variety by nutrition interaction apparent in the levels of botrytis leaf blight severity observed. Overall, Cabernet Sauvignon leaves were more susceptible than Chardonnay leaves, although the situation is usually reversed with Chardonnay considered to be more susceptible than Cabernet Sauvignon in the field situation.

In the second year of the glasshouse trial, there was no significant difference in the disease severity between the varieties and the data was pooled for analysis. Two nutrient treatments were observed to have a significant effect on the severity of leaf blight on potted vines in this trial; the addition of calcium significantly reduced the severity of botrytis leaf blight, while the severity tended ($P<0.1$) to be increased when the N:K ratio was 1:2.

Nutrient analysis showed that the addition of calcium significantly increased the level of calcium in the leaf tissues. The highest amount of calcium gave the least disease. This suggests that the reduction in botrytis leaf blight severity could be due to the accumulation of calcium in the leaf tissue. High Ca levels in plant tissues are thought to strengthen cell walls by crosslinking the middle lamella rendering cell walls less susceptible to degradation by pectolytic enzymes secreted by the fungi during infection (Kiraly 1976). Calcium in plant tissues may also have a direct detrimental effect on *B. cinerea* by thickening of the fungal cell wall, a consequence of the high osmotic pressure developed, which, in turn could retard hyphal extension and result in a loss of cell wall elasticity (Chardonnet, Sams *et al.* 1999).

A N:K ratio of 1:2 was shown in the second season glasshouse trial to increase the severity of botrytis leaf blight in both Cabernet Sauvignon and Chardonnay. This effect was not expected as increased levels of potassium are thought to increase disease resistance. However, analysis of petioles showed that N levels were deficient while K was optimal to excessive (Gartel, 1994). To our knowledge this effect has not been reported in grapevines (or other crops) and the reasons for this effect are still not understood. This finding does however, highlight the importance of considering nutrient ratios in addition to levels of individual nutrients alone.

In the glasshouse trial, cane length and weight increased in response to additional N in both varieties.
Chardonnay vines developed longer canes and more nodes with increasing nitrogen. Cabernet Sauvignon vines also had longer canes with increasing nitrogen but no increase in the number of nodes, suggesting elongation of the internodes. There may be the potential to decrease the canopy density of Cabernet Sauvignon by manipulation of nitrogen. The significance of this for field-grown vines needs to be tested.

Our results from the glasshouse trial indicated that manipulation of nutrients and their ratios can affect botrytis leaf blight severity, although it is unknown how this might translate to botrytis bunch rot severity.

There was some evidence from both the field and glasshouse trials that manipulation of calcium levels in particular may reduce susceptibility to *B. cinerea*. Higher levels of calcium resulted in reduced susceptibility of leaves to *B. cinerea* in the glasshouse and more open bunches in the field, warranting further investigation. Commercially available calcium products registered for use in *B. cinerea* control should be evaluated, including investigation of their effects on bunch compactness as well as on disease development and Ca accumulation in grapevine tissues.

The glasshouse trial indicated that altering nitrogen levels in Cabernet Sauvignon may influence canopy density, and this needs corroboration in the field. It appeared that nutrient effects may differ for different varieties, and this should be examined further. The use of different nutrient treatments for different varieties would not be difficult to manage since varieties are usually arranged in separate blocks in the vineyard. However, it is evident that future field trials undertaken to test the effects of the most promising treatments on disease expression cannot rely on natural weather events but require the stimulation of conducive conditions for disease development in the field, for example by overhead irrigation.

The results presented here, although preliminary, show promise that manipulation of vine nutrition has the potential to be used as part of an integrated disease management strategy to combat *B. cinerea* in Australian vineyards and reduce the reliance on chemical pesticides.

The comparison of organic and conventional vineyard practices on botrytis incidence.

Introduction

Organic wine grape growers report generally lower levels of botrytis bunch rot than neighbouring vineyards under traditional spray programs. As such, organic growers do not consider botrytis bunch rot to be a significant problem. Fruit grown under organic principles seems to be less susceptible to *B. cinerea* but these reports have not been adequately documented or scientifically validated.

Two types of long-term vineyard management regimes were compared, organic and conventional. The organically managed vineyard (OMV) chosen for this study was characterised by the use of cover crops and compost and no synthetic fertilisers or pesticides with the exception of sulphur and copper. A conventionally managed vineyard (CMV) as defined by the use of pesticides other than sulphur and copper and synthetic fertilisers was selected in the same region to best duplicate the climatic conditions of the OMV.

Methods and materials

Vineyard sites

Season 2003/04

The organic vineyard site at Balnarring consisted of 3 rows of 8 year-old spur pruned Chardonnay. The conventional vineyard site consisted of 20 rows of 10 year-old spur pruned Chardonnay. These sites were selected to investigate natural infection of *B. cinerea* (site history confident of high botrytis bunch rot). Artificial inoculation was not applied in keeping with the growers' wishes and previous experiences at the vineyards suggested that the availability of *B. cinerea* inoculum should not be a limiting factor. The vines at the conventional vineyard received a botryticide spray program; Scala at 80% capfall and Switch at pre-bunch closure.

Botrytis assessment

Vine inflorescences were collected at flowering and bunches at harvest. The samples were surface sterilised in 1% bleach and rinsed in sterile water then frozen at –20ºC overnight and moist incubated for 10 days at room temperature. The disease incidence (percentage of infected bunches) was determined on 25 bunches from the organic vineyard and 25 bunches from the conventional vineyard. It is recognised that the disease incidence measured is that of laboratory incubated bunches and does not always correlated with natural expression in the vienyard.

Vine growth parameters and canopy density

One shoot per vine was tagged for measurement of shoot length and bunch number per shoot on 25 vines. The tagged shoots were measured at approximately 10 cm shoot length (30/10/03), 8 leaves separated (6/11/03), 12 leaves separated (13/11/03), start of flowering (2/12/03) and 80% capfall (12/12/03). The growth rate was calculated based on shoot extension over time.

The canopy density was determined at 80% capfall and veraison in the bunch zone using a lux meter to determine the level of light penetration into the canopy. The percentage light penetration was based on the average of 3 readings at 25 panels and was proportioned against the average of the light intensity outside the rows prior to assessment and after assessment.

