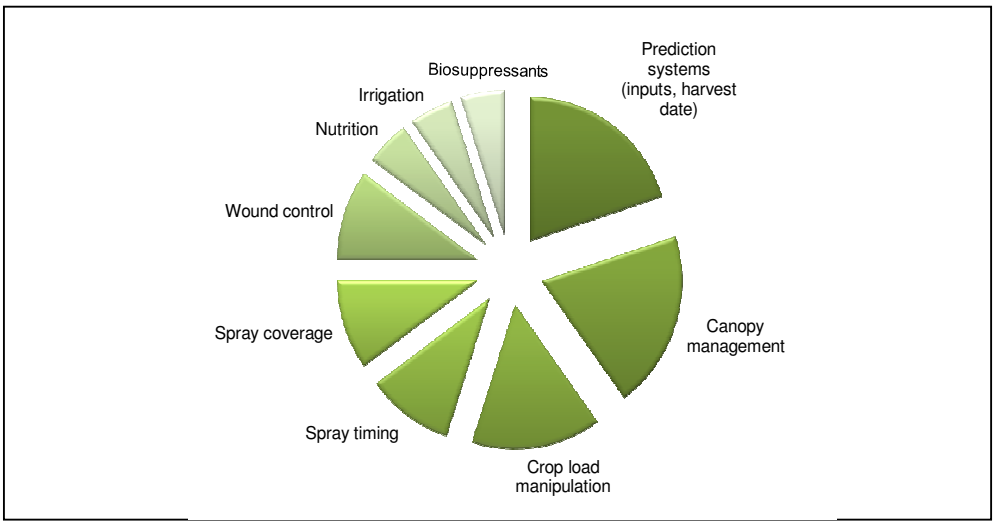
Effective management of botrytis bunch rot for cool climate viticulture.



FINAL REPORT to

GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

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Research Organisation: **University of Tasmania**

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Effective management of botrytis bunch rot for cool climate viticulture

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Effective management of botrytis bunch rot for cool climate viticulture

Abstract

Botrytis bunch rot, caused by *Botrytis cinerea*, continues to cause large seasonal variability in grape production and wine quality. This project identified pathogen, vine and weather factors that can be practically monitored during the season for predicting botrytis risk. Quantitative relationships between botrytis and individual risk factors were determined and these relationships incorporated into a prototype predictive model. The predictive model, known as the Botrytis Decision Support Model (BDSM), will be delivered in a decision support system for supporting advice from industry service providers and vineyard technical managers to vineyard staff, including:

- fungicide applications
- vine canopy management
- planning of harvest operations to minimise crop loss from botrytis
- retrospective analysis of the performance of botrytis control programs.

This report also describes

- quantitative methods that were validated for studies of botrytis bunch rot in grapevines,
- a new quantitative polymerase chain reaction (PCR) assay for quantifying *B. cinerea* DNA in grape berries,
- effects of fungicides and canopy manipulation on botrytis severity,
- whole-of-block experimentation for understanding spatial variation in treatment responses,
- a sampling strategy for monitoring botrytis as an input to the BDSM, and
- equipment, data, services, infrastructure and education required for adoption of the BDSM.

Executive Summary

Botrytis bunch rot, caused by *Botrytis cinerea*, causes significant loss of yield and quality in wine grapes grown in cool, temperate regions of the world. In Australia and New Zealand, synthetic fungicides continue to be used widely for protection of grape flowers and fruit when they are susceptible to infection by *B. cinerea*; however, there is increasing restriction on botryticides available for protection during berry ripening because of market specifications for low or no fungicide residues in wine. Dry seasons and low grape prices in recent years have seen spray programs reduced (less water and diesel) and canopy management minimised (less labour). When conditions then return to being highly favourable for disease, growers can suffer economic losses if they have not adjusted their inputs accordingly. Conversely, the same intensive, season-long spray program may be used regardless of actual botrytis risk. Prior to this project, The New Zealand Institute of Plant and Food Research Limited (P&FR, formerly HortResearch) had been developing a model that incorporated weather-related botrytis risk identified in existing models, with pathogen inoculum and vine factors integrated for determining overall botrytis risk.

This report describes results from 17 replicated, small-plot trials conducted in southern Tasmania and the Yarra Valley of Victoria in the period 2006-2009 for testing various fungicide programs and canopy management techniques for their impact on botrytis severity at harvest. Data from these trials and those from another 34 site-years conducted by P&FR in New Zealand in the period 2001-2009 were used to quantify botrytis risk relationships in relation to weather, vine factors and fungicide use. These risk relationships were integrated into the prototype **Botrytis Decision Support Model** (BDSM) with data from the final growing season (2008/09) used to calibrate the prototype model using different fungicide timings. The prototype BDSM is described by Beresford *et al.* (2009, Appendix 5).
The contents of each chapter in this report are summarised as follows:

Chapter 1: Quantitative methods for studies of botrytis bunch rot in grapevines
Methodologies for studying botrytis epidemics were developed with logistic models and Area Under the Disease Progress Curve (AUDPC) demonstrated as useful means for understanding how treatments impacted on botrytis epidemics. The following variables were related to botrytis severity at harvest for up to 51 site-years, and methods for measuring them were described:

Pathogen inoculum

- incidence of berries with latent *B. cinerea* at pre-bunch closure
- number of floral debris pieces colonised by *B. cinerea*

Vine factors

- leaf layer number (as an indirect measure of canopy density)
- yield (kg) of grapes per vine (as a measure of crop load)
- mean bunch weight (as an indirect measure of bunch compactness)
- bunch position on the vine

Weather factors

- The mean Bacchus risk index for defined crop-stage intervals. This index was calculated from the temperature during wet periods (surface moisture).

None of the individual pathogen or vine factors accounted for more than 32% of the variance in botrytis severity at harvest, suggesting that multiple factors interact with the weather to determine botrytis severity at harvest. A strong regional relationship was found between

mean late-season interval (days from veraison to harvest) and mean harvest botrytis severity; however, ripening period could not be isolated as a causative factor because several regional climatic factors were correlated with regional differences in botrytis severity.

Chapter 2: A technique for quantifying the amount of Botrytis cinerea *DNA in grape berries*
A quantitative real-time polymerase chain reaction (qPCR) technique for quantifying the amount of *Botrytis cinerea* DNA in grape berries was developed. A duplex assay that included probes and primers for both *Botrytis cinerea* DNA and *Vitis vinifera* DNA (internal control) ensured that lack of amplification of *B. cinerea* DNA indicated lack of detection rather than biochemicals inhibiting the PCR. Reliable standard curves for *B. cinerea* DNA were generated. Reaction efficiencies for *B. cinerea* DNA were estimated to be 81–113%, with accurate quantification of *B. cinerea* DNA for C_t values corresponding to DNA amounts ≥ 350 fg. The assay also worked for 278 of 300 single vine samples from a 2.4 ha block of Chardonnay. *B. cinerea* DNA was quantified accurately for 46 of the 278 samples in which *B. cinerea* DNA was detected. *B. cinerea* DNA as a percentage of total DNA for the 45 samples ranged from 0.004 to 2.5%.

Chapter 3: Botrytis trials in Tasmania 2006–2009
Application of the QuickStix™ Kit for Botrytis in Wine Grape Juice (EnviroLogix Inc.) complemented visual scoring of disease symptoms when assessing treatment effects in replicated small-plot trials in which botrytis severity in non-treated plots exceeded 3% at four of eight site-years. Switch® fungicide applied between pea-sized berries and pre-bunch closure generally resulted in a large effect in terms of reducing botrytis severity at harvest relative to non-treated grape bunches with >2% mean botrytis severity. Switch® applied at pea-sized berries also reduced the mean incidence of latent infection at pre-bunch closure relative to non-treated controls in one trial. The benefit of applying fungicide at 80% capfall depended on the fungicide tested and the season. Late-season application of Rovral® at two site-years with suspected dicarboximide resistance did not reduce botrytis severity significantly. Moreover, pre-bunch closure is the last opportunity to obtain good spray coverage inside the bunch, where latent infections often emerge. Bunch thinning, removal of bunch trash or leaf removal did not improve botrytis control and the reasons for these outcomes are explored in Chapter 3.

Chapter 4: Botrytis trials in Victoria 2006–2009
In seasons characterised by drought and concluding with bushfires, only two of nine site-years developed sufficient botrytis to evaluate treatment effects. Nevertheless, the conditions at sites with extremely low botrytis risk provided valuable information for developing the BDSM. When mean botrytis severity in the non-treated control was 2.3% in a Sauvignon Blanc trial, leaf removal on one side of the canopy between pre-bunch closure and veraison reduced botrytis severity to 0.4%, which was equivalent to the result for Scala® applied at 80% capfall (0.4% severity) and the full-season fungicide program (0.1% severity). When mean botrytis severity in the non-treated control was 2.0% in a Chardonnay trial, a full season fungicide program reduced botrytis severity to 0.3%, with shoot thinning (in the absence of fungicide treatment) resulting in 1.6% severity, which was a small and marginally significant reduction. Neither pre-veraison leaf removal (2.1% severity) nor bunch trash removal (2.7% severity) reduced botrytis severity in this trial, with speculation that the compressed air used to remove the trash damaged the bunches.

Chapter 5 Whole-of-block experimentation reveals spatial variation in the response to Switch® fungicide applied at flowering or pre-bunch closure for botrytis management

Switch® fungicide was applied at either 80% capfall or pre-bunch closure for the control of botrytis in a 2.4 ha Chardonnay block in southern Tasmania. Treatments were applied by the grower co-operator in alternate strips, each of six rows. Botrytis severity was assessed for 150 target vines per treatment, located in the centre two rows of each six-row treatment strip. Switch® applied at pre-bunch closure reduced botrytis severity relative to the application at flowering, but the response to each spray treatment was spatially variable, with higher vigour areas tending to be more severely affected by botrytis and treatment differences evident at $P < 0.05$. Spatial variation in response to the flowering treatment, when examined four times pre-harvest, provided evidence to support the hypothesis that the botrytis observed at this site-year was not the result of secondary spread.

Chapter 6: Sampling strategy and evaluation of sample sizes required for reliable estimation of botrytis severity in vineyard blocks in Tasmania
Using vineyard blocks of 1–2.5 ha at five site-years in Tasmania, a sampling strategy was developed for reliable estimation of botrytis severity as an input to the late-season BDSM. The sample unit was a panel of vines (or two panel sides facing each other in adjacent rows) from which mean botrytis severity was calculated from assessment of 20 grape bunches. The standard error to mean ratio for mean botrytis severity was used to demonstrate that 20–25 sample units were required to obtain a reliable estimate of mean botrytis severity for the block when severities were < 1%. Fewer sample units were needed as mean botrytis severity increased beyond 2%.

Chapter 7: Equipment and data needed to run the prototype Botrytis Decision Support Model (BDSM), and
Chapter 8: Service provision, infrastructure and education required for adoption of the Botrytis Decision Support Model
Chapter 7 describes the equipment and data needed to run the prototype BDSM, including a translation of the sampling strategy described in Chapter 6. Chapter 8 provides a discussion about service provision, infrastructure and education required for adoption of the BDSM.

Finally, implementation of the BDSM is expected to reduce seasonal variability in grape production and wine quality and reduce risks of fungicide residues in wine through accurate prediction of seasonal botrytis risk, allowing better targeting of early-season fungicides (or biocontrols) and optimum late-season management of vineyard operations to avoid botrytis losses. The strength of this trans-Tasman collaboration was the creation of a sufficiently large dataset encompassing the range of botrytis severities likely to be experienced in the cool climates studied. Adoption of standard, quantitative methods will allow researchers in future projects to collect compatible data, further refine models for predicting botrytis risk, and to compare new and existing treatments for botrytis control across multiple sites and regions.

The project outcomes have been communicated in three journal publications, two articles in *The Australian & New Zealand Grape Grower & Winemaker*, six conference proceedings including the *14th International Botrytis Symposium*, two workshops presentations at the Australian Wine Industry Technical Conference, and 13 (oral) presentations primarily to industry field days in cool climate regions of Victoria, Tasmania and New South Wales. Awards related to this project include the Wine Innovation Cluster – Best Student Poster prize awarded to K.J. Dunne *et al.* at the 14th AWITC and the Emerging Presenter Award to G.N. Hill at the New Zealand Plant Protection Society conference, 2010.

Background

Botrytis bunch rot, caused by *Botrytis cinerea*, causes significant loss of yield and quality in wine grapes grown in cool, temperate regions of the world (Nair and Hill 1992). In Australia, losses in the vineyard to botrytis and other bunch rots were estimated to cost $52 million/annum (Scholefield and Morison 2010). Furthermore, infection of grapes by *B. cinerea* can lead to a reduction in wine quality by causing oxidation, off-flavours and other biochemical changes (for example, La Guerche *et al.* 2006). Botrytis can also promote secondary rots, thus compounding undesirable traits in wine. Given the additional cost of remedial winemaking, many wineries in Australia and New Zealand impose a price penalty if a percentage of harvested grapes are botrytis-affected, the level of which is determined by individual wineries with crop rejection possible when percentages exceed 3–5%.

Synthetic fungicides continue to be used widely for protection of grape flowers and fruit when they are susceptible to infection by *B. cinerea*, but there is increasing restriction on botryticides available for protection during berry ripening because of market specifications for low or no fungicide residues in wine. Strategies to reduce reliance on fungicides include cultural and biological control measures and use of decision support models to allow fungicides to be used according to the level of disease risk in a given season. Dry seasons and low grape prices in recent years have seen spray programs reduced (less diesel) and canopy management minimised (less labour). When conditions then return to being highly favourable for disease, growers can suffer economic losses if they have not adjusted their inputs accordingly. Conversely, the same intensive, season-long spray program may be used regardless of actual botrytis risk.

Evans (2008) reviewed research relevant to managing botrytis bunch rot in Australia. The interaction between weather, pathogen inoculum and vineyard factors determines whether botrytis will cause a damaging epidemic in a given season, as illustrated in previous botrytis research supported by GWRDC (e.g. Cole *et al.* 2004; Emmett *et al.* 2005a,b). Further research was needed for quantifying seasonal risk and factors affecting botrytis control in areas prone to this disease. Although weather-based prediction models (e.g. the Nair, Broome and Bacchus models; Nair and Allen 1993; Broome *et al.* 1995; Kim *et al.* 2007) have been developed for botrytis, these are not currently used for practical disease management because risk thresholds have not been set and because weather is only one factor determining season-long botrytis risk. Furthermore, development of models for disease risk requires a consistent methodology, based on sound epidemiological principles, which can be applied in any grape growing region to allow effects of experimental disease control treatments to be compared and to analyse vineyard and climatic factors that influence botrytis development.

When project UT0601 was established, The New Zealand Institute of Plant and Food Research Limited (P&FR, formerly HortResearch) had developed an early version of their Botrytis Prediction Model. This model incorporated the weather-related botrytis risk identified in existing models and integrated it with pathogen inoculum and vine factors for determining overall botrytis risk. Australian researchers then sought to work with P&FR to generate standardised data across a range of cool-climate regions for further development and calibration of what is now called the prototype **Botrytis Decision Support Model** (BDSM) and the major output from this project. The trans-Tasman collaboration also sought to develop and further improve quantitative methods for botrytis so that future researchers could adopt standard procedures for collection of compatible data, further refine models predicting botrytis risk, and compare new and existing treatments for botrytis control across multiple sites and regions.

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Project Aims and Performance Targets

Sub-project A: Vineyard Information Systems for Botrytis Risk Management

The aims as outlined in the project proposal were to:

- Reduce seasonal variability in grape production and wine quality and reduce risks of fungicide residues in wine through accurate prediction of seasonal botrytis risk, allowing better targeting of early-season fungicides (or biocontrols) and optimum late-season management of vineyard operations to avoid botrytis losses
- Analyse regional botrytis field trial data from Australia, combine this with equivalent data from New Zealand and model the combined data to calibrate weather, inoculum and vine physiology parameters in the existing Botrytis Prediction Model developed by HortResearch (now Plant & Food Research) (Figure 2)
- Deliver the calibrated Botrytis Prediction Model to GWRDC by developing a prototype web-based Decision Support Model for botrytis risk management, including the weather and vineyard information databases required by the Decision Support Model
- Provide advice on weather data networks required to deliver a botrytis Decision Support System in Australia
- Note: Provision of database systems and definition of web programming requirements for implementation of the prototype Decision Support Model were part of this project, but the actual implementation of the model onto a web site as a Decision Support System for GWRDC was not part of this project.

The performance targets were:

- Generation of a data set suitable for further development of prototype botrytis risk models.
- Production of a functioning prototype Botrytis Prediction Model (now called the Botrytis Decision Support Model or BDSM) for web-based decision support
- Completion of a PhD thesis toward improved understanding of botrytis epidemiology upon which future botrytis risk models and management will be based
- Communication of results, including this final report, (oral) presentations to industry, an article in the technical issue of The Australian & New Zealand Grape Grower & Winemaker, at least one international conference paper and at least one paper submitted to a refereed, scientific journal.

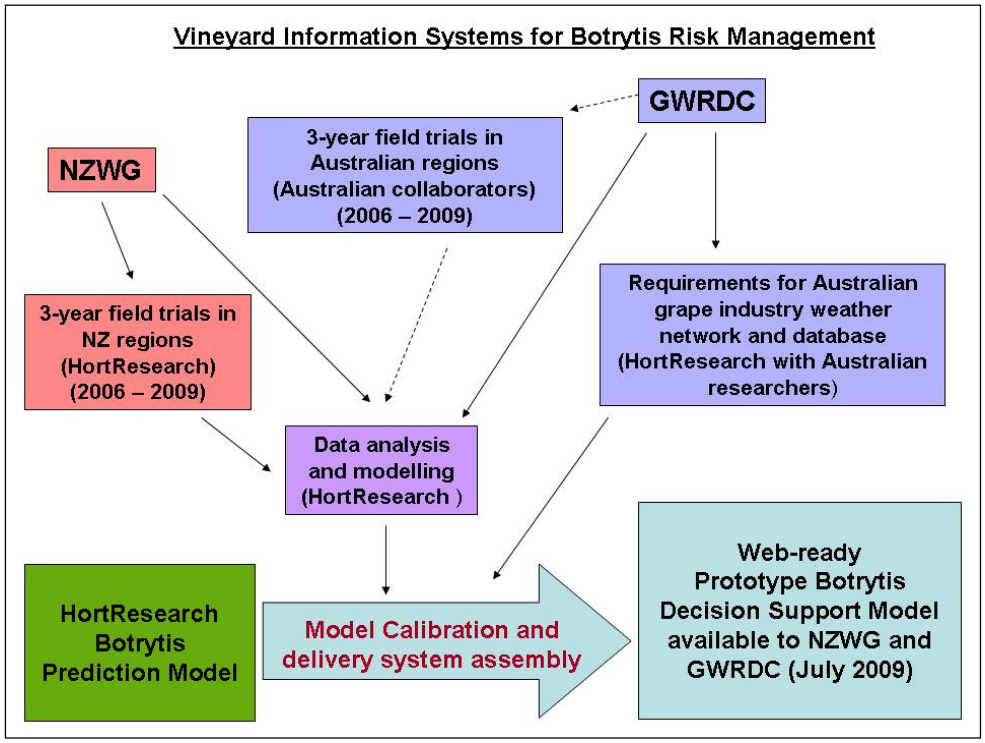


Figure 2. Development of the prototype botrytis Decision Support Model from the existing HortResearch (now Plant & Food Research) Botrytis Prediction Model, showing GWRDC and NZWG contributions to model calibration, delivery system design and weather networks.

Sub-project B: Bunch Rot Database for Decision Support

The overall aim was to utilise information generated in sub-project A to define variables for a bunch rot database for further development by Australian researchers and industry, allowing for continuous improvement of management strategies for bunch rot and for benchmarking.

The performance target was:

A document aimed at grower co-operators, consultants and technical managers in industry containing instructions for measurement of selected variables for the prototype bunch rot database.

General approach

Methods are outlined in detail in each chapter of this report, including quantitative PCR and whole-of-block experimentation developed or applied as part of Katie Dunne's PhD project.

The general approach for the main aim of developing the BDSM was to conduct replicated, small-plot trials in five grape-growing regions (Table 1) for testing various fungicide programs and canopy management techniques for their impact on botrytis severity at harvest. All researchers worked collaboratively to design and implement standard experimental protocols. Data from these trials, including data from non-treated plots, and those from trials conducting by P&FR in New Zealand from 2001 to 2003 were then used to quantify botrytis risk relationships in relation to weather, vine factors and fungicide use. In the third year of the project, the risk relationships identified were integrated into the prototype Botrytis Decision Support Model and further trials were conducted to calibrate the prototype model using different fungicide timings. In Victoria and Tasmania, sampling was extended to the whole vineyard block to develop botrytis monitoring methods required for implementing the BDSM.

Table 1. Number of replicated small-plot trials conducted in each region between 2006-2009.

		Number of trials sites in each region				
Year of vintage	Grapevine variety	Auckland, NZ[1]	Hawke's Bay, NZ	Marlborough, NZ	southern Tasmania	Yarra Valley, Victoria
2007	Sauvignon Blanc	1	1	2	1	1
2007	Chardonnay		1			2
2007	Riesling				1	
2008	Sauvignon Blanc	1	1	2	1	1
2008	Chardonnay		1		1	2
2008	Riesling				2	
2009	Sauvignon Blanc	1	1	1	1	1
2009	Chardonnay					2
2009	Riesling				1	

[1]New Zealand

Chapter 1: Quantitative methods for studies of botrytis bunch rot in grapevines

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1 Summary

Botrytis severity at harvest, together with other disease development processes, must be monitored in a way that allows the factors that influence them to be analysed and modelled quantitatively. Data were obtained from non-treated vine plots from up to 51-site years from three regions in New Zealand (Auckland, Hawke's Bay and Marlborough) and two regions in Australia (Yarra Valley, southern Tasmania). Days from veraison to harvest varied from 28 to 67 days and ≥ 3% mean botrytis severity at harvest was observed at 29 site-years. Variables were defined to describe weather, pathogen and vine factors that could be used for precise and consistent evaluation of the effects of experimental treatments applied in vineyard trials, and for description and prediction of disease progress in relation to causative factors. Variables measuring pathogen inoculum were the incidence of berries with latent *B. cinerea* at pre-bunch closure and the number of floral debris pieces colonised by *B. cinerea*. The vine variables, which could be measured routinely within the context of commercial vineyard operations, were grape yield as a measure of crop load and leaf layer number as an indirect measure of canopy density. Bunch weight also had potential to serve as a proximate measure of bunch compactness. Standard measurement of weather variables was also described, including the Bacchus index which was utilised for the prototype Botrytis Decision Support Model (Beresford *et al.* (2009) Appendix 5). The utility of the Area Under the Disease Progress Curve (AUDPC) and logistic models of disease progression were examined as a means for understanding how treatments impacted on a botrytis epidemic. The highest rate parameter for 30 botrytis epidemics was 0.25. AUDPC sometimes resulted in greater statistical separation of treatment effects than botrytis severity at harvest. Adoption of these quantitative methods will allow continued collection of compatible data, further refinement of the relationships defined here, and comparison of new and existing treatments for botrytis control across multiple sites and regions.

2 Introduction

Research into management strategies for botrytis bunch rot (botrytis) requires a consistent methodology to allow effects of experimental disease control treatments to be compared and to analyse vineyard and climatic factors that influence botrytis development. This methodology needs to be based on sound epidemiological principles and must be able to be readily applied in any grape growing region.

Even though botrytis appears relatively late in the growing season, infection may have established as early as the onset of flowering (McClellen and Hewitt 1973; Keller *et al.* 2003; Viret *et al.* 2003). Therefore, factors that affect disease progression need to be considered from no later than the beginning of cap fall (E-L stage 19; Coombe 1995) until grapes are ready for winemaking. In grapevines, there are two key processes by which *B. cinerea* infects fruit and which are able to be monitored using vineyard sampling and laboratory incubation methods. The first is latent infection of fruit (pathways I, IIa and IIb of Elmer and Michailides 2004), which occurs from flowering onwards. *B. cinerea* infects flowers or fruit but is inhibited by the natural resistance of the green grape berries (Hill *et al.* 1981; Stein and Blaich 1985; Jeandet *et al.* 1991; Bais *et al.* 2001, Bezier *et al.* 2002). When berry ripening occurs, the pathogen resumes growth and causes bunch rot. The second process involves the survival of *B. cinerea* as a saprobe on senescent tissues, including floral debris, stems and leaves, followed by infection of the ripening fruit after veraison by conidia or mycelia (pathways III, IV and V of Elmer and Michailides 2004). This second process involves *B. cinerea* as an opportunistic 'wound' pathogen that can gain entry through natural openings (Blaich *et al.* 1984; Mlikota Gabler *et al.* 2003) or wounds created by insects, birds, frost, hail, sunburn or the powdery mildew fungus (Gadoury *et al.* 2007).

Botrytis severity at harvest is a key variable of interest to grape growers and wine makers, because it influences the price for grapes and reflects the negative effect that botrytis has on wine quality. This variable, together with other disease development processes, must be monitored in a way that allows the factors that influence them to be analysed and modelled quantitatively. Variables influencing disease development must also be practical to measure in the vineyard, meaning that indirect measures of a process or influential factor may have to be obtained using surrogates for the variables of interest because of time and cost constraints.

The methods described here were developed to study botrytis bunch rot epidemiology and control in five geographic regions in Australia and New Zealand. Variables were defined to describe weather, pathogen and vine factors that could be used for precise and consistent evaluation of the effects of experimental treatments applied in vineyard trials, and for description and prediction of disease progress in relation to causative factors. Data from trials conducted in New Zealand prior to GWRDC project UT0601 were included in this study to increase the sample size for statistical analyses. Results of specific disease control treatments and/or application of factors or indices for estimation of botrytis risk are detailed elsewhere in this report.

3 Materials and methods

3.1 Trial design, sites and weather data

Botrytis severity and vine development were monitored in 51 small-plot trials carried out in two regions of Australia for three growing seasons and three regions of New Zealand for six growing seasons (Table 1). Each trial consisted of treatment plots comprising 5–7 vines in a panel (bay) and 5–8 replicates of each treatment, arranged in a randomised complete block. Experimental treatments included a control with no botrytis fungicides applied to vines and

other treatments such as the number and timing of fungicide applications and various canopy manipulations.

Weather stations were placed outside the vineyard block, rather than within vine canopies to ensure that the weather readings were standardised and not influenced by the peculiarities of individual canopies, and to avoid chemical spray deposits, which can alter the performance of surface wetness sensors. Stations were also positioned so that the nearest trees, buildings or other large objects were no closer than three times their height from the weather station. Sensors of electronic data loggers were located 1.4-1.6 m above ground level with temperature probes housed within a Stevenson screen or a suitable "stacked plate" thermometer screen. Tipping bucket rain gauges were adjusted to tip every 0.1 or 0.2 mm rainfall and the surface wetness sensing grid was adjusted to an angle of 10^{o} to prevent excess water pooling or run-off. The sensor scan interval was 1 min and the maximum recording interval was 10 min for calculation of hourly averages.

Surface wetness and temperature during wet periods were used to calculate the Bacchus risk index for each hour that plant surfaces remained wet (Kim *et al.* 2007). The hourly value of the Bacchus risk index is determined by a quadratic temperature response, with an optimum around 20°C. Hourly values were then summed for the duration of the wet period and the sums for all the wet periods within specified interval were averaged.

Table 1. Small-plot trials according to region, year of vintage and grape variety. The grape varieties were Chardonnay (C, 14 site-years), Pinot Noir (P, 1 site-year), Riesling (R, 5 site-years), Sauvignon Blanc (S, 29 site-years) and Semillon (Se, 2 site-years). The number subscript refers to a specific vineyard for that variety and region. Site-years in **bold** had ≥ 3% mean botrytis severity for non-treated plots at harvest.

Region	Year of vintage						Number of site-years	
	2001 (01)	2002 (02)	2003 (03)	2007 (07)	2008 (08)	2009 (09)	Total	≥ 3% botrytis severity
New Zealand								
Auckland (A)	**S_1**	**S_1**	**S_1**	**S_1**	S_1	**S_1**	6	5
Hawke's Bay (H)	**S_1**	**S_1**	**C_1, C_2, C_3, S_1, Se_1, Se_2**	**C_4, S_2**	**C_5, S_2**	**S_2**	13	13
Marlborough (M)	S_1	S_1	C_1, C_2, P_1, **R_1, S_1, S_2, S_3,** S_4	S_5, S_6	S_5, S_6	**S_5**	15	5
Australia								
southern Tasmania (T)	-	-	-	R_1, ***S_1***	**C_1**, **R_1**, R_2, ***S_1***	***R_1***, ***S_1***	8	6
Yarra Valley, Victoria (V)	-	-	-	C_1, C_2, S_1	C_2, C_3, S_1	C_2, C_3, S_1	9	0
Total site-years	3	3	15	10	12	8	51	29

3.2 Analysis of disease severity and progress

Botrytis incidence and severity. Unless specified otherwise, botrytis percentage severity was estimated for the same 20–50 randomly selected bunches per plot at 1–2 week intervals from veraison until harvest, based on the method of Beresford *et al.* (2006). The percentage area of berry tissue per bunch, viewed from the side, with botrytis symptoms was assessed for each bunch with the aid of a standard area diagram (Hill *et al.* 2010 or R. Emmett, DPI Vic., personal communication). Symptoms were assigned to botrytis if there were brown or pink-brown (discoloured) berries that were turgid or shrivelled, in a manner characteristic of botrytis rot, and if *B. cinere*a sporulation (grey mould) was visible on at least some berries or pedicels of the bunch. A separate assessment of the percentage surface area of berry tissue per bunch with sporulating *B. cinerea* was made if the effect of treatments or environmental conditions on sporulation were of interest. Disease incidence was derived from the data for severity.

Area under the disease progress curve. Each epidemic for all treatments at 15 site-years was described as the area under the plot of the mean percentage botrytis severity versus date of assessment, which was approximated by the midpoint rule and termed the area under the disease progress curve (AUDPC; Shaner and Finney 1977; Campbell and Madden 1990). AUDPC was calculated per plot to allow ANOVA of treatment means. The *P*-value resulting from one-way ANOVA of data from experimental treatments for each site-year was compared for the mean AUDPC per plot and the logit of the mean botrytis severity at harvest to determine which variable gave greater statistical separation of treatments.

Modelling disease progress. Botrytis epidemics usually show exponential increase (Beresford *et al.* 2006). Because percentage disease severity is theoretically bounded at 100%, a logistic model is conceptually more robust than an exponential model for describing botrytis disease progress (Campbell and Madden, 1990). In practice, severity seldom exceeds 40% in both field experiments and commercial vineyards. Disease progression in non-treated plots was quantified for 30 site-years (Table 5). At each assessment date, the mean percentage botrytis severity per bunch for each treatment was logit-transformed according to the equation:

$$\textit{logit severity} = \ln((\%\textit{severity} + 0.1) / (100.1 - \%\textit{severity}))$$

Linear regression of logit severity and date expressed as days from 1 January 1900 (Microsoft® Excel data convention) was calculated using GenStat version 11.1 (VSN International Ltd). The slope and intercept of the linear model described the rate and location in time of individual epidemics. Back-transformation of fitted logit severity was used to interpolate disease severity for any date during an epidemic or to estimate the date at which a certain severity was reached, using the following equation:

$$\textit{Back-transformed severity} = 100.2 / e^{(-\textit{logit severity})} - 0.1$$

3.3 Measurement of vine factors

Unless stated otherwise, linear regression (GenStat version 11.1) was used to determine the relationship between each variable described below and the mean botrytis severity at harvest in non-treated plots.

Vine phenology. Key stages of grapevine phenology, relevant to the development of botrytis bunch rot or timing of botrytis control treatments, were recorded at each trial site. The date and modified E-L number (Coombe 1995) of the following grapevine growth stages were recorded: 5% capfall (E-L 19-20), pre-bunch closure (PBC, E-L 31-32), veraison (E-L

34) and harvest (E-L 38). Other growth stages, such as 80% capfall (E-L 25) and pea-size berries (E-L 31) were recorded if experimental treatments were applied at these stages. The 5% and 80% cap fall stages were estimated according to the method of Evans and Gadoury (2008). The late-season interval, defined as the days from veraison to harvest, was recorded because this study was conducted across regions where large differences in this variable were likely to exist.

Vine canopy density. Canopy density was estimated as the leaf layer number (LLN) at veraison, measured by the point quadrat method developed for use by grape growers (Smart and Robinson 1991). In each experimental plot of trials at 16 site-years, a thin metal rod was inserted into the canopy ten times at the height of the fruiting zone and at 10-cm intervals. LLN was the total number of leaf contacts divided by the number of insertions.

Grape yield and bunch weight. The yield of grapes from the central vine per plot was determined near the date of commercial harvest for trials at 23 site-years by counting and weighing bunches to derive the mean bunch weight and total weight of grapes per vine.

Bunch position on the vine. The effect of bunch position on botrytis severity near the date of commercial harvest was examined using non-treated control plots in trials at six site-years in Tasmania. A sample of 14–16 bunches per plot were randomly selected from those at the first (basal) bunch position on vertically trained shoots (position 1). Another 14–16 bunches per plot were randomly selected from those positioned directly above the basal bunch (position 2). Botrytis severity per bunch was logit transformed and data for each bunch position was pooled across six plots (84–96 bunches per bunch position). The difference in location between the two samples was tested using the nonparametric Mann-Whitney U Test in GenStat version 11.1 (VSN International Ltd) because the data did not approximate a normal distribution.

Bunch openness. Bunch openness, as a percentage, was estimated at veraison for 11 site-years by a water displacement method (Shavrukov et al. 2003). Mean bunch compactness at a site was estimated by sampling one bunch from an end vine of each plot and alternating in the selection of a basal bunch or a distal bunch (refer to bunch position above) at each sample location.

3.4 Measurement of pathogen inoculum

Unless stated otherwise, linear regression (GenStat version 11.1) was used to determine the relationship between each variable described below and the mean botrytis severity at harvest in non-treated plots.

Latent *B. cinerea* infection of grape berries. Latent infection within grape berries was measured at pre-bunch closure by freezing and surface sterilising 20 berries from each of five or ten bunches selected at random from each non-treated control plots of trials from 44 site-years. Whole bunches were cut from the vine into polythene bags which were frozen as soon as possible at -18^{o}C for a minimum of 24 h. After freezing, bunches were surface sterilised with 70% ethanol for 10 s, followed by 1% sodium hypochlorite for 1 min. Twenty whole berries, with pedicels attached, were cut from each bunch onto a moist paper towel and placed in a plastic ‘fast food’ container (17.5 x 12 x 4 cm, approx. 500 ml) with a lid that allowed some air movement. A piece of ‘rug hold’ mesh underlay (1 x 1 cm), used to prevent carpet rugs from slipping, was placed on the paper towel and the berries were placed into the cells created by the ‘rug hold’ to prevent them rolling. The trays were placed on a laboratory bench at room temperature (15–20^{o}C) with exposure to daylight through windows, but not

direct sunlight. After a minimum of 6 days incubation, the incidence of berries with sporulating *B. cinerea* was recorded, with the aid of a stereomicroscope. Further assessments were made until the maximum incidence of berries expressing *B. cinerea* was observed, with the final assessment occurring within 12 days, before sporulation on berries was the result of the secondary spread of *B. cinerea*.

Survival of *B. cinerea* in bunch debris. It was assumed that a significant proportion of inocula contributing to survival of *B. cinerea* as a saprobe was likely to originate from *B. cinerea* on senescent floral debris trapped within grape bunches (Seyb 2004). Therefore, the amount of floral debris and its degree of colonisation by *B. cinerea* was assessed. Each of five of the grape bunches per non-treated control plot selected for detection of latent infection at pre-bunch closure, for trials from 10 site-years, were shaken inside separate polythene bags to dislodge the floral debris. This debris was spread, without freezing, on moist paper towels in plastic trays and incubated as described previously for the detection of latent infection. After 6–10 days, floral debris were categorised as green berries, necrotic aborted berries, calyptrae (floral caps) and 'other' debris, including stamens. The total number of calyptrae and aborted, necrotic berries per bunch with sporulating *B. cinerea* was counted with the aid of a stereomicroscope. Green berries rarely yielded sporulating *B. cinerea* (without freezing) and 'other' debris contributed little *B. cinerea* (data not presented). *B. cinerea* present within bunches as a saprobe was expressed as the total number of calyptrae and aborted berries showing sporulation.

4 Results

4.1 Botrytis epidemics in relation to region, vine phenology and weather

Mean botrytis severity at harvest for each region varied from 19.5% in Auckland to 0.6% in the Yarra Valley of Victoria (Table 2). The highest mean botrytis severity at harvest was 40% at site-year $H_{03}C_2$ (Hawke's Bay Chardonnay, Table 1). The mean botrytis severity at harvest was ≥ 3% for 29 of the 51 site-years, including all but one site-year from Auckland and Hawke's Bay, and 33% and 75% of site-years from Marlborough and Tasmania, respectively (Table 1, Figure 1). All nine site-years from Victoria had a mean botrytis severity at harvest of < 3%. Site-years in Victoria also had the earliest median dates for capfall and harvest, the shortest median interval for the number of days from veraison to harvest, and the highest mean daily maximum temperature between 1 November and 30 April (Table 2).

The number of days from veraison to harvest for the 51 site-years varied from 28 to 67 days, with a median interval of 43 days. The median late-season interval per region accounted for 83% of the variance in the mean (for the region) botrytis severity at harvest with the latter increasing exponentially with increasing late-season interval (Figure 2). Mean harvest severities exceeding 3% were predicted to occur when the median (regional) late-season interval exceeded 37.4 days.

The effect of various weather variables on botrytis severity at harvest among the five regions was reported by Beresford and Hill (2008). A modified table (Table 3) from that paper is presented to illustrate that regional means for botrytis severity at harvest are correlated significantly to regional mean Bacchus indices for specific crop-stage intervals. The mean Bacchus index over the early- season interval explained a greater proportion of the variation in harvest severity than the indices over either the mid- or late- season intervals (Table 3). The Victorian data for the early-season relationship (Figure 3) indicated a relatively high Bacchus index value.

Table 2. Mean botrytis severity at harvest, grapevine phenology and selected climatic variables summarised between 1 November and 30 April for 51 site-years, according to region. Numbers of sites and years studied in each region are shown in Table 1. Climatic data for the years studied in each region came from, for New Zealand, the National Institute of Water and Atmosphere (NIWA) (http://cliflo.niwa.co.nz/) and for Australia, from the Bureau of Meteorology (BOM) (www.bom.gov.au/climate/data/).