The lux index is

$$\frac{\text{lux in the bunch zone} \times 100}{\text{average lux outside the canopy}}$$

Higher lux index values indicate higher light penetration into the bunch zone. The amount of light that gets into the bunch zone may indicate the amount of air movement in the canopy and the likelihood of periods of high relative humidity in the bunch zone long enough to allow infection to occur.

Bunch compactness

At flowering and harvest, inflorescences and bunches, respectively, were collected and weighed and the bunch length (cm) recorded. A bunch compactness index was calculated as described previously. The larger the value, the tighter the bunch potentially leading to damage from splitting and increased probability of *B. cinerea* infection.

Results

The incidence of *B. cinerea* infection at flowering, following moist incubation, was the same at both the organic and the conventional vineyards with no infection being observed. There was no field expression on tagged bunches at either site. The incidence of botrytis bunch rot at harvest, following moist incubation in the laboratory, was similar for the organic and conventional vineyard (Figure 22) and quite low at 7-8% of bunches. The average relative growth rate was greater in the organic vineyard than the conventional vineyard (Figure 23). At flowering, the light penetration of the bunch zone was much greater in the organic vineyard than the conventional vineyard while at veraison, the light penetration of the organic vineyard was extremely high in comparison with the conventional vineyard (Figure 24). At flowering and at harvest the compactness of bunches was similar between organic and conventional site (Figure 25).

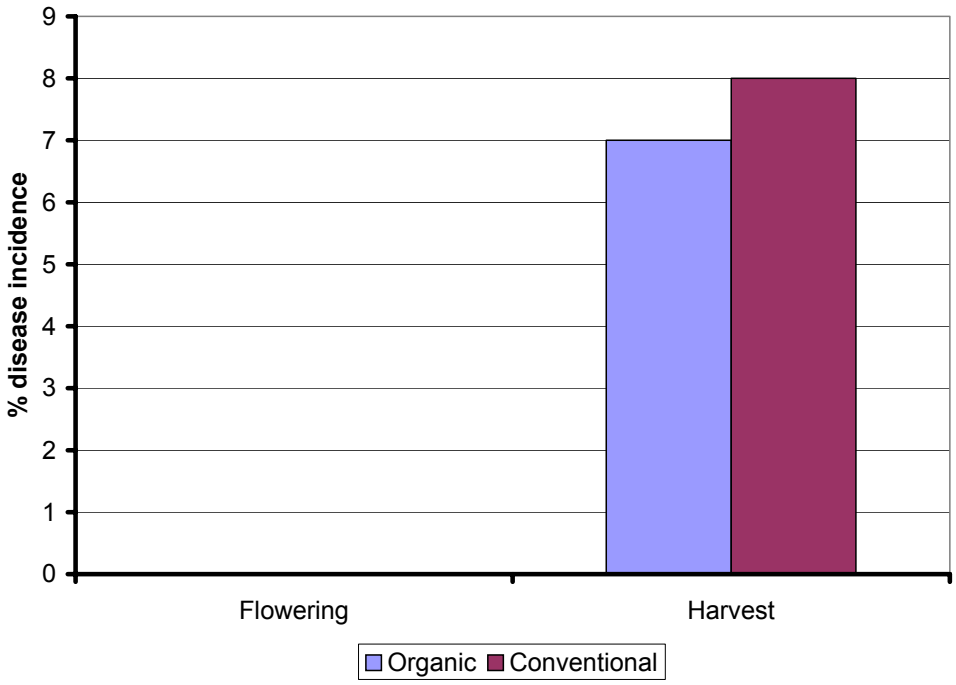


Figure 22 Percentage botrytis **bunch rot incidence in an organic and conventional vineyard in 2003/04 after surface sterilisation and moist incubation. Organic n=27, conventional n=25.**

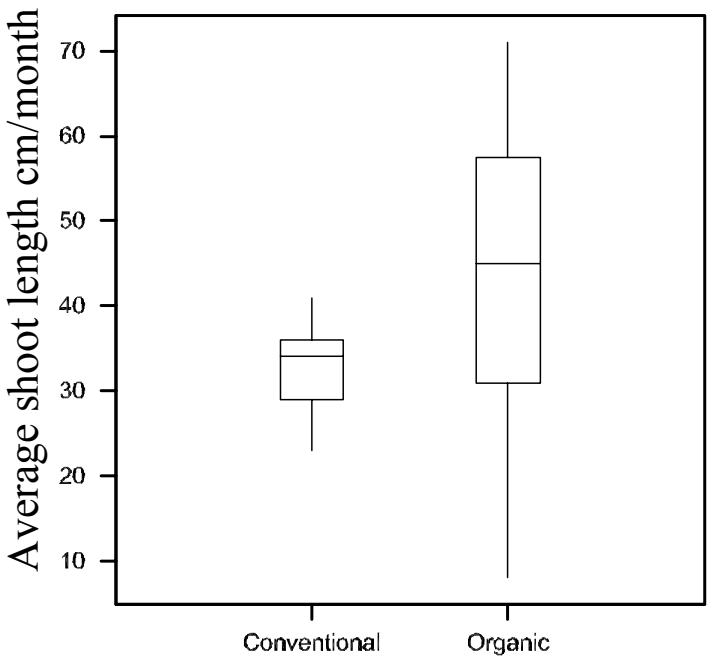


Figure 23 Shoot length growth over time in an organic and conventional vineyard for season 2003/04. Organic n=27, conventional n=25. Bars represent SE.

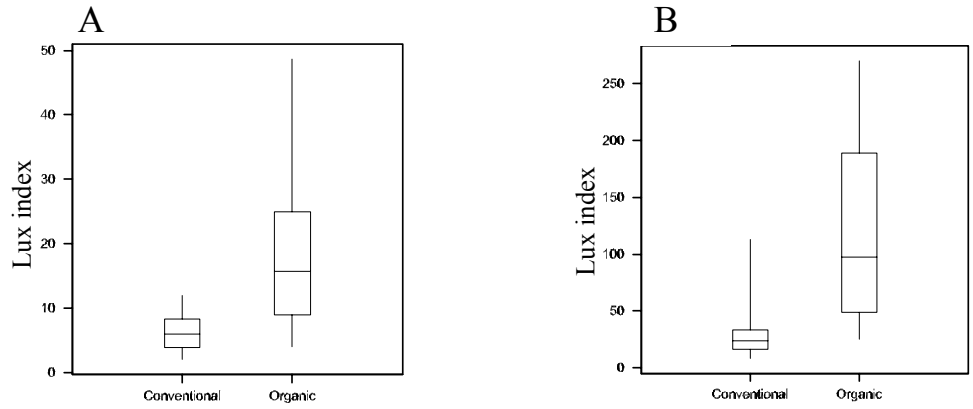


Figure 24 Light penetrations at A) flowering and B) veraison in an organic and conventional vineyard for season 2003/04. Organic n=27, conventional n=25.