Region	Mean botrytis severity at harvest (%)	Median date		Median interval (days)			Mean daily max. & min. temp. (°C)	Total rainfall (mm)
		5% capfall	harvest	5% capfall to PBC	PBC to veraison	veraison to harvest		
New Zealand								
[1]Auckland	19.5	7 Dec.	6 Apr.	43.5	37.5	44	21.8; 12.9	496
[2]Hawke's Bay	12.4	26 Nov.	8 Apr.	45	37	51	21.8;11.0	273
[3]Marlborough	4.6	1 Dec.	8 Apr.	45	39	43	21.5; 10.9	236
Australia								
[4]southern Tasmania	3.4	1 Dec.	28 Mar.	48	27	38	20.9; 10.9	226
[5]Yarra Valley, Victoria	0.6	10 Nov.	1 Mar.	46	35	29	24.9; 12.0	290

[1]NIWA agent no. 2006, Pukekohe EWS (altitude, 88m)
[2]NIWA agent no. 15876, Whakatu EWS (altitude, 5m)
[3]NIWA agent no. 12430, Blenheim research EWS (altitude, 4m)
[4]BOM station no. 094008, Hobart airport (altitude, 4m)
[5]BOM station no. 086104, Scoresby Research Institute (altitude, 80m)

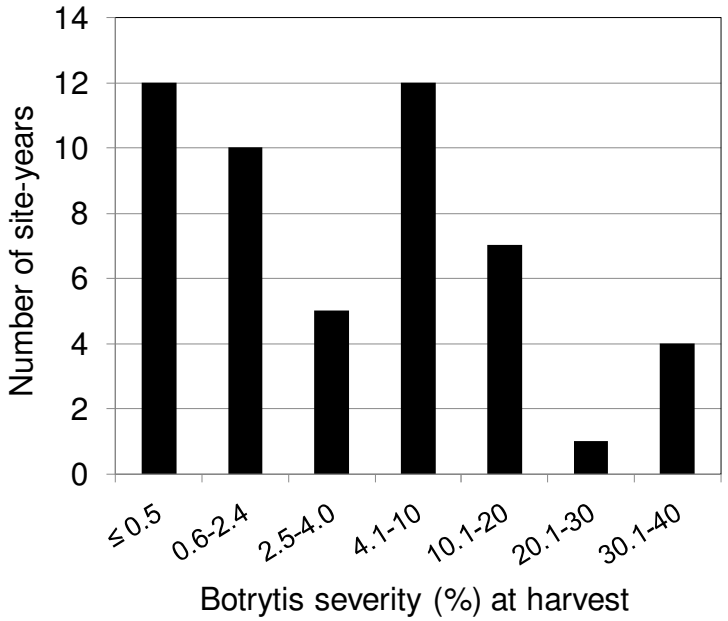


Figure 1. Frequency of sites in each category of botrytis severity at harvest for 51 site-years.

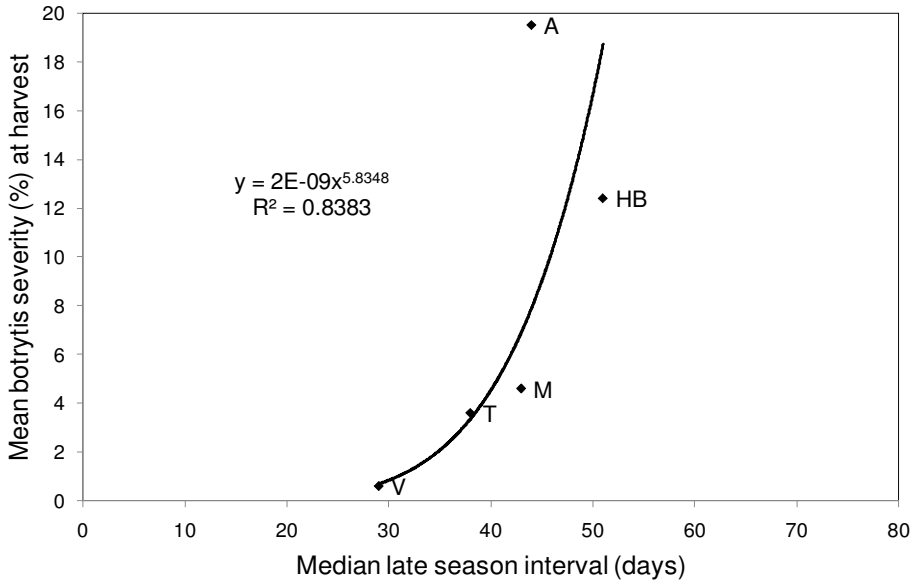


Figure 2 Median late-season interval versus mean harvest botrytis severity at harvest for 51 site-years. Regions are, Victoria (V), Tasmania (T), Marlborough (M), Hawke's Bay (HB) and Auckland (A).

Table 3. Parameters for the linear regression (significant at $P = 0.05$) of regional means of botrytis severity at harvest on the mean Bacchus index for three crop-stage intervals, using 44 site-years of data from five regions (Auckland, Hawke's Bay, Marlborough, southern Tasmania and Yarra Valley, Victoria). Table modified from Beresford and Hill (2008).

Crop-stage interval	Slope	Intercept	R^2	Predicted mean Bacchus index at 3% severity
5% capfall to pre-bunch closure (early season)	38.287	-19.009	0.74	0.57
pre-bunch closure to veraison (mid season)	29.607	-12.680	0.42	0.53
veraison to harvest (late season)	32.652	-16.573	0.58	0.60

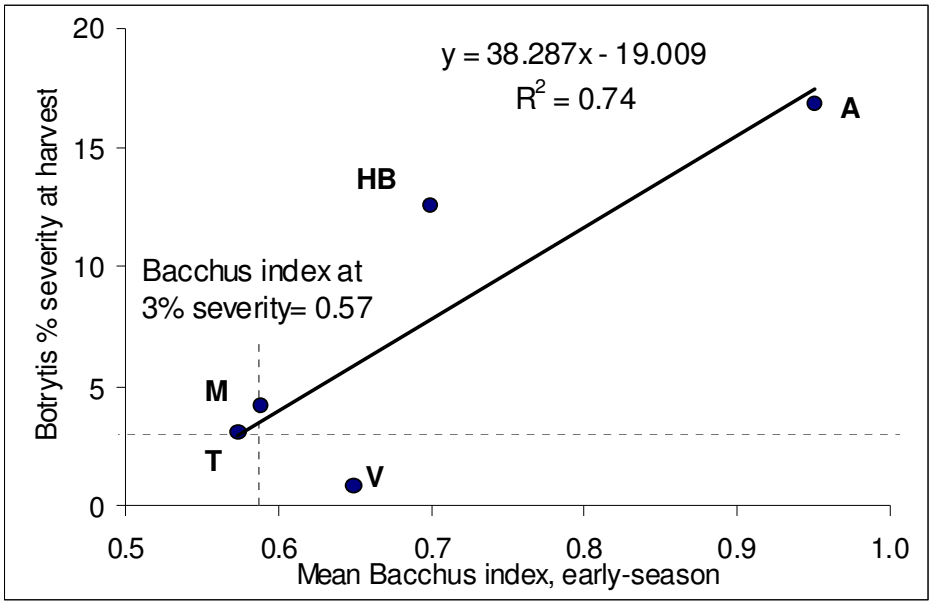


Figure 3. Regional means of early-season Bacchus index versus harvest botrytis severity. Regions are, Victoria (V), Tasmania (T), Marlborough (M), Hawke's Bay (HB) and Auckland (A). Figure reproduced from Beresford and Hill (2008).

4.2 Utility of AUDPC as a response variable

Based on the P-value for the F-statistic from ANOVA, statistical separation of treatments using either the logit of the mean botrytis severity at harvest ('harvest severity') or AUDPC as the disease severity variable was equivalent for nine out of 15 trials where 'harvest severity' ranged from 0.8 to 25% (Table 4). Either variable separated the treatments when large treatment differences resulted in $P < 0.001$ (six trials). When $P \geq 0.001$, AUDPC resulted in a lower *P*-value than logit 'harvest severity' for five trials (1–6.4% 'harvest severity'). Two of these five trials were at the same site over two years ($T_{07}R_1$, $T_{08}R_1$) where AUDPC resulted in $P < 0.05$, whereas logit 'harvest severity' resulted in $P > 0.05$. Logit 'harvest severity' resulted in a lower *P*-value than AUDPC at site $T_{08}S_1$, although both *P*-values were < 0.05.

Table 4. Statistical detection of treatment effects in small-plot vineyard trials with replicated treatments using either logit mean botrytis severity or AUDPC as the response variable for data from 15 site–years. Bold text indicates where *P* values appear to differ numerically.

Site-year[1]	Highest mean botrytis severity[2] (%)	ANOVA residual df	*P* value logit botrytis severity	*P* value LN AUDPC
$V_{08}C_2$	0.8	36	<0.001	<0.001
$M_{08}S_5$	2.1	35	<0.001	<0.001
$V_{08}C_3$	2.7	28	<0.001	<0.001
$H_{08}S_2$	8.4	12	<0.001	<0.001
$H_{08}C_4$	9.5	15	<0.001	<0.001
$A_{07}S_1$	25.3	28	<0.001	<0.001
$H_{07}S_2$	4.5	12	**0.001**	**<0.001**
$V_{08}S_1$	2.3	36	0.005	0.005
$A_{08}S_1$	1.6	28	**0.007**	**0.042**
$H_{07}C_4$	9.6	12	0.007	0.008
$T_{08}R_2$	1.9	35	0.094	0.110
$T_{07}R_1$	3.8	30	**0.165**	**0.045**
$M_{08}S_6$	1.5	35	**0.224**	**0.138**
$T_{08}R_1$	6.4	35	**0.231**	**0.041**
$M_{07}S_6$	1.0	56	**0.335**	**0.162**

[1]Refer to Table 1 for site-year codes.
[2]for a treatment in the field trial, usually the non-treated control.

4.3 Analysis of disease progress

The linear regressions to generate disease progress curves (logistic models) for 27 of 30 botrytis epidemics were significant ($P < 0.05$) and R^2 values were > 0.90 for 18 of these analyses (Table 5). The highest value of the rate parameter (Campbell and Madden 1990), or slope of the linearized disease progress curve, was 0.248 for site-year $M_{03}S_1$, which also had 12% botrytis severity at harvest.

Table 5. Regression parameters for logit mean severity of botrytis in non-treated plots, as a function of days from 1 January 1900[1] for data from 30 site-years.

Site-year[2]	n[3]	Slope	Intercept	*P*-value	R^2
$A_{01}S_1$	6	0.038	-1402	0.006	0.847
$A_{02}S_1$	6	0.094	-3504	<0.001	0.977
$A_{03}S_1$	6	0.085	-3223	<0.001	0.994
$A_{07}S_1$	5	0.168	-6574	<0.001	0.980
$A_{08}S_1$	5	0.076	-3005	0.023	0.815
$A_{09}S_1$	6	0.062	-2462	0.007	0.836
$H_{01}S_1$	4	0.081	-3010	0.001	0.997
$H_{02}S_1$	6	0.080	-2981	0.012	0.782
$H_{03}S_1$	3	0.043	-1617	0.294	0.602
$H_{07}C_4$	4	0.039	-1530	0.115	0.674
$H_{07}S_2$	5	0.060	-2342	0.002	0.959
$H_{08}C_5$	4	0.124	-4908	0.063	0.817
$H_{08}S_2$	4	0.111	-4372	0.009	0.974
$H_{09}S_2$	5	0.091	-3618	0.002	0.959
$M_{01}S_1$	6	0.006	-243	0.027	0.682
$M_{02}S_1$	6	0.031	-1172	0.015	0.758
$M_{03}S_1$	4	0.248	-9362	0.026	0.924
$M_{08}S_2$	5	0.117	-4622	0.004	0.941
$M_{08}S_5$	4	0.119	-4692	0.009	0.974
$M_{09}S_5$	7	0.072	-2880	0.001	0.885
$T_{07}S_1$	4	0.132	-5183	0.018	0.947
$T_{08}C_1$	6	0.092	-3631	0.009	0.812
$T_{08}R_1$	7	0.094	-3706	<0.001	0.977
$T_{08}R_2$	4	0.090	-3571	0.022	0.934
$T_{08}S_1$	3	0.205	-8105	0.100	0.951
$T_{09}R_1$	7	0.111	-4423	<0.001	0.903
$T_{09}S_1$	5	0.115	-4610	0.016	0.856
$V_{08}C_2$	6	0.100	-3960	0.001	0.930
$V_{08}C_3$	5	0.074	-2917	0.003	0.953
$V_{08}S_1$	6	0.067	-2647	<0.001	0.948

[1] Microsoft Excel data convention.

[2] Refer to Table 1 for site-year codes.

[3] n = number of time points when assessments made

4.4 Vine factors affecting disease severity

Vine canopy density. The mean leaf layer number (LLN) ranged from 1.3-5.2 across 16 site-years (Fig. 4a). The mean LLN accounted for 18% of the variance in botrytis severity at harvest according to the positive and marginally significant linear regression (Fig. 4a).

Grape yield and bunch weight. The mean yield of grapes per vine at harvest ranged from 0.88 to 10.2 kg across 23 site-years (Fig. 4b). The mean yield of grapes per vine accounted for 20% of the variance in botrytis severity at harvest in non-treated plots according to the positive and significant linear regression (Fig. 4b).

The mean weight of bunches per vine at harvest ranged from 50 to 176 g across 23 site-years. There was no apparent relationship between mean bunch weight and botrytis severity at harvest in non-treated plots. Linear regression of logit botrytis severity in non-treated plots against bunch weight was not significant (P = 0.117, R^2 = 0.07; n = 23). In contrast, Beresford *et al.* (2009, Appendix 5) reported a significant relationship between bunch weight and botrytis severity at harvest when bunch weight was subjected to a logarithmic transformation.

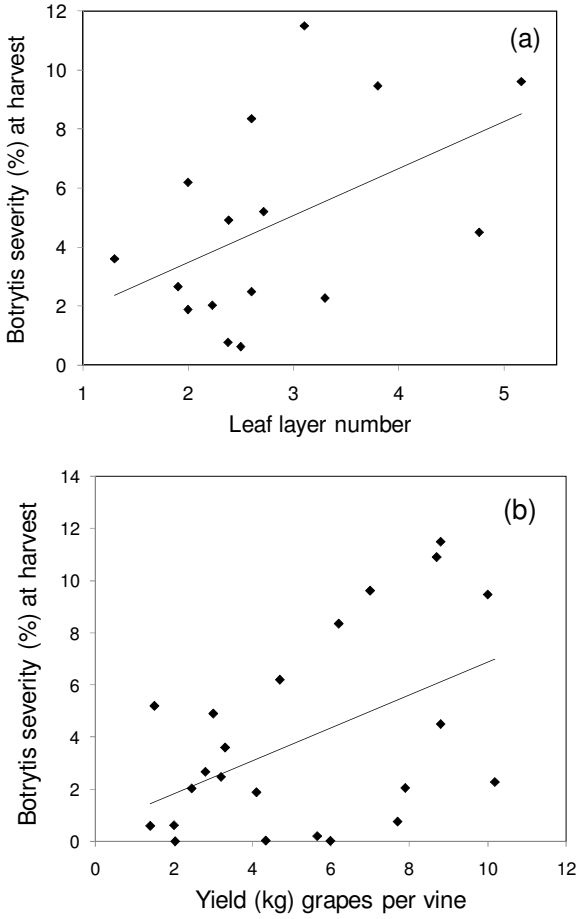


Figure 4. The relationship between (a) grapevine canopy leaf layer number, or (b) yield (kg) of grapes per vine and botrytis severity at harvest in non-treated plots. According to linear regression: (a) $y = 0.31x + 1.59$; $R^2 = 0.18$; $P = 0.058$; n = 16, and (b) $y = 0.631x + 0.57$; $R^2 = 0.20$; $P = 0.019$; n = 23. The site-years were (a) $H_{07}C_4$, $H_{07}S_2$, $H_{08}C_5$, $H_{08}S_2$, $H_{09}S_2$, $T_{07}R_1$, $T_{07}S_1$, $T_{08}C_1$, $T_{08}R_1$, $T_{08}R_2$, $T_{08}S_1$, $T_{09}R_1$, $T_{09}S_1$, $V_{08}C_2$, $V_{08}C_3$ and $V_{08}S_1$, and (b) $H_{07}C_4$, $H_{07}S_2$, $H_{08}C_5$, $H_{08}S_2$, $H_{09}S_2$, $M_{07}S_5$, $M_{07}S_6$, $M_{08}S_5$, $M_{09}S_5$, $T_{07}R_1$, $T_{07}S_1$, $T_{08}C_1$, $T_{08}R_1$, $T_{08}R_2$, $T_{08}S_1$, $T_{09}R_1$, $T_{09}S_1$, $V_{08}C_2$, $V_{08}C_3$, $V_{08}S_1$, $V_{09}C_2$, $V_{09}C3$ and $V_{09}S_1$.

Bunch position on the vine. The position of the bunch on vertically trained shoots influenced botrytis severity at harvest for non-treated bunches in four of six trials conducted in Tasmania (Table 6). The mean botrytis severity on bunches at position 1 (basal) was significantly lower ($P < 0.05$) than observed at position 2 for three trials using Riesling grapes and significantly higher than observed at position 2 for one trial using Sauvignon Blanc grapes. Mean botrytis severity on bunches at position 1 was also higher than observed at position two for Chardonnay bunches, but the difference was marginally significant ($P = 0.079$).

Bunch openness. The mean percentage bunch openness on a per site-year basis ranged from 25 to 75% across 11 site-years. There was no relationship between mean percentage bunch openness and botrytis severity at harvest in non-treated plots ($P > 0.05$).

Table 6. Difference in mean botrytis severity between bunches sampled according to position on shoots. Position 1 is the position of the basal bunch and position 2 is the bunch position above the basal bunch on vertically positioned shoots. Bunches were sampled from non-treated control plots and six site-years in Tasmania.

	Bunch position						
	One (1)		Two (2)				
Site-year[3] (Tasmania)	Mean logit severity	Mean severity (%)	Mean logit severity	Mean severity (%)	number of bunches per position	U[1] statistic for logit severity	*P*-value[2] 1 = 2
$T_{07}R_1$	-6.39	0.6	-6.70	0.7	90	3663	0.029
$T_{08}R_1$	-4.63	5.6	-4.15	6.7	90	3511	0.112
$T_{09}R_1$	-5.12	3.6	-4.20	6.2	96	3539	0.004
$T_{08}R_2$	-5.96	3.9	-5.22	4.6	90	3136	0.004
$T_{07}S_1$	-4.79	3.5	-5.74	1.4	90	2937	<0.001
$T_{08}C_1$	-3.98	4.7	-4.46	2.5	84	2984	0.079

[1]Mann-Whitney U test for differences in location between two samples of data.
[2]Adjusted for ties.
[3]Refer to Table 1 for site-year codes

4.5 Relationships between pathogen inoculum and disease severity

Latent *B. cinerea* infection in grape berries. The mean incidence of latent infection at PBC ranged from 0–38% across 44 site-years, with 30 site-years resulting in a mean incidence of ≤ 5%. There was a weak but marginally significant linear relationship between the logarithm of the mean incidence of latent *B. cinerea* at PBC and the logit mean severity of botrytis at harvest (Fig. 5a). The six site-years that had greater than 15% incidence of berries with latent botrytis at PBC (Fig. 5a) also had more than 3% botrytis severity at harvest (3% = a logit of –3.444).

Survival of *B. cinerea* in bunch debris. The mean number of pieces of floral debris colonised by *B. cinerea* per bunch at PBC ranged from 1.4–45 across 10 site-years (Fig. 5b). The number of debris pieces colonised by *B. cinerea* accounted for 32% of the variance in botrytis severity at harvest according to the positive and marginally significant linear regression (Fig. 5b).

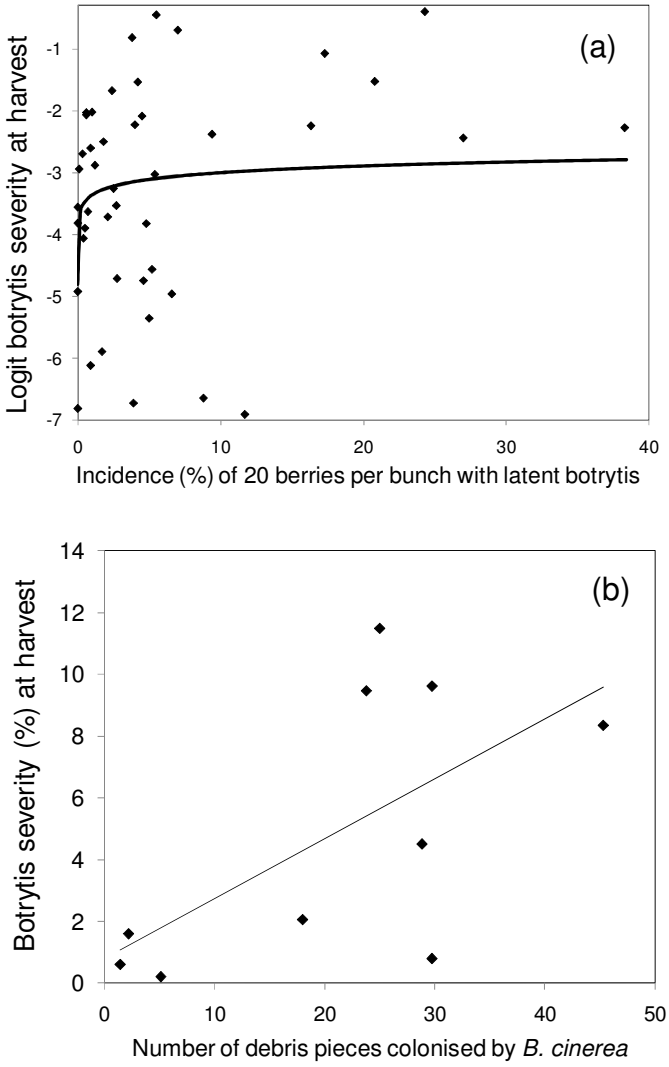


Figure 5. The relationship between (a) incidence (%) of 20 berries with latent *B. cinerea* at pre-bunch closure, or (b) number of debris pieces colonised by *B. cinerea* at pre-bunch closure and botrytis severity at harvest. According to linear regression: (a) $y = 0.359\log_{10}x - 3.359$; $R^2 = 0.006$; $P = 0.059$; n = 44, and (b) $y = 0.194x + 0.80$; $R^2 = 0.32$; $P = 0.052$; n = 10. The site-years were (a) all 51 site-years excluding $A_{01}S_1$, $H_{01}S_1$, $M_{01}S_1$, $M_{07}S_5$, $Y_{07}C_1$, $Y_{07}C_2$ and$Y_{07}S_1$, and (b) $A_{08}S_1$, $H_{07}C_4$, $H_{07}S_2$, $H_{08}C_5$, $H_{08}S_2$, $H_{09}S_2$, $M_{07}S_5$, $M_{07}S_6$, $M_{08}S5$ and $M_{08}S_6$.

5 Discussion

This research revealed the status of botrytis epidemics from a large number of sites, seasons and regions that span the likely range of botrytis severity at harvest in cool-climate regions of southern Australia and New Zealand. Assuming that 3% botrytis severity is a common threshold level for grape price penalties, then more than half the sites in this study would have experienced a negative economic impact if the unsprayed control plots in the study represented disease risk in a commercial situation. These results demonstrated that botrytis control was almost always required at the site-years in Auckland and Hawke's Bay, and was often required at sites in Marlborough and southern Tasmania. The site-years in Victoria were studied during a period of protracted drought (2006-2009) culminating in one of the

hottest and driest harvest periods (2009) in recorded history. A return to weather patterns in Victoria closer to the long-term average might result in a greater risk of botrytis in some years.

It was confirmed that the logistic model provided a reliable description of the location (in time) and rate of disease progression for most botrytis epidemics. Another epidemiological descriptor, the area under the disease progress curve (AUDPC), is a variable that accounts for both the severity and duration of an epidemic. Comparison of this variable with the logit of mean botrytis severity at harvest revealed that it occasionally provided greater statistical separation of treatments applied to small-plot trials when *P*-values for at least one of the response variables were > 0.001 but < 0.05 (eg. sites $T_{07}R_1$ and $T_{08}R_1$). Treatments with large differences in AUDPC, but smaller differences in botrytis severity at harvest, are likely to occur when one or more treatments delay the onset of the epidemic, but the epidemic then develops at a greater rate relative to other treatments. Conversely, differences in the location of the epidemics in time might lead to treatments with similar AUDPC, but larger differences in harvest severity. The multiple disease assessments required for calculation of AUDPC makes it more labour intensive than assessment of botrytis severity at harvest. Collection of such data may not always be warranted for identifying the most effective treatments for botrytis control, but the AUDPC and other epidemiological descriptors derived from the disease progress curve, such as the rate parameter and time of occurrence of a specified disease severity, are likely to be useful for understanding how treatments impact on the botrytis epidemic.

The wide range of botrytis severities observed in this study provided sufficient data for identifying variables correlated to botrytis severity at harvest. It has often been assumed that prolonging the ripening period for grapes in cool climates increases the risk of severe botrytis rot if the crop is exposed to multiple rain events. The strong regional relationship found in this study between mean late-season interval and mean harvest botrytis severity appears to agree with that assumption. However, ripening period could not be isolated as a causative factor, because several regional climatic factors were correlated with the regional differences in botrytis severity. Accumulated rainfall and the number of days with rain were correlated significantly with botrytis severity at harvest for 44 site-years (Beresford and Hill 2008); however, these variables were also correlated to the late-season interval. Mean daily rainfall in the late-season interval, which removes the time element, was also correlated to botrytis severity at harvest. Nevertheless, areas with long late-season intervals can also experience dry weather conditions. The Bacchus index, which deals with the interaction between temperature and surface wetness and which was demonstrated to influence botrytis severity from the early season onwards, has since been used extensively in developing algorithms for the Botrytis Decision Support Model (Beresford *et al.* (2009), Appendix 5).

None of the individual pathogen or vine factors accounted for more than 32% of the variance in botrytis severity at harvest, suggesting that multiple factors interact to determine botrytis severity at harvest. The weak relationship between the incidence of latent infection at PBC and botrytis severity at harvest demonstrated appropriate methodology but confirmed results of previous studies that latent infection is a poor predictor of botrytis severity at harvest (de Kock and Holz 1991; Dubos and Roudet 2000; Fermaud and Pieri 2000). More site-years with > 15% incidence of berries with latent botrytis at PBC are required to see if they consistently lead to > 3% botrytis severity at harvest. PBC was selected as the standardised crop stage for measuring latent infection so that there would be sufficient time for this information, if appropriate, to be used to support decisions about late-season disease management. Clearly, latent infection needs to be combined with other factors for predictive

purposes (Beresford 2007) and to account for the potential for many infections to remain latent through to harvest (Zitter 2005). There is also potential for latent infection frequency to increase after PBC, as observed in a block of *V. vinifera* variety Gamay in Switzerland where the frequency increased from 20% to 100% between veraison and harvest (Keller et al. 2003). Nair *et al.* (1995) found that a high level of latent infection during a season, regardless of the amount of disease expressed at harvest, was correlated to high levels of carry-over infection at the beginning of the following season. This finding is a reminder that in a perennial crop such as grapevine, inoculum in the current season might have an impact in the subsequent season.

The number of pieces of floral debris colonised by *B. cinerea* per bunch at PBC accounted for a higher percentage of the variance in botrytis severity than latent infection, although data from more sites, years, regions and grapevine varieties, potentially with different rates of debris retention, need to be investigated. This finding was consistent with the report by Seyb (2004) who found positive linear relationships between the number of berries infected per bunch post-véraison and the total number, as well as number infested, of the aborted berries within each bunch. Wounding of fruit can lead to more botrytis rot (eg. Kretschmer et al. 2007) and the extent of berry wounds and splits across sites was not estimated. Thin-skinned berries of Sauvignon Blanc and Riesling at some sites were prone to splitting after sudden and excessive uptake of water (Considine and Kriedemann 1972).

The density of the vine canopy is an indirect measure of bunch microclimate. Bunches surrounded by many leaves experience less air movement, take longer to dry and are at greater risk of botryis infection than those surrounded by few leaves. Differences in leaf canopy density are caused by differences in trellis system, pruning technique and/or canopy manipulation (English *et al.* 1989). Botrytis severity at harvest was related to canopy density, as estimated by leaf layer number (LLN), in this study. Valdéz-Gómez *et al.* (2008) reported a significant relationship between LLN and botrytis incidence at harvest at a single vineyard over 3 years, although the maximum mean incidence of botrytis reported was relatively low at 20%. These results suggest that LLN might be a useful variable for interpreting results within sites where canopy density is manipulated.

Crop load, as measured by the yield of grapes per vine, was related to harvest botrytis severity and is known to influence the development of total soluble solids in berries within a particular site (Bennett *et al.* 2006). However, it was not possible to isolate the effect of total soluble solids on botrytis severity in relation to crop load among sites. Crop load might contribute to bunch crowding within a canopy, which presumably facilitates bunch-to-bunch spread of *B. cinerea* or pooling or condensing of water in natural wells created by adjoining bunches, thus creating a favourable microclimate for fungal colonisation.

It was found that bunch position on vertically trained shoots could influence botrytis severity at harvest. Presumably, significant differences in botrytis severity were related to differences in average berry characters between bunch positions that impacted on the infection efficiency or rate of colonisation by *B. cinerea*. Peterson (1974), for example, reported that berries of basal bunches (position 1) in Cabernet Sauvignon grapevines were heavier and had higher juice soluble solids and lower acidity than those of distal bunches (position 2). At some sites, therefore, bunch position is likely to be a factor contributing to the generally large variation observed among bunches within plots for botrytis severity. This variation was also the reason why the same bunches per plot were assessed over time for generation of the disease progress curve. The additional, but small, task of identifying and noting bunch

position during botrytis assessments could be used to partition variance in ANOVA, which when combined with a factorial design, should aid statistical separation of treatments.

Botrytis severity can be promoted by bunch compactness (Vail and Marois 1991; Vail *et al.* 1998). The relatively time-consuming method of determining bunch openness by volume displacement (Shavrukov *et al.* 2003) was reliable in the sense that it discriminated bunch openness at different positions on vertically positioned shoots (data not presented). The lack of a relationship between bunch openness and botrytis severity at harvest across site-years might have reflected the relatively small sample size, variation among collaborators in the application of the method, and/or large variation among grapevine cultivars for this character. Bunch weight within a particular grapevine cultivar has been correlated positively with bunch compactness in several studies (for example, Vail and Marois 1991; Intrieri *et al.* 2008; Poni *et al.* 2008) and a weak correlation between bunch weight and botrytis severity was observed in this project (Beresford *et al.* (2009), Appendix 5). Bunch weight is measured routinely in many vineyards, either at harvest or pre-harvest for yield prediction (Coombe 1992). The number of berries per cm of rachis has been correlated to bunch compactness, as estimated by the code OIV 204, and to botrytis severity in *Viti*s interspecific hybrid 'Vignoles' (Hed *et al.* 2009), suggesting that comparison of the effects of bunch compactness on botrytis severity within sites is more meaningful than across site-years.

This multi-regional study of botrytis epidemiology in commercial vineyards meant that it was impractical to structure data collected in terms of cultivar, sites and years. Even if variance was partitioned in this way, it is likely that a similar conclusion would be drawn; namely, that multiple factors are correlated to botrytis severity at harvest. This study identified key variables that could be measured cost-effectively, with vine variables, in particular, being measured routinely within the context of commercial vineyard operations. The variables measuring pathogen inoculum were the incidence of berries with latent *B. cinerea* at pre-bunch closure and the number of floral debris pieces colonised by *B. cinerea*. The vine variables were grape yield as a measure of crop load and leaf layer number as an indirect measure of canopy density. Bunch weight also had potential to serve as a proximate measure of bunch compactness. Practical measurement of these variables means that some have been incorporated to the first version of the Botrytis Decision Support Model for predicting the in-season risk of botrytis (Beresford *et al.* (2009) Appendix 5). This model utilises the Bacchus index as the key weather variable. Adoption of these standard quantitative methods will allow continued collection of compatible data, further refinement of the relationships defined here, and comparison of new and existing treatments for botrytis control across multiple sites and regions.

6 Acknowledgements

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Chapter 2: A technique for quantifying the amount of *Botrytis cinerea* DNA in grape berries.

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1 Summary

Quantitative real-time polymerase chain reaction (qPCR) is a laboratory technique used to amplify and simultaneously quantify a targeted DNA molecule. The Grape Genetics Research Unit of USDA-ARS published a TaqMan® qPCR technique for *Botrytis cinerea* which illustrated the potential for using qPCR to identify the relative extent of grape cluster colonisation by *B. cinerea* well before symptoms of bunch rot become evident. In the process of transferring this technique to TIAR at the University of Tasmania, an improved assay was developed in which new probes and primers were designed. An internal control for *Vitis vinifera* DNA was also included to ensure that lack of amplification of *B. cinerea* DNA indicated lack of detection rather than biochemicals inhibiting the PCR. Reliable standard curves for *B. cinerea* DNA in the duplex assay were generated. Reaction efficiencies for *B. cinerea* DNA were estimated to be 81–113%, with accurate quantification of *B. cinerea* DNA for C_t values corresponding to DNA amounts ≥ 350 fg. The assay also worked for 278 of 300 single vine samples from a 2.4 ha block of Chardonnay. *B. cinerea* DNA was quantified accurately for 46 of the 278 samples in which *B. cinerea* DNA was detected. *B. cinerea* DNA as a percentage of total DNA for the 46 samples ranged from 0.004 to 2.5%. The technique has been applied successfully to grape juice and *B. cinerea* DNA has also been amplified successfully from grape berries processed from pre-bunch closure to maturity (to be reported in K. Dunne's thesis). Two potential opportunities for this research tool are (i) further research to provide insight on the timing and extent of colonisation of grape berries after latency for different cultivars and environmental conditions, and (ii) the use of qPCR in the development (calibration) of a more rapid, cost-effective tool for assessing botrytis risk in the vineyard well before harvest.

2 Introduction

Infections of grape berries by *Botrytis cinerea* can be established as early as flowering and months before disease symptoms become visible, usually after veraison. The mechanism by which the fungus resumes growth after latency is unknown, nor is the rate at which the fungus colonises the berry before symptoms become visible. While the amount of latent infection is correlated weakly to botrytis severity at harvest (Chapter 1), the ability to quantify temporal changes in fungal colonisation of grape berries should help identify factors correlated to these changes and help fine-tune decision support tools that are based on measuring the level of disease risk. The late-season component of the Botrytis Decision Support Model (BDSM) is based on knowledge of how the weather determines the shape of the curve that predicts the progression of visible disease. Disease progress curves for 'unseen' botrytis colonisation might allow botrytis risk to be estimated much earlier, thus providing more time to implement changes to botrytis management. Improvements to the quantification of latent infection, which is currently determined by the labour-intensive ONFIT (overnight freezing incubation technique, Chapter 1), might also improve the model relating latent infection to botrytis severity at harvest, especially if other factors such as weather are

considered. If so, then a rapid and cost-effective measure of latent infection could be practically incorporated to the BDSM.

Quantitative real-time polymerase chain reaction (qPCR) is a laboratory technique which is used to amplify and simultaneously quantify a targeted DNA molecule. It enables both detection and quantification of one or more specific sequences in a DNA sample. The technique reported here uses un-modified oligonucleotides as primers and an oligonucleotide probe, which is labelled with a donor fluorophore (molecule that absorbs light energy and becomes excited) and a quencher (acceptor dye) (Schena *et al.* 2004). The primers and probe bind to specific target DNA sequences permitting detection of a target DNA sequence only after hybridization of the probe with the target and nuclease activity, which causes dissociation of the fluorophore and the quencher and emission of fluorescence of a specific wavelength. The amount of fluorescence increases exponentially as the target DNA sequence is amplified exponentially by PCR. A threshold value for fluorescence is established, below which samples are considered negative for the target DNA molecule. The number of cycles required for a sample to reach the threshold is the cycle threshold (C_t) value (Ward *et al.* 2004; Smith and Osborn 2008). The higher the concentration of target DNA, the fewer the number of cycles required to reach the fluorescence threshold and the lower the C_t value will be for that sample (Figure 1).

For the purpose of this project, it was postulated that the amount of target DNA in a sample would be proportional to the biomass of the organism under study; namely, *B. cinerea* hyphae and spores. It should be noted that the technique does not discriminate live and dead cells, as long as the integrity of the DNA has been maintained. With regard to the grape and wine industry, the cost of conducting the assay and the technical expertise needed to do so, means that this technique is likely to be primarily a research tool. However, it potentially provides a robust and accurate method of quantifying non-visible and visible botrytis (total disease) for studies of disease epidemiology and for calibrating more cost-effective and laboratory-independent diagnostic tools such as infrared spectroscopy (Scott *et al*. 2010).

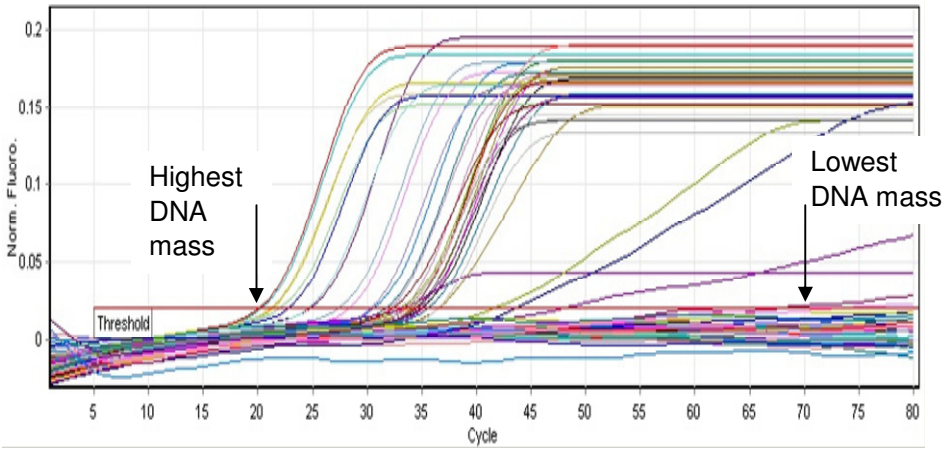


Figure 1. An illustration of results from quantitative PCR where each line represents a different DNA sample. The level of fluorescence is shown on the y-axis and the number of cycles of PCR on the x-axis. The number of cycles at which the sample's fluorescence exceeds the threshold (baseline) level is called the Cycle Threshold (C_t) value. The lower the C_t value, the higher the concentration of the target DNA in the sample. The negative control generally does not exceed the baseline, or if it does, it occurs many cycles later than a positive sample.