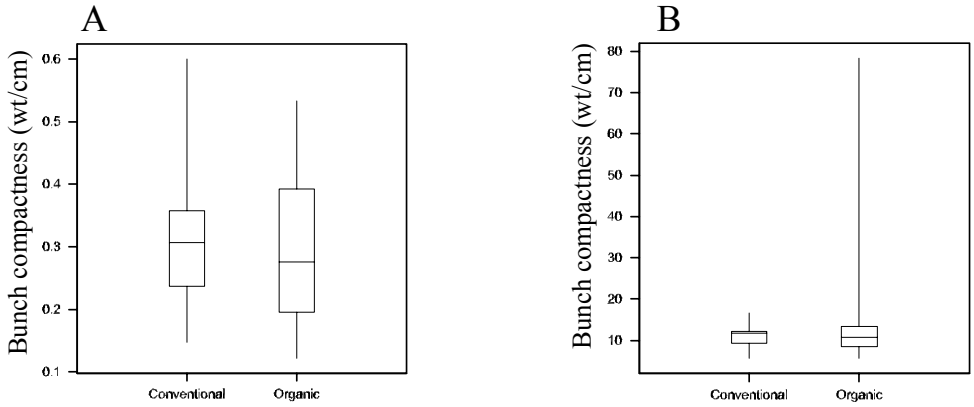


Figure 25 Bunch compactness at A) flowering and B) harvest in an organic and conventional vineyard for season 2003/04. Organic n=27, conventional n=25.

Discussion

Botrytis bunch rot is not considered to be a significant problem in organic viticulture (D. Bruer, pers. comm.). Organic growers generally report lower levels of botrytis bunch rot than neighbouring vineyards using conventional spray programs, suggesting that fruit grown under organic protocols is less susceptible to botrytis rot. Anecdotal evidence also suggests that infection is not the limiting factor, as incidence monitoring (ie moist incubation) is often the same between organic and non-organic systems. However, most of these reports are neither adequately documented nor scientifically validated. There is increasing evidence that solvent-based wetters recommended for chemical sprays may increase susceptibility to Botrytis infection. It is also suggested that the increased health of organic vines resulting from increased mulch and nutrition, protects the vines against infection by Botrytis and its later expression in conducive weather conditions. Characteristics of organic vineyards are low yields, pruning and vine management systems that result in smaller more open bunches and less inter-bunch congestion, the use of organic soil amendments and minimal to no pesticide use which encourage natural predators.

In season 2003/04, the weather conditions were not conducive for botrytis bunch rot expression at harvest at either vineyard. Even after moist incubation of bunches in laboratory to mimic a rain event, the disease incidences were relatively low (8 to 9% of bunches with sporulating *B. cinerea*) suggesting that weather during the season was not conducive for infection in the region. Disease incidence was similar for the organic and conventional vineyards, despite the conventional vineyard receiving spray applications of Scala at 80% capfall and Switch at pre-bunch closure for *B. cinerea* control, while the organic vineyard received no sprays other than sulphur and copper.

Many environmental factors influence the microclimate around bunches. If we compare the grapevine parameters that are different between the organic vineyard and the conventional vineyard we find that the organic vineyard had increased shoot elongation up to flowering, increased light penetration at flowering and veraison and an increase in bunch compactness or more tightly packed berries at harvest.

Disease development is favoured by shaded conditions, which is usually associated with reduced irradiation, reduced temperature and increased humidity. The increase in shoot elongation up to flowering observed in the organic vines may aid in reducing the congestion and shading in the bunch zone and thus reduce the time required for bunches to dry after a moisture event.

The increased light penetration at veraison may increase the amount of epicuticular wax on bunches or reduce the viability of *B. cinerea* spores that land on bunches during this susceptible period. Epicuticular wax protects plant parts from desiccation and plant pathogens. Exposed bunches have more epicuticular wax and cuticle than shaded bunches. Rosenquist and Morrinson (1989) and Percival *et al.* (1993) demonstrated that bunches developed in the canopy shade had thinner cuticles and less epicuticular wax, respectively, than berries from sun exposed positions. The production of wax is increased when plants are subject to high light intensities and temperatures, and low relative humidity (Percival, Sullivan *et al.* 1993). Rosenquist and Morrinson (1989) also found that the amount of cuticle was variety dependent and that this correlated with the *B. cinerea* susceptibility of the varieties.

The factors affecting spore viability of *B. cinerea* are not well known. Spores recovered from bunch washes in field experiments have generally had low viability. Several factors may affect the viability of spores including excessive temperatures >35ºC, high or prolonged solar radiation levels. Studies of several aerial fungal pathogens have showed radiation can limit fungal growth and infection. For example, direct sunlight has been shown to reduce viability of *Alternaria solani* spores (Rotem 1968). Rotem, Wooding *et al.* (1985) and Stevenson and Pennypacker (1988) were able to separate the effects of sunlight and temperature to showed that UV radiation rather than elevated temperature was responsible for spore death. Given that spores of *A. solani* have melanised cell walls and those of *B. cinerea* do not, sunlight would be expected to have at least a similar detrimental effect on the survival of *B. cinerea* spores. Therefore, canopy management techniques that allow direct sunlight to penetrate into the bunch zone may have a negative impact on the spore viability of *B. cinerea*.

Compact bunch structures with more tightly packed berries generally predispose bunches to increased damage from splitting, increased humidity within the bunch and in turn increased botrytis bunch rot. At harvest the bunches from the organic vineyard were slightly more compact than those from the conventional vineyard, although this difference was not significant and disease levels were the same in the two systems.

An important difference between these two systems is the exposure of grapevine tissues including berries to pesticides other than sulphur and copper, in the conventional vineyard. Levels of infection were relatively low in

both vineyards. The use of a recommended spray program for botrytis bunch rot control in the conventional vineyard did not reduce the incidence of *B. cinerea* following moist incubation relative to the organic vineyard.

Results from laboratory bioassays on field sprayed bunches show that application of sublethal chemical doses, either through inefficient application or presence of resistant strains of *B. cinerea*, may actually increase the amount of botrytis bunch rot at harvest compared with unsprayed bunches (Murphy and Warren 2000; Riches, Warren *et al.,* 2003). This may be due to chemical sprays killing or inhibiting other microflora on the berry, which normally suppress or compete with *B. cinerea,* or due to damage of the berry skin, or a combination of factors.

Dr Rogiers is currently investigating the effects of agrochemicals on the berry wax layer, susceptibility to *B. cinerea* and surface populations of other microorganisms. Scanning electron microscopy of berries grown in the vineyard without exposure to pesticide sprays showed that waxes on the surface are arranged in intricate upright platelets with a lacey fringe often lining the ends of these platelets (Rogiers *et al*. 2003). Exposure of the berry surface to fungicide alone slightly affected the lacy structure of the wax, while the wetters and spreaders, commonly added to fungicides, generally had an even harsher effect on the wax structure, potentially creating more entry points for infection by *B. cinerea*. Some of the adjuvants tested, when applied alone (without the antifungal), increased *B. cinerea* growth on the berry, but when combined with an antifungal compound this effect was eliminated. However, in these trials bunches were sprayed individually by hand-sprayer ensuring better coverage of berries with fungicide than would normally be achievable under spray application in the field (Murphy, Warren 2000; Riches, Warren *et al*. 2003). Surfactants and fungicides may not only have a direct effect on the waxes of the berry but also on the natural microflora on the berry. A significant decrease in yeast population on berries which had received sprays was also reported by Rogiers *et al* (2003) and many of these yeasts populations are known to be inhibitory to growth of *B. cinerea*.

Murphy and Warren (2000) have shown that it is often difficult to deposit a lethal dose of chemical onto berries, particularly in dense canopies and later in the season when bunches close and berries touch at bunch closure. This would suggest that the spray program itself, particularly if not well targeted, could potentially create greater susceptibility to *B. cinerea* infection and severity of expression. Therefore applications of pesticides including botryticides may result in higher levels of botrytis bunch rot due to damage to wax layers, reduction in population of other microorganisms when sublethal doses of the fungicide are applied or when resistance strains are prevalent and the efficacy of the fungicide wears off over time.

The effect of spur-pruned and cane-pruned systems on botrytis bunch rot incidence.

Introduction

Many environmental factors affect the microclimate around the grape bunches. Excessive fertilisation and irrigation can increase vine vigour and the canopy density that increases the time required to dry bunches after a rain event. *B. cinerea* infection is promoted by high humidity (>90%) that often occurs in a congested canopy in the fruit zone.

Savage and Sall (1983) initially demonstrated that vine trellising does have an effect on the level of botrytis bunch rot at harvest under dry and moist conditions. A two wire vertical trellis system consistently had less disease than a cross-arm trellis system. Further studies on the canopy microclimates of the two systems found that temperature and moisture were similar for both systems. However, during each day there were distinct periods where the canopy temperature between the two systems was different. This was correlated with the ambient wind speed above the canopy suggesting differential wind penetration of the two canopies (Savage and Sall 1984).

In Australia, Emmett, Clingeleffer *et al.* (1994) found that, in warm regions minimal pruned vines had less botrytis bunch rot than mechanically hedged and cane-pruned vines. Factors that contributed to lower bunch rot on minimal pruned vines were smaller, less compact bunches that were generally located on the outer part of the canopy and, thus more exposed. However, minimally pruned vines often ripen their crop later than conventionally pruned vines. In cooler regions, delayed maturity could lead to greater risk of the crop being subject to a rain event.

Many trellis designs and canopy management systems are used in modern viticulture. Those that are successful in reducing the severity of bunch rot have the least amount of vine material per unit volume of canopy (Guilbaud-Oulton 2000). The VSP trellising system is the most common in Australian vineyards in cooler regions.

The objective of this study was to investigate the effect of spur and cane-pruned vines under a VSP trellising system on vine vigour and the incidence and severity of botrytis bunch rot.

Methods and Materials

Trial site

The trial site consisted of 12 rows of spur-pruned Chardonnay and 12 rows of cane-pruned Chardonnay (Figure 26). The vines were 6 years old. Twenty-five vines were selected across each treatment for sampling.

	Row/Panel	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	Row/Panel
	1																								1
	2		5		1						6					19							25		2
	3																		20						3
S	4							14																	4
P	5			13									7							2					5
U	6							8		15					18								24		6
R	7				12																21				7
	8										16					3									8
	9			11														9				22			9
	10												17												10
	11		10					4															23		11
	12																								12
	13																								13
	14		1										9								18				14
	15													10			21								15
C	16							24												17					16
A	17			2							8				11										17
N	18												22						16						18
E	19															12						20			19
	20				3					7															20
	21																13		15						21
	22			25		4			6				23										19		22
	23							5										14							23
	24																								24

Figure 26 Canopy management trial site at Mornington Estate vineyards.

Vine growth parameters and canopy density

One shoot per vine of 25 vines was measured at approximately 10 cm shoot length (30/10/03), 8 leaves separated (6/11/03), 12 leaves separated (13/11/03), start of flowering (2/12/03) and 80% capfall (12/12/03). The growth rate was calculated based on shoot extension over time.

The canopy density was determined at 80% capfall and harvest in the bunch zone using a lux meter to measure the level of light penetration into the canopy. The percentage light penetration was based on the average of 3 readings at 25 panels and was proportioned against the average of the light intensity outside the rows prior to assessment and after assessment.

The lux index is

$$\frac{\text{lux in the bunch zone} \times 100}{\text{average lux outside the canopy}}$$

The higher the value the more light that gets into the bunch zone.
The amount of light that gets into the bunch zone may indicate the amount of air movement in the canopy and the likelihood of periods of high relative humidity in the bunch zone long enough to allow infection to occur.