Quantitative PCR has been developed for the detection and quantification of *B. cinerea* DNA in *Arabidopsis thaliana* (Brouwer *et al.* 2003; Gachon and Saindrenan 2004), ornamental plants (Suarez *et al.* 2005), strawberry (Mehli *et al.* 2005), wine grapes (Cadle-Davidson 2008) and table grapes (Celik *et al.* 2009). The approach of Cadle-Davidson (2008) was tested because it used a fluorogenic sequence-specific DNA probe based on TaqMan® chemistry, which, in theory, binds only to the target amplification product. An alternative qPCR assay, for example Celik *et al.* (2009), uses SYBR® Green dye which binds to any double-stranded DNA, thus potentially generating false positive signals. Cadle-Davidson (2008) used a standard curve of *B. cinerea* DNA diluted in *Vitis vinifera* DNA to show that *Vitis vinifera* DNA was not amplified and that the TaqMan®-based assay quantified as little as 32 pg of *B. cinerea* DNA accurately, with a detection limit of 100 fg. Grape clusters were harvested from 32 *Vitis* accessions at E-L stages 31 (pea-sized), 33 (bunch closure), 35 (veraison) and 38 (mature). Five berries were sampled randomly per cluster, with two samples of five berries from four replicate clusters subjected to qPCR. For *Vitis vinifera* interspecific hybrids, the mean incidence of five-berry samples with *B. cinerea* DNA detected by qPCR increased from 7.7% at pea-sized berries to 46% for mature grape clusters. This result supported the use of qPCR to identify the relative extent of grape cluster colonisation by *B. cinerea* over time.

In late 2007, Katie Dunne visited Lance Cadle-Davison's laboratory at USDA-ARS, Geneva, New York, to learn the qPCR (TaqMan®) technique for *Botrytis cinerea* (Cadle-Davidson 2008). An attempt was then made to adjust the technique to suit equipment available at the University of Tasmania (UTas). Despite many preliminary experiments and modifications to Cadle-Davidson's method (to be reported in K. Dunne's thesis), a reproducible technique was not achieved. A state-of-the-art RotorGene PCR cycler was used at UTas and this rotor-type machine was thought to have a more stable temperature profile than the 'block' type PCR cycler used by Cadle-Davidson. Differences in the PCR cycler or other unknown factors meant that non-specific binding of the probe was observed when the technique was applied at UTas. Given this situation, a new assay was designed as described below. Furthermore, there was an opportunity to improve the assay by including an internal control that would indicate that a negative result was a consequence of *B. cinerea* DNA being below detectable levels and not because of the presence of biochemicals inhibiting the PCR.

This chapter outlines the improved qPCR technique and provides information on its reliability and application.

3 Methods

3.1 DNA extraction from grape berries (field samples)

Samples of 50 berries were collected randomly from bunches on each of the 150 target vines per treatment tested in the whole-of-block experiment (refer to Chapter 5). The treatments were: application of Switch® at 80% cap fall ('flowering') or at pre-bunch closure ('PBC').

Each 50-berry sample, contained in a linen-calico cloth bag, was frozen in liquid nitrogen and stored at -80°C for at least 24 h before being pummelled with a rubber mallet to break the berries into smaller pieces. The tissue pieces were then transferred to liquid nitrogen in a mortar and pestle for further grinding. Sub-samples of ground tissue, each 500 µL in volume, were stored at -80°C of up to 7 days prior to DNA extraction.

The ground tissue was transferred to 750 µL of extraction buffer described by Lin and Walker (1997), with 3% rather than 2% soluble PVP-40 and amendment with sodium bisulphite (36.6 mM final concentration) and sodium borate (200 mM final concentration). Samples were

homogenised using a Retsch MM200 shaker (Retsch, Germany) for 30 s at the highest setting (30 vibrations/s), refrozen with liquid nitrogen and stored at -80°C overnight. Samples were then thawed prior to shaking for 10 min as before. A pellet, obtained by centrifugation at 5,180 x *g* for 20 min at 4ºC (Sorvall Super T21 SL5OR centrifuge, Thermo Fisher Scientific, Waltham MA, USA), was suspended in a solution containing 200 µL each of the extraction buffer minus the PVP, the lysis buffer described by Lin and Walker (1997) and 5% sarcosyl (N-lauroylsarcosine). Samples were then mixed using the Retsch shaker for 1 min at 30 vibrations/s and then incubated in a water bath at 75°C for 30 min, with samples mixed gently for 1 sec every 10 min. Samples were then left to cool briefly before adding 200 µL of 24:1 (v/v) chloroform:isoamyl alcohol, mixed gently and then left to settle for 5 min. The samples were mixed again and left to settle for another 5 min before centrifugation at 5,180 x *g* for 20 min at 4ºC. The top layer of the mixture (approximately 300 µL) was transferred to new tubes and extracted again with 200 µL of 24:1 (v/v) chloroform:isoamyl alcohol before centrifugation at 5,180 x *g* for 20 min at 4ºC. Approximately 400 µL of the aqueous layer was transferred to new tubes. Nucleic acids were precipitated with two volumes 95% ethanol and the pellet washed twice with 70% ethanol. Pellets in wells of 96-well plates were dried in an oven set at 37°C and pellets in microfuge tubes were dried using a vacuum dehydrator (DNA mini dehydrator, Imbros, Pty Ltd). Pellets were dissolved in 35 µL of sterile Milli Q water and nucleic acids purified further using an Ultra Clean DNA kit (MoBio Pty Ltd, Carlsbad, CA, USA) followed by a second clean-up with a Sure Clean kit (Bioline Pty Ltd, UK).

Total DNA was quantified using the Quant-iT PicoGreen Assay (Invitrogen, Australia) and a real-time PCR machine (RotorGene 6000) (formerly Corbett Life Sciences, Qiagen), following the manufacturer's recommendations. The quantification was performed using 3 µL of DNA sample, which was diluted with 47 µL of 1X TE buffer. The DNA concentration of samples was then adjusted to 5 ng/µL (if it was > 5 ng/µL) using sterile mQ water.

3.2 DNA extraction from B. cinerea mycelia and V. vinifera leaves

A pure culture of *B. cinerea*, isolated from infected rachides remaining on grapevines in a commercial vineyard over winter, was grown on Malt Extract Agar (50g/L) (Oxoid Australia Pty Ltd, Adelaide) at room temperature and a 12-h photoperiod. *B. cinerea* conidia and mycelia were scraped from a culture covering a 9-cm Petri plate using a razor blade and transferred to a 2 ml microfuge tube and snap frozen with liquid nitrogen. Four 3 mm-diameter metal beads were then added to the frozen fungal material which was then pummelled using a Retsch MM200 shaker (Retsch, Germany) for 2 min at the highest setting and then placed back into liquid nitrogen prior to DNA extraction as described previously.

Healthy, small and unexpanded leaves of *Vitis vinifera* cultivar Chardonnay, were ground using liquid nitrogen and a mortar and pestle. DNA was extracted from a 500 µL volume of ground tissue as described previously.

3.3 Probes and primers for duplex qPCR assay

A duplex assay, including primers designed for detection of *Vitis vinifera* DNA, was designed with the assistance of Dr Fabrice Magnino from Integrated Sciences (Sydney) based on the existing *B. cinerea* TaqMan® assay (Cadle-Davidson, 2008). New primers for *B. cinerea* DNA were from the same region of the intergenic spacer used by Cadle-Davidson (2008) and were designed to have a similar optimal annealing temperature as the primers designed for *V. vinifera* DNA. The sequences for the primers and probes are not described here because of plans to publish the work. Individuals may contact Kathy Evans for these sequences if they are intended to be used for research purposes only. The primer sequences

(BcF and BcR) for *B. cinerea* DNA produced a 150 bp product. The grape DNA primer sequences (GF and GR) were based on *V. vinifera* chromosome 10 (Jaillon *et al.*, 2007) (NCBI NC_012016.2) and produced a product of 113 bp. The *B. cinerea* probe (BcP) incorporated the FAM dye and the *V. vinifera* probe (GP) incorporated the ROX dye. All primers and probes were synthesised by Integrated Sciences Pty Ltd (Sydney).

3.4 Polymerase chain reaction conditions

All qPCR reactions were performed using a RotorGene 3000 (formerly Corbett Life Sciences, now Qiagen Pty Ltd). The reaction volume was 25 µL containing 12.5 µL 2X StrataGene Brilliant® II qPCR Master Mix (StrataGene, Agilent Technologies, California, USA), 0.3 µM of each primer (BcF, BcR, GF and GR), 0.2 µM of each probe (BcP and GP) and 2.5 µL of a 5 ng/µL (total) DNA solution. There was an initial activation step of 95°C for 10 min followed by 40 cycles of 95°C for 30 s, 50°C for 1 min and 72°C for 15 s. The qPCR machine was set to detect fluorescence and acquire images after the annealing temperature of 50°C for both the FAM (Green, *B. cinerea* probe) and ROX (Orange, *V. vinifera* probe) channels. The cycle threshold (C*t*) was the number of cycles required for the fluorescence signal to exceed values in the range 0.02–0.05 (automatically adjusted by the RotorGene software). The threshold fluorescence value reflected that the amount of DNA had exceeded the background level but was still in the range where the PCR was in the log-linear phase.

Standard curves for qPCR assay

Stock solutions of 5 ng/µL *B. cinerea* or *V. vinifera* DNA (negative control) were prepared. The *V. vinifera* DNA was diluted to 0.2 ng/µL. A standard curve was created by mixing equal volumes of 5 ng/µL *B. cinerea* and 0.2 ng/µL *V. vinifera* DNA because preliminary experiments revealed that higher concentrations of *V. vinifera* DNA reduced the sensitivity and efficiency of detecting *B. cinerea* in the duplex qPCR assay. This mixture of *B. cinerea* and *V. vinifera* DNA was then used for a 5-fold dilution series with 0.2 ng/µL *V. vinifera* DNA as the diluent. Standard solutions in a total volume of 2.5 µL are specified in Table 1. Using the qPCR conditions described above, a standard curve was generated (for example, Figure 2) by plotting the C_t value for *B. cinerea* DNA against the amount of *B. cinerea* in the standard solution (expressed as femtograms (fg) = ng x 10^6) transformed according to the equation: Log_{10}(DNA amount (fg)).

Table 1. Amounts of DNA in 2.5 µL per qPCR for each standard DNA solution used to generate the standard curve for *B. cinerea* DNA mass.

Standard solution	DNA amount (ng) per reaction		Total DNA (ng) per reaction
	B. cinerea	*V. vinifera*	
B. cinerea	12.5	0	12.5
Standard 1	6.25	0.25	6.5
Standard 2	1.25	0.45	1.7
Standard 3	0.25	0.49	0.74
Standard 4	0.05	0.498	0.548
Standard 5	0.01	0.4996	0.5096
Standard 6	0.002	0.49992	0.50192
Standard 7	0.0004	0.499984	0.500384
V. vinifera	0	0.5	0.5

In addition to producing a suitable standard curve, an assay was considered valid in the case where no *B. cinerea* DNA was amplified if *V. vinifera* DNA was amplified with a C_t value of ≤ 40 (internal control). If *B. cinerea DNA* was amplified, then the assay was considered positive for the detection of *B. cinerea* DNA if the C_t value was ≤ 40. *B. cinerea* DNA was quantified only when the C_t value corresponded to a DNA mass in the range used for the standard curve and in the range where the response was linear. *B. cinerea* DNA mass was expressed as a percentage of total DNA in the sample to account for the fact that some DNA extractions from grape berries resulted in < 5 ng total DNA/µl.

3.5 Data analyses

Linear regressions, two-sample and Chi-square tests from contingency tables were calculated using GenStat Release 12.1 (VSN International Ltd). The reaction efficiency of the qPCR was estimated as E = 10(-1/(slope of the standard curve))-1 expressed as a percentage of 2.0 (i.e. 100% efficiency when the product doubles each cycle).

4 Results and Discussion

4.1 Validation of qPCR assay

At total of 15 standard curves were generated for analyses of 50-berry samples from the whole-of-block experiment (Chapter 5). Log_{10}(*B. cinerea* DNA (fg)) explained more than 98% of the variance in C_t value for all standard curves (Figure 2, Table 2), with an R^2 of ≥ 0.99 for nine out of fifteen standard curves. The slopes of the linear regressions (Table 2) indicated that reaction efficiencies for *B. cinerea* DNA in the duplex assay were 81–113%, with all but one regression indicating ≥ 91% efficiency. The linear response for all amounts of *B. cinerea* DNA used to generate the standard curves indicated that *B. cinerea* could be quantified accurately for C_t values corresponding to DNA amounts ≥ 350 fg (Log_{10}(350) = 2.54 in Figure 2).

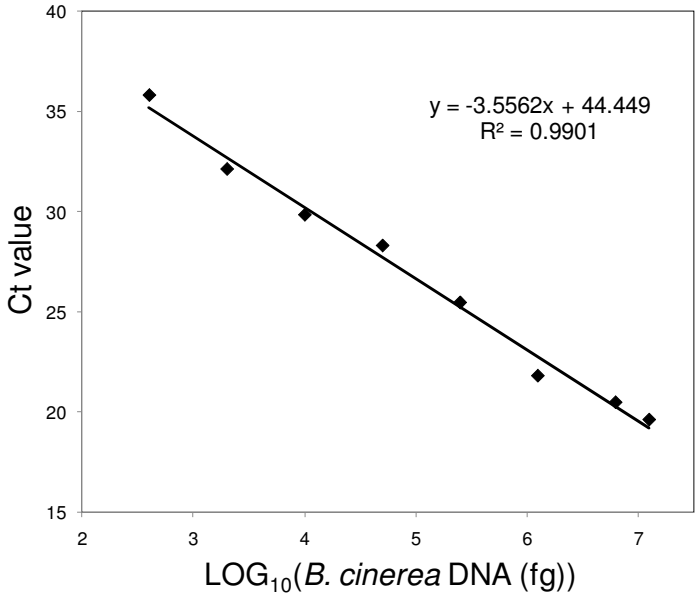


Figure 2. An example of a standard curve (number 13 in Table 2) for *B. cinerea* DNA where C_t (cycle threshold) is the number of cycles required for the fluorescent signal to exceed the background level. The slope of the linear regression was -3.5562, suggesting an efficiency of 91% for the PCR.

Table 2. Standard curve parameter estimates from linear regressions. All regressions were significant at $P < 0.001$ for 8 observations.

Curve number	Constant estimate	Slope estimate	R^2	Reaction efficiency estimate
1	47.130	-3.8300	0.975	81
2	43.646	-3.4324	0.996	96
3	43.995	-3.4230	0.989	96
4	43.615	-3.3450	0.983	99
5	43.282	-3.3580	0.984	99
6	43.237	-3.4420	0.990	95
7	44.929	-3.4170	0.994	96
8	44.359	-3.4530	0.980	95
9	42.956	-3.2730	0.993	103
10	44.539	-3.4231	0.996	96
11	43.829	-3.2590	0.978	103
12	45.211	-3.3550	0.991	99
13	44.449	-3.5560	0.989	91
14	42.645	-3.317	0.989	101
15	42.482	-3.0700	0.980	113

4.2 Application of qPCR to samples from the vineyard

A total of 278 of 300 samples resulted in valid qPCR assay in which *B. cinerea* DNA was detected or not (Table 3). When samples were allocated to categories according to whether or not the *B. cinerea* DNA mass exceed the amount that could be quantified accurately, then the 'Flowering' treatment resulted in marginally more samples with ≥ 350 fg *B. cinerea* DNA (Table 4, $P = 0.085$ for a likelihood chi-square value from a 2 x 2 contingency table). Among the 278 samples in which *B. cinerea* DNA was detected, DNA mass was quantified accurately for 46 samples (29 + 17 samples from Table 5).

Table 3. Number of samples in which *B. cinerea* DNA was detected ($C_t \leq 40$)

Treatment	Number of samples		
	$C_t > 40$ (not detected)	$C_t \leq 40$ (detected)	Total number of samples
Flowering	31 (23%)	105 (77%)	136
PBC	27 (19%)	115 (80%)	142

Table 4. Number of samples with sufficient *B. cinerea* DNA for accurate quantification (≥ 350 fg)

Treatment	Number of samples with the indicated amount of *B. cinerea* DNA (femtograms)		
	< 350 fg	≥ 350 fg	Total number of samples
Flowering	107 (79%)	29 (22%)	136
PBC	125 (88%)	17 (12%)	142

The amounts of *B. cinerea* DNA expressed as a percentage of total DNA among samples with ≥ 350 fg are summarised in Table 5 and illustrated in box plots for the specified values (Figure 3).

Table 5. Summary statistics for the amounts of *B. cinerea* DNA expressed as a percentage of total DNA for samples with ≥ 350 fg. A value of 2.5% was removed from the data set for the 'Flowering' treatment because it was an extreme outlier.

	Treatment	
Summary statistic	Flowering	PBC
number of values	28	17
mean	0.0509	0.109
median	0.0149	0.02050
minimum	0.00439	0.00627
maximum	0.418	0.898
range	0.413	0.892
lower quartile	0.0115	0.0148
upper quartile	0.0387	0.0368
standard deviation	0.0930	0.240
variance	0.00865	0.0578
sum of values	1.426	1.853

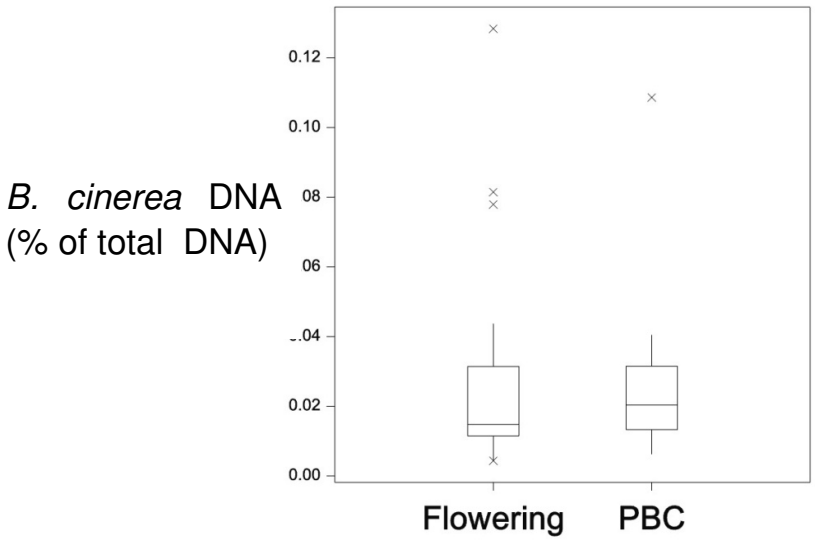


Figure 3. Box plot for data presented in Table 3 with outliers > 0.2% removed (for clarity of presentation). The three outliers for the flowering treatment were 2.5. 0.42 and 0.30% and the two outliers for the PBC treatment were 0.90 and 0.55%.

There were no significant differences between treatments for % *B. cinerea* DNA after a non-parametric Mann-Whitney U test was applied because of heterogeneity in sample variance. This result was in contrast to the statistical separation of treatments when mean botrytis severity per 12 bunches per vine was scored (Chapter x). Switch® applied at 80% cap fall (flowering) or PBC resulted in an overall mean botrytis severity at harvest of 3.5% and 1.5%, respectively. The results for qPCR indicated that while the flowering treatment resulted in a marginally higher incidence of 50-berry samples (vines) with levels of *B. cinerea* DNA that could be quantified accurately, once DNA levels reached the quantifiable range differences between the treatments were not detected. Given the potential for large variation among grape bunches for botrytis severity (by visual scoring) (refer to Chapter 1), then the sampling strategy for qPCR might have been insufficient to provide an accurate estimate of *B. cinerea* DNA mass per vine. However, it was not practical to process (by hand) more than 50 berries at a time, meaning that direct application of the technique, in its current form, to vineyard samples would be limited by sample size, cost and technical capacity. The technique has been applied successfully to grape juice (to be reported in K. Dunne's thesis) with the potential for juice to be applied to Whatman FTA® cards (Whatman Inc., USA) for a more streamlined assay. Even so, such an assay would be restricted to a laboratory offering a commercial service. A more practical application of the technique might be for the calibration of more rapid diagnostic tools, with infrared spectroscopy (Scott *et al.* 2010) considered a potential candidate for further development.

In summary, this new, sensitive TaqMan® qPCR assay was reproducible and ensured that lack of amplification was due to lack of *B. cinerea* DNA. This assay is ready to be tested further as a research tool and for transferability to other laboratories. *B. cinerea* DNA has been amplified successfully from grape berries processed from pre-bunch closure to maturity (to be reported in K. Dunne's thesis). Two potential opportunities are (i) research to provide

insight on the timing and extent of colonisation of grape berries after latency for different cultivars and environmental conditions, and (ii) the use of qPCR in the development of a more rapid, cost-effective tool for assessing botrytis risk in the vineyard well before harvest.

5 Acknowledgments

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Chapter 3: Botrytis trials in Tasmania 2006–2009

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1 Summary

Eight small-plot, replicated botrytis trials in Chardonnay, Riesling or Sauvignon Blanc were conducted in southern Tasmania during the period 2006–2009. Mean botrytis severity at harvest in non-treated plots exceeded 3% at four site-years. The highest mean botrytis severity in non-treated plots was 6.2% and the median botrytis severity was 3.2%. There was a positive and significant linear relationship between the mean incidence of latent infection at pre-bunch closure and cumulative rainfall in the 14 days prior to 100% capfall, although this response variable was not correlated positively with botrytis severity at harvest. In the Chardonnay trial in 2008, Switch® applied at pea-sized berries reduced the mean incidence of latent infection at pre-bunch closure when compared to non-treated controls.

Analyses of grape juice for antigens of *B. cinerea* using the QuickStix™ Kit for Botrytis in Wine Grape Juice (EnviroLogix Inc.) complemented visual scoring of disease symptoms to give an overall picture of 'botrytis load' in grapes and juice at harvest. The QuickStix™ test separated treatment means in some trials where visual scoring did not result in statistically significant difference. Considering both the severity of botrytis rot at harvest and the level of *B. cinerea* antigens in grape juice, application of Switch® once or twice during the mid-season reduced 'botrytis load' in five of seven trials where disease or antigen levels were high enough for statistical separation of treatments. Mid-season fungicide applications also increased grape yield (kg/vine) in one trial. Application of Bravo® and Scala® during flowering of Sauvignon Blanc in 2006/2007 did not reduce mean botrytis severity at harvest (from 2.5% in non-treated plots), whereas Switch® applied at 80% capfall at the same site but in 2008/2009 reduced mean botrytis severity to 2.4%, relative to the mean of 5.2% in non-treated plots. Application of Switch® at pre-bunch closure at the same site in 2008/2009, reduced mean severity to 1.0%. Late season application of Rovral® at this site did not reduce mean botrytis severity significantly at harvest in either 2007 or 2009.

With regard to modification to the canopy, there was limited evidence from one trial that bunch thinning at pre-bunch closure increased the severity of botrytis rot significantly relative to no bunch thinning. The reason for this result was obscure but might have been related to the effect on botrytis severity of an interaction between weather and berry sugar development. The use of compressed air to remove bunch trash at 100% capfall in one trial and also at pre-bunch closure in another trial did not reduce the incidence or severity of botrytis at harvest significantly. These treatments may have damaged the inflorescence. The lack of a response to leaf removal treatments was explained by the fact that the respective canopies had relatively low leaf layer numbers and moderate canopy vigour prior to leaf removal.

Differences between treatment means for some trials appeared to be large (eg. Riesling in 2009); however, large variation in botrytis severity on bunches within and between plots meant that statistical separation of treatments was not always achieved. Across all trials, botrytis severity was either significantly higher, lower or similar for basal bunches when

compared with bunches positioned distally, supporting the use of bunch position as a factor during ANOVA.

2 Introduction

The objective of this research was to determine the effect of fungicide timing and certain canopy manipulations on the severity of botrytis at harvest. Pathogen, weather and vine variables associated with the resulting botrytis epidemics, with or without treatment, were integrated with results from trials from New Zealand and Victoria for the development of the Botrytis Decision Support Model (Attachment 5). Information on disease progression in non-treated plots is presented in Table 5 of Chapter 1. The focus of this chapter was the effect of treatments on botrytis severity at harvest in replicated, small-plot trials.

The fungicide timing trials were based on spray programs commonly adopted in Tasmania at the time of this study so that the effectiveness of current practices could be evaluated. Market specifications for low or no fungicide residues in wine meant that the use of the same fungicide for each spray timing would have meant little in practice because of severe restrictions on fungicides that apply mid to late season. Nevertheless, the same fungicide was applied at different times throughout the growing season in one small-plot trial (2008-2009) to see which timing gave the best botrytis control.

After capfall, decaying flower parts provide the perfect habitat for colonisation and sporulation by *B. cinerea*. A specific objective of two of the field trials was to investigate the importance of floral debris on the severity of botrytis rot at harvest by reducing its quantity in grape bunches.

3 Materials and methods

3.1 Trial design

Eight botrytis trials were conducted in southern Tasmania in the seasons 2006-2007, 2007-2008 and 2008-2009. Each trial consisted of treatment plots comprising a panel of 5–7 vines and six replicates per treatment arranged in a randomised complete block.

3.2 Trial site location and viticultural characters

Table 1 lists the location and viticultural characteristics of each trial site. The altitude of trial sites in Tasmania ranged from 30 to 90 m above sea level. All vines had shoots that were positioned vertically, except for vines at site S43CH08, which were trellised according to the Scott Henry method. The height of the fruit zone was generally 0.8 to 1.1 m, except for the Scott Henry trellis where the height of the fruit zone ranged from 0.2 to 1.6 m. Fungicides for pathogens other than *B. cinerea*, pesticides and foliar nutrients were applied to trial sites by the grower co-operator, according to standard, conventional practices. At site S43R07, all fungicide applications for powdery mildew control were applied by hand (as described below) to allow inclusion of a control treatment for powdery mildew.

The weight of bunches from the central vine of each plot was used to determine the mean yield per vine for each trial site. The mean pruning weight for 18 vines per trial site was estimated at sites indicated in Table 1, according to the method described by Smart and Robinson (1991). The ratio of the yield to pruning weight (trimmed vines) and the mean cane weight was used to rate crop vigour as low, moderate or high (Smart and Robinson, 1991).

Sites S43R07, S43R08 and S43R09 were in the same block of Riesling but site S43R07 was located at the opposite end of the block. Damage caused by severe frost in October 2006 necessitated the re-location of trial S43R07 to the higher end of the block.

Table 1. Location and viticultural characteristics of trial sites in southern Tasmania.

Site-year code	Vintage year	District	Variety	Latitude; longitude	Row orientation	Row x vine spacing (m)	Slope[a] down rows (degrees)	LLN	Crop vigour[b]
S43R07	2007	Coal River Valley	Riesling	42° 36′ 59″ S; 147° 26′ 11″ E	NW to SE	2.5 x 1.2	3–4	2.5	2.8; 58; high
S43SB07	2007	Coal River Valley	Sauvignon Blanc	42° 48′ 39″ S; 147° 25′ 37″ E	N to S	2.1 x 1.5	0	2.6	4.3; 44; moderate
S43R08	2008	Coal River Valley	Riesling	42° 36′ 59″ S; 147° 26′ 11″ E	NW to SE	2.5 x 1.2	3–4	2.0	3.0; 65; high
S43SB08	2008	Coal River Valley	Sauvignon Blanc	42° 48′ 39″ S; 147° 25′ 37″ E	N to S	2.1 x 1.5	0	1.9	3.9; 35; moderate
S43CH08	2008	Rokeby	Chardonnay	42° 52′ 39″ S; 147° 25′ 25″ E	E to W	2.4 x 1.35	5–6	1.3	not estimated
S43R08_2	2008	Derwent River Valley	Riesling	42° 45′ 04″ S; 147° 10′ 33″ E	N to S	2.1 x 1.4	7–8	2.0	not estimated
S43R09	2009	Coal River Valley	Riesling	42° 36′ 59″ S; 147° 26′ 11″ E	NW to SE	2.5 x 1.2	3–4	2.4	5.0; 41; moderate
S43SB09	2009	Coal River Valley	Sauvignon Blanc	42° 48′ 39″ S; 147° 25′ 37″ E	N to S	2.1 x 1.5	0	2.7	3.7; 38; moderate

[a]Slope was measured using a clinometer (Suunto, Finland)

[b] The three values are the ratio of mean yield (kg/vine) / mean pruning weight (kg); mean cane weight (g) and the vigour ranking according to Smart and Robinson (1991).

3.3 Weather data

Average air temperature, relative humidity, surface moisture and rainfall were recorded at 10 min intervals using Tinytag data loggers (Gemini Data Loggers (UK) Ltd) located 1.6 m above the ground on a headland near the trial site according to the methods of Beresford and Spink (1992). The Tinytag Ultra 2 (dual channel) data logger for temperature and relative humidity was housed in a plastic shelter (Hastings Data Loggers (HDL), Port Macquarie, Australia). The tipping bucket rain gauge (Rain Collector II, Davis Instruments, USA) was adjusted by HDL to tip every 0.2 mm of rainfall. In 2006-07, the leaf wetness sensor (Model 237, Campbell Scientific, Inc., Utah, USA) was mounted at a 45^{o} angle to mimic the position of a leaf. However, in the remaining two seasons the sensor was mounted at 10^{o} to minimise run-off of surface moisture and for standardising measurement across sites in the larger trans-Tasman study (RM Beresford, HortResearch, personal communication). The cable of the leaf wetness sensor was modified by HDL for connection to a Tinytag data logger.

3.4 Application of fungicides to vines

Details of fungicides used in trials are presented in Table 2. All fungicide and biological control treatments were applied to the crop canopy with a hand gun connected to a hose reel and diaphragm pump mounted on an all-terrain vehicle (ride-on quad bike). In 2006/2007, the spray was propelled with a pump pressure of 1034 kPa, delivering approximately 108 ml/s. In 2007/2008, the spray was propelled with a pump pressure of 689 kPa, delivering approximately 80 ml/s. The spray was directed to the fruiting zone of the crop canopy. In 2006/2007, each plot received an average of 3.2 L of spray, respectively, which was reduced to 2.4 L of spray per plot in the remaining seasons.

Table 2. Fungicides and rates used in trials.

Trade name	Active constituent	Supplier	Rate of product/ 100 L
Bravo®	720 g/L chlorothalonil	Syngenta Group Company	210 ml
Captan®	800 g/kg captan	Crop Care Australasia Pty Ltd	125 g
Flint®	500 g/kg trifloxystrobin	Bayer CropScience	15 g
Scala®	400 g/L pyrimethanil	Bayer CropScience	200 ml
Switch®	375 g/kg cyprodinil 250 g/kg fludioxinil	Syngenta Group Company	80 g plus 0.02% non-ionic surfactant[a]
Rovral®	500 g/L iprodione	Bayer CropScience	100 ml

[a] Spraymate® Activator® Surfactant, 900 g/L non-ionic surfactants, Nufarm Australia Limited.

3.5 Treatments applied at each trial site

Tables 4, 9, 14, 20, 24, 31, 37 and 40 list the treatments applied at each trial site and are located before the tables of results for each trial for ease of reading. Crop stages, for the purpose of timing treatments, were defined as early, mid or late season. Early season applications ('early') were timed for 5% and/or 80% capfall, mid season applications were timed for pea-size berries and/or pre-bunch closure (PBC, 'mid') and late season applications were timed for veraison and/or pre-harvest ('late'). Percentage capfall was determined according to the methods described by Evans and Gadoury (2008).

The application of Flint late in the season in trial S43CH08 was an experimental treatment to determine whether or not this fungicide inhibited the sporulation of *B. cinerea*.

Materials A and B applied at trial site S43R07 were materials under development, as was Material C at trial sites S43R07 and S43R08. Material C was applied at a rate of 10 g/100 L.

3.6 Leaf plucking pre-flowering at site S43R08

Prior to application of material C at 80% capfall, selected leaves were removed if the leaf was directly in front of the bunch and if its position may have prevented adequate spray coverage of that bunch. The level of leaf plucking was very light and applied to treatments indicated in Table 15.

3.7 Leaf plucking at pre-bunch closure at sites S43SB08, S43SB09 and S43R09

Prior to the application of fungicide at pre-bunch closure, leaves were removed in selected treatments to achieve approximately 70% bunch exposure on the eastern side of the trellis only for site S43SB08 and on both sides of the canopy for sites S43R09 and S43SB09.

3.8 Bunch thinning at site S43R07

The number of bunches per vine was counted pre-flowering in selected treatments for estimating crop yield and to impose a treatment whereby bunches were removed from vines at pre-bunch closure in order to achieve a target yield of 5t/ha.

3.9 Removal of dead and decaying plant matter from the fruiting zone

At site S43SB07 compressed air was used to blow a significant proportion of bunch trash out of the fruiting zone and onto the ground in selected treatments. This treatment was performed at 100% capfall, in the middle of the day and using an air pressure of 345 kPa. At site S43CH08, bunch trash was removed in selected treatments at 100% capfall and before treatment with fungicide at pre-bunch closure, in the middle of the day and using a compressed air pressure of 500 kPa.

3.10 Assessment of bunch compactness

Bunch compactness was estimated by a water displacement method, which expresses compactness as 'percentage openness' (Shavrukov *et al.* 2003). In 2007, bunch compactness was estimated at harvest for five basal and five distal bunches per plot, prior to juice extraction as described below. These bunches were on vertically positioned shoots. A basal bunch (position 1) was the first bunch on the shoot, closest to the vine arm (cordon), of cane-pruned vines. A distal bunch was the bunch above the basal bunch (position 2). In 2008, mean bunch compactness of basal and distal bunches for the trial site was estimated by sampling one bunch from an end vine of each plot and alternating in the selection of a basal bunch or a distal bunch at each sample location.

3.11 Assessment of latent infection at pre-bunch closure

Latent infection within grape berries was measured at pre-bunch closure by freezing and surface sterilising 20 berries from each of 10 bunches selected at random from each non-treated control plots of all trials and from all treatment plots in trials conducted in 2006-07 and 2007-08. Whole bunches were cut from the vine into polythene bags which were frozen as soon as possible at -18°C for a minimum of 24 h. After freezing, bunches were surface sterilised with 70% ethanol for 10 s, followed by 1% sodium hypochlorite for 1 min. Twenty whole berries, with pedicels attached, were cut from each bunch onto a moist paper towel and placed in a plastic 'fast food' container (17.5 x 12 x 4 cm, approx. 500 ml) with a lid that allowed some air movement. A piece of 'rug hold' mesh underlay (1 x 1 cm), used to prevent carpet rugs from slipping, was placed on the paper towel and the berries were placed into the cells created by the 'rug hold' to prevent them rolling. The trays were placed on a laboratory bench at room temperature (15–20°C) with exposure to daylight through windows, but not direct sunlight. After a minimum of 6 days incubation, the incidence of berries with sporulating *B. cinerea* was recorded, with the aid of a stereomicroscope. Further assessments were made until the maximum incidence of berries expressing *B. cinerea* was observed, with the final assessment occurring within 12 days, before sporulation on berries was the result of the secondary spread of *B. cinerea*.

3.12 Disease assessment at harvest

The severity of botrytis bunch rot for each of 30 bunches on the central 3-4 vines per plot was assessed visually according to a standard area diagram (R.W. Emmett, Department of Primary Industries, Victoria, personal communication). Fifteen of the 30 bunches were selected at random among bunches at the basal position on the shoot and the other 15 were selected at random among bunches at the distal position on the shoot. At site S43CH08, 28 tagged bunches were assessed: 14 basal and 14 distal bunches. Severity was defined as the area of brown or pink-brown berries that were turgid, semi-turgid and/or shrivelled in a manner that was characteristic of botrytis bunch rot. It was assumed that the symptoms scored were caused by *B. cinerea* because a proportion of symptomatic bunches showed signs of *B. cinerea* sporulation.

3.13 Extraction of grape juice at harvest for assay or storage

In 2007, five basal and five distal bunches of the 30 tagged bunches per plot were harvested. Bunch compactness was measured for each bunch prior to juice extraction. Juice from all 10 bunches was extracted in a 14 x 14 cm aluminium stainless steel fruit press (Ferrari group, Italy) for sampling two lots of 50 ml juice. The first 50 ml sample was used for determination of total soluble solids (°Brix), pH and titratable acidity, with the latter determined according to the method of Iland et al. (2004). Total soluble solids (°Brix) were measured by placing a 1 ml sub-sample of juice on the well of a digital refractometer (Pocket PAL-1, Atago, Japan). Sodium metabisulfite was added to the second 50 ml juice sample at a concentration of 200 mg/L SO_2. This juice was then stored at -20°C.

In 2008, a similar procedure for juice preparation was followed, except that basal and distal bunches were kept separate for preparing the 50 ml juice samples. The intention was to prepare juice using five bunches per bunch position per plot, but in practice and unintentionally, the sample size varied from five to nine bunches, mostly five to seven bunches per bunch position per plot. At trial site S43SB08, there were many shoots with only one bunch and so bunch position was not recorded for the bunches sampled per plot. Nevertheless, two 50 ml juice samples were prepared using two samples of at least five bunches. At trial site S43CH08, six basal and six distal bunches were processed. Each

sample of six bunches was divided into two lots of three bunches for juice preparation and storage.

3.14 Analysis of grape juice with QuickStix™

The QuickStix™ Kit for Botrytis in Wine Grape Juice (EnviroLogix Inc.) screens for the presence of antigens of *Botrytis cinerea*. Preliminary testing of QuickStix™ (EnviroLogix Inc.), using juice from harvested grapes, was integrated into existing trials in an *ad hoc* manner noting that these trials were not designed specifically for evaluating QuickStix™.

Juice samples were thawed and assayed according to the manufacturer's instructions for the QuickStix™ Kit for Botrytis in Wine Grape Juice (EnviroLogix Inc.), with the exception that the juice sample was diluted 1:5 with EB8 buffer before assay. After completion of the QuickStix™ strip test, the strip was inserted to the QuickStix™ reader to measure the strength of the test signal, hereafter referred to as the signal intensity (SI). SI is proportional to the amount of *B. cinerea* in grape juice. If the juice is not diluted sufficiently, then SI values plateau as they approach the upper limit of about 55 for an individual strip test. Assays were conducted in duplicate for sites S43R08, S43SB08, S43R08_2 or in triplicate for other sites where data are presented. For site S43CH08, two 1 ml sub-samples of juice from each of the two lots of juice prepared per three bunch sample was thawed, mixed and returned to storage at -20^{o}C for 48 h prior to analysis. During this 48 h period, the freezer door was not shut properly and the juice samples did not freeze. The samples were assayed even though there may have been some growth of *B. cinerea* in the juice during the 48 h storage period.

3.15 Data analyses

One-way or factorial analysis of variance (ANOVA) was used to identify treatment means that were significantly different at P = 0.05. Mean disease severity (y) per plot was transformed according to Logit 'mean severity' = ln((y%+0.1)/(100.1-y%)) (Beresford et al. 2006), prior to ANOVA, to reduce heterogeneity of variances among means. Bunch position (basal or distal) was included as a factor where possible.