Bunch compactness

Bunch compactness was determined in the same manner as for the nutrition trial.

Botrytis assessment

Vine inflorescences were collected at flowering and bunches at harvest. The samples were surface sterilized in 1% bleach and rinsed in sterile water then frozen at –20ºC overnight and moist incubated for 10 days at room temperature in a takeaway food container. The disease incidence (percentage of infected clusters) and disease severity (percentage of infected berries per cluster) was estimated on 25 clusters from each vineyard.

Results

At flowering, no inflorescences from either treatment were infected with *B. cinerea*. At harvest cane pruning resulted in a higher incidence of botrytis bunch rot after moist incubation in the laboratory compared with the spur pruned vines (Figure 27). The average shoot length was greater in spur pruned than in cane-pruned vines (Figure 28). At flowering, the cane pruning system had less light penetration into the bunch zone than the spur pruned vines, but by veraison the light penetration in the cane system was similar to the spur pruned vines (Figure 29). At flowering and at harvest the degree of bunch compactness was very similar in the cane and spur pruned systems (Figure 30).

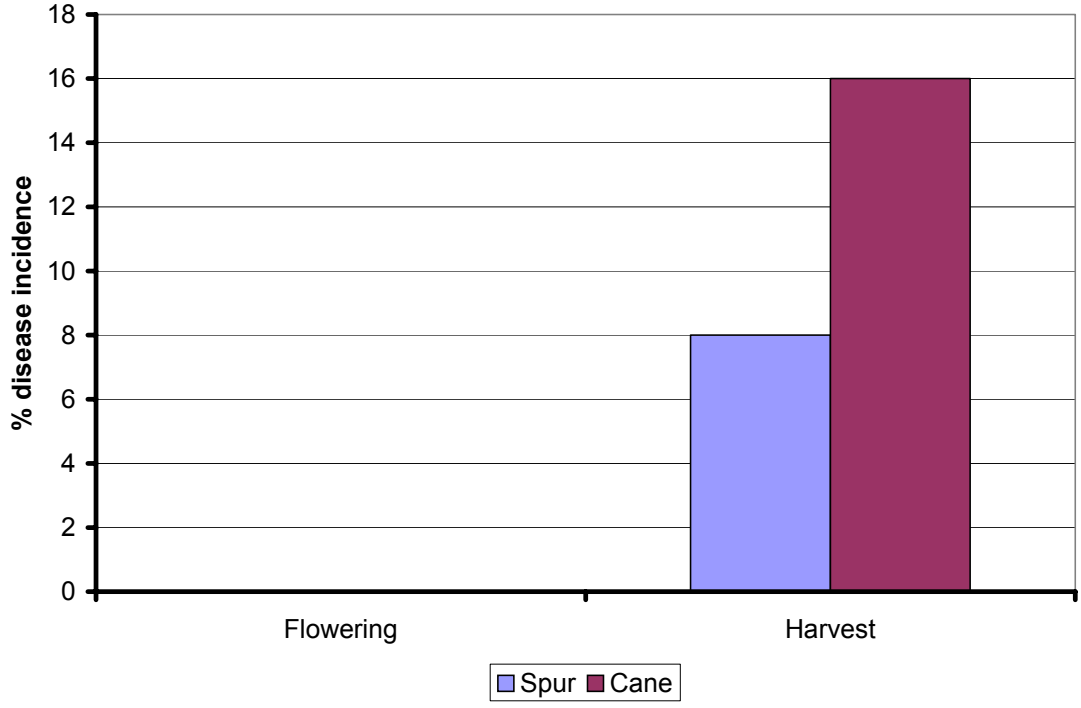


Figure 27 Comparison of disease incidence between spur and cane-pruned vines after surface sterilisation and moist incubation. Data represent the mean of 25 measurements.

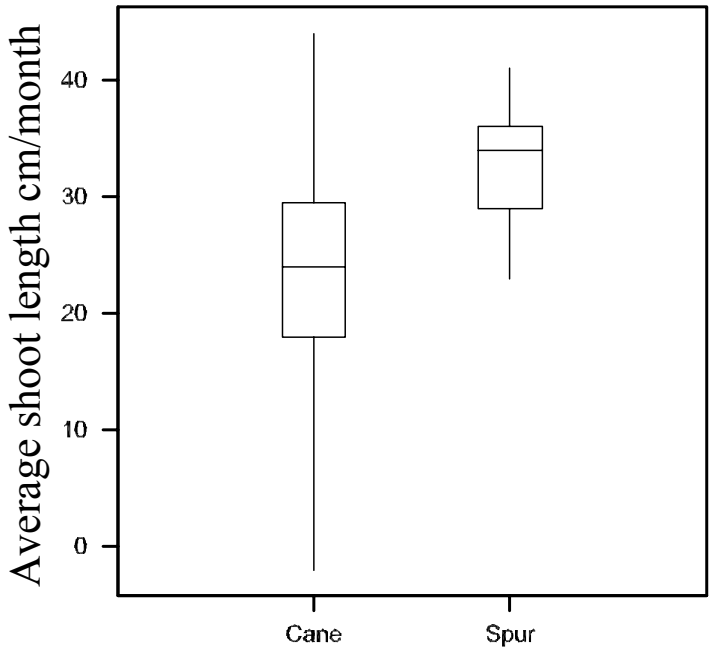


Figure 28 The average shoot length of spur and cane-pruned vines. Data represent the mean of 25 measurements.

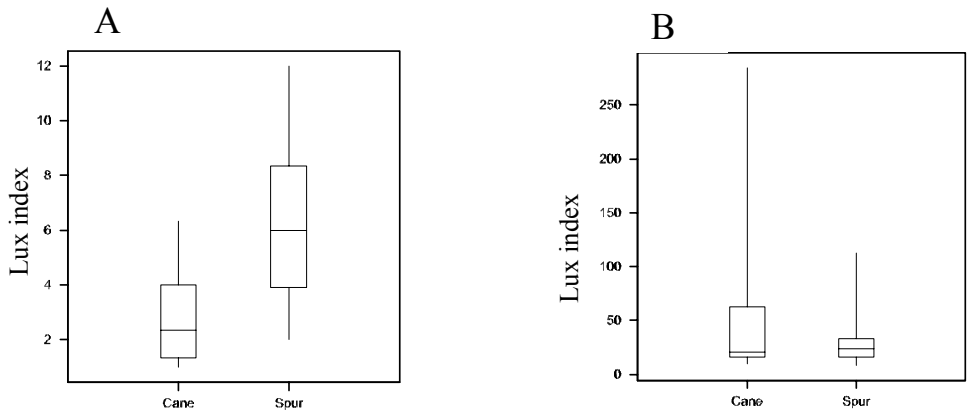


Figure 29 Light penetrations at A) flowering and B) veraison in spur and cane-pruned vines. Data represent the mean of 25 measurements.

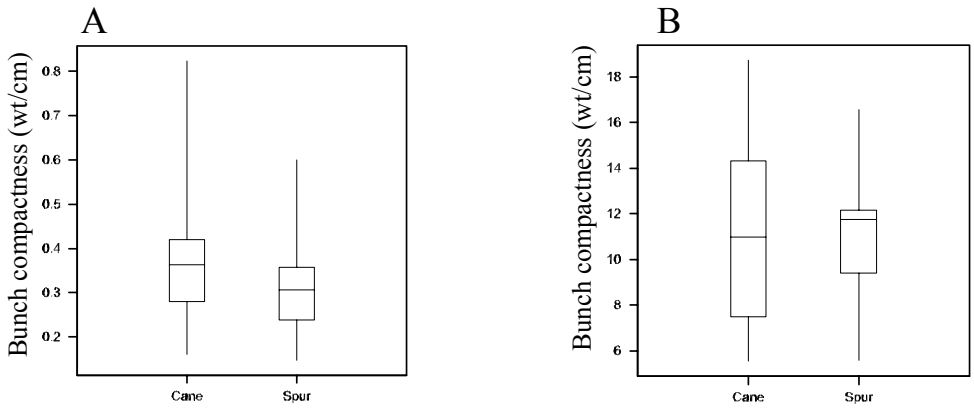


Figure 30 Bunch compactness at flowering and harvest after spur and cane pruning. Data represent the mean of 25 measurements.

Discussion

There was no botrytis bunch rot in the field at harvest suggesting conditions leading up to harvest were not conducive to disease expression.

The pruning systems had an effect on the level of latent infections at harvest and therefore potential disease incidence should environmental conditions be suitable. Spur-pruned vines had lower latent infection (8%) than cane-pruned vines (16%) at harvest. The spur-pruned vines had longer average shoot lengths and higher light penetration into the bunch zone at flowering than the cane-pruned vines, but by veraison, the spur-pruned vines had less light penetration into the bunch zone than the cane-pruned system. This suggests spur-pruning promotes faster spring shoot growth resulting in a more open canopy at flowering, possibly increasing light penetration and reducing humidity at a critical stage for *B. cinerea* infection. In this present study, however, moist incubation of inflorescences collected at flowering indicated that no infection had occurred at this stage in either pruning system, but instead occurred sometime between the end of flowering and harvest. However, a more open canopy is likely to result in less disease by allowing greater light penetration (thicker cuticles on berries, direct effect on spore survival), more air movement (lower relative humidity, quicker drying times) and better penetration of sprays into bunch zones (more spray deposits on berries, better disease control).

The effects of different pruning systems on field expression should be investigated in future using trials with artificial weather events favourable to botrytis bunch rot at harvest factored into the experimental design.

Outcome and Conclusion

Outcomes are presented in context of best practice in Appendix 5

- These results from the glasshouse trial indicated that manipulation of nutrients and their ratios can affect Botrytis leaf blight severity, although it is unknown how this might translate to botrytis bunch rot severity.

- There was some evidence from both the field and glasshouse trials that manipulation of calcium levels in particular may reduce susceptibility to *B. cinerea*. Higher levels of calcium resulted in reduced susceptibility of leaves to *B. cinerea* in the glasshouse and more open bunches in the field, warranting further investigation. Commercially available calcium products registered for use in botrytis bunch rot control should be evaluated, including investigation of their effects on bunch compactness as well as on disease development and calcium accumulation in grapevine tissues.

- The glasshouse trial indicated that altering nitrogen levels in cabernet sauvignon may influence canopy density, and this needs corroboration in the field. It appeared that nutrient effects may differ for different varieties, and this should be examined further. The use of different nutrient treatments for different varieties would not be difficult to manage since varieties are usually arranged in separate blocks in the vineyard.

- It is evident that future field trials undertaken to test the effects of the most promising treatments on disease expression cannot rely on natural weather events but require the stimulation of conducive conditions for disease development in the field, for example by overhead irrigation.

- The results presented here, although preliminary, show promise that manipulation of vine nutrition has the potential to be used as part of an integrated disease management strategy to combat botrytis bunch rot in Australian vineyards and reduce the reliance on chemical pesticides.

- Spur pruning stimulated faster shoot growth prior to flowering, and created a more open canopy and less botrytis bunch rot. Increasing the growth rate up to flowering may create a more open bunch zone.

- The use of botryticides did not always result in less disease than no sprays. This may indicate that the spray application itself may damage the berries, rendering the berries more prone to developing botrytis bunch rot.

Conclusions

Cool and warm temperatures favour botrytis infection and expression, high humidity, which may lead to long periods of free moist on berries. In some seasons in cool climate vineyards these conditions are present much of the season.

It is important to note that the measured Botrytis expression in this project is attributed to artificial expression through laboratory incubation. This does not directly equate to natural field expression.

Managing bunch wetness:

Avoid compact bunches by producing bunches with loosely packed berries.
In warm climate vineyards or in years when rain is deficient in cool climate vineyards, irrigation scheduling can have an effect on the bunch architecture that results in loose bunches with small berries. However, in wet seasons where irrigation scheduling is not necessary, other methods are required to reduce the size of berries.

Reduce canopy density and fruit congestion, increase fruit exposure:

It is now known that Botrytis is endemic in the vineyard and will always be a potential threat from veraison to harvest so one management regime is to accelerate ripening. Factors that delay ripening are high rainfall, high altitude, heavy cropping and large canopy (excessive shading reduces light levels and temperature in the bunch zone). In most cases, the grower is already established at a site so it is not possible to control the rainfall and temperature of the site. Therefore, management of cropping levels and canopy density is necessary.