It was not possible to compare the data for mean logit severity of botrytis rot directly with the mean signal intensity (SI, QuickStix™ assay) of grape juice, given the difference in sample sizes per plot. However, the correlation between the two response variables was compared. The treatment means for SI, for juice from 10-12 bunches per plot, for all treatments in a trial, were transformed to a natural logarithm (LN) and used as an independent variable in linear regression. The response variable was the treatment mean for logit mean severity of botrytis rot for 28-30 bunches per plot. Trial S43R07 was excluded from regression analysis because the QuickStix™ assay was applied to juice from five (and not six) replicates in the randomized complete block design.

4 Results

The mean severity of botrytis at harvest and mean incidence of latent infection at pre-bunch closure in non-treated plots, plus the cumulative rainfall for various crop stage intervals and at each trial site are presented in Table 3. Four of the eight sites exceeded 3% botrytis severity, assumed to be an upper threshold severity for negative economic impact. There did not appear to be any relationship between mean botrytis severity at harvest (over the range observed in Tasmania) and mean incidence of latent infection at pre-bunch closure. However, there was a positive and significant linear relationship between the mean incidence of latent infection at pre-bunch closure and cumulative rainfall in the 14 days prior to 100% capfall (P = 0.006, R^2= 0.71 for the linear regression).

Table 3. Mean severity of botrytis rot in non treated plots near harvest and cumulative rainfall for various crop stage intervals at trial sites in southern Tasmania.

						Cumulative rainfall (mm)			
Site-year code	Vintage year	Mean severity (%) of botrytis rot	Mean incidence (%) of latent infection at pre-bunch closure	Date of harvest disease assessment	14 days prior to 100% capfall	5% capfall to PBC	PBC to veraison	veraison to harvest	5% capfall to harvest
S43R07	2007	0.6	0.0	March 22	2.8	60.2	89.4	93.0	243
S43SB07	2007	2.5	0.7	March 26	12.0	133.2	5.0	25.2	171
S43R08	2008	6.2	0.3	April 21	13.3	56.6	36.0	58.6	151
S43SB08	2008	2.7	0.0	March 17	28.8	36.0	23.6	42.4	102
S43CH08	2008	3.6	2.5	April 7	57.6	53.2	27.8	87.0	168
S43R08_2	2008	1.9	0.5	March 24	18.2	51.6	22.0	36.0	104
S43R09	2009	4.9	0.1	April 20	9.4	42.2	42.4	91.8	174
S43SB09	2009	5.2	1.2	March 30	31 .0	46.8	41.2	45.0	133

4.1 Site S43R07 (Riesling)
Refer to tables 4-8.

There were no significant differences among treatments for the mean incidence of latent infection at pre-bunch closure (Table 5, data analyses not presented).

The non-treated control had a relatively low mean botrytis severity near harvest of 0.6%, whereas the highest mean botrytis severity (2.5%) was recorded for two of the fungicide treatments (treatments 2 and 6, Table 5).

The yield of grapes at this site was below average and berry size was uneven. Bunch thinning in treatments 1 and 2 (also with mid-season fungicide treatment) resulted in approximately 70% of the yield of non-thinned plots in treatments 3 and 4 (Table 5). The mean weight of grapes (kg/vine) among these mid-season fungicide treatments was not significantly different ($P = 0.198$) according to factorial ANOVA.

By the third week of February, many of the smallest berries were turning brown and presented symptoms that were atypical of botrytis bunch rot. By mid March, these symptoms had worsened and were most noticeable in the three blocks (rows) of the trial located nearer the edge of the block, where soil moisture was possibly sub-optimal throughout the growing season. Assessing the severity of botrytis rot was challenging in this trial, given the presence of other (abiotic) symptoms.

The only treatment that reduced the logit mean severity of botrytis rot significantly, when compared with the non-treated control, were multiple applications of material A (Table 6). Fungicide application mid season (Captan® then Switch®), when combined with bunch thinning, increased the logit mean severity of botrytis rot significantly, when compared with the non-treated control, but not when compared with the water control (Table 6). In the latter treatment, powdery mildew was first observed on leaves at pea-size berries and bunches appeared to escape infection by *Erysiphe necator*. Multiple applications of materials A or B reduced logit mean severity of botrytis rot significantly when compared with the water control (Table 6).

When compared with the non treated and water controls, all treatments where fungicides were applied mid season, except where bunch thinning was applied in treatment 2, significantly reduced the level of *B. cinerea* antigens detected (SI, Table 6). The lowest levels of *B. cinerea* antigens were observed in the full season fungicide treatment and the fungicides applied mid season, with or without application of both material C and bunch thinning (treatments 1 and 4, Table 6).

Factorial ANOVA of the mid-season fungicide treatments indicated that bunch thinning increased the logit mean botrytis severity significantly (Table 7). The mean SI for bunch thinning appeared to be higher than without thinning, but the difference was not significant (Table 8). Material C did not have any impact on either logit mean botrytis severity or the mean SI (Tables 7 and 8) when combined with mid-season fungicide treatment.

There were no significant differences among means of treatments for bunch weights or juice quality in terms of total soluble solids, pH or titratable acidity (data analyses not presented).

Table 4. Treatments applied at trial site S43R07 in 2006-2007.

Treatment	Fungicide timing	Material C at 80% capfall	Bunch thinning to 5 t/ha	Material applied at each crop stage: 5% capfall 24 Nov. 06	80% capfall 30 Nov. 06	Pea size 27 Dec. 06	PBC 16 Jan. 07	Veraison 10 Feb. 07	Pre-harvest 13 Mar. 07
1	mid	+	+	-	material C	Captan	Switch	-	-
2	mid	-	+	-	-	Captan	Switch	-	-
3	mid	+	-	-	material C	Captan	Switch	-	-
4	mid	-	-	-	-	Captan	Switch	-	-
5	non treated	-	-	-	-	-	-	-	-
6	early	-	-	Bravo	Scala	-	-	-	-
7	late	-	-	-	-	-	-	Rovral	Rovral
8	full	-	-	Bravo	Scala	Captan	Switch	Rovral	Rovral
9	full, 5% capfall spray omitted	-	-	-	Scala	Captan	Switch	Rovral	Rovral
10	material A	-	-	material A	material A	material A	material A	material A	material A
11	material B	-	-	material B	material B	material B	material B	material B	material B
12	water[a] control, non treated for powdery mildew	-	-	water	water	water	water	water	water

[a] The water was dechlorinated by circulating it with a pond pump for 24 h

Table 5. Mean severity of botrytis rot on March 22, 2007, and mean grape yield and juice quality (na = not assayed) at harvest on March 26, 2007 at trial site S43R07. A yield of 5 t/ha was equivalent to 1.5 kg grapes/vine.

Treatment	Fungicide timing	Material C	Bunch thinning to 5 t/ha	Mean incidence (%) of latent infection at pre-bunch closure	Mean severity (%) of botrytis rot	Mean weight grapes (kg)/vine	Mean bunch weight (g)	Mean total soluble solids of juice (^{o}Brix)	Mean pH of juice	Mean titratable acidity (g/L) of juice
1	mid	+	+	0.08	1.2	1.7	69	20.8	3.0	10.2
2	mid	-	+	0.08	2.5	1.3	54	20.9	3.0	9.7
3	mid	+	-	0.00	1.3	2.1	57	20.8	3.0	9.7
4	mid	-	-	0.33	0.95	2.2	54	20.6	2.9	10.2
5	non treated	-	-	0.00	0.62	2.0	55	20.6	2.9	10.5
6	early	-	-	0.00	2.46	na	na	na	na	na
7	late	-	-	na	1.63	na	na	na	na	na
8	full	-	-	0.00	0.78	na	na	na	na	na
9	full, 5% capfall spray omitted	-	-	0.00	0.53	2.2	59	20.6	2.9	10.3
10	material A	-	-	na	0.0	2.2	63	20.4	3.0	9.9
11	material B	-	-	na	0.12	2.4	64	20.3	2.9	10.4
12	water control, non treated for powdery mildew	-	-	na	1.4	2.2	57	20.6	2.9	10.4

Table 6. Results of ANOVA for treatments at trial site S43R07 for logit mean severity of botrytis rot on March 22, 2007, and the mean signal intensity (SI) of the QuickStix™ test for *B. cinerea* antigens in grape juice (1:5 dilution), at harvest on March 26, 2007. Grape juice was thawed for assay on June 12, 2008, and na = not assayed. Means with the same letter are not significantly different at $P = 0.05$.

Treatment	Fungicide timing	Material C	Bunch thinning to 5 t/ha	Logit mean severity of botrytis rot (n = 6; 30 bunches/plot)		Mean SI (n = 5, blocks 2-6; juice from 10 bunches/plot)	
1	mid	+	+	-4.83	cde	13.3	abc
2	mid	-	+	-4.03	e	22.7	cde
3	mid	+	-	-5.35	cd	18.3	bcd
4	mid	-	-	-5.24	cd	8.3	ab
5	non treated	-	-	-5.56	bcd	30.5	de
6	early	-	-	-4.72	de	na	
7	late	-	-	-4.82	cde	na	
8	full	-	-	-5.84	abc	na	
9	full, 5% capfall spray omitted	-	-	-5.76	bcd	5.3	a
10	material A	-	-	-6.91	a	28.9	de
11	material B	-	-	-6.64	ab	25.9	de
12	water control, non treated for powdery mildew	-	-	-4.92	cde	34.1	e
				P	<0.001	*P*	<0.001
				residual df	115	residual df	32
				lsd	1.102	lsd	12.29

Table 7. Results of factorial ANOVA (n = 6) for fungicide treatments applied mid-season (residual df = 35) at trial site S43R07 for logit mean severity of botrytis rot on March 22, 2007.

		Bunch thinning to 5 t/ha *P* = 0.037 lsd = 0.811		
		+	-	Mean
Material C *P* = 0.261	+	-4.83	-5.35	-5.09
	-	-4.03	-5.24	-4.64
	Mean	-4.43	-5.30	Interaction: *P* = 0.392

Table 8. Results of factorial ANOVA (n = 5, blocks 2-6) of means for signal intensity (SI) of the QuickStix™ test for *B. cinerea* antigens in grape juice (1:5 dilution), for fungicide treatments applied mid-season (residual df = 12), at harvest on March 26, 2007, at trial site S43R07.

		Bunch thinning to 5 t/ha *P* = 0.177		
		+	-	Mean
Material C *P* = 0.937	+	13.3	18.3	15.8
	-	22.7	8.3	15.5
	Mean	18.0	13.3	Interaction: *P* = 0.013

4.2 Site S43SB07 (Sauvignon Blanc)
Refer to tables 9-13.

There were no significant differences among treatments for the mean incidence of latent infection at pre-bunch closure (Table 10, data analyses not presented).

The highest mean botrytis severity in this trial was 3.8% (Table 10). Berry splitting (fresh wounds) was observed in some bunches across the trial site generally on February 23, 2007. Sporulating *B. cinerea* appeared to emerge from split berries on March 5, 2007.

When compared with the non treated control, application of fungicide mid season (Captan® then Switch®) significantly reduced logit mean botrytis severity, the total soluble solids and titratable acidity (Table 11). The mean SI was also reduced by this treatment, but the difference from the non treated control was significant only at the 1% level (Tables 10 and 11).

Removal of trash at 100% capfall did not affect either logit mean botrytis severity or mean SI when means were subjected to factorial ANOVA with fungicides timed 'early', 'late' or 'non treated' (Tables 12 and 13). This factorial ANOVA was repeated for the mean incidence of botrytis rot, which was 37 and 40%, with and without trash removal, respectively. The effect of trash removal on botrytis incidence was not significant ($P = 0.560$, data not presented).

Grape yield (kg/vine) was increased significantly by mid-season fungicide treatment (Table 10). There were no significant differences among means of treatments for bunch weights or juice quality in terms of total soluble solids, pH or titratable acidity (Table 10).

Table 9. Treatments applied at trial site S43SB07 in 2006-2007.

Treatment	Fungicide timing	Trash removal at 100% capfall on 15 Dec. 06	Material applied at each crop stage: 5% capfall 5 Dec. 06	80% capfall 11 Dec. 06	Pea size 2 Jan. 07	PBC 23 Jan. 07	Veraison 10 Feb. 07	Pre-harvest 9 Mar. 07
1	early	+	Bravo	Scala	-	-	-	-
2	early	-	Bravo	Scala	-	-	-	-
3	mid	-	-	-	Captan	Switch	-	-
4	late	+	-	-	-	-	Rovral	Rovral
5	late	-	-	-	-	-	Rovral	Rovral
6	non treated	+	-	-	-	-	-	-
7	non treated	-	-	-	-	-	-	-

Table 10. Mean severity of botrytis rot on March 26, 2007, mean grape yield and juice quality, including the mean signal intensity (SI) of the QuickStix™ test for *B. cinerea* antigens in grape juice (1:5 dilution), at harvest on March 28, 2007, at trial site S43SB07. Grape juice was thawed for assay by QuickStix™ on June 4, 2008. Refer to Tables 10-11 for ANOVA of logit means for severity of botrytis rot. For the remaining grape or juice variables, NS = not significant at $P = 0.05$. Means with the same letter are not significantly different at $P = 0.05$.

Treatment	Fungicide timing	Trash removal at 100% capfall on 15 Dec. 06	Mean incidence (%) of latent infection at pre-bunch closure	Mean severity (%) of botrytis rot (30 bunches /plot)	Mean SI (juice from 10 bunches /plot)	Mean weight grapes (kg)/vine	Mean bunch weight (g)	Mean total soluble solids of juice (^{o}Brix)	Mean pH of juice	Mean titratable acidity (g/L) of juice
1	early	+	0.42	2.46	12.5	3.0 c	95	20.3	2.9	10.4
2	early	-	0.67	3.79	18.5	3.0 c	105	20.0	2.9	10.5
3	mid	-	0.00	0.89	3.1	4.2 a	102	19.6	2.9	9.7
4	late	+	-	2.44	11.8	3.9 ab	105	20.4	2.9	10.5
5	late	-	-	2.37	17.4	3.4 abc	96	20.0	2.9	10.3
6	non treated	+	0.25	2.51	9.9	3.1 bc	97	20.5	2.9	10.6
7	non treated	-	0.71	2.48	18.2	3.2 bc	106	20.6	2.9	10.7
					$P = 0.084$	$P = 0.037$	NS	NS	NS	NS
					residual df = 29	residual df = 29				
						lsd = 0.829				

Table 11. Results of ANOVA for logit mean severity of botrytis rot on March 26, 2007, the mean signal intensity (SI), total soluble solids and titratable acidity in grape juice at harvest on March 28, 2007, at trial site S43SB07, for treatments where there was no trash removal. Means with the same letter are not significantly different at $P = 0.05$.

Treatment	Fungicide timing	Logit mean severity of botrytis rot (30 bunches/plot)		Mean SI (10 bunches/plot)		Mean total soluble solids of juice (°Brix)		Mean titratable acidity (g/L) of juice	
2	early	-3.58	b	18.5	b	19.98	ab	10.517	a
3	mid	-4.69	a	3.1	a	19.57	b	9.717	b
5	late	-4.11	ab	17.4	b	19.95	ab	10.283	ab
7	non treated	-3.88	b	18.2	b	20.63	a	10.733	a
		P	0.028	*P*	0.086	*P*	0.040	*P*	0.012
		residual df	35	residual df	15	residual df	15	residual df	15
		lsd	0.734	lsd	13.84	lsd	0.707	lsd	0.5780

Table 12. Results of factorial ANOVA (residual df = 58, two missing values) for trash removal at 100% capfall at trial site S43SB07 for logit mean severity of botrytis rot on March 26, 2007.

		Trash removal at 100% capfall *P* = 0.586		
		+	-	Mean
Fungicide timing *P* = 0.333	early	-3.89	-3.58	-3.74
	late	-4.18	-4.11	-4.14
	non treated	-3.87	-3.88	-3.87
	Mean	-3.98	-3.86	Interaction: *P* = 0.840

Table 13. Results of factorial ANOVA (residual df = 24, one missing value) of the means for signal intensity (SI), at harvest on March 28, 2007, at trial site S43SB07.

		Trash removal at 100% capfall *P* = 0.122		
		+	-	Mean
Fungicide timing *P* = 0.978	early	12.5	18.5	15.5
	late	11.8	17.4	14.6
	non treated	11.1	18.2	14.7
	Mean	11.8	18.0	Interaction: *P* = 0.987

4.3 Site S43R08 (Riesling)
Refer to tables 14-19.

There were no significant differences among treatments for the mean incidence of latent infection at pre-bunch closure (Table 15, data analyses not presented).

The highest mean botrytis severity in this trial was 6.4% (Table 15). There was 7.8 mm rainfall within 24 h after application of material C at 80% capfall, and 4 mm rainfall in the following 24 h. Wasps were observed feeding on some rotting berries on March 28, 2008.

When compared with the non-treated controls, Switch® applied twice mid season significantly reduced logit mean botrytis severity and mean SI (Tables 16 and 17). Material C applied during flowering did not affect these variables significantly. Application of material C reduced total soluble solids and increased titratable acidity significantly (Tables 18 and 19), although there was a significant interaction between fungicide timing and material C for total soluble solids (Table 18). Application of fungicides mid season reduced titratable acidity significantly (Table 19).

There were no significant differences among means of treatments for grape yield (kg/vine), bunch weights or juice quality in terms pH (Table 15, data analyses not presented).

Table 14. Treatments applied at trial site S43R08 in 2007-2008.

			Material applied at each crop stage			
Treat-ment	Fungicide timing	Light leaf pluck on 3 Dec.07	5% capfall 29 Nov. 07	80% capfall 3 Dec. 07	Pea size 31 Dec. 07	PBC 8 Jan. 08
1	mid	+	-	-	Switch	Switch
2	mid	-	-	-	Switch	Switch
3	non treated	+	-	-		
4	non treated	-	-	-	-	-
5	mid	+	material C	material C	Switch	Switch
6	mid	-	material C	material C	Switch	Switch
7	non treated	+	material C	material C	-	-
8	non treated	-	material C	material C	-	-

Table 15. Mean severity of botrytis rot on April 21, 2008, mean grape yield and juice quality, including the mean signal intensity (SI) of the QuickStix™ test for *B. cinerea* antigens in grape juice (1:5 dilution), at harvest on April 22, 2008, at trial site S43R08. Grape juice was thawed for assay by QuickStix™ on July 8, 2008.

Treatment	Fungicide timing	Light leaf pluck on 3 Dec.07	Material C	Mean incidence (%) of latent infection at pre-bunch closure	Mean severity (%) of botrytis rot (30 bunches /plot)	Mean SI (juice from ≥ 10 bunches /plot)	Mean weight grapes (kg)/vine	Mean bunch weight (g)	Mean total soluble solids of juice (^{o}Brix)	Mean pH of juice	Mean titratable acidity (g/L) of juice
1	mid	+	-	0.00	2.0	8.4	4.5	115	20.7	3.2	5.7
2	mid	-	-	0.33	2.6	7.9	5.4	142	20.3	3.2	5.6
3	non treated	+	-	1.25	6.4	35.5	4.9	123	21.9	3.2	6.2
4	non treated	-	-	0.33	6.2	31.8	4.7	118	21.6	3.3	5.9
5	mid	+	+	0.42	2.7	12.2	5.5	141	20.6	3.2	6.2
6	mid	-	+	0.00	2.8	14.6	5.8	130	20.4	3.2	5.9
7	non treated	+	+	0.00	4.5	42.3	5.9	135	20.2	3.2	6.2
8	non treated	-	+	0.17	5.5	28.8	5.4	131	20.2	3.2	6.4

Table 16. Results of factorial ANOVA (residual df = 75) for logit mean severity of botrytis rot on April 21, 2008, at trial site S43R08. Means for the effect of light leaf plucking were not significantly different (P = 0.284) and the interactions between light leaf plucking and the other factors were not significant.

		Material C at 5% and 80% capfall P = 0.517		
		+	-	Mean
Fungicide timing P = <0.001 lsd = 0.3725	mid	-3.935	-4.012	-3.974
	non treated	-3.180	-3.346	-3.263
	Mean	-3.557	-3.679	Interaction: P = 0.812

Table 17. Results of factorial ANOVA (residual df = 75) for the means of signal intensity (SI), at harvest on April 22, 2008, at trial site S43R08. Means for the effect of light leaf plucking were not significantly different (P = 0.222) and the interactions between light leaf plucking and the other factors were not significant.

		Material C at 5% and 80% capfall P = 0.251		
		+	-	Mean
Fungicide timing P = <0.001 lsd = 6.13	mid	13.4	8.1	10.8
	non treated	35.6	33.7	34.6
	Mean	24.5	20.9	Interaction: P = 0.855

Table 18. Results of factorial ANOVA (residual df = 75) for the means of total soluble solids (°Brix), at harvest on April 22, 2008, at trial site S43R08. Means for the effect of light leaf plucking were not significantly different (*P* = 0.487) and the interactions between light leaf plucking and the other factors were not significant.

		Material C at 5% and 80% capfall *P* = 0.012, lsd = 0.588		
		+	-	Mean
Fungicide timing *P* = 0.130	mid	20.54	20.50	20.52
	non treated	20.19	21.75	20.97
	Mean	20.36	21.13	Interaction: *P* = 0.008

Table 19. Results of factorial ANOVA (residual df = 75) for the means of titratable acidity (g/L), at harvest on April 22, 2008, at trial site S43R08. Means for the effect of light leaf plucking were not significantly different (*P* = 0.489) and the interactions between light leaf plucking and the other factors were not significant.

		Material C at 5% and 80% capfall *P* = 0.023, lsd = 0.3099		
		+	-	Mean
Fungicide timing *P* = 0.022 lsd = 0.3099	mid	6.061	5.608	5.834
	non treated	6.334	6.063	6.198
	Mean	6.197	5.835	Interaction: *P* = 0.560

4.4 Site S43SB08 (Sauvignon Blanc)
Refer to tables 20-23.

There were no significant differences among treatments for the mean incidence of latent infection at pre-bunch closure (Table 21, data analyses not presented).

The highest mean botrytis severity in this trial was 2.9% (Table 21). A moderate level of berry splitting and an infestation of European wasps were observed across the trial site generally on March 3, 2008, when the symptoms of botrytis rot were first observed. The wasps appeared to feed on split berries, exacerbating berry damage.

When compared with the non-treated controls, application of fungicides did not reduce the logit mean botrytis severity near harvest significantly (Table 22), although fungicides applied early or mid season reduced the mean SI of grape juice (Table 23). Leaf plucking on the eastern side of the trellis at pre-bunch closure did not reduce the logit mean botrytis severity nor mean SI significantly (Tables 22 and 23).

There were no significant differences among means of treatments for grape yield (kg/vine), bunch weights or juice quality in terms of total soluble solids, pH or titratable acidity (Table 21, data analyses not presented).

Table 20. Treatments applied at trial site S43SB08 in 2007-2008.

Treatment	Fungicide timing	Leaf pluck on 21 Jan.08[a]	Material applied at each crop stage: 5% capfall 4 Dec. 07	80% capfall 13 Dec. 07	Pea size 8 Jan. 08	PBC 22 Jan. 08	Veraison 18 Feb. 08	Pre-harvest 13 Mar. 08
1	early	-	Scala	Scala	-	-	-	-
2	mid	-	-	-	Switch	Switch	-	-
3	late	-	-	-	-	-	Rovral	Rovral
4	non treated	-	-	-	-	-	-	-
5	early	+	Scala	Scala	-	-	-	-
6	mid	+	-	-	Switch	Switch	-	-
7	late	+	-	-	-	-	Rovral	Rovral
8	non treated	+	-	-	-	-	-	-

[a]Leaves removed on eastern side of trellis only.

Table 21. Mean severity of botrytis rot on March 17, 2008, and mean grape yield and juice quality, including the mean signal intensity (SI) of the QuickStix™ test for *B. cinerea* antigens in grape juice (1:5 dilution), at harvest on March 19, 2008, at trial site S43SB08. Grape juice was thawed for assay by QuickStix™ on July 7, 2008.

Treatment	Fungicide timing	Leaf pluck on 21 Jan.08	Mean incidence (%) of latent infection at pre-bunch closure	Mean severity (%) of botrytis rot (30 bunches /plot)	Mean SI (juice from ≥ 10 bunches /plot)	Mean weight grapes (kg)/vine	Mean bunch weight (g)	Mean total soluble solids of juice (^{o}Brix)	Mean pH of juice	Mean titratable acidity (g/L) of juice
1	early	-	0.00	2.9	10.1	2.7	83	22.5	3.0	12.8
2	mid	-	0.33	1.9	7.3	2.8	97	22.5	3.1	11.0
3	late	-	0.33	1.9	15.7	2.4	93	22.2	3.0	11.9
4	non treated	-	0.00	2.7	22.8	2.8	92	22.4	3.0	11.3
5	early	+	0.83	1.8	11.0	3.3	105	21.0	3.0	11.6
6	mid	+	0.67	1.6	6.4	2.8	95	21.7	3.0	10.9
7	late	+	0.00	2.9	18.1	2.5	85	22.1	3.0	11.4
8	non treated	+	0.33	2.2	15.3	2.7	93	22.2	3.0	11.4

Table 22. Results of factorial ANOVA (residual df = 35) for logit mean severity of botrytis rot on March 17, 2008, at trial site S43SB08.

		Leaf pluck at pre-bunch closure *P* = 0.182		
		-	+	Mean
Fungicide timing *P* = 0.745	early	-4.093	-3.566	-3.829
	mid	-4.073	-3.953	-4.013
	late	-3.756	-3.932	-3.844
	non treated	-3.973	-3.606	-3.790
	Mean	-3.974	-3.764	Interaction: *P* = 0.406

Table 23. Results of factorial ANOVA (residual df = 35) for the mean signal intensity (SI), at harvest on March 19, 2008, at trial site S43SB08.

		Leaf pluck at pre-bunch closure *P* = 0.574		
		-	+	Mean
Fungicide timing *P* = 0.001 lsd = 6.32	early	11.0	10.1	10.5
	mid	6.4	7.3	6.8
	late	18.1	15.7	16.9
	non treated	15.3	22.8	19.0
	Mean	12.7	14.0	Interaction: *P* = 0.422

4.5 Site S43CH08 (Chardonnay)
Refer to tables 24-30.

Fungicide applied at pea-sized berries reduced the mean incidence of latent infection at pre-bunch closure when compared to non-treated controls (Tables 25 and 26a).

The highest mean botrytis severity in this trial was 4.8% (Table 25). The grower cooperator removed leaves mechanically from the fruiting zone across the whole trial site on December 22, 2007.

When compared with the non-treated controls, application of fungicides or trash removal did not reduce the logit mean botrytis severity near harvest significantly (Table 26b). This factorial ANOVA was repeated for the mean incidence of botrytis rot, and, again, the effects were not significant (Table 27). However, there was a significant interaction between trash removal and fungicide timing treatments for the mean incidence of botrytis rot. Factorial ANOVA without data for the fungicide treatment at pea-size berries resulted in a significant effect (P = 0.021) for trash removal. The mean incidence with and without trash removal, was 65 and 72%, respectively.

Fungicides applied mid season (pea size or PBC) or late season reduced the mean SI of grape juice (Table 28). There was also a significant interaction between trash removal and fungicide timing treatments for mean SI (Table 28) and a marginally significant interaction for mean total soluble solids (Table 29), although the mean effect of trash removal was not significant for either grape juice variable. Factorial ANOVA of means for SI without data for the fungicide treatment at PBC resulted in a significant effect of trash removal (P = 0.019): the mean SI with and without trash removal was 29 and 23, respectively.

Switch® applied at pre-bunch closure had a higher mean pH of grape juice when compared with Switch® applied at pea-size berries (Table 30).

Table 24. Treatments applied at trial site S43CH08 in 2007-2008.

Treat-ment	Fungicide timing	Trash removed at 100% capfall (12 Dec. 07) & before PBC (15 Jan. 08)	Material applied at each crop stage			
			Pea size 2 Jan. 08	PBC 22 Jan. 08	Veraison 18 Feb. 08	Pre-harvest 13 Mar. 08
1	non treated	+	-	-	-	-
2	non treated	-	-	-	-	-
3	mid, pea	+	Switch	-	-	-
4	mid, pea	-	Switch	-	-	-
5	mid, PBC	+	-	Switch	-	-
6	mid, PBC	-	-	Switch	-	-
7	late	+	-	-	Flint	Flint
8	late	-	-	-	Flint	Flint

Table 25. Mean severity of botrytis rot on April 7, 2007, and mean grape yield and juice quality, including the mean signal intensity (SI) of the QuickStix™ test for *B. cinerea* antigens in grape juice (1:5 dilution), at harvest on April 9, 2008, at trial site S43CH08. Grape juice was thawed for assay by QuickStix™ on May 13, 2008.

Treatment	Fungicide timing	Trash removed at 100% capfall (12 Dec. 07) & before PBC (15 Jan. 08)	Mean incidence (%) of latent infection at pre-bunch closure	Mean severity (%) of botrytis rot (30 bunches /plot)	Mean SI (juice from 12 bunches /plot)	Mean weight grapes (kg)/vine	Mean bunch weight (g)	Mean total soluble solids of juice (°Brix)	Mean pH of juice	Mean titratable acidity (g/L) of juice
1	non treated	+	3.44	4.8	42.2	4.3	105	23.1	3.2	7.2
2	non treated	-	2.50	3.6	38.7	3.3	90	23.0	3.2	7.1
3	mid, pea	+	0.63	3.3	15.8	3.9	101	23.4	3.2	7.0
4	mid, pea	-	1.77	3.2	14.6	4.6	100	23.1	3.2	7.3
5	mid, PBC	+	-	2.4	18.8	5.0	106	22.4	3.2	7.2
6	mid, PBC	-	-	3.4	25.6	4.3	98	23.1	3.3	6.8
7	late	+	-	2.4	30.2	3.9	105	22.7	3.2	7.4
8	late	-	-	2.8	16.0	4.2	99	23.1	3.2	7.2

Table 26a. Results of factorial ANOVA for mean incidence of latent infection at pre-bunch closure, at trial site S43CH08.

		Trash removal at 100% capfall and before PBC *P* = 0.955		
		+	-	Mean
Fungicide timing *P* = 0.04	mid, pea	0.63	1.77	1.20
	non treated	3.44	2.50	2.97
	Mean	2.04	3.14	Interaction: *P* = 0.05

Table 26b. Results of factorial ANOVA (residual df = 75) for logit mean severity of botrytis rot on April 7, 2008, at trial site S43CH08.

		Trash removal at 100% capfall and before PBC *P* = 0.794		
		+	-	Mean
Fungicide timing *P* = 0.065 lsd = 0.2894	mid, pea	-3.458	-3.538	-3.498
	mid, PBC	-3.749	-3.356	-3.553
	late	-3.706	-3.643	-3.675
	non treated	-3.150	-3.419	-3.284
	Mean	-3.516	-3.489	Interaction: *P* = 0.145

Table 27. Results of factorial ANOVA (residual df = 75) for mean incidence of botrytis rot on April 7, 2008, at trial site S43CH08.

		Trash removal at 100% capfall and before PBC *P* = 0.363		
		+	-	Mean
Fungicide timing *P* = <0.001 lsd = 6.87	mid, pea	79.8	67.3	73.5
	mid, PBC	58.3	69.6	64.0
	late	60.1	67.9	64.0
	non treated	75.0	77.4	76.2
	Mean	68.3	70.5	Interaction: *P* = 0.005

Table 28. Results of factorial ANOVA (residual df = 75) for the means of signal intensity (SI) at harvest on April 9, 2008, at trial site S43CH08.

		Trash removal at 100% capfall and before PBC spray *P* = 0.203		
		+	-	Mean
Fungicide timing P = <0.001 lsd = 7.29	mid, pea	15.8	14.6	15.2
	mid, PBC	18.8	25.6	22.2
	late	30.2	16.0	23.1
	non treated	42.2	38.7	40.4
	Mean	26.7	23.7	Interaction: *P* = 0.047

Table 29. Results of factorial ANOVA (residual df = 75) for the mean total soluble solids (°Brix) of grape juice at harvest on April 9, 2008, at trial site S43CH08.

		Trash removal at 100% capfall and before PBC spray $P = 0.263$		
		+	-	Mean
Fungicide timing $P = 0.110$	mid, pea	23.39	23.09	23.24
	mid, PBC	22.42	23.10	22.76
	late	22.73	23.13	22.93
	non treated	23.11	22.97	23.04
	Mean	22.91	23.07	Interaction: $P = 0.054$

Table 30. Results of factorial ANOVA (residual df = 74, one missing value) for the mean pH of grape juice at harvest on April 9, 2008, at trial site S43CH08.

		Trash removal at 100% capfall and before PBC spray $P = 0.213$		
		+	-	Mean
Fungicide timing $P = 0.017$ lsd = 0.0488	mid, pea	3.20	3.17	3.18
	mid, PBC	3.24	3.29	3.26
	late	3.23	3.24	3.23
	non treated	3.20	3.24	3.22
	Mean	3.22	3.23	Interaction: $P = 0.374$

4.6 Site S43R08_2 (Riesling)
Refer to tables 31-36.

There were no significant differences among treatments for the mean incidence of latent infection at pre-bunch closure (Table 32, data analyses not presented).

The highest mean botrytis severity in this trial was 1.4% (Table 32). There was 5.4 mm of rainfall between 12 and 24 h after application of Scala® at 80% capfall. Bunches on the western side of the canopy were sunburned on January 8, 2008, with the lower one third of the bunch dry and shrivelled in some cases. The severely damaged bunches were removed by the grower co-operator on January 16, 2008.

There were no significant differences among means of treatments for logit mean severity of botrytis rot near harvest nor mean SI of grape juice (Tables 33 and 34). There were significant differences among treatment means for grape yield (kg/vine) or total soluble solids (Tables 35 and 36), but the differences from the non treated control were not unidirectional.

Table 31. Treatments applied at trial site S43R08_2 in 2007-2008.

Treatment	Fungicide timing	Material applied at each crop stage		
		80% capfall 2 Dec. 07	Pea size 31 Dec. 07	PBC 8 Jan. 08
1	mid, pea	-	Switch	-
2	mid, PBC	-	-	Switch
3	mid, pea & PBC	-	Switch	Switch
4	non treated	-	-	-
5	mid, pea	Scala	Switch	-
6	mid, PBC	Scala	-	Switch
7	mid, pea & PBC	Scala	Switch	Switch
8	non treated	Scala	-	-

Table 32. Mean severity of botrytis rot on 24 March, 2008, and mean grape yield and juice quality, including the mean signal intensity (SI) of the QuickStix™ test for *B. cinerea* antigens in grape juice (1:5 dilution), at harvest on March 26, 2008, at trial site S43R08_2. Grape juice was thawed for assay by QuickStix™ on July 5, 2008.

Treatment	Fungicide timing	Scala applied at 80% capfall	Mean incidence (%) of latent infection at pre-bunch closure	Mean severity (%) of botrytis rot (30 bunches /plot)	Mean SI (juice from ≥ 10 bunches /plot)	Mean weight grapes (kg)/vine	Mean bunch weight (g)	Mean total soluble solids of juice (°Brix)	Mean pH of juice	Mean titratable acidity (g/L) of juice
1	mid, pea	-	0.3	1.0	2.4	4.0	103	20.4	3.0	8.5
2	mid, PBC	-	0.0	0.84	3.2	4.6	120	19.6	3.0	8.3
3	mid, pea & PBC	-	0.0	1.4	7.3	3.7	112	21.2	3.1	8.1
4	non treated	-	0.5	1.9	15.3	4.1	112	20.5	3.0	8.5
5	mid, pea	+	0.0	0.84	2.2	4.5	126	20.3	3.0	8.6
6	mid, PBC	+	0.0	1.4	4.8	4.7	117	20.2	3.1	8.3
7	mid, pea & PBC	+	0.0	0.77	4.8	3.4	102	20.7	3.0	8.2
8	non treated	+	0.0	0.78	5.1	3.4	97	20.4	3.1	8.1

Table 33. Results of factorial ANOVA (residual df = 75) for logit mean severity of botrytis rot on March 24, 2008, at trial site S43R08_2.

		Scala applied at 80% capfall *P* = 0.112		
		+	-	Mean
Fungicide timing *P* = 0.787	mid, pea	-4.928	-4.731	-4.830
	mid, PBC	-4.359	-4.964	-4.662
	mid, pea & PBC	-4.999	-4.389	-4.694
	non treated	-5.011	-4.227	-4.619
	Mean	-4.824	-4.578 *P* = 0.107 for ANOVA without capfall spray treatments, residual df = 35	Interaction: *P* = <0.001

Table 34. Results of factorial ANOVA (residual df = 75) for the means of signal intensity (SI), at harvest on March 26, 2008, at trial site S43R08_2.

		Scala applied at 80% capfall *P* = 0.240		
		+	-	Mean
Fungicide timing *P* = 0.112	mid, pea	2.2	2.4	2.3
	mid, PBC	4.8	3.2	4.0
	mid, pea & PBC	4.8	7.2	6.0
	non treated	5.1	15.3	10.2
	Mean	4.2	7.1 *P* = 0.103 for ANOVA without capfall spray treatments, residual df = 35	Interaction: *P* = 0.321

Table 35. Results of factorial ANOVA (residual df = 35) for the mean grape weight (kg) per vine, at harvest on March 26, 2008, at trial site S43R08_2.

		Scala applied at 80% capfall *P* = 0.694		
		+	-	Mean
Fungicide timing *P* = 0.061 lsd = 0.81	mid, pea	4.5	4.0	4.2
	mid, PBC	4.7	4.6	4.6
	mid, pea & PBC	3.4	3.9	3.6
	non treated	3.4	4.1	3.8
	Mean	4.0	4.1	Interaction: *P* = 0.395

Table 36. Results of factorial ANOVA (residual df = 75) for the mean total soluble solids (^{o}Brix), at harvest on March 26, 2008, at trial site S43R08_2. Means with the same letter are not significantly different at *P* = 0.05.

		Scala applied at 80% capfall *P* = 0.988		
		+	-	Mean
Fungicide timing *P* = <0.001 lsd = 0.3857	mid, pea	20.3	20.4	20.3 bc
	mid, PBC	20.2	19.6	19.9 c
	mid, pea & PBC	20.7	21.2	20.9 a
	non treated	20.4	20.5	20.4 b
	Mean	20.4	20.4	Interaction: *P* = 0.075

4.7 Site S43R09 (Riesling)
Refer to tables 37-39.

The highest mean botrytis severity in this trial was 5.6% (Table 38). This site was characterised by relatively large bunches with even berry size and very little berry damage. Botrytis symptoms began to increase significantly at the start of April, but then there was fine, dry weather from the second week of April. In the week before harvest, European wasps were seen feeding on berries that had sporulating *B. cinerea*. Indeed, the wasps seemed to remove the contents of these berries. The numerical differences among treatments for mean botrytis severity were not statistically significant (Table 39).