Excessive shading also promotes a thinner cuticle on the berry that increases the fruit susceptibility to Botrytis.

Growers can start to manage their Botrytis problem as early in the season as pruning. Too many buds left after pruning may increase yield and delay maturity, however, leaving a large number of buds at pruning can increase competition among clusters for assimilates and reduce compactness by reducing berry set. Post fruit set thinning can correct the yield and accelerate ripening. Post fruit set thinning also enables growers to create a more open bunch zone that can reduce the bunch wetness and increase spray penetration.

Finger and thumb pruning of spur-pruned vines can increase the height of the bunch zone reducing bunch congestion.

Since Botrytis preferentially infects damaged or dying tissue, growers need to identify practices that damage bunches and eliminate them. Controlling other pests, such as LBAM, and pathogens is integral to Botrytis management as pests cause damage to bunches that enable a Botrytis infection to establish .With the increasing exposure of bunches, sunburn damage may become a problem and inturn excessive opening up the canopy may be detrimental to Botrytis control. Consideration should be given to the effect of solvent-based wetters on the cuticular wax layer. Many viticulturists have abandoned the use of additives and maintain successful application rates of the chemicals (Stonier's Winery, pers. Comm.)

Recommendations

In unfavourable weather conditions, growers must be aware that their best efforts of Botrytis management may not be successful.

Manage canopies to reduce the period of leaf and fruit wetness after an event that favours botrytis development, eg rain, fog, dews.

Manage canopies to provide the most open and accessible fruit zone for light and spray application.

Monitor nutritional status, particularly nitrogen levels, with petiole analyses.

Maintain a vineyard regime that results in earlier ripening of fruit.

Make use of the Botrytis Best Practice Booklet and CD ROM (see references) for management advice at critical times in vine development.

Appendix 1: Communication

Communication / Extension activities

Papers and Poster presentations

Cole, M. (2004) Botrytis and its place in the vineyard. Part 2: Distribution of Botrytis in the vineyard Australian & New Zealand Grapegrower & Winemaker **482**:15-18).

Cole, M., Whiting, J & Braybrook, D. (2004) Botrytis Best Practice Protocols, Monash University.

Cole, M., Whiting, J., & Braybrook, D. (2004) Botrytis Best Practice Check List

Keller, M., Viret, O., Cole, M *(*2003) *Botrytis cinerea* in grape flowers: defence reaction, latency and disease expression, *Phytopathology* **93:** 316-322.

Wiechel, T.J.,Thomson, G.E., Holmes, R., Joyce, D.C., Warren, M.A. and Cole, F.M. (2004). Investigating the potential effect of grapevine nutrition on Botrytis severity. *Proceedings of the 12th Australian Wine Industry Technical Conference, Melbourne.*

Wiechel, T.J., Cole, F.M. and Joyce, D.C. (2003). Management of botrytis bunch rot in cool climate vineyards. *Proceeding of Cool Climate Viticulture Forum, DPI Knoxfield, Melbourne*.

Wiechel, T.J., Thomson, G.E., Joyce, D.C., Warren, M.A. and Cole, F.M. (2003). The effect of grapevine mineral nutrition on botrytis bunch rot. *Proceedings of Combio2003, Melbourne, Australia.*

Conference Presentations on related projects

Cole, M (2003) Distribution and compatibility of *Botrytis cinerea* isolates. Botrytis workshop proceedings, 8th International Congress of Plant Pathology, Christchurch, NZ.1-2 February 2003

T. J. Wiechel (2003) The occurrence of *Botrytis cinerea* subspecies *transposa* and *vacuma* in Australian vineyards. Botrytis workshop proceedings, 8th International Congress of Plant Pathology, Christchurch, NZ.1-2 February 2003

T. J. Wiechel, S. R. Whitmore, A. Agarwal and F. M. Cole (2003). A survey of *Botrytis cinerea* from budburst to post harvest in regional vineyards in Victoria. *Proceedings of the 8th International Congress of Plant Pathology, Christchurch, New Zealand.*

F. M. Cole, T. J. Wiechel, S. R. Whitmore and A. Agarwal (2003). Phyloplane fungi in Victorian vineyards. *Proceedings of the 8th International Congress of Plant Pathology, Christchurch, New Zealand.*

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T. J. Wiechel, F. M. Cole, M. Warren and R. W. Emmett (1999). Investigating genetic variation in *Botrytis cinerea* populations within a vineyard. *Proceedings of the 12th Biennial Australasian Plant Pathology Conference Canberra, Australia.*

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Viret, O., Pezet, R., Cole, M., and Keller, M. (2001) Latency, disease incidence and localisation of *Botrytis cinerea* Pers. during the early stages of bloom infection of grapes. 11th Congress of the Mediterranean Phytopathological Union and 3rd Congress of the Sociedade Portuguesa de Fitopatologia (17-20 Sept, Evora, Portugal

Appendix 2: Intellectual Property

Appendix 3: References

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Appendix 4: Staff

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The following people assisted with the project:

- Richard Shenfield and staff, Fernhill Vineyard, Coldstream.
- Chris Messerle and staff, Yarra Glen Vineyards, Yarra Glen.
- Hugh Robinson, Craig Fletcher and Murray Lyons, Mornington Estate Vineyards, Tuerong.
- Don and Sandra Lazzar, Lazzar Organic Wines, Balnarring.
- Linda Gilson Wesfarmers for previous grapevine nutrition information.
- Phil Deveral, Orlando Wyndam Nursery, Rowland Flat, SA for grapevine cuttings.