Table 37. Treatments applied at trial site S43R09 in 2008-2009. The date for 5% capfall was 28 Nov. 08 and pea-size berries were evident by 7 Jan. 09.

Treatment	Fungicide timing	Material applied at each crop stage			
		Leaf Plucking 7 Jan. 09 (both sides)	80% capfall 9 Dec. 08	PBC 14 Jan. 09	Veraison 26 Feb. 09
1	nil	-	-	-	-
2	nil	+	-	-	-
3	early	-	Switch	-	-
4	mid	-	-	Switch	-
5	late	-	-	-	Rovral
6	full	-	Switch	Switch	Rovral

Table 38. Mean severity of botrytis rot on 20 April 2009, and mean grape yield and juice quality (na = not assayed) at harvest on 21 April, 2009 at trial site S43R09.

Treatment	Fungicide timing	Leaf plucking (both sides)	Mean severity (%) of botrytis rot	Mean weight grapes (kg)/vine	Mean bunch weight (g)	Mean total soluble solids of juice (°Brix)	Mean pH of juice	Mean titratable acidity (g/L) of juice
1	nil	-	4.9	3.0	103.6	20.6	2.92	8.35
2	nil	+	2.2	3.2	105.3	20.4	2.99	8.49
3	early	-	5.6	3.3	109.9	20.5	na	na
4	mid	-	2.8	3.6	116.5	20.5	na	na
5	late	-	3.7	3.0	98.5	20.8	na	na
6	full	-	1.7	3.1	108.5	20.7	na	na

Table 39. Results of ANOVA for logit mean severity of botrytis rot on April 20, 2009, at trial site S43R09.

Treatment	Fungicide timing	Leaf plucking (both sides)	Logit mean severity of botrytis rot
1	nil	-	-3.31
2	nil	+	-3.80
3	early	-	-3.16
4	mid	-	-3.83
5	late	-	-3.53
6	full	-	-4.26
			$P = 0.403$
			residual df = 25

4.8 Site S43SB09 (Sauvignon Blanc)
Refer to tables 40-42.

The highest mean botrytis severity in this trial was 6.4% (Table 41). Widespread splitting of berries was observed 2-5 March, 2009. Symptoms of botrytis were first observed on split berries March 9, 2009. When compared with the non-treated control, Switch® applied early, mid or late season reduced the logit mean botrytis severity to an equivalent level (Table 42). Switch® was more effective than Rovral® when applied late season, although there are restrictions on applying Switch® at this time (Table 42). Leaf plucking did not reduce logit mean botrytis severity at this trial site (Table 42).

Table 40. Treatments applied at trial site S43SB09 in 2008-2009. The date for 5% capfall was 10 Dec. 08 and pea-size berries were evident by 16 Jan. 09.

Treatment	Fungicide timing	Leaf plucking 23 Jan. 09 (both sides)	Material applied at each crop stage 80% capfall 17 Dec. 08	PBC 3 Feb. 09	Veraison 26 Feb. 09
1	nil	-	-	-	-
2	nil	+	-	-	-
3	early	-	Switch	-	-
4	mid	-	-	Switch	-
5	late	-	-	-	Switch
6	late	-	-	-	Rovral

Table 41. Mean botrytis severity on March 30, 2009, and mean grape yield and juice quality (na = not assayed) at harvest on March 31, 2009 at trial site S43SB09.

Treatment	Fungicide timing	Leaf plucking	Mean severity (%) of botrytis rot	Mean weight grapes (kg)/vine	Mean bunch weight (g)	Mean total soluble solids of juice (^{o}Brix)	Mean pH of juice	Mean titratable acidity (g/L) of juice
1	nil	-	5.2	1.5	50.1	22.0	2.94	12.1
2	nil	+	6.4	2.0	59.4	21.7	2.99	11.4
3	early	-	2.4	2.3	62.3	21.7	na	na
4	mid	-	1.0	2.1	62.3	22.3	na	na
5	late (Switch)	-	0.6	2.0	57.8	22.6	na	na
6	late (Rovral)	-	3.6	2.0	55.3	22.2	na	na

Table 42 . Results of ANOVA for logit mean severity of botrytis rot on March 30, 2009, at trial site S43SB09.

Treatment	Fungicide timing	Leaf plucking (both sides)	Logit mean severity of botrytis rot	
1	nil	-	-2.91	c
2	nil	+	-3.07	c
3	early	-	-4.19	ab
4	mid	-	-4.69	ab
5	late (Switch)	-	-5.61	a
6	late (Rovral)	-	-3.94	bc
			P	<0.001
			residual df	25
			lsd	1.052

4.9 Correlation of means for logit severity of botrytis rot and SI in grape juice

There was a positive and significant correlation between treatment means for logit severity of botrytis rot and LN mean SI across trial sites (Figure 1). LN mean SI explained 78.9% of the variation in logit mean severity of botrytis rot and the slope and the intercept of the linear regression were significantly different from zero.

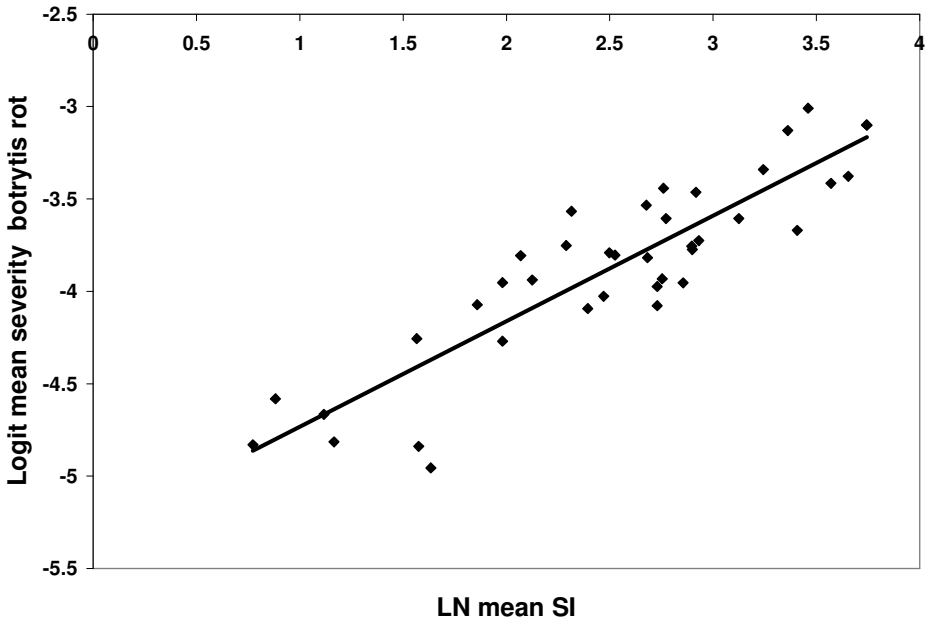


Figure 1. Linear relationship between LN mean SI (x) and logit mean severity of botrytis rot (y) for all treatment means in all trials, except trial S43R07. The linear regression was significant (P<0.001) and LN(mean SI) explained 78.9% of the variation in logit mean severity of botrytis rot. The slope and intercept of the fitted line (y = -5.305 + 0.5715x) were significantly different (P<0.001) from zero.

4.10 Botrytis severity, juice characters and compactness of bunches according to bunch position

Depending on the year of testing, botrytis severity may be higher, lower or similar for basal bunches when compared with bunches positioned distally (Table 43). Distal bunches appeared to be more compact than basal bunches at the six trial sites assessed. The difference in compactness was statistically significant at three sites (Table 43).

Unlike botrytis severity, mean SI did not vary significantly according to bunch position, although the numerical values of SI for site S43R08_2 corresponded to the means observed for botrytis severity.

Basal bunches had significantly higher total soluble solid content at two of the three sites tested (Table 43). There was no evidence that botrytis severity, according to bunch position, was correlated positively to the total soluble solid content of juice (Table 43). For example, the lower botrytis severity for basal bunches at site S43R08 was associated with a higher total soluble solid content, relative to distal bunches. Basal bunches had either a higher or lower pH of grape juice than distal bunches for the three sites tested (Table 43). Basal bunches had a higher titratable acidity of grape juice than distal bunches at one of three sites tested (Table 43).

Table 43. Means of the grape or juice variables indicated for bunches in either the basal or distal position on vertically positioned shoots. For all variables apart from bunch openness, the means were calculated using data from all treatments in which the variable was estimated. The residual df refers to the ANOVA of treatments in which bunch position was used as a factor. NS = means not significantly different at $P = 0.05$.

Trial	Basal bunch (position 1)	Distal bunch (position 2)	*P*	residual df	lsd
Logit mean severity of botrytis rot (15 bunches/position/plot					
S43R07	-5.29	-5.48	0.397	115	NS
S43SB07	-3.787	-4.241	0.021	63	0.3843
S43R08	-3.970	-3.266	<0.001	75	0.3725
S43CH08	-3.428	-3.577	0.150	75	NS
S43R08_2	-5.049	-4.353	<0.001	75	0.3052
Mean bunch openness (%)					
S43R07	57.1	53.3	0.075	n = 225/sample for t-test	
S43SB07	19.9	13.5	0.023	n = 225/sample for t-test	
S43R08	64.3	60.3	0.154	n = 24/sample for t-test	
S43SB08	59.3	41.8	<0.001	n = 24/sample for t-test	
S43CH08	79.2	73.5	0.005	n = 24/sample for t-test	
S43R08_2	65.4	63.2	0.229	n = 24/sample for t-test	
Mean signal intensity (SI, 5-6 bunches/position/plot)					
S43R08	22.4	23.0	0.855	75	NS
S43CH08	23.6	26.6	0.248	75	NS
S43R08_2	3.7	7.5	0.115	75	NS
Mean total soluble solids (^{o}Brix)					
S43R08	21.16	20.33	0.006	75	0.588
S43CH08	23.10	22.88	0.113	75	NS
S43R08_2	20.75	20.04	<0.001	75	0.2728
Mean pH					
S43R08	3.246	3.157	0.028	75	0.07
S43CH08	3.195	3.252	0.001	75	0.03451
S43R08_2	2.981	3.0987	<0.001	75	0.03626
Mean titratable acidity (g/L)					
S43R08	5.949	6.083	0.392	75	NS
S43CH08	7.224	7.085	0.256	75	NS
S43R08_2	8.751	7.899	<0.001	75	0.3239

5 Discussion

5.1 Fungicide timing

Considering both the severity of botrytis rot at harvest and the level of *B. cinerea* antigens in grape juice, application of Switch® once or twice during the mid-season was beneficial for the management of botrytis rot in five of the eight trials conducted. At site S43CH07, the mid-season fungicide applications also increased grape yield (kg/vine). The effect of wasp activity in several trials was not quantified, but potential interactions with botrytis could be investigated further.

At site S43R08_2, results were inconclusive for investigating the timing of Switch® mid season, at either pea size berries or PBC, because both the severity of botrytis rot and the level of *B. cinerea* antigens in grape juice from non treated plots was relatively low. At site S43CH08, one application of Switch® at either pea size berries or PBC resulted in similar means for the severity of botrytis rot and level of *B. cinerea* antigens in grape juice. The optimum time for applying one application of Switch® mid-season requires further investigation.

No significant differences were observed among fungicide timing treatments at site S43SB08, which might be explained by general wounding of the berries (splitting and wasp damage) at the onset of symptoms of botrytis rot. These wounds may have provided entry sites for *B. cinerea* and an additional infection pathway that may not have been prevented by the fungicides applied. Similarly, no significant differences were observed among treatments at site S43R09, even though numerical differences between some means might have been significant in practice. Large variation among bunches within plots for botrytis severity might explain this result, which highlights the challenges of designing small-plot trials for botrytis that lead to statistical separation of treatments.

There was no benefit for the management of botrytis rot in applying Bravo® and Scala® at flowering at site S43SB07, whereas two applications of Scala® at flowering at the same site in 2008 (S43SB08) reduced the level of *B. cinerea* antigens in grape juice. There was less rain during flowering in 2007 in comparison to 2008 (Table 3), meaning that conditions may not have favoured infection at flowering by *B. cinerea* at this site in 2007. The results for fungicides applied at flowering for sites S43R07 and S43R08_2 were inconclusive.

There was no benefit for the management of botrytis rot in applying two applications of Rovral® late season at sites S43SB07 and S43SB08, nor one application of Rovral® late season at site S43SB09. This result suggests that Rovral® is not necessary in years when conditions in southern Tasmania are moderately favourable for the development of botrytis rot and when harvest is timely and not delayed by excessive crop loads. There may have also been an (unknown) level of dicarboximide resistance in the population of *B. cinerea* that reduced the effectiveness of Rovral® at this site.

It was not possible to test the hypothesis that Flint® reduced the severity of sporulation of *B. cinerea*, because the severity of sporulation at site S43CH08 was very low (data not presented). Nevertheless, Flint® reduced the severity of botrytis rot (at $P = 0.065$) and the level of *B. cinerea* antigens in grape juice, when compared with the non treated control.

5.2 Effectiveness of developmental materials

Based on results of two trials in Riesling, there was no evidence that material C applied once or twice during flowering, with or without two applications of fungicide mid season, reduced the severity of botrytis rot significantly. In one of the Riesling trials (S43R08), rainfall after

application of material C at 80% capfall may have influenced the effectiveness of the treatment. In this same trial, material C reduced total soluble solids and increased titratable acidity in grape juice significantly, but the reason for this effect is unknown.

Materials A and B, when applied to Riesling at site S43R07, appeared to reduce the visual symptoms of *B. cinerea* infection, although the antigens of *B. cinerea* in juice from these treatments were as abundant as those detected in the non treated and water controls. The mechanism of action of materials A and B requires further investigation.

5.3 Effect of bunch thinning

There was limited evidence from one trial that bunch thinning increased the severity of botrytis rot significantly. The reason for this result is obscure, given that bunch thinning did not increase the mean total soluble solid content of berries significantly and, potentially, the infection efficiency of *B. cinerea* (Hill *et al.* 1981).

5.4 Effect of bunch trash removal

Removal of floral debris at early fruit set has been shown to reduce the incidence (Rozario *et al.* 2005) and severity (Wolf *et al.* 1997) of botrytis confirming that this source of inoculum can be important for infection of fruit in some epidemics. Removal of floral debris, however, may have little impact on severity of botrytis rot at harvest when conditions are highly favourable for disease expression (Rozario *et al.* 2005). Unlike the studies of Rozario *et al.* (2005) and Wolf *et al.* (1997), removal of bunch trash in two trials of this study did not reduce the incidence or severity of botrytis rot significantly. At trial site S43CH08, the significant interaction between trash removal and fungicide timing treatments for the mean incidence of botrytis implied that the trash removal might reduce disease incidence slightly if combined with fungicide treatment after trash removal. However, the mean SI, which was correlated to botrytis severity, appeared to increase in three of the fungicide timing treatments, which might be explained if the compressed air caused damage to setting and immature berries. Given that conidia of *B. cinerea* are dispersed mainly by air, then the results might also reflect some inter-plot interference. Furthermore, bunch trash is not removed completely by the use of compressed air and there may still be sufficient inoculum within the canopy and in the immediate vineyard environment for significant disease development.

A more reliable, but more laborious, method for determining the role of bunch trash in the epidemiology of botrytis rot, is quantification of the incidence of infected floral debris as reported by Seyb (2004) and in Chapter 1. In the Marlborough region of New Zealand, the mean number of trash pieces per bunch and the number infected with *B. cinerea*, especially aborted berries and infected caps (calyptrae), has been correlated positively with the number of berries infected with *B. cinerea* post veraison (Seyb 2004).

5.5 Effect of leaf removal

Leaf removal (plucking) had no impact on botrytis severity at harvest for two trials conducted in 2008-2009. The leaf layer numbers (2.4 and 2.7 for sites S43R09 and S43SB09, respectively) and the 'moderate' crop vigour rating (Table 1) suggested that the respective canopies were not dense prior to leaf removal. Leaf removal on one side of the canopy also had no impact on botrytis severity the previous season at site S43SB08, with a leaf layer number of 1.9 (Table 1). Leaf removal treatments were included at sites in 2008-09 so that comparisons could be made across the regions (Tas., Vic. and NZ).

5.6 Effect of treatments on grape juice quality

Significant differences were observed among treatments in some trials for grape juice quality in terms of total soluble solids, pH and/or titratable acidity. The reasons for the differences observed are obscure.

5.7 Preliminary evaluation of QuickStix™

Analyses of grape juice for antigens of *B. cinerea* using the The QuickStix™ Kit for Botrytis in Wine Grape Juice (EnviroLogix Inc.) complemented visual scoring of disease symptoms to give an overall picture of 'botrytis load' in grapes and juice. Indeed, the QuickStix™ test separated treatment means in some trials where visual scoring did not result in statistically significant difference. In other cases, the statistical separation provided by QuickStix™ was less than for visual scoring, which might be explained by the lower sample size used for the juice analyses when compared with visual scoring of grape bunches. Given the potential for large variation among grape bunches in expression of botrytis rot during mild epidemics, it is recommended that QuickStix™ be applied to juice from 30 bunches/plot, so that direct comparison with visual scoring can be made.

The negative exponential (or monomolecular) shape of the fitted curve for logit mean severity (visual scoring) as a function of the mean signal intensity, which becomes linear with logarithmic transformation of SI (Figure 1), reflected that the SI plateaus as it approaches the upper limit of about 55 for an individual strip test. A higher rate of dilution with EB8 buffer would be required for juice from trials where the severity of botrytis rot was higher than observed in this study.

The utility of the QuickStix™ test is likely to be greatest when treatment differences are not detected visually because the epidemic of botrytis rot has not progressed sufficiently for adequate expression of disease symptoms. The QuickStix™ test is also likely to be useful when some symptoms on grape berries are inadvertently scored as botrytis rot but the symptoms are caused by abiotic stress and/or a different bunch rot pathogen. Again, the likelihood of falsely scoring symptoms is greatest in the early stages of a botrytis epidemic and when the incidence and severity of sporulating *B. cinerea* is very low.

5.8 Botrytis severity, juice characters and compactness of bunches according to position

Given the trend for distal bunches to be more compact than basal bunches in this study, differences in botrytis severity according to bunch position might, in some cases, be influenced by the architecture of the bunch. Bunch compactness has also been found to influence botrytis severity in a number of studies; for example, Vail and Marois (1991), Vail *et al.* (1998) and Hed *et al.* (2009). Significant differences in grape juice characters at the different bunch positions suggests that botrytis severity at a particular bunch position might be influenced by multiple factors, including bunch compactness, bunch microclimate and/or light exposure. Whatever the explanation for the differences observed, these results suggest that statistical separation of treatment means in small-plot botrytis trials might be improved if variation in botrytis severity according to bunch position is accounted for. Furthermore, the statistical design could be improved by comparing basal and distal bunches from the same grapevine shoot.

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Chapter 4: Botrytis trials in Victoria 2006–2009

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1 Introduction

When environmental conditions are favourable for disease, botrytis bunch rot may cause severe economic losses to Victorian winegrape growers. As a result, over recent years there has been considerable research investment into understanding and managing this disease.

Emmett et al. (2005) "Effects of grapevine canopy management practices on disease development and spray deposition and distribution." GWRDC Project DAV 92/1 examined the effects of different canopy management such as minimal pruning, mechanical hedging, cane pruning and spur pruning on the development of botrytis bunch rot, and on the effectiveness of spraying. Less bunch rot developed on the smaller looser bunches of minimum pruned vines, even though these vines carried more potential inoculum (bunch remnants). They found that botrytis severity was strongly associated with bunch weight and bunch compactness. There was also a clear association between light brown apple moth (LBAM) damage and botrytis bunch rot such that control of LBAM was sufficient to also control botrytis, unless conditions were particularly favourable for botrytis.

This research was followed by an extensive National project led by DPI Victoria: Emmett et al. (2005) "Strategic management of Botrytis bunch rot and lightbrown apple moth on grapevines." GWRDC Project DAV 95/1, that aimed to produce an integrated pest management (IPM) strategy minimising chemical use for the control of botrytis bunch rot and LBAM in grapevines in Australia. As part of this research, a botrytis prediction model developed by Nair et al. (1995) for the Lower Hunter Valley was validated across other geographical regions. The model was shown to have low predictive value in vineyards outside of the Hunter Valley, but improvements were suggested such as inclusion of other parameters such as length of the season in days, the cumulative number of rain days and days over 35^{o}C. In addition, several botrytis monitoring techniques were evaluated and botrytis severity in incubated bunches was found to have the best predictive value for disease potential at harvest.

Other Victorian-based research has included the investigation of disease control using adjuvants (McGregor et al. 2006. GWRDC Project DNR 02/04), nitrogen and calcium manipulation (Cole et al. 2004. GWRDC Project MOU 01/02) and the development of a grower checklist to help manage the disease (Whiting et al. 2004. Greater Victoria Wine Grapes Industry Development Committee).

This current research project, (GWRDC project UT0601), aimed to build on the outcomes of this previous research, focussing on cool climate viticulture. Victoria accounts for 13% of Australia's cool climate winegrape crop, and in a bad botrytis year, botryticides alone do not provide sufficient control to prevent losses (Braybrook, pers comm.). A recommendation from Project DAV 95/1 was that with further systematic research, an improved botrytis bunch rot disease risk model could be developed. Therefore, the objectives of this project were to continue the development of a robust disease prediction model that would allow growers to make management decisions based on the botrytis risk they faced in their vineyard for that

season. Our previous experience, however, showed that reliance on natural disease development was very unreliable, made worse as Victoria experienced drought conditions during this project. The strength of this current project approach is the creation of a large dataset of numerous site years across a range of cool climate regions in Victoria, Tasmania and New Zealand.

In Victoria, botrytis-prone trial sites were chosen at four commercial vineyards in the Yarra Valley located in: Wantirna in South East Melbourne (cv Chardonnay), Coldstream (cv Chardonnay), Woori Yallock (cv Chardonnay) and Yarra Junction (cv Sauvignon blanc). The varieties were chosen on the basis of botrytis susceptibility and, where possible, to be consistent with varieties in the trials in New Zealand and Tasmania. The trials were conducted over three seasons from 2006-2009. The Victorian data set was combined with the New Zealand and Tasmanian data sets for development of the Botrytis Decision Support Model (BDSM).

2 Summary and Recommendations

While the primary purpose of these trials was to gather data for the development of botrytis bunch rot predictive models (covered elsewhere in this report), data on the effectiveness of botrytis bunch rot control measures was also generated. Bunch rot severity was generally low over the three seasons in Victoria relative to trials in New Zealand and southern Tasmania over the same period. The Victorian seasons were characterised by low rainfall over the ripening period, relatively high temperatures and early maturity.

Only one season (2007-08) saw the development of significant levels of botrytis bunch rot in unsprayed plots in the trials by harvest. In this season, maximum botrytis severities reached 2% in unsprayed plots at two of the three trial sites. This level of bunch rot, while below the level of 3% which was considered to be a standard threshold for price penalty, may still be of concern to some growers and winemakers. In the 2007-08 season, fungicide treatments resulted in significantly lower disease at harvest. Leaf removal in the bunch zone also reduced botrytis severity at harvest at sites where there was initially a dense canopy but provided no benefit at sites with sparser canopies or high levels of bunch damage due to light brown apple moth infestation. The experimental inoculum reduction treatment that involved using compressed air to blow necrotic bunch tissue out of bunches did not reduce botrytis bunch rot at harvest. Estimations of latent infection by collecting immature bunches at pre-bunch closure and moist incubating in the laboratory appeared to have little value for predicting bunch rot severity at harvest.

In the other two seasons (2006/07 and 2008/09), bunch rot severities at harvest were below 0.1 % even in unsprayed plots and significant treatment effects did not occur. One conclusion of this work is that applying disease reduction treatments in cool climate Victorian vineyards may not produce any measurable benefits in some seasons. A risk model for botrytis bunch rot may allow lower input disease control regimes when conditions are not conducive to bunch rot while identifying higher risk seasons that require more intensive disease control programmes.

3 Materials and Methods

3.1 Trial design
All trials were randomized complete block designs with 5 replicates (2006-07 and 2007-08) or 6 replicates (2008-09). Plot size was two panels of vines (6-8 vines per plot).

3.2 Trial site location and viticultural characters
The Victorian trials were conducted at three vineyards in the Yarra Valley south east of Melbourne and one vineyard in Wantirna in South East Melbourne. Table 1 lists the location and viticultural characteristics of each trial site. The altitude of trial sites in Victoria ranged from 90 to 200 m above sea level. All vineyard blocks had shoots that were positioned vertically, most with upward trained shoots in a VSP (vertical shoot positioned) canopy.

Vines at the Woori Yallock site (S38CH08 and S38CH09) were trellised according to the Scott-Henry method and while Yarra Junction (S38SB07, S38SB08 and S38SB09) had a Smart-Dyson trellis system. The height of the fruit zone was between 0.8 to 1.1 m. Fungicides for pathogens other than *B. cinerea*, other pesticides and foliar nutrients were applied to trial sites by the grower co-operator, according to standard, conventional practices.

The weight of bunches from the central vine of each plot was used to determine the mean yield per vine for each trial site. The mean pruning weight for the central vine from each plot in the trial site was estimated at sites indicated in Table 1, according to the method described by Smart and Robinson (1991). The ratio of the yield to pruning weight (trimmed vines) and the mean cane weight was used to rate crop vigour as low, moderate or high (Smart and Robinson, 1991).

At Yarra Junction (S38SB07), a high degree of variability in canopy size and fruit yield was observed in the block used in 2006-07 trial and necessitated the re-location of the trial for the 2007-08 and 2008-09 seasons. The Yarra Junction 2008 (S38SB08) and 2009 (S38SB09) trials were conducted in a different block of Sauvignon Blanc located within the same vineyard.

3.3 Weather data recording
At each site, a Model T weather station (Western Electric Design, SA) was installed at a height of 1.5 m. Leaf wetness, temperature, relative humidity were recorded at 10 min intervals. Data was converted into hourly readings prior to analysis with either Metwatch software (Hortplus NZ) or Microsoft Excel.

Table 1. Location and viticultural characteristics of trial sites in southern Victoria.

Site-year code	Vintage year	Location	Variety	Latitude; longitude	Row orientation	Row x vine spacing (m)	Crop vigour[a]
S38CH07_2	2007	Coldstream	Chardonnay	37° 42′ 12″ S; 145° 28′ 49″ E	E to W	3 x 1.5	1.4; 37; high/mod
S38CH07_3	2007	Wantirna	Chardonnay	37° 52′ 3″ S; 145° 15′ 29″ E	E to W	2.5 x 2.5	not estimated
S38SB07	2007	Yarra Junction	Sauvignon Blanc	37° 47′ 59″ S; 145° 37′ 23″ E	NNE to SSW	3.0 x 1.6	10.8; 18; moderate
S38CH08	2008	Woori Yallock	Chardonnay	37° 48′ 36″ S; 145° 32′ 46″ E	NNE to SSW	2.3 x 2	9.9; 46; moderate
S38CH08_2	2008	Coldstream	Chardonnay	37° 42′ 12″ S; 145° 28′ 49″ E	E to W	3 x 1.5	3.4; 33; moderate
S38SB08	2008	Yarra Junction	Sauvignon Blanc	37° 47′ 59″ S; 145° 37′ 23″ E	NNE to SSW	3.0 x 1.6	8.2; 24; moderate
S38CH09	2009	Woori Yallock	Chardonnay	37° 48′ 36″ S; 145° 32′ 46″ E	NNE to SSW	2.3 x 2	4.3; 40; moderate
S38CH09_2	2009	Coldstream	Chardonnay	37° 42′ 12″ S; 145° 28′ 49″ E	E to W	3 x 1.5	2.2; 33; high/mod
S38SB09	2009	Yarra Junction	Sauvignon Blanc	37° 47′ 59″ S; 145° 37′ 23″ E	NNE to SSW	3.0 x 1.6	4.7; 20; moderate

[a] The three values are the ratio of mean yield (kg/vine) / mean pruning weight (kg); mean cane weight (g) and the vigour ranking according to Smart and Robinson (1991).

3.4 Treatments applied at each trial site

Crop stages, for the purpose of timing fungicide treatments, were defined as early, mid or late season. Early season fungicide applications were timed for 80% capfall, mid season applications were timed for pre-bunch closure (PBC) and late season applications were timed for veraison and/or pre-harvest. Percentage capfall was determined according to the methods described by Evans and Gadoury (2008).

3.4.1 Application of fungicides

All fungicides were applied with either a backpack or trailed 12 V battery powered sprayer (model KV14-2 and model SP20 SLVTR2 respectively, Silvan Australia, Vic). The sprayers were calibrated to deliver approximately 1.1 L/min. Sprays were only applied to the fruiting zone of the canopy. Fungicides used in the trial and application rates are shown in Table 2. Approximately 2-3 L of spray mixture was applied per treatment plot (approximately 0.17 L/metre of row).

Table 2. Fungicides used in vineyard field trials in 2006-2009

Product	Active ingredient	Manufacturer	Rate/100L
Scala®	400 g/L pyrimethanil	Bayer CropScience	200 mL
Switch®	375 g/Kg cyprodinil 250 g/Kg fludioxinil	Syngenta	80 g + 0.02% non-ionic surfactant*
Rovral® WG	750 g/L iprodione	Bayer CropScience	67 g

*Hortiwett (Nipro, Qld)

3.4.2 Canopy management 2006-07 (Shoot thinning)

Shoot thinning was carried out on Nov 3 2006 at the Coldstream vineyard (S38CH07_2) and on Nov 6 2006 at the Wantirna vineyard (S38CH07_3) by removing non-fruitful shoots in the canopy to leave a shoot density of approximately 15 shoots/m.

3.4.3 Canopy management in 2007-08 (Leaf plucking)

Around veraison, leaves were removed from selected treatments to reduce canopy density and increase bunch exposure on the least exposed side of the trellis only (east or south sides). A maximum of two leaves per shoot were removed in the bunch zone.

3.4.4 Canopy management in 2008-09 (Leaf plucking)

In the 2008-09 season, leaf plucking was performed earlier than in the 2007-08 season. Prior to the application of fungicide at pre-bunch closure, leaves were removed from both sides of the canopy for selected treatments. A maximum of two leaves per shoot were removed in the bunch zone.

3.4.5 Removal of "bunch trash" from the fruiting zone

At the Coldstream site (S38CH07_2 and S38CH08_2) in 2006-07 and 2007-08, compressed air was used to blow a significant proportion of senescent floral matter or "bunch trash" out of the fruiting zone in selected treatments. This treatment was performed shortly after 100% capfall, using an air gun connected to a petrol driven air compressor.

3.5 Measuring canopy density and bunch exposure

Canopy density was determined at veraison using the point quadrat method (Smart and Robinson 1991). Ten canopy measurements were made for the central vine in each

treatment plot. From these measurements, leaf layer number, percent interior leaves and percent interior clusters were calculated.

3.6 Assessment of bunch compactness

Bunch compactness was estimated by a water displacement method, which expresses compactness as 'percentage openness' (Shavrukov *et al.* 2003). In 2006-07, bunch compactness was estimated at harvest for five basal and five distal bunches per plot, prior to juice extraction. In 2007-08, mean bunch compactness was estimated for the trial site by sampling one bunch from an end vine of each plot and alternating in the selection of a basal bunch or a distal bunch at each sample location.

3.7 Latent botrytis assessment at pre-bunch closure

Bunches were collected at PBC for assessment of latent botrytis incidence. Bunches were frozen over night before surface sterilization. Bunches were removed from the freezer and surface sterilized for 10 sec in 70% ethanol followed by 1 minute in 1% sodium hypochlorite. Bunches were then rinsed in distilled water before being placed in plastic take away food containers. In 2006-07, whole bunches were incubated. In 2007-08 and 2008-09, 20 berries were cut from surface sterilized bunches and placed in take away food containers containing a grid of "rug hold" mesh to keep the berries in position. Bunches or berries were incubated at room temperature and assessments made at 7-8 and 10-15 days. For each assessment, the number of berries with visible botrytis sporulation was recorded and the incidence of berries with sporulation was calculated as a percentage.

3.8 Disease assessment at harvest

In 2006-07 and 2007-08, the severity of botrytis bunch rot for each of 30 bunches on the central 3 vines per plot was assessed visually according to a standard area diagram (R.W. Emmett, Department of Primary Industries, Victoria, personal communication). Thirty bunches were selected at random, 15 from the basal positions on shoots and the other 15 were from the distal position on the shoots. In 2008-09, 32 tagged bunches were assessed: 16 basal and 16 distal bunches. Severity was defined as the area of brown or pink-brown berries that were turgid, semi-turgid and/or shrivelled in a manner that was characteristic of botrytis bunch rot. It was assumed that the symptoms scored were caused by *B. cinerea* because a proportion of symptomatic bunches showed signs of *B. cinerea* sporulation.

3.9 Extraction of grape juice at harvest and analysis

Ten bunches were collected from the central vine from each plot for juice analysis at harvest. Juice was collected by crushing grapes and pressing using a small basket press. Total soluble solids (ºBrix) were measured using a digital refractometer (Pocket PAL-1, Atago Japan). Measurements of juice pH and titratable acidity were determined according to the method of Iland et al (2004). YAN (yeast assimilable nitrogen) was determined using enzymatic kits for amino nitrogen (4A110) and ammonia (4A120) according to the manufacturer's directions (Vintessential laboratories, Dromana, Vic).

3.10 Estimation of bunch size (Lag phase)

This measurement was only made in the 08-09 season. Lag phase measurements of bunch weight were made after pre-bunch closure when seeds had hardened (indicated by resistance when by slicing berries with a razor blade). Bunches were collected from either half a vine or a full vine on the end of every second plot (so not to interfere with the central vines used for assessments at harvest) and bunches were counted and weighed.

3.11 Evaluation of grower botrytis sampling techniques

This sampling technique was evaluated in the 2008-09 season only. Samples of twenty grape bunches were sampled randomly from panels (bays) of grapevines. Bunches were selected without looking at the bunch first, trying to choose half of the bunches from the outer canopy and other half inside, spaced along the panel. Each bunch was inspected and the presence (+) or absence (-) of botrytis bunch rot was recorded and as well as severity of botrytis if present. Sampling protocols were designed to sample 5% of the block and a subset of these samples was chosen for 1% of the block by taking every 5th sampling point. Monitoring started and finished with the 3rd row from either end of the block. This prevents monitoring rows that are possibly atypical due to an edge effect on vine health or microclimate. In the selected row, monitoring started in panel number 3 (from the end of the row) and then included every 5th panel, with the last panel monitored being at least the third panel in from the end of the row. Therefore, the panel numbers monitored were 3, 8, 13, 18, 23..... depending on how many panels there were. Monitoring began after approximately 1500 degree hours accumulation (base 0º C) from 5% cap-fall which was around the time of veraison. Sampling was repeated approximately every two weeks until harvest.

4 Results

Cumulative rainfall for various crop stage intervals and the mean severity of botrytis rot in non-treated plots at each trial site are presented in Table 3. Assuming that the threshold severity of botrytis bunch rot for negative economic impact is 3%, none of the nine trials exceeded this threshold severity. The 2007-08 season was the only season where botrytis was present at harvest above trace amounts and had the highest amount of late season rain (veraison-harvest). In 2007-08 there were approximately half as many days with the maximum temperature above 35 ºC as the other two seasons (2006-07 and 2008-09).

4.1 Latent botrytis infection

Latent botrytis infection varied between 0.1 – 16 % berry incidence over the three seasons at the four trial sites (Table 4). In the 2006-07 season a slightly different incubation technique was used (whole bunches incubated instead of the 20 berries used in 2007-08 and 2008-09) so these results cannot be directly compared with the following two seasons. Latent infections were approximately twice as high in 2008-09 compared to 2007-08 at all sites. The highest latent infections were recorded at Coldstream (S38CH09_2) and Woori Yallock (S38CH09. Yarra Junction (S38SB08 and S38SB09) had the lowest levels of latent botrytis in all years. The application of early season fungicides only resulted in reduced latent botrytis at the Coldstream trial in 2007 (S38CH07_2). At Woori Yallock in 2008 (S38CH08), only the treatment receiving two fungicide sprays had significantly lower latent botrytis severity than the control (unsprayed) treatment.

Table 3. Mean severity of botrytis rot at harvest in non-treated plots, cumulative rainfall and maximum daily temperatures for various crop stage intervals, at trial sites in southern Victoria.

Site-year code	Vintage year	Mean severity (%) of botrytis rot	Date of harvest disease assessment	Cumulative rainfall (mm)					5% capfall-harvest	
				14 days prior to 100% capfall	5% capfall to PBC	PBC to veraison	veraison to harvest	5% capfall to harvest	No. of days >30 ºC	No. of days > 35 ºC
S38SB07	2007	0.09	1 March	na	74.9	47.5	27.2	150.5	28	9
S38SB08	2008	2.27	6 March	36.3	142.3	65.2	42.0	249.5	26	5
S38SB09	2009	0.02	5 March	84.8	185.4	26.4	29.0	240.8	24	10
S38CH07_2	2007	0.01	28 Feb	19.5	74.8	30.0	48.0	164.9	43	13
S38CH08_2	2008	2.03	26 Feb	48.9	61.9	94.7	99.3	255.9	29	6
S38CH09_2	2009	0.00	2 March	34.8	121.1	21.3	8.0	184.5	34	13
S38CH07_3	2007	0.00	23 Feb	20.0	109.5	36.8	16.6	162.9	34	9
S38CH08	2008	0.76	21 Feb	4.8	85.9	122.9	34.4	243.2	24	5
S38CH09	2009	0.03	8 March	51.1	158.1	33.5	28.4	304.9	32	10

Table 4. Latent botrytis measurements for 2006-2009 Victorian vineyard trials.

Site-year code	Vintage year	Location	Variety	Date of sample collection (growth stage)	Latent botrytis incidence (%) on berries		
					Control	80% capfall fungicide	80% capfall and PBC fungicide
S38SB07	2007[a]	Yarra Junction	Sauvignon Blanc	8/1/07 (PBC)	0.1	0.02 n.s.	-
S38SB08	2008	Yarra Junction	Sauvignon Blanc	3/1/08 (PBC)	2.1	0 n.s.	0 n.s.
S38SB09	2009	Yarra Junction	Sauvignon Blanc	3/1/09 (PBC)	3.9	-	-
S38CH07_2	2007[a]	Coldstream	Chardonnay	2/1/07 (bunch closure)	15.9	3.0 s	-
S38CH08_2	2008	Coldstream	Chardonnay	7/12/07 (PBC)	4.8	3.9 n.s.	-
S38CH09_2	2009	Coldstream	Chardonnay	10/12/08 (PBC)	11.7	-	-
S38CH07_3	2007[a]	Wantirna	Chardonnay	27/12/06 (PBC)	9.4	5.5 n.s.	-
S38CH08	2008	Woori Yallock	Chardonnay	20/12/07 (PBC)	4.6	4.7 n.s.	0.6 s
S38CH09	2009	Woori Yallock	Chardonnay	15/12/08 (PBC)	8.8	-	-

[a] Whole bunches were used for incubation rather than trays of 20 berries
PBC = pre-bunch closure
s = significantly different to the control treatment at the 5% level
n.s.= not significantly different to the control treatment at the 5% level

4.2 Site S38CH07_2 (Chardonnay)

Treatments applied at the trial are shown in Table 5. Botrytis was not observed until the harvest assessment and even then, only at the very low severity of 0.01% in the control (nil fungicide) treatment (Table 6). Fungicide application did not have a significant effect on botrytis severity at harvest (p=0.154). Trash removal and canopy treatments did not significantly affect botrytis incidence or severity at harvest (Table 7). There were significant differences in pH and TA and YAN between treatments but no significant differences in bunch weight or Brix (Table 6). Canopy modification treatments did not have a significant effect on any of the canopy parameters measured (Table 8). There was a moderate level of light brown apple moth infestation in this trial (11% of bunches affected).

Table 5. Treatments applied at trial S38CH07_2 (Coldstream, 2007 vintage).

					Fungicide applied		
Treatment	Fungi-cide timing	Trash removal 15 Dec 06	Shoot thinning 3 Nov 06	80% capfall 14 Nov 06	PBC 2 Jan 07	Veraison 30 Jan 07	Pre-harvest 14 Feb 07
1	nil	-		-	-	-	-
2	early	-	-	Scala	Switch	-	-
3	early	+	-	Scala	Switch	-	-
4	early	-	+	Scala	Switch	-	-
5	early	+	+	Scala	Switch	-	-
6	late	-	-	-	-	Rovral	Rovral
7	late	+	-	-	-	Rovral	Rovral
8	late	-	+	-	-	Rovral	Rovral
9	late	+	+	-	-	Rovral	Rovral
10	full	-	-	Scala	Switch	Rovral	Rovral

The characteristics of the canopy measured by the point quadrat method are presented in Table 8.

Table 6. Mean severity of botrytis rot, mean grape yield and juice quality at harvest on 27 February, 2007 for the S38CH07_2 trial (Coldstream).

Treatment	Fungicide timing	Trash removal 15 Dec 06	Shoot thinning 3 Nov 06	Severity of botrytis rot (%)	Yield (kg/vine)	Mean bunch weight (g)	Total soluble solids (°Brix)	pH	Titratable acidity (g/L)
1	nil	-		0.01	1.4	49	21.6	3.3	5.4
2	early	-	-	0	1.3	43	21.2	3.3	5.3
3	early	+	-	0.01	1.4	48	21.6	3.4	5.1
4	early	-	+	0	0.9	41	21.4	3.3	5.6
5	early	+	+	0	1.1	43	21.4	3.3	5.6
6	late	-	-	0	1.1	39	21.6	3.3	5.4
7	late	+	-	0	1.0	44	21.4	3.4	5.0
8	late	-	+	0	1.0	49	21.7	3.3	5.7
9	late	+	+	0	1.3	44	21.5	3.3	5.5
10	full	-	-	0	1.2	44	21.3	3.4	5.1
P value					0.304	0.549	0.880	0.007	0.028
LSD (5%)					-	-	-	.0495	0.394

Table 7. Results of factorial ANOVA for canopy modification and trash removal treatment effects on mean severity of botrytis bunch rot at harvest for the S38CH07_2 trial (Coldstream).

(With Fungicide)		Trash Removal +	Trash Removal -	Means	
Shoot Thinning	+	0.00	0.00	0.00	p=0.318
	-	0.01	0.00	0.00	
	Means	0.00	0.00	Interaction: p=0.318	
		p=0.318			

Table 8. Canopy parameter for the S38CH07_2 trial (Coldstream) on 24 Jan 2007 and botrytis bunch rot severity at harvest.

Treatment	Fungicide timing	Trash removal 15 Dec 06	Shoot thinning 3 Nov 06	Leaf layer number	Interior leaves (%)	Interior bunches (%)	Exposed bunches (%)	Severity of botrytis rot at harvest (%)
1	nil	-	-	2.5	27.2	96.7	3.3	0.01
2	early	-	-	2.7	28.9	50.0	50.0	0
3	early	+	-	2.6	25.6	94.7	5.3	0.01
4	early	-	+	2.9	33.9	63.8	36.2	0
5	early	+	+	2.6	29.3	70.0	30	0
6	late	-	-	2.6	28.9	62.0	38.0	0
7	late	+	-	3.2	37.2	96.0	4.0	0
8	late	-	+	2.4	27.6	76.7	23.3	0
9	late	+	+	2.9	34.9	70.0	30.0	0
10	full	-	-	2.8	32.7	66.7	33.3	0
P value				0.131	0.342	0.280	-	-

4.3 Site S38CH07_3 (Chardonnay)
Treatments applied at the trial are shown in Table 9. No botrytis was observed in this trial, even at harvest (Table 10). There was severe bird damage close to harvest resulting in extremely low yields at harvest.

Table 9. Treatments applied at S38CH07_3 trial (Wantirna, 2007 vintage).

Treatment	Fungicide timing	Shoot thinning 6 Nov 06	80% capfall 17 Nov 06	Fungicide applied PBC 27 Dec 06	Veraison 23 Jan 07	Pre-harvest 8 Feb 07
1	nil	-	-	-	-	-
2	nil	+	-	-	-	-
3	early	-	Scala	Switch	-	-
4	early	+	Scala	Switch	-	-
5	late	-	-	-	Rovral	Rovral
6	late	+	-	-	Rovral	Rovral
7	full	-	Scala	Switch	Rovral	Rovral
8	full	+	Scala	Switch	Rovral	Rovral

Shoot thinning resulted in significantly lower leaf layer number and percent interior leaves relative to the non-shoot thinned treatment (Table 11 and Table 12). The percentage of interior bunches was not significantly different between canopy treatments.

4.4 Site S38SB07 (Sauvignon Blanc)
Treatments applied at the trial are shown in Table 13. With the exception of a single botrytis infected berry with sporulation observed on Feb 9, no other botrytis was observed in assessments conducted before harvest. At harvest, botrytis severities were low (maximum of approximately 0.1% severity) and there were no significant differences between any of the fungicide treatments (Tables 14 and 15). There were no significant differences between treatments for the juice quality measures total soluble solids, pH or titratable acidity. There was a low level of light brown apple moth infestation in this trial (4.5% of bunches affected). The grape bunches in this trial were badly affected by sunburn (8.4 % damage severity), relative to other trials.

The characteristics of the canopy measured by the point quadrat method are presented in Table 16. There were no significant differences in any of the canopy parameters measured for the different treatments.

There was very high variability in yield between plots (ranged from 1.6 – 14 kg/vine). Due to this high variability, a different block of Sauvignon Blanc was used for trials for the 2008 and 2009 vintages.

4.5 Site S38CH08_2 (Chardonnay)

Treatments applied at the trial are shown in Table 17. Botrytis was first observed on 31 Jan 2008, approximately 3 ½ weeks prior to harvest, and severity increased at each sampling (approximately weekly) until harvest (Figure 1).

Table 10. Mean severity of botrytis rot and mean grape yield and juice quality at harvest on 23 February, 2007 at the S38CH07_3 trial (Wantirna).

Treatment	Fungicide timing	Shoot thinning 6 Nov 06	Severity of botrytis rot (%)	Yield (kg/vine)*	Mean bunch weight (g)*	Total soluble solids (°Brix)	pH	Titratable acidity (g/L)
1	nil	-	0	0.35	18.9	21.1	3.5	6.2
2	nil	+	0	0.39	18.7	22.9	3.4	6.7
3	early	-	0	0.30	12.5	23.4	3.5	6.4
4	early	+	0	0.31	14.9	22.9	3.3	7.0
5	late	-	0	0.49	15.1	23.5	3.3	6.5
6	late	+	0	0.31	12.8	23.3	3.4	6.6
7	full	-	0	0.39	13.8	23.3	3.4	6.2
8	full	+	0	0.64	17.7	23.4	3.3	6.6

* trial was severely affected by bird damage and statistical analysis of juice quality parameters was not performed

Table 11. Canopy parameters at the S38CH07_3 trial (Wantirna) on 25 Jan 2007 and bunch rot severity at harvest.

Treatment	Fungicide timing	Shoot thinning 6 Nov 06	Leaf layer number	Interior leaves (%)	Interior bunches (%)	Exposed bunches (%)	Severity of botrytis rot at harvest
1	nil	-	2.6	32.2	70.0	30.0	0
2	nil	+	2.0	21.5	32.0	68.0	0
3	early	-	3.1	39.5	85.0	15.0	0
4	early	+	2.3	29.5	36.7	63.0	0
5	late	-	2.6	34.1	42.3	57.7	0
6	late	+	2.0	25.8	53.3	46.7	0
7	full	-	3.0	40.9	52.0	48.0	0
8	full	+	2.4	34.5	60.6	39.4	0

Table 12. ANOVA results for the effect of shoot thinning on canopy parameters for the S38CH07_3 trial (Wantirna).

Treatment	Shoot thinning 6 Nov 06	Leaf layer number	Interior leaves (%)	Interior bunches (%)
1	-	2.835	36.7	62.3
2	+	2.180	27.8	45.6
P value		<0.001	0.002	0.107
LSD (5%)		0.331	5.39	-

Table 13. Treatments applied at the S38SB07 trial (Yarra Junction, 2007 vintage).

Treatment	Fungicide timing	5% capfall	80% capfall 29 Nov 06	Fungicide applied PBC 8 Jan 07	Veraison 31 Jan 07	Pre-harvest 9 Feb 07
1	nil	-	-	-	-	-
2	early	-	Scala	Switch	-	-
3	late	-	-	-	Rovral	Rovral
4	full	-	Scala	Switch	Rovral	Rovral

Table 14. Mean severity of botrytis rot and mean grape yield and juice quality at harvest on 1 March, 2007 at the S38SB07 trial (Yarra Junction).

Treatment	Fungicide timing	Severity of botrytis rot (%)	Yield (kg/vine)	Mean bunch weight (g)	Total soluble solids (^{o}Brix)	pH	Titratable acidity (g/L)
1	nil	0.09	5.4	80	19.3	2.9	5.9
2	early	0.02	6.2	69	19.7	3.0	5.9
3	late	0.01	3.4	64	20.6	3.0	5.9
4	full	0	6.1	76	18.6	2.9	6.0
P value		0.141	0.440	0.679	0.479	0.945	0.997

Table 15. Results of ANOVA for the effect of fungicide timing on mean severity of botrytis rot at harvest for the S38SB07 trial (Yarra Junction).

Fungicide Timing			
Nil	0.09	b	p=0.141
Early	0.02	ab	
Mid	0.01	ab	
Late	0	a	

Table 16. Canopy parameters at the S38SB07 trial (Yarra Junction) measured on 2 Feb 07 and botrytis bunch rot severity at harvest.

Treatment	Fungicide timing	Shoot thinning	Leaf layer number	Interior leaves (%)	Interior bunches (%)	Exposed bunches (%)	Severity of botrytis rot at harvest (%)
1	nil	-	3.1	39	67	33	0.09
2	early	-	3.1	39	86	14	0.02
3	late	-	2.8	37	81	19	0.01
4	full	-	3.4	45	73	27	0
P value			0.235	0.200	0.814	-	-

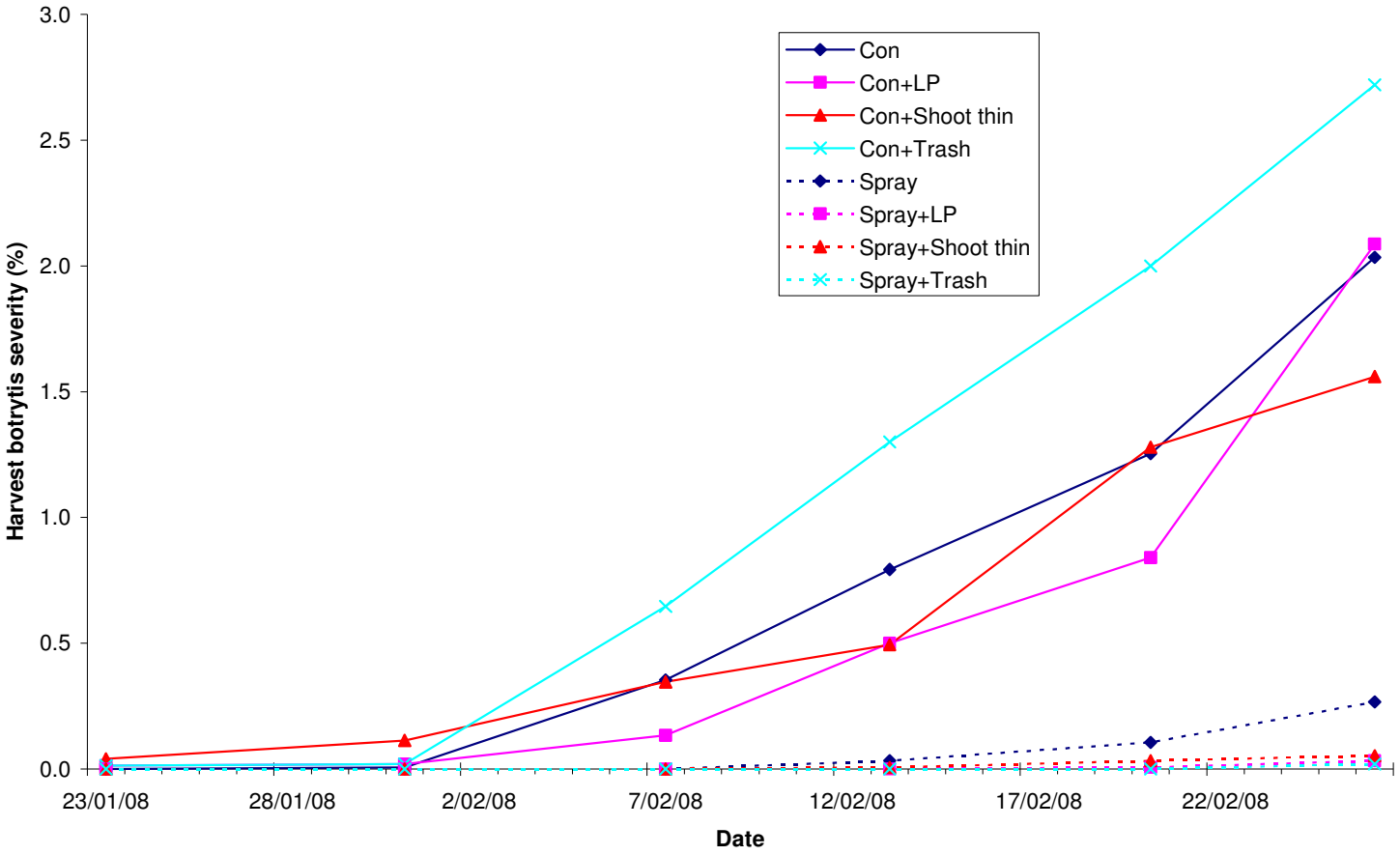


Figure 1. Botrytis bunch rot development during the season for trial S38CH08_2 (Coldstream). LP = leaf plucked, Trash = bunch trash removed, Shoot thin = Shoot thinning pre flowering.

Table 17. Treatments applied at the S38CH08_3 trial (Coldstream, 2008 vintage).

Treatment	Fungicide timing	Shoot thinning	Leaf plucking	Trash removal	Fungicide applied 80% capfall 15 Nov 07	Fungicide applied PBC 12 Dec 07
1	nil	-	-	-	-	-
2	nil	-	+	-	-	-
3	nil	+	-	-	-	-
4	nil	-	-	+	-	-
5	full	-	-	-	Scala	Switch
6	full	-	+	-	Scala	Switch
7	full	+	-	-	Scala	Switch
8	full	-	-	+	Scala	Switch

All treatments that received the fungicide program had significantly lower botrytis severity at harvest relative to the unsprayed treatments (Table 18). Canopy modifications and bunch trash removal did not significantly affect botrytis severity at harvest (Table 19). However, water stress due to a failure of the irrigation system caused severe defoliation prior to harvest. Therefore, even bunches in the treatments without canopy modification had very high levels of bunch exposure before harvest. Light brown apple moth incidence was very high in this block (36 % bunches infested). There were no significant differences between treatments for the juice quality measures total soluble solids, pH or titratable acidity (Table 18).

The leaf plucked treatments had higher cluster exposure (i.e. lower percentage of interior clusters) than the unmodified canopy (control) (Tables 20 and 21). Leaf layer number was not significantly reduced relative to the untreated control by leaf plucking or shoot thinning treatments. Other canopy parameters and bunch openness were not significantly affected by canopy treatments.

Table 18. Mean severity of botrytis rot and mean grape yield and juice quality at harvest on 26 February, 2008 at the S38CH08_3 trial (Coldstream).

Treatment	Fungicide timing	Shoot thinning	Leaf plucking	Trash removal	Severity of botrytis rot (%)	Yield (kg/vine)	Mean bunch weight (g)	Total soluble solids (°Brix)	pH	Titratable acidity (g/L)
1	nil	-	-	-	2.03 a	3.37	97	21.8	3.3	7.3
2	nil	-	+	-	2.09 a	2.16	94	22.2	3.3	7.0
3	nil	+	-	-	1.56 a	1.91	105	22.6	3.3	7.3
4	nil	-	-	+	2.72 b	2.36	93	22.6	3.4	7.2
5	full	-	-	-	0.27 c	2.33	105	22.0	3.3	6.7
6	full	-	+	-	0.03 c	2.49	102	22.4	3.3	6.9
7	full	+	-	-	0.05 c	2.35	110	21.7	3.4	7.2
8	full	-	-	+	0.02 c	2.67	94	22.0	3.3	6.9
P value					<0.001	0.454	0.934	0.853	0.314	0.569

Table 19. Results of factorial ANOVA for canopy modification and trash removal on mean severity of botrytis rot at harvest for the S38CH08_3 trial (Coldstream).

		Fungicide				
		+	-	Means		
Canopy	Nil	0.27	2.03	1.15	a	p=0.094
	Leaf Plucking	0.03	2.09	1.06	ab	
	Shoot Thinning	0.05	1.56	0.81	b	
	Trash Removal	0.02	2.72	1.37	ab	
	Means	0.09	2.10	Interaction: p=0.181		
		p<0.001				

Table 20. Canopy parameters S38CH08_2 (Coldstream) on 31 Jan 2008 and botrytis bunch rot severity at harvest.

Treatment	Fungicide timing	Shoot thinning	Leaf plucking	Trash removal	Leaf layer number	Interior leaves (%)	Interior bunches (%)	Exposed bunches (%)	Severity of botrytis rot at harvest (%)
1	nil	-	-	-	2.2	24.0	68.2	31.8	2.03
2	nil	-	+	-	2.2	28.6	41.4	58.6	2.09
3	nil	+	-	-	2.4	31.2	66.8	33.2	1.56
4	nil	-	-	+	2.5	31.4	66.3	33.7	2.72
5	full	-	-	-	2.3	23.7	68.7	31.3	0.27
6	full	-	+	-	2.2	28.5	52.5	47.5	0.03
7	full	+	-	-	2.6	34.2	57.6	42.4	0.05
8	full	-	-	+	2.6	28.4	81.1	18.9	0.02

Table 21. ANOVA results for effect of canopy modification treatments on canopy parameters for S38CH08_2 (Coldstream) trial.

Canopy treatment	Leaf layer number	Interior bunches (%)	Interior leaves (%)
Control	2.30 ab	68.4 a	23.8
Leaf plucked	2.20 a	46.9 b	28.5
Shoot thinned	2.47 ab	62.2 ab	32.7
Trash removal	2.57 bc	73.7 a	29.9
P value	0.043	0.030	0.047
LSD (5%)	0.299	18.17	6.21

4.6 Site S38CH08 (Chardonnay)

Treatments applied at the trial are shown in Table 22. Botrytis was first observed in the field on 23 Jan and reached approximately 0.7 % severity by harvest in the control treatment (Figure 2). Botrytis severities, yields and quality measurements are presented in Table 23. Leaf plucking did not have a significant effect on botrytis incidence or severity at harvest (Table 24). All sprayed treatments, with the exception of the veraison fungicide treatment, had significantly lower botrytis incidence and severity than the unsprayed treatment (control).

Table 22. Treatments applied at the S38CH08 trial (Woori Yallock, 2008 vintage).

Treatment	Fungicide timing	Leaf plucking 22 Jan 08	Fungicide applied 80% capfall 16 Nov 07	PBC 19 Dec 07	Veraison 24 Jan 08
1	nil	-	-	-	-
2	nil	+	-	-	-
3	early	-	Scala	-	-
4	early	+	Scala	-	-
5	mid	-	-	Switch	-
6	mid	+	-	Switch	-
7	late	-	-	-	Rovral
8	late	+	-	-	Rovral
9	full	-	Scala	Switch	Rovral
10	full	+	Scala	Switch	Rovral

There was a moderate level of light brown apple moth infestation in this trial (7.5% of bunches affected).There were no significant differences between treatments for any juice quality measures, total soluble solids, pH or titratable acidity.

Leaf layer number in the bunch zone decreased and cluster exposure increased significantly in the leaf plucked treatments relative to the treatments with no canopy modifications. All other canopy parameters and bunch openness were not significantly affected by leaf plucking (Tables 25 and 26).

4.7 Site S38SB08 (Sauvignon Blanc)

Treatments applied at the trial are shown in Table 27. Botrytis bunch rot was first observed in the field on 4 February (approximately 1 month prior to harvest) and had reached 2.3% severity by harvest in the control treatment (Table 28, Figure 3). Canopy modification treatments (+/- leaf plucking) and fungicide treatments both significantly affected botrytis severity at harvest (Table 29) but the interaction was not significant. There were no significant differences in botrytis severity between different spray programs for the leaf plucked treatments with all having less disease than the control (unsprayed) treatment without leaf plucking. Of all the treatments that received a single fungicide application, those

that received the 80% cap-fall spray had the lowest botrytis severity although this was not statistically significant.

There were no significant differences between any juice quality measures between treatments in terms of total soluble solids, pH or titratable acidity. Leaf layer number in the bunch zone decreased and cluster exposure increased significantly in the leaf plucked treatments relative to the treatments with no canopy modifications (Tables 30 and 31). All other canopy parameters were not significantly affected by leaf plucking.

Table 23. Mean severity of botrytis rot and mean grape yield and juice quality at harvest on 21 February, 2008 at S38CH08 trial (Woori Yallock).

Treatment	Fungicide timing	Leaf plucking 22 Jan 08	Severity of botrytis rot (%)	Yield (kg/vine)	Mean bunch weight (g)	Total soluble solids (°Brix)	pH	Titratable acidity (g/L)
1	nil	-	0.76 a	8.0	150	18.4	3.0	8.1
2	nil	+	0.61 a	7.5	149	19.7	3.0	7.8
3	early	-	0.28 ab	7.4	141	19.6	3.0	8.3
4	early	+	0.06 b	7.5	152	19.8	3.0	8.0
5	mid	-	0.03 b	7.8	148	19.8	3.0	8.2
6	mid	+	0.00 b	8.0	153	19.3	3.0	7.9
7	late	-	0.23 b	7.7	159	19.0	3.0	8.0
8	late	+	0.47 ab	7.3	139	19.4	3.0	8.0
9	full	-	0.00 b	7.2	131	19.3	3.0	7.9
10	full	+	0.01 b	8.3	156	19.0	3.0	8.0
P value			0.029	0.992	0.166	0.2458	0.565	0.934

Table 24 Results of factorial ANOVA for canopy modification and fungicide treatments on mean severity of botrytis rot at harvest for the S38CH08 trial (Woori Yallock).

		Leaf Plucking		Means	
		+	-		
Fungicide Timing	Nil	0.61	0.76	0.69 a	**p<0.001**
	80% CF	0.06	0.28	0.17 bc	
	PBC	0.00	0.03	0.02 cd	
	Veraison	0.47	0.23	0.35 ab	
	Full	0.01	0.00	0.00 d	
	Means	0.23	0.26	Interaction:	
		p=0.606		p=0.779	

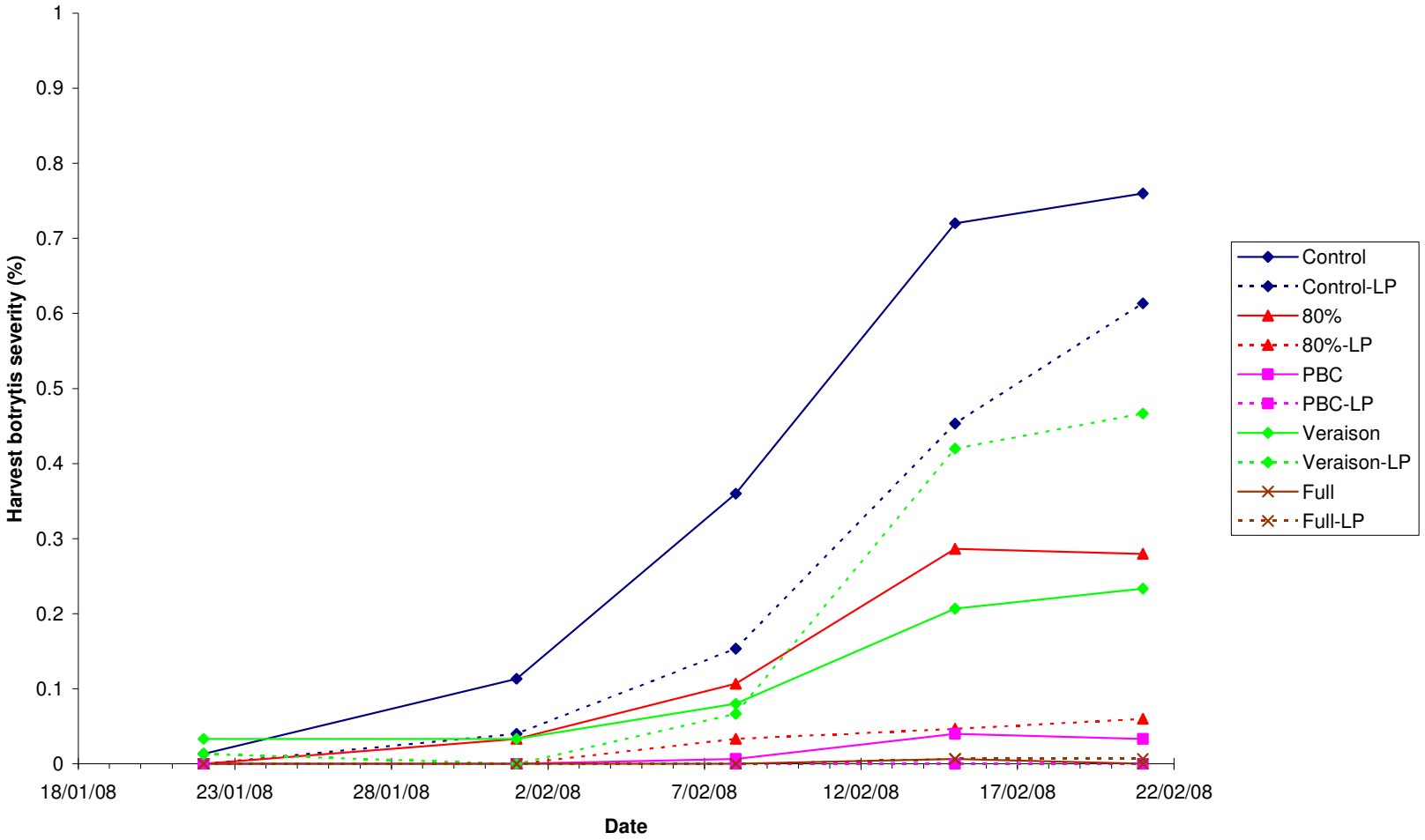


Figure 2 Botrytis development at S38CH08 (Woori Yallock) trial during the 2007-2008 season. Control = untreated, 80% = Scala applied at 80% cap-fall, PBC = Switch applied at pre-bunch closure, veraison = Rovral applied at veraison, Full = all three fungicide applications, LP = leaf plucked.

Table 25. Canopy parameters at the S38CH08 trial (Woori Yallock) on 8 Feb 2008 and botrytis bunch rot severity at harvest.

Treatment	Fungicide timing	Leaf plucking (one side) 22 Jan 08	Leaf layer number	Interior leaves (%)	Interior bunches (%)	Exposed bunches (%)	Severity of botrytis rot at harvest (%)
1	nil	-	2.4	33.3	70.6	29.4	0.76
2	nil	+	2.1	29.2	60.7	39.3	0.61
3	early	-	2.5	31.9	73.8	26.2	0.28
4	early	+	1.9	31.0	40.4	59.6	0.06
5	mid	-	2.4	32.2	67.8	32.2	0.03
6	mid	+	1.6	28.0	45.1	54.9	0.00
7	late	-	2.1	33.0	55.9	44.1	0.23
8	late	+	1.6	24.4	49.0	51.0	0.47
9	full	-	2.2	25.7	65.7	34.3	0.00
10	full	+	2.2	35.9	50.0	50.0	0.01

Table 26. ANOVA results for effect of canopy modification treatments on canopy parameters for the S38CH08 trial (Woori Yallock).

Canopy treatment	Leaf layer number	Interior bunches (%)	Interior leaves (%)
Control	2.31	66.8	31.2
Leaf plucked	1.87	49.1	29.7
P value	0.012	<0.001	0.635
LSD (5%)	0.339	8.32	-

Table 27. Treatments applied at the S38SB08 trial (Yarra Junction, 2008 vintage).

Treatment	Fungicide timing	Leaf plucking 25 Jan 08	Fungicide applied 80% capfall 27 Nov 07	PBC 2 Jan 08	Veraison 4 Feb 08
1	nil	-	-	-	-
2	nil	+	-	-	-
3	early	-	Scala	-	-
4	early	+	Scala	-	-
5	mid	-	-	Switch	-
6	mid	+	-	Switch	-
7	late	-	-	-	Rovral
8	late	+	-	-	Rovral
9	full	-	Scala	Switch	Rovral
10	full	+	Scala	Switch	Rovral

Table 28. Mean severity of botrytis rot and mean grape yield and juice quality at harvest on 6 March, 2008 at the S38SB08 trial (Yarra Junction).

Treatment	Fungicide timing	Leaf plucking 25 Jan 07	Severity of botrytis rot (%)	Yield (kg/vine)	Mean bunch weight (g)	Total soluble solids (°Brix)	pH	Titratable acidity (g/L)
1	nil	-	2.27	8.6	89	18.8	2.8	7.5
2	nil	+	0.43	10.0	76	19.2	2.9	7.0
3	early	-	0.37	11.7	80	18.5	2.8	7.1
4	early	+	0.05	11.7	77	19.5	2.8	7.6
5	mid	-	1.25	9.2	70	18.7	2.8	6.9
6	mid	+	0.18	10.6	72	19.1	2.9	6.7
7	late	-	1.49	9.5	74	20.1	2.8	6.7
8	late	+	0.62	10.7	79	18.4	2.8	7.5
9	full	-	0.05	10.5	89	19.9	2.8	7.4
10	full	+	0.60	11.3	76	18.6	2.8	6.3
p value				0.407	0.819	0.620	0.217	0.608

Table 29. Results of factorial ANOVA for canopy modification and trash removal on mean severity of botrytis rot at the S38SB08 trial (Yarra Junction). CF = capfall; PBC = pre-bunch closure.

		Leaf Plucking				
		+	-	Means		
Fungicide Timing	Nil	0.43	2.27	1.35	a	
	80% CF	0.05	0.37	0.21	b	
	PBC	0.18	1.25	0.72	ab	**p=0.013**
	Veraison	0.62	1.49	1.06	a	
	Full	0.60	0.05	0.33	b	
	Means	0.38	1.09	Interaction: p=0.178		
		p=0.024				

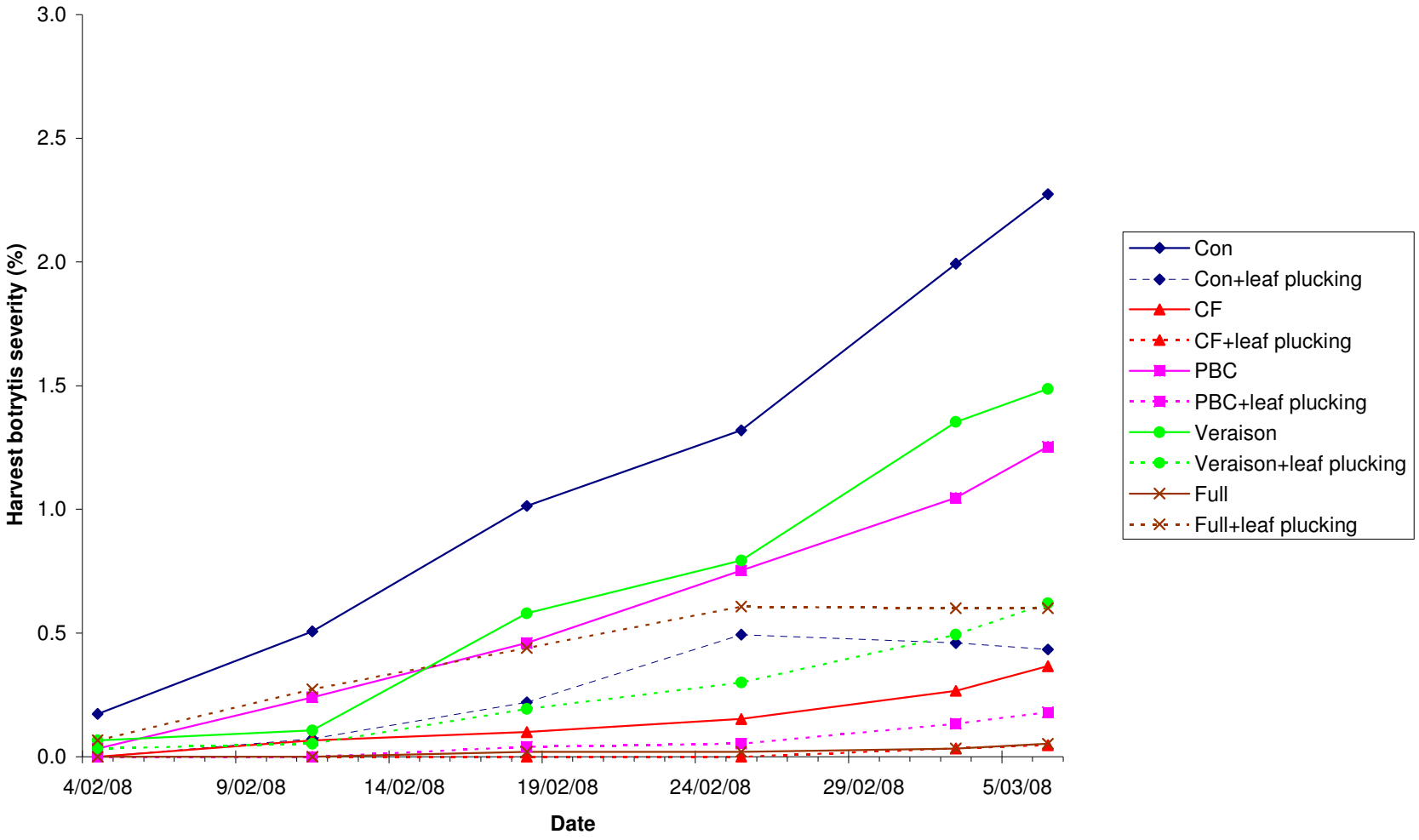


Figure 3 Botrytis development at trial S38SB08 (Yarra Junction) during the 2007-2008 season. CF = cap fall; PBC = pre-bunch closure.

Table 30. Canopy parameters at the S38SB08 trial (Yarra Junction) on 11 Feb 2008 and botrytis bunch rot severity at harvest.

Treatment	Fungicide timing	Leaf plucking (one side) 25 Jan 08	Leaf layer number	Interior leaves (%)	Interior bunches (%)	Exposed bunches (%)	Severity of botrytis rot at harvest (%)
1	nil	-	3.3	37.5	98.7	1.3	2.27
2	nil	+	2.8	33.4	88.4	11.6	0.43
3	early	-	3.4	41.2	93.7	6.3	0.37
4	early	+	2.9	36.1	82.8	7.2	0.05
5	mid	-	3.0	36.4	89.3	10.7	1.25
6	mid	+	2.8	35.8	79.3	20.7	0.18
7	late	-	3.0	37.0	86.1	13.9	1.49
8	late	+	3.0	39.8	76.7	23.9	0.62
9	full	-	3.2	41.7	89.7	10.3	0.05
10	full	+	3.1	39.8	88.2	1.8	0.60

Table 31. ANOVA results for effect of canopy modification treatments on canopy parameters for the S38SB08 trial (Yarra Junction).

Canopy treatment	Leaf layer number	Interior bunches (%)	Interior leaves (%)
Control	3.17	91.5	38.8
Leaf plucked	2.90	83.1	37.0
P value	0.017	0.025	0.336
LSD (5%)	0.221	7.31	-

4.8 Site S38CH09_2 (Chardonnay)

Treatments applied at the trial are shown in Table 32. No botrytis was observed at this site all season in both the small plot trial (Table 33) and the whole block monitoring program. Extensive leaf fall was noticed on Jan 28, and was subsequently attributed to water stress due to problems with the irrigation pump. The February 7th bush fires burnt through the vineyard and resulted in the loss of the irrigation system for the rest of the season. There were no significant differences between any juice quality measures between treatments in terms of total soluble solids, pH or titratable acidity.

Point quadrat measurements showed that leaf plucked treatment did not explain any of the differences in leaf layer number (the leaf plucked treatment actually had the highest leaf layer number) (Table 34). However, the widespread leaf fall due to water stress due to a malfunctioning irrigation pump may have masked any canopy effects. There were no significant differences between percent interior bunches or percent interior leaves between treatments.

Table 32. Treatments applied at the S38CH09_2 trial (Coldstream, 2009 vintage).

Treatment	Fungicide timing	Leaf Plucking 10 Dec 08 (both sides)	Fungicide applied 80% capfall 13 Nov 08	PBC 15 Dec 08	Veraison 29 Jan 09
1	nil	-	-	-	-
2	nil	+	-	-	-
3	early	-	Switch	-	-
4	mid	-	-	Switch	-
5	late	-	-	-	Rovral
6	full	-	Switch	Switch	Rovral

Table 33. Mean severity of botrytis rot and mean grape yield and juice quality at harvest on 2 March, 2009 at the S38CH09_2 trial (Coldstream).