Appendix 5: Table of outcomes and their relation with current best practice

Best practice control options	**Contributing factors**	**Expected effect**	**Expected effect on *Botrytis cinerea* Infection/expression**	**Observed Effect**	**Observed effect on *Botrytis cinerea* infection/expression**	**Additional comments**
Site selection	Low lying and subjected to flooding. Previously under pasture.	Favourable climate for *B. cinerea* infection and expression. High nitrogen content in soil. High nitrogen content in grapevine petioles and berries.	Build up of inoculum. Higher levels of *B. cinerea* infection and expression.	Soil nitrogen levels at the high side of normal. Measured petiole nitrogen levels adequate.	**Meroo trial:** High levels of B. cinerea infection (90% of berries with *B. cinerea* following moist incubation) and potential expression. History of high level of Botrytis bunch rot. Compared to Fernhill in the same season, which had significantly lower levels of infection and potential expression when the same number of spores was applied at flowering.	
Avoid compact bunches	Genetics, environment, cultural factors such as pruning level, availability of water (Winkler et al 1974), trellis system and temperature (Mullins et al 1992)	Less compact bunches	Less Botrytis bunch rot. Berries less susceptible to *B. cinerea* infection and expression (Savay & Sall, 1983; Gubler et. al., 1987 & Ellison et al 1998). More humid micro-climate provides more favourable	**Meroo trial:** CaK treatment had significantly less compact bunches. **Organic vs traditional:**	**Meroo trial**: Effects on field expression could not be determined because weather conditions were not conducive. Treatments with looser bunches did not have less Botrytis bunch rot following moist incubation. Effects on field expression could not	Evidence that CaK treatment resulted in less compact bunch

			environment for infection, production of mycelium and conidia (Vail & Marios, 1991). Berries more susceptible because more berry-to-berry contact sites where cuticle is poorly developed (Marois et. al. 1986). More difficult to penetrate with sprays.	Organic had more compact bunches than those from the traditional vineyard.	be determined because weather conditions were not conducive. Organic bunches did not have more disease following moist incubation than traditional bunches.	
Produce an open bunch zone	Pruning systems Spur vs cane	Effect canopy architecture and openness of bunch zone.	Less Botrytis bunch rot in canopies with a more open bunch zone.	Spur pruning vines increased shoot length up to flowering, and created a more open canopy at flowering.	Effects on field expression could not be determined because weather conditions were not conducive. The severity of *B. cinerea* expression at harvest following moist incubation was significantly lower in the spur than cane-pruned vines.	A more open canopy at flowering may also allow more efficient spray penetration onto flowers/bunches.
Control vine vigour	Nitrogen fertilisation	Leads to increased vine vigour and a dense canopy. Increased nitrogen in tissues makes more "soft" and predisposes tissues to *B. cinerea* infection.	Increased susceptibility to disease. By making tissues themselves more susceptible to infection. It has been speculated	**Meroo and Fernhill trials:** Nitrogen treatments did not result in different analytical Nitrogen levels in the petioles or berries.	**Meroo and Fernhill trials:** Effects on field expression could not be determined because weather conditions were not conducive. Nitrogen treatments did not affect the	

			that excessive nitrogen fertilisation promotes the development of Botrytis bunch rot but there is no definitive data to support this (Dubos 2000). A denser canopy provides a more favourable microclimate for disease development since the bunch zone take longer to dry out. More difficult to get sprays into bunch zones of denser canopy types.		severity of *B. cinerea* at harvest following moist incubation.	
Apply Potassium	Potassium fertilisation	Increase K content of leaves and bunches	Decrease susceptibility to Botrytis bunch rot (Kiraly, 1976). High levels of K may reduce wound injury and accelerate wound healing (Kiraly, 1976 & Huber 1981)	**Meroo and Fernhill trials:** Potassium treatments did not result in different analytical Potassium levels in the petioles or berries.	**Meroo and Fernhill trials:** Effects on field expression could not be determined because weather conditions were not conducive. Potassium treatments did not affect the severity of *B. cinerea* at harvest following moist incubation.	
Apply Calcium	Foliar Calcium sprays	Increased Calcium in leaf and berry tissues	Reduced incidence and severity to	**Meroo and Fernhill trials:** Foliar sprays	**Meroo and Fernhill trials:**	

		(Winter & Nicol, 1998).	Botrytis bunch rot (Winter & Nicol, 1998). Reduced mechanical damage by strengthening cell walls. Calcium makes cell walls more resistant to enzymatic degradation (Huber 1981).	did not significantly increase the amount of Calcium in leaf or bunch tissues. This suggests that very little if any of the applied Calcium was taken up by the plant cells. It also appears that applied Calcium has been removed from leaf and bunch surfaces by harvest.	Effects on field expression could not be determined because weather conditions were not conducive. Calcium sprays did not result in lower levels of *B. cinerea* expression following moist incubation.	
Apply Potassium and Calcium in balance	KCa balance	Increased Calcium and Potassium in petiole and berry tissues	Reduced incidence and severity to Botrytis bunch rot As for Calcium and Potassium above	**Meroo and Fernhill trials:** Calcium and Potassium treatments did not significantly increase the amount of Calcium or Potassium in petiole or bunch tissues. **Meroo trial:** CaK treatment had significantly less compact bunches than other treatments.	**Meroo and Fernhill trials:** Effects on field expression could not be determined because weather conditions were not conducive. Calcium and Potassium treatments did not result in lower levels of *B. cinerea* expression following moist incubation.	Potential competition between these two cations affecting uptake and function in Botrytis bunch rot development Shown that for rose blight that calcium reduced the severity of post harvest Botrytis blight but increasing potassium negated the ability of calcium to reduce Botrytis blight probably due to competition of for cation uptake (Volpin & Elad, 1991)
Fungicide spray program	Application of Botryticides at flowering and pre-	Deposition of fungicide and formulation additives	Disease control Lower incidence and severity of *B. cinerea*		**Meroo:** In this trial a disease severity of 70-90% of berries	There is some evidence that chemical sprays (even

	bunch closure	such as adjuvants (wetters, stickers, spreaders etc)	bunch rot		infected after moist incubation at harvest was observed despite a spray program of Teldor at flowering being applied. **Organic vs convential**: Incidence of botrytis bunch rot at harvest following moist incubation was similar for organic (7%) and conventional (8%) despite a spray program being applied to the conventional vineyard (Scala @ flowering and Switch @ pre-bunch closure).	botryticides) may be a source of damage to berry surface by affecting the delicate lacy structure of the wax layer rendering it more susceptible to infection (Rogiers, 2003). By decreasing populations of other natural microflora on the berry such as yeasts some of which are antagonist's or competitive with *Botrytis cinerea*. (Rogiers, 2003).