Treatment	Fungicide timing	Leaf plucking (both sides)	Severity of botrytis rot (%)	Yield (kg/vine)	Mean bunch weight (g)	Total soluble solids (°Brix)	pH	Titratable acidity (g/L)
1	nil	-	0	2.0	75.3	20.5	3.3	6.0
2	nil	+	0	2.2	82.8	20.4	3.3	5.9
3	early	-	0	2.1	81.0	20.5	3.3	6.2
4	mid	-	0	2.7	79.3	20.2	3.3	6.0
5	late	-	0	2.1	71.8	20.4	3.3	6.0
6	full	-	0	2.0	72.1	20.4	3.3	5.8
P value			-	0.538	0.598	0.991	0.702	0.663

Table 34. Canopy parameters at the S38CH09_2 trial (Coldstream) on 29 Jan 2009 and botrytis bunch rot severity at harvest.

Treatment	Fungicide timing	Leaf plucking (both sides)	Leaf layer number	Interior leaves (%)	Interior bunches (%)	Exposed bunches (%)	Severity of botrytis rot at harvest (%)
1	nil	-	2.0 ac	15.4	56.2	43.8	0
2	nil	+	2.2 a	21.0	68.1	31.9	0
3	early	-	1.8 ac	9.1	66.1	33.9	0
4	mid	-	1.5 bc	10.4	58.4	41.6	0
5	late	-	2.1 a	15.3	75.8	24.2	0
6	full	-	1.6 c	8.4	53.5	46.5	0
P value			0.042	0.078	0.365		
LSD (5%)			0.449	-	-		

4.9 Site S38CH09 (Chardonnay)

Treatments applied at the trial are shown in Table 35. No botrytis was observed in either the small plot trial or the whole block sampling prior to harvest. At harvest, botrytis severity in the control treatment was extremely low (0.03%) while all other treatments had no botrytis (Table 36). There were no significant differences between any juice quality measures between treatments in terms of total soluble solids, pH or titratable acidity.

Table 35. Treatments applied at trial the S38CH09 trial (Woori Yallock, 2009 vintage).

Treatment	Fungicide timing	Fungicide applied			
		Leaf plucking 15 Dec 08 (both sides)	80% capfall 17 Nov 08	PBC 19 Dec 08	Veraison 5 Feb 09
1	nil	-	-	-	-
2	nil	+	-	-	-
3	early	-	Switch	-	-
4	mid	-	-	Switch	-
5	late	-	-	-	Switch
6	late	-	-	-	Rovral

Leaf layer number in the bunch zone decreased and percentage of interior clusters increased in the leaf plucked treatment relative to the treatments with no canopy modifications, but only the decrease in interior bunches was significant (Table 37).

4.10 Site S38SB09 (Sauvignon Blanc)

Treatments applied at the trial are shown in table 38. No botrytis was detected until the harvest assessment where mean severity in the untreated control was 0.02 % (one bunch with botrytis was detected) (Table 39). All other treatments had no botrytis bunch rot at harvest. There were no significant differences between any juice quality measures between treatments in terms of total soluble solids, pH or titratable acidity.

Table 36. Mean severity of botrytis rot on March 30, 2009, and mean grape yield and juice quality (na = not assayed) at harvest on March 31, 2009 at the S38CH09 trial (Woori Yallock).

Treatment	Fungicide timing	Leaf plucking	Severity of botrytis rot (%)	Yield (kg/vine)	Mean bunch weight (g)	Total soluble solids (°Brix)	pH	Titratable acidity (g/L)
1	nil	-	0.03	4.3	82.9	19.2	3.0	7.2
2	nil	+	0	3.8	83.1	19.8	2.9	6.9
3	early	-	0	4.6	81.0	19.0	3.0	7.3
4	mid	-	0	4.5	80.3	19.3	2.9	7.0
5	late	-	0	4.9	83.1	19.1	3.0	7.4
6	Full	-	0	4.5	84.5	19.5	3.0	7.1
P value				0.569	0.989	0.335	0.831	0.621

Table 37. Canopy parameters at the S38CH09 trial (Woori Yallock) on 29 Jan 2009 and botrytis bunch rot severity at harvest.

Treatment	Fungicide timing	Leaf plucking (both sides)	Leaf layer number	Interior leaves (%)	Interior bunches (%)	Exposed bunches (%)	Severity of botrytis rot at harvest (%)
1	nil	-	2.4	22.6	74.4 a	25.6	0.03
2	nil	+	1.9	16.5	48.3 b	51.7	0
3	early	-	2.3	21.7	64.4 ab	35.6	0
4	mid	-	2.4	25.5	71.9 a	28.1	0
5	late	-	2.3	26.4	66.9 ab	33.1	0
6	full	-	2.5	26.3	77.9 a	22.1	0
P value			0.199	0.558	0.047		
LSD (5%)			-	-	18.77		

Table 38. Treatments applied at the S38SB09 trial (Yarra Junction, 2009 vintage).

Treatment	Fungicide timing	Leaf Plucking 5 Jan 09 (both sides)	Fungicide applied 80% capfall 25 Nov 08	PBC 2 Jan 09	Veraison 6 Feb 09
1	nil	-	-	-	-
2	nil	+	-	-	-
3	early	-	Switch	-	-
4	mid	-	-	Switch	-
5	late	-	-	-	Rovral
6	full	-	Switch	Switch	Rovral

Leaf layer number was significantly lower in the leaf plucked treatment than in some of the non-leaf plucked treatments (but not all of them) (Table 40). Percent interior bunches was lower in the leaf plucked treatment than in all the non-leaf plucked treatments but this was not statistically significant. Leaf plucking had no significant effect on percent interior leaves although the leaf plucked treatment had the lowest value.

4.11 Estimation of bunch size at harvest from lag phase measurements

The ratio between mean bunch weight at harvest and the mean bunch weight during lag phase was different for the different vineyards. At Yarra Junction (S38SB09) the ratio was the highest, followed by Woori Yallock (S38CH09) with Coldstream (S38CH09_2) the lowest (Table 41).

Table 39. Mean severity of botrytis rot and mean grape yield and juice quality at harvest on 2 March, 2009 at the S38SB09 trial (Yarra Junction).

Treatment	Fungicide timing	Leaf plucking (both sides)	Severity of botrytis rot (%)	Yield (kg/vine)	Mean bunch weight (g)	Total soluble solids (°Brix)	pH	Titratable acidity (g/L)
1	nil	-	0.02	6.0	49.0	19.9	2.9	7.9
2	nil	+	0.05	4.8	47.6	19.5	2.9	7.8
3	early	-	0.02	6.6	51.7	19.8	3.0	7.5
4	mid	-	0	5.6	49.7	19.7	2.9	7.9
5	late	-	0	6.5	52.9	19.6	2.9	7.9
6	full	-	0	5.6	47.9	19.7	2.9	7.1
P value			0.175	0.360	0.659	0.977	0.390	0.638

Table 40. Canopy parameters at S38SB09 trial (Yarra Junction) on 11 Feb 2009 and botrytis bunch rot severity at harvest.

Treatment	Fungicide timing	Leaf plucking (both sides)	Leaf layer number	Interior leaves (%)	Interior bunches (%)	Exposed bunches (%)	Severity of botrytis rot at harvest (%)
1	nil	-	3.5	35.8	87.4	12.6	0.02
2	nil	+	2.5	31.1	71.8	28.2	0.05
3	early	-	4.2	39.7	94.5	5.5	0.02
4	mid	-	3.7	38.4	100	0	0
5	late	-	2.9	35.6	98.6	1.4	0
6	full	-	3.1	41.6	95.4	4.6	0
P value			0.006	0.102	<0.001		0.175
LSD (5%)			0.459	-	8.41		-

Table 41. Lag phase estimate of harvest bunch weight for 2008-09 trials.

Trial	Number of bunches/vine	Sampling date	Lag phase mean bunch weight (g)	Actual harvest mean bunch weight (g)	Harvest weight/lag phase weight
S38CH09	61.0	16/1/09	45.6	82.5	1.8
S38CH09_2	23.2	12/1/09	51.3	77.1	1.5
S38SB09	110.6	21/1/09	22.4	49.4	2.2

4.12 Evaluation of grower botrytis sampling protocols

At Coldstream (S38CH09_2), water stress caused much of the foliage to drop over a month prior to harvest. This loss of canopy, combined with fire damage to the block meant that the whole block sampling was discontinued after the first assessment (Table 42).

Table 42. Botrytis severity measurement using different sampling protocols in 2008-2009 season at the three Victorian trials.

Trial site	Assessment	Assessment date	Estimate of Botrytis severity when sampling		
			1% of vines	5% of vines	Small plot
S38CH09_2[a]	1	29 Jan 09	0	0	0
S38CH09	1	5 Feb 09	0	0	0
S38CH09	2	19 Feb 09	0	0	0
S38CH09	3	8 Mar 09[b]	0	0	0.03
S38SB09	1	6 Feb 09	0	0	0
S38SB09	2	18 Feb 09	0	0	0
S38SB09	3	5 Mar 09	0.05	0.02	0.02

[a] No further assessments were made due to fire damage to the block

[b] Incomplete assessment as part of block had been already been harvested

At Woori Yallock (S38CH09) no botrytis bunch rot was detected prior to harvest by sampling either 5% or 1% of vines in the trial block. At harvest, botrytis was only detected in the untreated control in the small plot trial so the failure to detect botrytis in whole block sampling may have been due to the fungicides applied by the grower reducing the botrytis severity to an undetectable level.

At the Yarra Junction trial (S38SB09), no botrytis was detected by the first two whole block assessments. During the third assessment, 1 day prior to harvest, 0.05% and 0.02% botrytis severity was estimated by sampling 1% of vines and 5% of vines respectively. These figures were both close to the 0.02 % severity in the control treatment of the small plot experiment.

5 Discussion

Very little botrytis bunch rot developed in any of the Victorian vineyards during these trials. At the time of this study, Victoria was experiencing drought conditions with 12 consecutive years of below average rainfall in many parts of the state. In addition to the low rain, extreme high temperature events are becoming more frequent such as those experienced in 2009. There were 24 to 4 days above 30°C, and 5-13 days above 35°C. Emmett et al. (2005) identified days above 35°C as having a significant negative impact on Botrytis development. The second season (2007-2008) was most conducive to botrytis rot, with considerable late season rainfall and cooler temperatures. Even in this higher disease season, the highest botrytis bunch rot severity recorded was 2.3% (trial S38SB09), which is still below the 3% severity threshold.

These results indicate there is probably a lot of unnecessary cost being incurred by Victorian winegrape growers in seasons when the botrytis risk is low. The development of the botrytis risk model will ensure that control measures can be targeted to higher risk seasons. This will reduce costs to growers while still minimizing the risk of serious crop losses.

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7 Acknowledgements

The assistance of the following vineyard managers: Tim McCarthy and David Amerlaan (Fosters Group), Mathew Carter (Bulong Estate), David Smith (Old Orchard winery) and Andrew Smith (Shelmerdine vineyards) is gratefully acknowledged.
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Chapter 5: Whole-of-block experimentation reveals spatial variation in the response to Switch® fungicide applied at flowering or pre-bunch closure for botrytis management.

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1 Background

This chapter summarises information, and includes figures, presented in a manuscript* to be submitted for publication. This manuscript is currently being reviewed internally and will be supplied to GWRDC in due course. Co-authors Dr Rob Bramley and Mr David Gobbett, CSIRO Ecosystem Sciences, while not formal collaborators on project UT0601, provided input to the experimental design employed and applied the complex geo-statistics required for the spatial analyses described below. The manuscript describes the value of collecting spatially distributed data as a means of better understanding the incidence, spread, progression and control of fungal grapevine diseases. As such, it provides a new application for the whole-of-block experimental approach in which spatial variability is used as an experimental tool. Additional data derived from this experiment will be presented in K. Dunne's thesis (in preparation) towards improved understanding of botrytis epidemiology.

**Manuscript:*
Bramley R.G.V., Evans K.J., Dunne K.J. and Gobbett, D.L. Spatial variation in response to 'reduced input' spray programs for powdery mildew and botrytis identified through whole-of-block experimentation.

2 Introduction and Methods

Replicated small-plot trials conducted in southern Tasmania during 2006-07 and 2007-08 suggested that application of Switch® fungicide between pea-size berries and pre-bunch closure was a critical treatment in that it reduced botrytis severity significantly relative to non-treated vines and, on one occasion, relative to vines treated with Scala® fungicide during the flowering period. As different fungicides were used at different crop stages in these small-plot trials, consistent with standard grower practice, it is possible that the differences observed between treatments may have been the result of the material applied rather than the timing of application. It was therefore of interest to examine the effect of the same fungicide applied at different crop stages, and in particular, to examine any difference in the mean severity of botrytis at harvest when the fungicide was applied either at 80% cap fall or at pre-bunch closure.

The objective was to evaluate the effect of Switch® fungicide applied either at 80% capfall (15th December, 2008) or pre-bunch closure (E-L 31–32, 27th January, 2009) for the control of botrytis bunch rot (*Botrytis cinerea*) in a 2.4 ha Chardonnay vineyard in the Rokeby region of southern Tasmania. Switch® (Syngenta Group, Basel, Switzerland) is a formulation of 375 g/kg cyprodinil and 250 g/kg fludioxinil, and for each treatment, was applied at 80 g/100 L with 0.02% non-ionic surfactant (Spraymate® Activator®, Nurfarm Australia Limited) and a spray volume of 780 L/ha. Treatments were applied, by the grower co-operator using a Silvan Turbo Sprayer, to a whole-of-block experimental design, in alternate strips, each of six rows. Botrytis severity on bunches was assessed with the aid of a standard area diagram (B. Emmett, Department of Primary Industries, Victoria, personal communication) for 150 target vines per treatment. These vines were located in the centre two rows of each six-row

treatment strip. For each vine, a total of 12 bunches were tagged for assessment and comprised six basal and six distal bunches selected randomly. Marked variation in berry size within many grape bunches was noted and splits in some smaller berries were noted on 25th February 2009, with most wounds becoming dry and necrotic by 13th March 2009. Bunch rot severity was assessed for the same 12 bunches per vine at approximately weekly intervals on March 10th, 18th and 24th, with the final bunch rot assessment conducted over three days from April 2nd because rain interfered with the assessment. All bunches on target vines were harvested on 8th April 2009 for table wine production.

3 Results and Discussion

On March 24, vines treated with Switch® at pre-bunch closure had a mean disease severity of 1.6%, which was significantly lower than the mean of 3.6% for vines treated with Switch® at 80% cap fall ($P < 0.001$, one-sided t test of logit transformed data). However, the response to each spray treatment was spatially variable. According to the geo-statistical analysis, Switch® applied at pre-bunch closure reduced disease severity relative to the application at flowering in locations of the block where $P < 0.05$ was assumed as the rule for deciding one spray strategy over another (Figure 1). Some rotten berries may have fallen to the ground between the two assessment dates, as evidenced by the higher levels of disease severity observed on March 24 compared to April 3. Comparison of the significance of difference and flowering treatment maps for March 24 data suggest a disease severity of about 3.6% (square-root transformed value of 1.9% in Figure 1) is the threshold above which the two spray programs were statistically separated. A similar comparison for the April maps suggests a threshold of about 2.3% (1.5% in Figure 1). Note, however, that back-transformation of kriged data may be problematic with the consequence that the map value may not equate precisely to an actual botrytis severity.

Botrytis severity was related to topographic variation, with Figure 1 representing an east facing slope with an elevation range of approximately 23 m. Comparison of Figure 1 with Figure 2 suggests that there was an association between vine vigour and botrytis severity; higher vigour areas tended to be more severely affected by botrytis. Vines were visually more vigorous at upslope sites where the soil was a clay loam, with apparently better texture and water holding capacity than the lighter silty soil further downslope.

Spatial variation in the response to the flowering treatment, when examined four times pre-harvest, illustrated that disease severity generally increased relative to the severity and location first observed. These observations suggested that secondary spread may not have been an important mechanism for increasing disease severity over time (Figure 3). This result provides evidence to support the hypothesis that the severity of botrytis on a single grapevine bunch is the result of growth of the fungus after latency or a single direct infection event (usually via wounds) in one or more berries followed by berry to berry spread within the bunch. As some berry splitting was observed in this vineyard, direct infection of berries was a possible infection pathway at this site.

4 Conclusion

This experiment revealed valuable information about apparent interactions between spray effectiveness and topography. The results obtained also related directly to the capability of the commercial equipment, in contrast to small-plot trials that use sprayers that do not readily simulate commercial equipment. The timing of fungicide applications were also subject to the normal operational constraints of the weather, availability of labour and equipment, the preferences of the vineyard managers and their judgment about crop phenology. Indeed, the

co-operators readily allocated the whole block to experimentation, and the ease of implementation meant that they neither needed to understand the complex nature of the spatially distributed design nor the geo-statistical analysis of results. With repeated measurement of treatment response during the season, the spatially distributed experimental design also provided important insights as to the spread of botrytis, with the results suggesting that secondary spread of botrytis, relative to initial infestation, may have been of minor significance for this site and season. As such, this approach will provide an avenue of important investigation for other sites and seasons. Regardless of disease epidemiology, spatial mapping can indicate where a management program is failing to maintain disease severity below a threshold level, above which there are price penalties for reduced crop quality. Similarly, such mapping can also assist in identification of disease 'hot spots', and also those areas with little disease risk and which may either not need to be sprayed at all, or which may respond to either reduced spray regimes or application of sprays at specific times.

5 Acknowledgments

The input of Rob Bramley and David Gobbett to this work was funded by CSIRO. We are most grateful to Matt Barwick and staff (Clarence House Estate) for co-operating with us and providing the whole block of Chardonnay to this experiment. Thanks also to Neil Meadows (Terrapix), for conducting the EM38 and elevation surveys and geo-referencing of target vines, and to Dr Tom Bishop (University of Sydney) who allowed us to use his code for geostatistical analysis of experimental treatment responses.

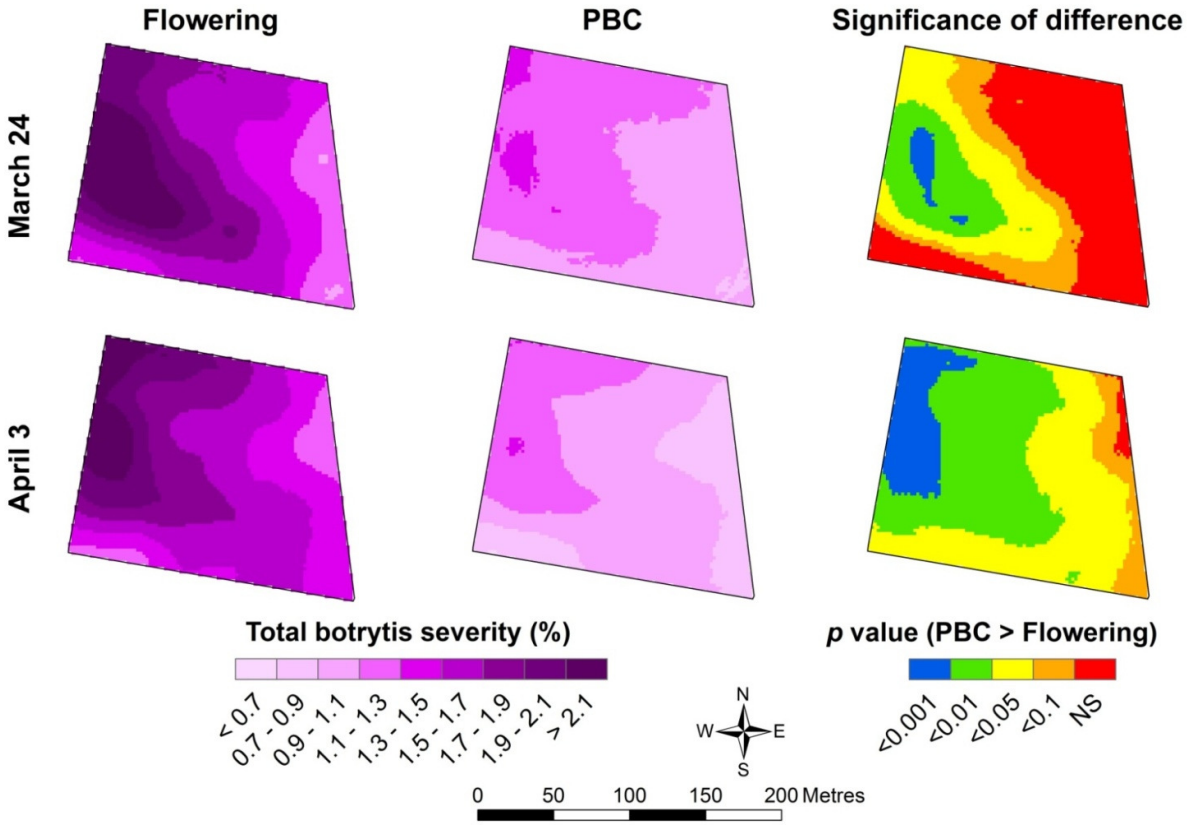


Figure 1. Spatial variation in the severity of botrytis (%; square root transformed data) in a 2.4 ha Tasmanian Chardonnay vineyard treated with fungicide either at flowering or pre-bunch closure (PBC) assessed immediately prior to harvest (April 3) and approximately 10 days earlier. The block was planted on own roots in 1998 on an east facing slope with an elevation range of approximately 23 m. The row and vine spacings were 2.4 and 1.35 m and vines were trained to a Scott-Henry trellis; row orientation is approximately E-W. (From Bramley *et al.*).

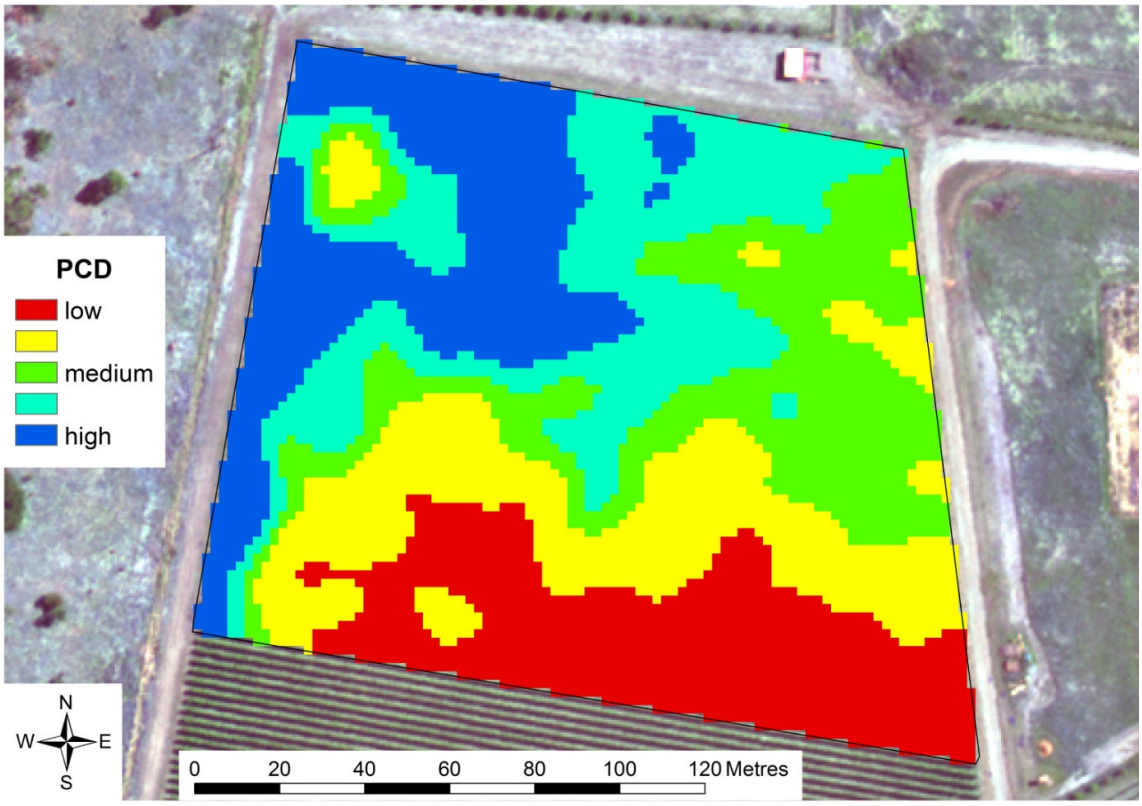


Figure 2. Variation in vine vigour at the botrytis trial site as measured by the 'plant cell density' index (PCD) using remote sensing. This image was obtained at veraison in 2010 – one year after the trial. (From Bramley *et al.*).

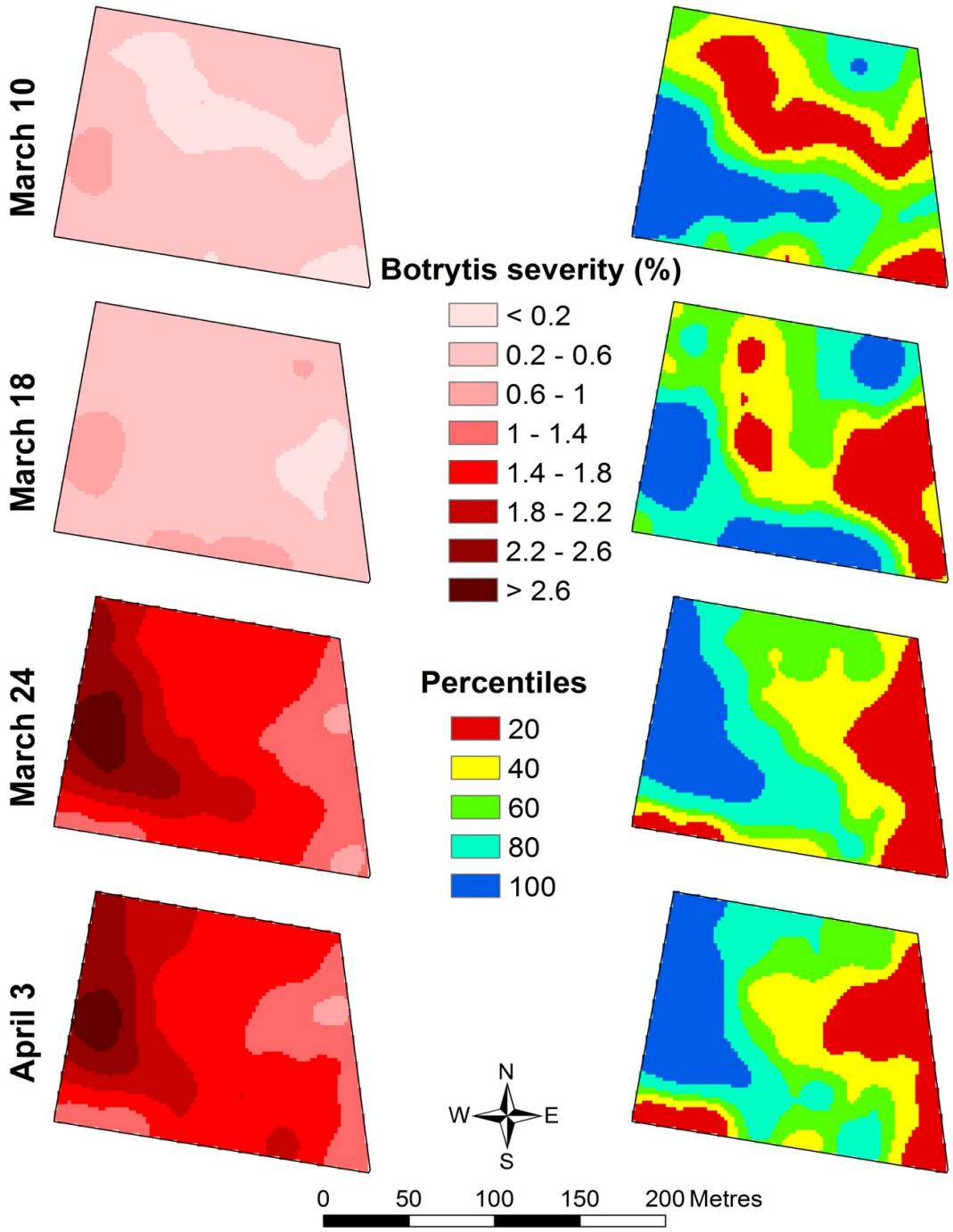


Figure 3. Change in the spatial distribution of botrytis severity over time in a 2.4 ha Chardonnay vineyard in Tasmania, vintage 2009. Note that the two panels of maps show exactly the same data for botrytis severity (%), but are classified in terms of either equal intervals (left panel) or 20th percentiles (right panel). These maps were derived from the Flowering treatment data only and were interpolated using a common variogram for all dates. (From Bramley *et al.*).

Chapter 6: Sampling strategy and evaluation of sample sizes required for reliable estimation of botrytis severity in vineyard blocks in Tasmania.

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1 Summary

A sampling strategy was developed for reliable estimation of botrytis severity in vineyard blocks, particularly during the early stages of a botrytis epidemic when severity is used as an input variable to the late-season Botrytis Decision Support Model (BDSM). The sample unit was equivalent to a panel of vines, with 10 grape bunches assessed for botrytis severity on each of two panel sides facing each other in adjacent rows (for ease of assessment). Using vineyard blocks of 1–2.5 ha at five site-years in Tasmania, 20–25 sample units were located using a systematic, semi-grid system for ease of implementation. The standard error to mean ratio for mean botrytis severity at various sample sizes was used to demonstrate that 20–25 sample units were required to obtain a reliable estimate of mean botrytis severity when severities were < 1%. For these low botrytis severities, standard error to mean ratios were often 18–38%, which corresponded to ratios observed for botrytis severities ≥ 2.5% in a small-plot trial in Riesling. Sample sizes of 10–15 panels should be sufficient when botrytis severities are > 2%. This method, when used in conjunction with the Bunch Rot Assessment Trainer of Hill *et al.* (2010), can be applied during implementation of the BDSM.

2 Introduction

Data used to develop the prototype Botrytis Decision Support Model (Beresford *et al.* (2009), Appendix 5) were generated from replicated small plots in trials located in sub-sections of vineyard blocks that were typically 1–2.5 ha. The late-season BDSM requires estimates of average botrytis severity across an entire vineyard block in the early stages of the botrytis epidemic for prediction of future disease progression.

Any vineyard sampling strategy needs to reflect a balance between practical considerations, such as ease of implementation and labour cost, and the need to obtain reliable input data to the BDSM. In theory, any sampling strategy developed for application of the BDSM should also serve to provide reliable estimates of botrytis severity near harvest for assessment of grape quality. With these purposes in mind, Hill *et al.* (2010) described the Bunch Rot Assessment Trainer (BRAT) and explored the question of sample size. The objective of this study was to design a practical sampling strategy and assess its reliability for sample means derived at different levels of mean botrytis severity assessed from the early stages of a botrytis epidemic through to harvest.

3 Methods

3.1 Sample unit and variation among units from small-plot trials

The vine panel was selected as the *sampling unit* because it was the unit used in replicated small-plot trials for developing the BDSM. A range of mean botrytis severities from non-treated plots at site-year $R_1$09 (refer to Chapter 1 and coded as S43R09 in chapter 3) were used to examine the coefficient of variation of the mean (CV_{mean}), which is the ratio of the standard error to the mean expressed as a percentage (Campbell and Madden, 1990). The coefficient of variation is useful for comparing how precisely a population mean is estimated because it is a dimensionless number that allows comparison of data sets with different units or widely different means. The target CV_{mean} for reliable sample estimates is defined in

Section 3.3. Mean botrytis severity for each plot was determined from botrytis severities for the same 32 bunches assessed at each of six assessment dates between veraison and harvest. Site-year $R_1$09 was selected from all the trials conducted in Tasmania because it had the greatest number of assessment dates and disease progressed to nearly 5% mean severity in a manner typical for Riesling.

3.2 Sampling strategy for whole vineyard blocks

For sampling whole vineyard blocks, the *sampling unit* was a single panel of four to six grapevines. Mean botrytis severity for each vine panel was determined from botrytis severities for 20 grape bunches sampled semi-randomly as described below. The number of grape bunches assessed per panel was less than used in small-plot trials to reduce the amount of time on disease assessment. To avoid having to climb under vines to assess both sides of the panel, 10 grape bunches were sampled on one side of the panel in the selected row and 10 bunches were sampled from the panel directly opposite in the adjacent row. If facing north in vineyard rows oriented north-south, for example, then the panel on the west side in one row was assessed before assessing the panel on the east side in the adjacent row. The 10 bunches per panel side were selected without looking at the bunch first, with half the bunches selected from the outer canopy and the other half inside the canopy.

Each bunch was inspected and the presence or absence of botrytis bunch rot. Presence was confirmed when sporulating *B. cinerea* was observed. The severity of botrytis for each bunch was assessed as described in Chapter 1, and average botrytis severity calculated for the 20-bunch sample.

Up to 25 sampling units (500 bunches) were selected in vineyard blocks up to 2.5 ha, with this sample size reflecting the maximum assessment that could be conducted in practice, with the aim of reducing the sample size further if a reliable estimate of the mean botrytis severity in the block was produced. Figure 1 illustrates the grid-style sampling pattern used. Each row in the block was numbered from left to right (or right to left), with the first two rows at either end of the block avoided in case there were 'edge effects'. The first and second panels at the end of a row were also avoided for this same reason. When walking between two rows to be sampled, every 5th panel in the row was sampled. If starting at panel 3, for example, then panels 3, 8, 13, 18, 23, and so on, were sampled until the end of the row, as long as there were at least two panels at the end of the row that were not sampled. The spacing between rows to be sampled in the block was based on the size of the block and (often variable) row lengths, and was determined with the aid of an algorithm in an Excel® spreadsheet which can be obtained from the author. A simpler procedure, especially for 0.7–1 ha blocks of rectangular shape, might be to assess every third corridor, as illustrated in Figure 1.

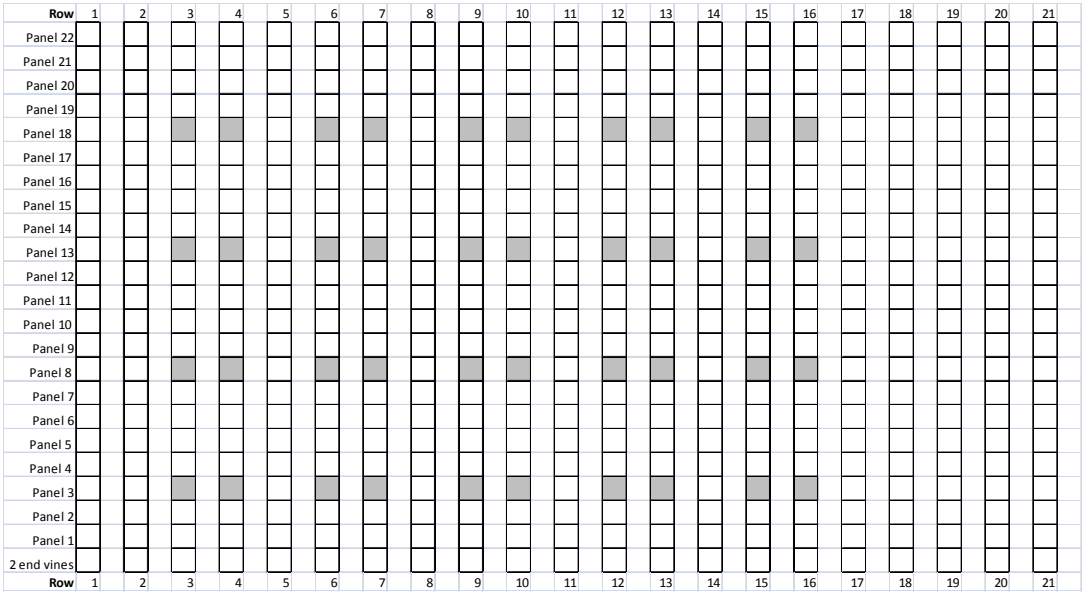


Figure 1. Schematic representation of panels and rows in a 0.7 ha vineyard block with 21 rows. Each 'cell' or square is a panel of 4 vines and a shaded square represents a panel selected for assessment, noting that only one side of each adjacent panel was assessed. In this case, 20 sampling units (400 bunches) are illustrated.

The vineyard blocks and sampling regimes for this study are described in Table 1. All blocks were managed for botrytis bunch rot using standard commercial practices, apart from site $C_1$09 which was used for the 'whole block' experiment described in Chapter 5. Site $R_1$09 also had small-plot trial S43R09 (Chapter 3) located in the south-western section of the block.

Similar protocols were implemented in the Yarra Valley in Victoria in 2008/2009 but there was insufficient botrytis to test the strategy (Chapter 4).

Table 1. Sampling regime and details for site-years in Tasmania.

Site code	Vintage year	Variety	Location	Block size (ha)	Rows (corridors) selected	Panels selected in each row
$R_1$09	2009	Riesling	N of Campania[1]	0.7	3-4, 6-7, 9-10, 11-12, 14-15	3,8,13,18, 23
$R_1$10	2010	Riesling	N of Campania[1]	0.7	3-4, 6-7, 9-10, 11-12, 14-15	3,8,13,18, 23
$R_3$10	2010	Riesling	Kayena[2]	0.7	3-4, 5-6, 8-9, 11-12, 14-15	3, 8, 13, 18
$C_1$09	2009	Chardonnay	Rokeby[1]	2.2	'Flowering' treatment[3]: 9-10, 22-23, 34-35, 44-45, 47-48.	3,8,13,18, 23
$C_1$09	2009	Chardonnay	Rokeby[1]	2.2	'PBC' treatment[3]: 3-4, 16-17, 28-29, 40-41, 51-52	3,8,13,18, 23
$C_1$10	2010	Chardonnay	Rokeby[1]	~1	64-65, 69-70, 74-75, 79-80, 84-85	3,8,13,18, 23

[1] southern Tasmania
[2] northern Tasmania
[3]Site of 'whole block' experiment, refer to Chapter 5.

3.3 Reliability of sample estimates

The sampling strategy resulted in up to 25 means for botrytis severity on each assessment date. Because CV_{mean} is inversely proportional to the number of samples (*n*), then the smallest sample size for a reliable estimate of the mean is selected in the range of the response where CV_{mean} does not vary significantly as the sample size increases.

CV_{mean} (%) was calculated at various assessment dates pre-harvest (refer to results) for the sites indicated in Table 1. At each assessment date for site $R_1$10 in 2010, CV_{mean} (%) was also calculated for 20, 15, 10 or 5 sub-samples for 10 samples of each sub-sample size taken randomly from the 25 sample units. The average CV_{mean} (%) for each sub-sample size and the standard error of this average was plotted against the sub-sample size to identify the range of CV_{mean} (%) in which it was no longer declining steeply.

4 Results and Discussion

As mean botrytis severity increased gradually from 0.02% to 4.9% for the six non-treated plots at site-year $R_1$09 (small-plot trial), the standard error to mean ratio ($CV_{mean,}$ %) declined from 63% to 31% between 16 March and 20 April, 2009 (Figure 1). Therefore, the lowest CV_{mean} observed in small-plot trials was around 30%.

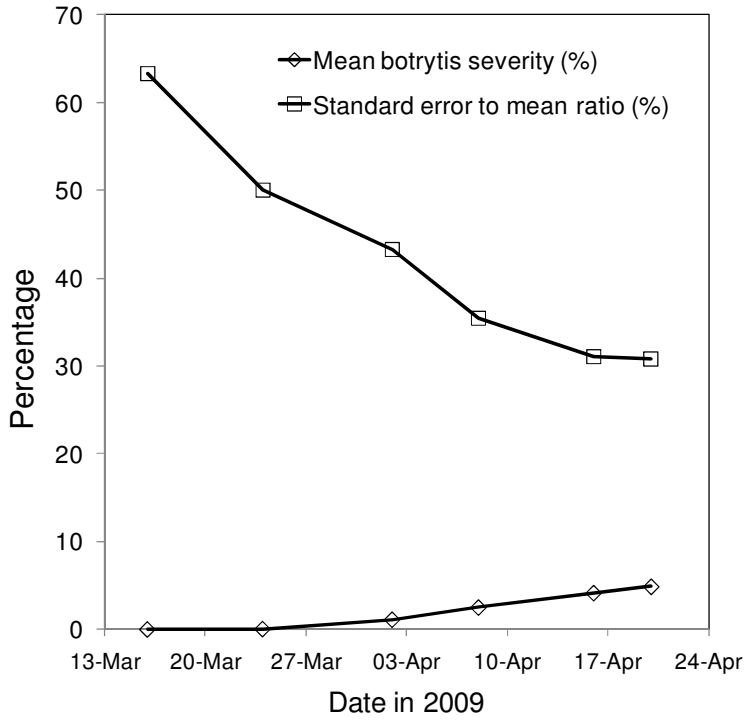


Figure 1. Epidemic of botrytis bunch rot in non-treated plots at site $R_1$09 (site S43R09 in Chapter 3). Each point represents the mean botrytis severity for six plots (single vine panel). The mean for each plot was determined from botrytis severities for the same 32 bunches assessed at each date. The corresponding standard error to mean ratio ($CV_{mean,}$ %) for n = 6 plots declined with increasing mean botrytis severity.

When 25 sample units were used for the whole block at this same site, the CV_{mean} ranged from 21 to 38% for average botrytis severities for the block ranging from 0.06 to 1.2% (refer to site $R_1$09 in Table 2). This range in CV_{mean} corresponded to CV_{mean}'s for botrytis severities ≥ 2.5% in the small-plot trial.

Different sample sizes were examined in the same Riesling block in 2010 (site-year $R_1$10), where the relationship between CV_{mean} and sample size varied according to the average botrytis severity for the block (Figure 2). When average botrytis severities were 7.7% or 2.2%, a sample size > 15 resulted in a flatter response to CV_{mean} than sample sizes of ≤ 10. In contrast, an average botrytis severity of 0.4% did not lead to a flattening in the response to CV_{mean} across the range of sample sizes evaluated. Moreover, the values of CV_{mean} were higher, although for n ≥ 15 they were still less than the CV_{mean} of 43 and 50% for botrytis severities of 1.1 and 0.06%, respectively, in the small-plot trial (n = 6, site-year $R_1$09). For this block of Riesling, it was concluded that a sample size of 25 should be attempted for the early stages of the botrytis epidemic when mean botrytis severities are lower than 1%, whereas a sample size of 15 is likely to be adequate for later stages in the epidemic. CV_{mean} values for Riesling at site-year $R_3$10 in northern Tasmania responded in a similar manner to increasing mean botrytis severity, with smaller sample sizes feasible at later stages in the epidemic.

The CV_{mean} in relation to mean botrytis severity appeared to be site and/or cultivar specific, with site-year $C_1$10 (Chardonnay) resulting in higher standard error to mean ratios than other

sites with equivalent mean botrytis severities. A sample size of 25 should be maintained for assessments made in the early stages of the botrytis epidemic at this site. In contrast, there was little variation among the smaller values for CV_{mean} for Chardonnay at site-year $C_1$09 (Table 2).

Table 2. Standard error to mean ratios ($CV_{mean,}$ %) for mean botrytis severities assessed at five site-years from Tasmania.

Site code	Date	Sample size[1]	Mean botrytis severity (%)	$CV_{mean,}$ (%)
$R_1$09	March 16, 2009	25	0.06	20.5
$R_1$09	March 24, 2009	25	0.09	37.8
$R_1$09	April 2, 2009	25	0.77	30.7
$R_1$09	April 8, 2009	25	1.2	21.3
$R_1$10	March 19, 2010	25	0.38	29.0
$R_1$10	March 31, 2010	25	2.2	12.3
$R_1$10	April 9, 2010	25	7.7	9.4
$R_3$10	March 9, 2010	20	4.6	13.1
$R_3$10	March 23, 2010	20	33	6.9
$R_3$10	April 9, 2010	20	81	2.2
$C_1$09 'Flowering'	March 18 2009	25	0.1	17.8
$C_1$09 'Flowering'	March 24, 2009	25	1.2	17.4
$C_1$09 'PBC'	March 18 2009	25	0.14	20.0
$C_1$09 'PBC'	March 24, 2009	25	1.2	21.6
$C_1$10[2]	March 22, 2010	25	0.13	76.6
$C_1$10[2]	March 31, 2010	25	0.29	55.3

[1]Each sample unit was a panel of vines. Botrytis severity was assessed for 20 bunches per vine panel.
[2]Different section of the block used for botrytis assessments when compared with site-year $C_1$09.

At site-year $C_1$09, the sampling regime for March 24 resulted in a significant difference in mean botrytis severity between the treatments imposed for the whole-of-block experiment (Chapter 5), with $P = 0.045$ for a one-sided *t*-test. This test illustrated that the approach can also be used to examine treatment effects when applied to alternating blocks of rows across a whole vineyard block.

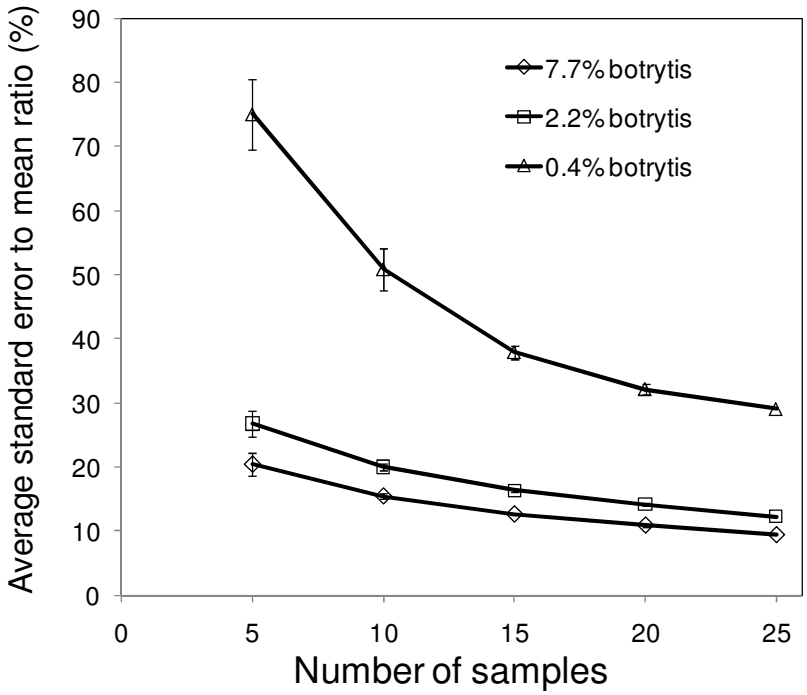


Figure 2. Average standard error to mean ratio ($CV_{mean,}$ %) for the 10 samples taken randomly from the 25 sample units for the sample size indicated on the x-axis. Error bars represent the standard error for the average CV_{mean}. Each line represents a date in 2010 in Riesling at site $R_1$10, southern Tasmania: March 19, March 31 or April 9 with mean botrytis severities for the 25 sample units of 0.4, 2.2 and 7.7%, respectively.

Hill *et al.* (2010) used samples of 150–250 bunches from replicated small-plot trials in five vineyards near harvest in 2008/2009 and selected random subsets of increasing sample size to calculate a coefficient of variation (CV) for mean botrytis severity. Sample sizes of about 100 bunches or more were within 5% of the CV for all bunches, which Hill *et al.* (2010) concluded was a starting point for designing a botrytis sampling regime for the vineyards used in their study. The sampling regime reported here used the vine panel as the sampling unit rather than single bunches, with up to 500 bunches in 25 panels needing to be assessed in order to obtain a reliable estimate of mean botrytis severity in the early stages of an epidemic. If data from site-year $C_1$10 were excluded, then this sample size (n = 25) resulted in a standard error to mean ratios of 18–38% for mean botrytis severities of < 1%. This range in CV_{mean} was similar to that reported by McSorely and Parrado (1983), who selected 20–25% CV_{mean} as the sample size for bioassays of soil samples for root-knot nematode.

It was concluded that the sampling strategy for vineyard blocks using a sample size of 20 or 25 panels generally resulted in standard error to mean ratios that were equivalent to or smaller than observed for similar mean botrytis severities observed in the replicated small-plot trial (Figure 1, Table 2). While it is acknowledged that many wineries already have standard protocols for assessing botrytis severity pre-harvest, it is not known if they provide similar levels of precision as the sampling strategy proposed here, which was designed specifically for implementing the BDSM. This sampling regime is described and translated further in Chapter 7 ('*Equipment and data needed to run the prototype BDSM*').

The next step is to develop a more rapid assessment of botrytis severity in the early stages of an epidemic, which in the first instance could be derived from the relationship between disease incidence and severity described by Hill *et al.* (2010). Disease incidence is the number of grape bunches that show botrytis symptoms and is much faster to assess than disease severity. The accuracy of model by Hill *et al.* (2010) in predicting low levels of botrytis severity from botrytis incidence needs to be evaluated for site-years not used in model development.

5 Acknowledgments

Special thanks to Justin Direen, TIAR, for conducting disease assessments at a number of the sites and also to David Riches, DPI Victoria, and Gareth Hill, Peter Wood and Dion Mundy of P&FR NZ, for discussions about sampling techniques.

6 References

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Hill GN, Beresford RM, Evans KJ (2010) Tools for accurate assessment of botrytis bunch rot (*Botrytis cinerea*) on wine grapes. *New Zealand Plant Protection*, **63**:174–181. Available online from http://www.nzpps.org/journal/contents.php?vol=63

McSorely, R. and Parrado, J.L. (1983) A bioassay sampling plan for *Meloidogyne incognita*. *Plant Disease* **67**: 182–184.

Chapter 7: Equipment and data needed to run the prototype Botrytis Decision Support Model (BDSM)

Information compiled by K. J. Evans in consultation with R.M. Beresford

1 Introduction

The following information indicates the equipment and data needed to run the prototype Botrytis Decision Support Model (BDSM), which currently operates via an Excel® spreadsheet. This spreadsheet has been developed from collaborative research supported by the GWRDC and New Zealand Winegrowers (NZW), with the model algorithms owned by The New Zealand Institute for Plant and Food Research Limited (P&FR). The spreadsheet version of the BDSM is not yet available to the grape and wine industry in Australia because more work is needed to make it 'user friendly'; ideally, software needs to be developed and tested with industry input before the 'final package' can be delivered. Model parameters also need to be calibrated for regional climates using data beyond that used for model development.

The purpose of supplying the following information is to highlight what information will be needed in order to use the BDSM (when it becomes available). It also provides an opportunity for interested parties to collect information now for running the BDSM sometime in the future, and for retrospective analysis of botrytis development and management. A fully implemented BDSM would be run 'in real time' to allow changes to management 'in season'.

2 When is the BDSM used?

Between 5% capfall (start of capfall) and harvest.

3 Weather data collection

The BSDM uses:

1. Average hourly temperature and
2. Duration of surface moisture (leaf wetness sensor: flat plate that detects change in resistance across an electronic circuit).

Rainfall is not used in the model but it is useful to observe the year-to-year variations in relation to botrytis development.

3.1 Weather station location:

Weather stations are sited outside, rather than within, vine canopies to ensure that the weather readings are not influenced by the peculiarities of individual canopies, and to avoid chemical spray deposits, which can alter the performance of surface wetness sensors.

The weather station should be located at a site that is representative of the vineyard block where the BDSM will be applied. Ideally, it should be placed outside the vineyard block of interest, with the nearest trees, buildings or other large objects no closer than three times their height from the weather station.

3.2 Weather station setup:

Many types of electronic data loggers are capable of measuring these weather variables, such as Tinytag data loggers (Gemini Data Loggers (UK) Ltd) or Campbell CR10 dataloggers (Campbell Scientific, Inc., Utah, USA). Sensors should be located 1.4-1.6 m above ground

level. Hourly data are used by the BDSM and the sensor scan interval should be 1 min. The surface wetness sensing grid should be at an angle of 10^{o} to the horizontal to prevent water pooling. Tipping bucket rain gauges should be sensitive enough to tip with every of 0.1 or 0.2 mm of rainfall. Temperature probes should be housed within a Stevenson screen or a suitable "stacked plate" thermometer screen.

It is very important that sensors are calibrated and checked regularly for damage. Wetness sensors should be cleaned and rain gauges checked for blockage regularly. Check data downloads to see if the information makes sense. Absence of positive records after a rain event (at the site) is a sign that equipment is not working.

4 Vineyard block records that need to be kept:

Crop (block) stages (dates)

1. 5% capfall (start of capfall in the block of interest)
    a. Between E-L 19 and 20
2. Pre-bunch closure
    a. Between E-L 31 and 32
3. Veraison (ideally when 50% of bunches are at veraison)
    a. E-L 34
4. Expected harvest date (when known)

Crop (block) status

1. Vineyard name
2. Vineyard manager and contact details (phone, email)
3. Block location
4. Grape variety
5. Year of planting
6. Between-row and between-vine spacings
7. Crop yield or target yield (harvest tonnes/ha or kg/vine)
8. Target ^{o}Brix for the block (if known)
9. A rating of botrytis severity last season: minor (< 3% severity) or major (≥ 3% severity)
10. A rating of current crop load: low, medium (normal for this site), high
11. A rating of current canopy vigour: low, medium (normal for this site), high

Management actions (applied to this block)

1. The dates of all fungicide applications for botrytis control
2. The date/s of leaf removal (plucking), if any, to achieve about 70% fruit exposure.

5 Monitoring botrytis development

Objectives

1. To assess the average severity of botrytis bunch rot in the vineyard block at a particular date
2. To assess botrytis severity when symptoms are first seen in the block and then 1-2 weeks later to initiate the BDSM prediction of botrytis development leading up to harvest
3. To continue botrytis assessments at 1-2 week intervals leading to harvest to check accuracy of the BDSM prediction (for the 'testing' phase only).

4. To obtain an accurate assessment of botrytis severity immediately before harvest

Method

When to start looking for botrytis

The first step is to 'walk the block' regularly to detect the first symptoms of botrytis. If it has been a very wet season, then check for the presence of 'green fruit rot' (Figure 1) at pre-bunch closure and again at veraison. Botrytis symptoms typically occur after veraison and in the pre-harvest period (Figures 2 & 3).



Figure 1. Green fruit rot can occur before veraison when conditions are extremely wet. Photo: Chris Haywood





Figure 2. Symptoms often first appear as one or several discoloured berries. The 'grey mould' often emerges from inside the bunch (pedicel area), so spread berries apart to check for botrytis spores. Photos: Kathy Evans and Katie Dunne





Figure 3. Check that the rot is caused by *Botrytis cinerea*. Using magnification, the clusters of botrytis spores (the grey mould) are borne on dark, branched stalks. Photos: Kathy Evans

Assessment of botrytis severity across the block

The following method is intended to be used by a well trained 'crop scout' or vineyard technician. Recording sheets can be developed, and there may be scope to streamline the assessment further (fewer bunches assessed and less time per assessment), once more data become available.

Sample unit

The sample unit is a single panel of four to six grapevines. Average botrytis severity for each vine panel is determined, as described below, from botrytis severities for 20 grape bunches per panel. To avoid having to climb under vines to assess both sides of the panel, 10 grape bunches are sampled on one side of the panel in the selected row and 10 bunches are sampled from the panel directly opposite in the adjacent row. These two panel sides in adjacent rows constitute 'one panel'. If facing north in vineyard rows oriented north-south, for example, then the panel on the west side in one row is assessed before assessing the panel on the east side in the adjacent row. The 10 bunches per panel side are selected without looking at the bunch first, with half the bunches selected from the outer canopy and the other half inside the canopy.

Sampling regime

The aim is to assess botrytis severity for 20 panels across an area of approximately 0.5 ha within a vineyard block that has a total area of up to 2.5 ha. If the block is > 2.5 ha, then the block should be subdivided into two or more blocks so that additional areas of 0.5 ha are assessed. The 0.5 ha area to be sampled should cover the range of vine vigour, soil and microclimate observed in the block; for example, vines from the top of the slope to those in the lowest lying area. If the 0.5 ha area is not representative of the whole block, then use the alternative method listed below.

Each row in the block is numbered from left to right (or right to left, depending on which end you start from). To avoid 'edge effects', the first row that can be monitored is row 3 and the last row that can be monitored is the third row in from the other end of the block.

Once the row to be monitored is selected, then avoid assessing the first two panels or the last two panels in the row, again, to avoid the 'edge effect'. Think about the number '3' for the boundary to the sample area: the third row in and the third panel from the end of the row.

If row 3 is the first row to be assessed, start at panel number 3 in rows 3 and 4 and sample every 5th panel in the row; that is, panels 3, 8, 13, 18, 23, and so on, until the third last panel at the other end of the row. Move across 3 rows and start walking in the corridor between rows 6 and 7. Again, sample every 5th panel in the row starting at panel 3; that is, panels 3, 8, 13, 18, 23 and so on. Move across another 3 rows and start walking in the corridor between rows 9 and 10, and repeat the process until about 20 panels are assessed over an area of about 0.5 ha. Think about the number '5' as well as the number '3': every 5th panel within a row, every 3rd corridor between rows and the block boundary area represented by the 3rd row in and the 3rd panel from the end of the row (Figure 4).

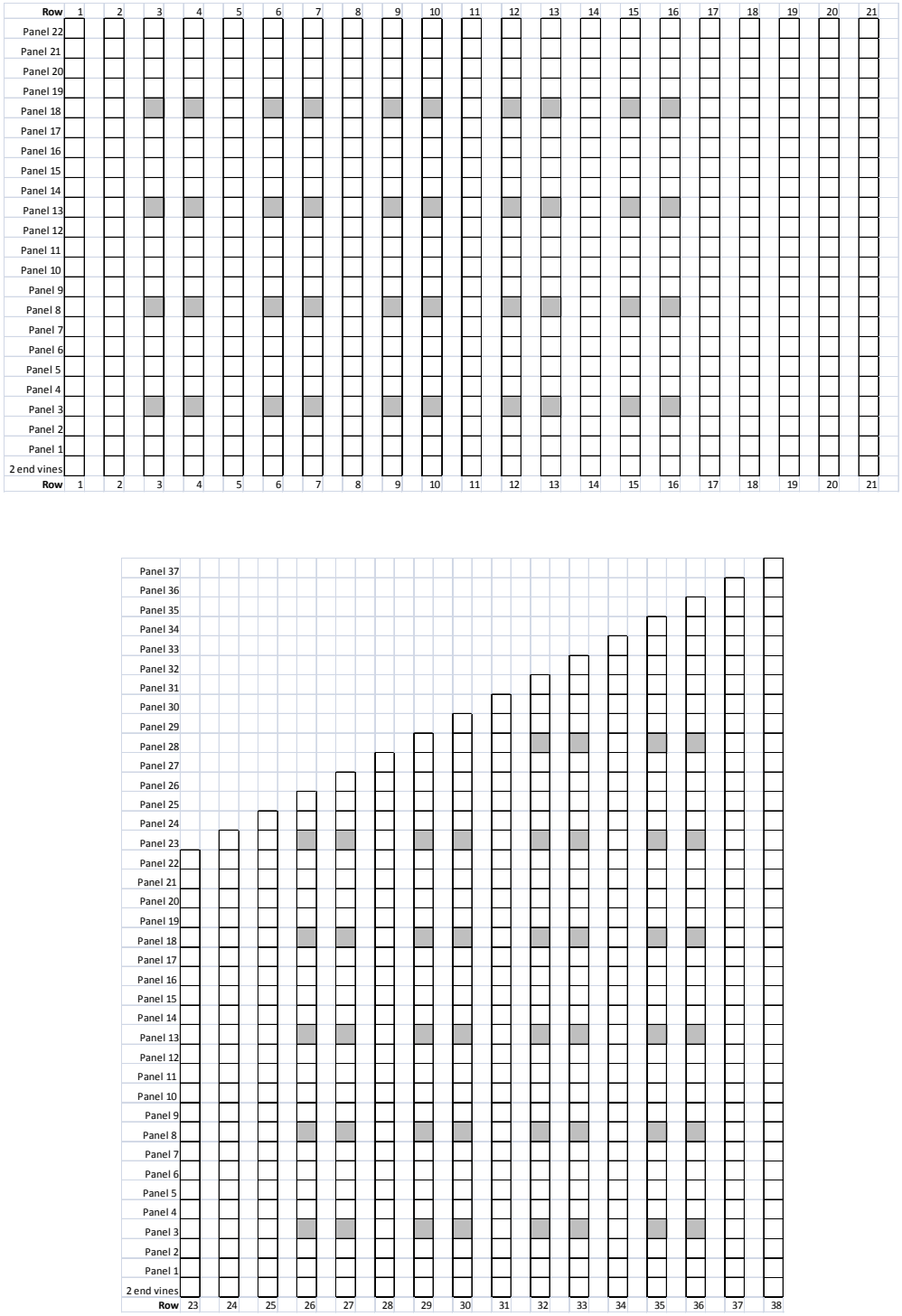


Figure 4 Schematic representation of panels and rows in two vineyard blocks of different shapes, row numbers and total area (top: 21 rows of about 0.7 ha; bottom: rows 23–36 in a 2.3 ha block of 60 rows). Each 'cell' or square is a panel of 4–6 vines and a shaded square represents a panel selected for botrytis assessment, noting that only panel sides facing each other in adjacent rows are selected for assessment.

Alternative method for determining sample area

An alternative to monitoring in the 0.5 ha area (for blocks up to 2.5 ha), is to space the monitoring rows further apart to utilise the whole block area, especially if botrytis

development is very patchy. An Excel® spreadsheet to help calculate the spacing of rows to be monitored can be obtained from K. Evans.

Assessment of botrytis severity at each panel

Each grape bunch is inspected and the presence (+) or absence (-) of botrytis bunch rot is recorded. In the early stages of an epidemic, berries can begin rotting and turn a pinkish brown colour (in white varieties) before grey mould (fungal spore masses) is obvious. In this case, the bunch could be scored as putative botrytis and the (+/-) notation used to indicate rotting berries in the absence of grey mould. Presence (+) is confirmed when grey fungal mould (spore masses) can be seen. Grey spore masses are often most obvious in the inner part of the bunch (rachis and pedicel-end of the berry). Gently pull brownish/pink rotting berries on compact bunches open to see inside. Spore masses also often emerge from splits in the berry.

If the bunch is scored as + or +/-, then assess the severity of botrytis on each bunch using a botrytis assessment key (Figure 5).

Calculating average botrytis severity in the sample area for any particular assessment date
Sum the severity scores (percentages) for each bunch scored and divide this sum by the number of bunches scored. Botrytis incidence per sample area can also be calculated by counting the number of bunches scored as (+) and express this count as a percentage of the total number of bunches scored.

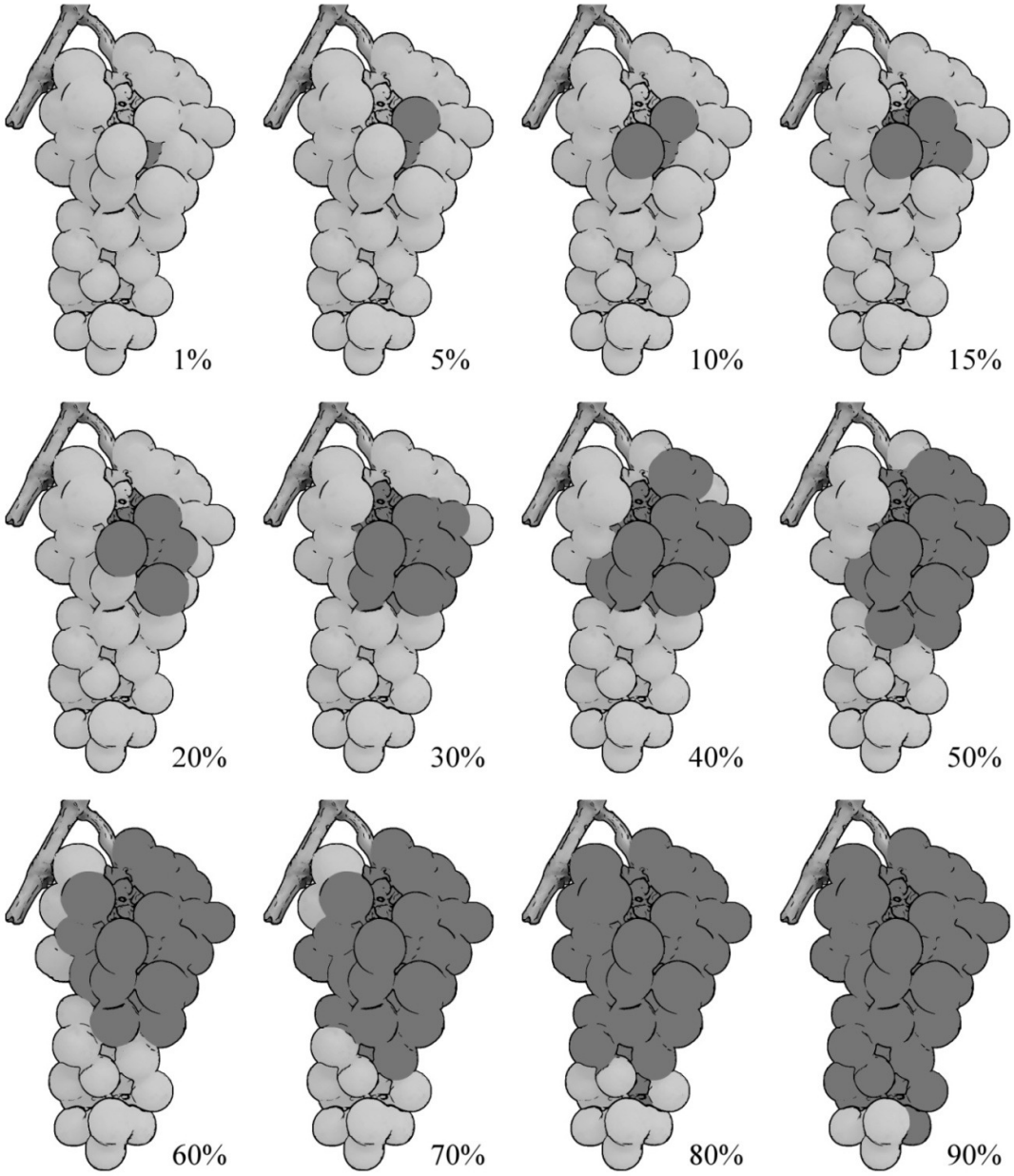


Figure 5. Assessment key for the severity of botrytis bunch rot. Lighter areas represent healthy berries; darker areas represent diseased berries. Numbers indicate the percentage of the visible side of the bunch occupied by diseased berries.
Reproduced from Hill GN, Beresford RM, Evans KJ (2010) Tools for accurate assessment of botrytis bunch rot (*Botrytis cinerea*) on wine grapes. *New Zealand Plant Protection* **63**:174–181. Available online from http://www.nzpps.org/journal/contents.php?vol=63

6 Monitoring for berry sugar development (°Brix)

The prototype BDSM also has an algorithm for predicting berry sugar development.

Objectives

1. To obtain a sample of fruit that represents the concentration of soluble solids (°Brix) in the vineyard block at a particular date
2. To repeat samples at 1-2 week intervals from just before veraison to harvest, in order to track °Brix development leading up to harvest.

Winery sampling protocols can be used, but the same method must be used for each sample date. Consider the following to increase precision of the measurement:

- Sample across the range of vine vigour in the block (not just very vigorous vines that can ripen later)
- Sample each side of the row as aspect may affect °Brix
- Sample all bunch positions, as bunches near the trunk can have different °Brix than those higher up the shoot
- Avoid sampling diseased or damaged berries
- Avoid sampling second-set berries or sample them separately
- Sample when the bunches are dry

If a winery sampling protocol is unavailable, then the following method can be used:

Either sample 100 individual berries (one berry each from 5 bunches on each of 20 vines) or 20 whole bunches from 20 different vines. These 20 vines should be distributed widely across the block and represent the full range of vine vigour/aspect as described above.

Collect berries or whole bunches into a 'zip-lock' plastic bag and store out of the sun, preferably in a cooler box (esky). Squash all the berries inside the bag and filter the juice through a coarse mesh to remove large particles. Place a drop of juice on the well of a refractometer to measure the °Brix.

Reference:

Grape Futures 2010. Grape Maturity (°Brix) Prediction Model v1.2 February 2010. Fact Sheet prepared for the project "Implementing Ultra-Low Residue Wine Grape Production" (GrapeFutures) funded by New Zealand Winegrowers and MAF Sustainable Farming Fund 2008-2010.

Chapter 8: Service provision, infrastructure and education required for adoption of the Botrytis Decision Support Model

Katherine J. Evans

1 Web-based delivery to regions

1.1 General requirements

An efficient method for delivering the BDSM is by a centrally managed web-based service tailored to the needs of particular region or district. Examples of services for web-based decision support include Crop Watch in South Australia (http://www.cropwatch.com.au), HortPlus™ in New Zealand and Australia (http://www.hortplus.com), AgWeatherNet (http://weather.wsu.edu) in Washington State, USA, and the Australian Wine Research Institute (http://www.awri.com.au/commercial_services/spectral_technologies/tannin_portal/). These services require access to a network of well-maintained and well-positioned weather stations plus a computer server that collects the weather data in a central location for further processing.

Data from weather stations can be downloaded via telephone landlines, the Next G mobile phone network, and/or, as technology improves, via wireless-directed internet access. Weather stations can vary in type, as long as a communication protocol is developed to enable a common file format to be delivered to the central server. Weather stations in Australia include those managed by the Bureau of Meteorology (BOM), water catchment authorities, private companies and individual vineyards. Calculation of the Bacchus risk index for the BDSM requires temperature and surface (leaf) wetness data. Some weather stations may not have a leaf wetness sensor. There is potential to estimate leaf-wetness duration using other weather variables or by interpolation models (for example, Kim *et al.* 2010).

1.2 'Push' and 'Pull' methods of delivery

The media and method for delivering model outputs depends on the client. Methods of delivery can be classified broadly as 'push' or 'pull' systems. Information can be 'pushed' via faxes, automated emails and/or text messages that are sent directly to clients at regular intervals such as daily or weekly reports. These reports are 'mass customised' with warnings or suggestions about the timing of crop monitoring. Information can also be 'pulled' by clients directly from web-sites, either on-screen or via a text message. One benefit of the 'pull' system is that the internet site can be used to create a 'favourites' report where clients can configure a report (by mouse clicking) to run specific models for a particular site, single models at multiple sites or multiple models at multiple sites. The 'favourite' report can also be delivered to the client at the time they specify. Given that every grower perceives risk differently and will have specific preferences for how information is received, then the decision support service appears to work best when a combination of 'push' and 'pull' systems are provided.

Some disease models require technical staff or 'scouts' to monitor crops near weather stations to 'ground truth' a model if it has predicted an outbreak of disease. An example is the downy mildew operated by Crop Watch in the Riverland. The BDSM does not require crop scouts at a district level, although crop monitoring for botrytis rot 'on site' will be required in order run the late-season BDSM.

1.3 Funding and provision of web-based delivery in cool climate viticulture

Widespread adoption of decision-support services is likely if individual growers do not have to pay for the service 'up front'. A 'user pays' system at the level of an individual grower could lead to the situation where certain growers who need to change suboptimal management would forgo such a service. Adoption of Crop Watch in the Riverland of South Australia was made possible by support from a regional grape industry development corporation (RWIDC), who paid for the service through a grape levy and provided the service 'free' to all members. Similarly, Pipfruit NZ buys a MetWatch licence *en masse* and delivers it to apple growers. AgWeatherNet in Washington State is supported by the State government, a situation that is unlikely to occur in Australia unless growers and land managers lobby government intensely. Alternatively, government support through schemes such as 'Caring for our Country' or 'adapting to climate change' programs might help initiate a scheme. In this context, the service would need to provide decision support across a wide range of environmental threats, including disease, frost, evapo-transpiration, fire risk and so on.

1.4 Current situation in cool climate viticulture

There is scope for Crop Watch to expand to cool climate regions in Australia that are currently not serviced. HortPlus™ also have the potential to establish a service for viticulture, given that they currently have systems running in the Goulburn Valley and the Batlow regions for pome-fruit growers.

Current weather stations in many cool climate regions need to be networked and managed centrally for efficient web-based delivery. Additional weather stations may be required for any gaps in coverage, especially in high altitude regions and Tasmania where multiple microclimates are created by hills and valleys.

2 Delivery of a 'stand alone' product to individual businesses

For regions that currently lack the infrastructure and service for web-based delivery, then the BDSM could be delivered to individual businesses, including large wine companies, by provision of an enhanced Excel® spreadsheet or software, plus appropriate technical support. In this scenario, the vineyard will need a suitable on-site weather station or a BOM or private weather station in a location that is representative of on-site conditions. For efficient operation, weather data should be downloaded directly (eg. by telemetry) to the office computer. The BDSM spreadsheet or software will need to account for the likely range in data files that will be downloaded to office computers from various types of weather stations. The output will relate specifically to decision support for botrytis management, although there may be scope to integrate the software into existing company platforms for all aspects of vineyard management.

3 Education and adoption

The mechanism of delivery of the BDSM will determine the nature of the education required for its uptake. A stand-alone product would need significant 'self help' and adoption guidelines, such as weather station specifications and location, disease diagnosis and a guide to monitoring botrytis. Large wine companies could implement the system internally using their own technical staff. Alternatively, a commercial provider/consultant could deliver either the stand-alone product or web-based decision support, including additional technical support to assist product uptake.

One model that has been proposed for industry implementation is that relevant organisations in Australia would enter into a support agreement with P&FR. This support agreement would

cover training in the use of the system and some validation of data. Any agreements would be for a fixed period and offer defined benefit to the contracted organisations. Such a support agreement may be an effective way of ensuring uptake of the model by industry and ensuring correct use of the model.

4 Reference

Kim, K.S., Hill, G.N. and Beresford R.M. (2010) Remote sensing and interpolation methods can obtain weather data for disease prediction. *New Zealand Plant Protection* **63**, 182–186.

5 Acknowledgements

Thanks to Peter Magarey, Andrew Hodson, Gary Grove, Rob Beresford and other colleagues for informing this discussion.

Outcome/Conclusion

The strength of this trans-Tasman collaboration was the creation of a sufficiently large dataset encompassing the range of botrytis severities likely to be experienced in the cool climates studied. Development and application of quantitative methods with this large dataset enabled the major outcome of this project, which was the prototype Botrytis Decision Support Model (BDSM).

The BDSM, as described by Beresford *et al.* (2009, Appendix 5), has two component models to assess the risk of a botrytis outbreak:

- The early-season model (E-BDSM, Figure 1) uses weather data and vineyard management actions against botrytis (e.g. fungicides) between flowering and veraison to assess the risk of major botrytis damage leading up to harvest. The model accepts inputs of actual dates for management actions and also for possible future actions anytime between flowering and veraison and it shows the effects these would have on botrytis risk (including traffic light warnings: low, moderate or high risk).

- The late-season model (L-BDSM, Figure 2) is used when the first sign of botrytis bunch rot appears in a grape block (usually after veraison). Botrytis severity is measured according to an assessment protocol and the severity readings and dates are entered into the model, which provides a graph of how future botrytis severity will increase (or not). Grape sugar development (^{o}Brix) is also monitored and a separate model predicts the date that a target ^{o}Brix for the grape block will be reached. This allows botrytis severity at harvest to be predicted and shows the effect that harvesting at different dates would have on botrytis severity.

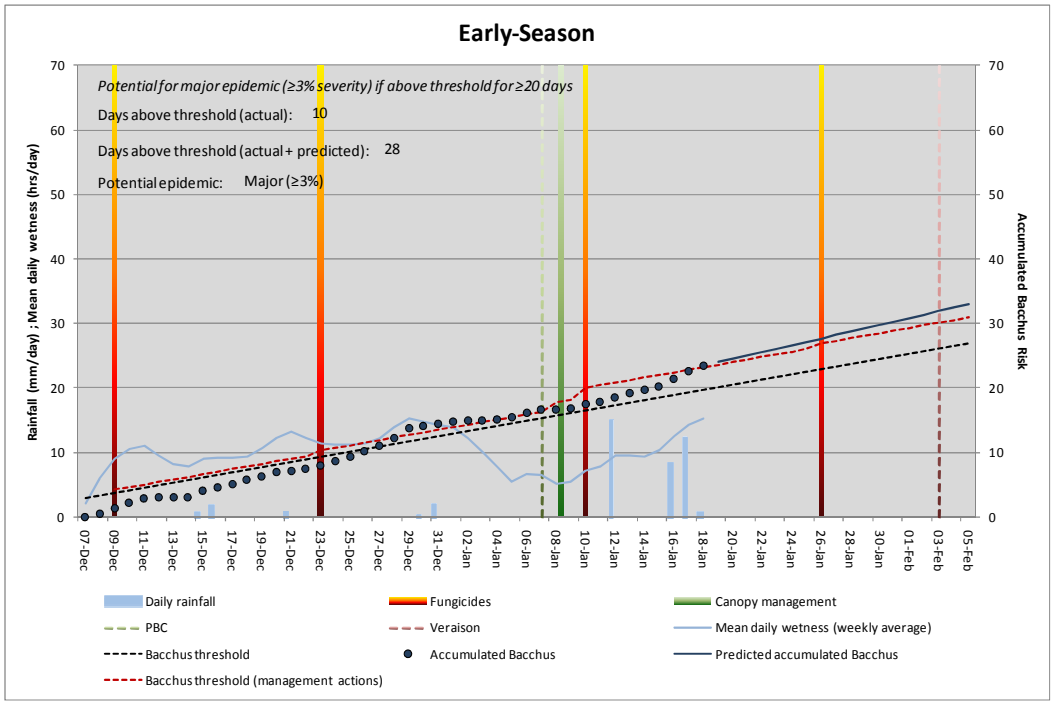


Figure 1. (Courtesy of P&FR). Output screen (Excel® spreadsheet version) for the Early-season Botrytis Decision Support Model showing weather variables, management actions, phenological stages and accumulating Bacchus risk in relation to the epidemic threshold.

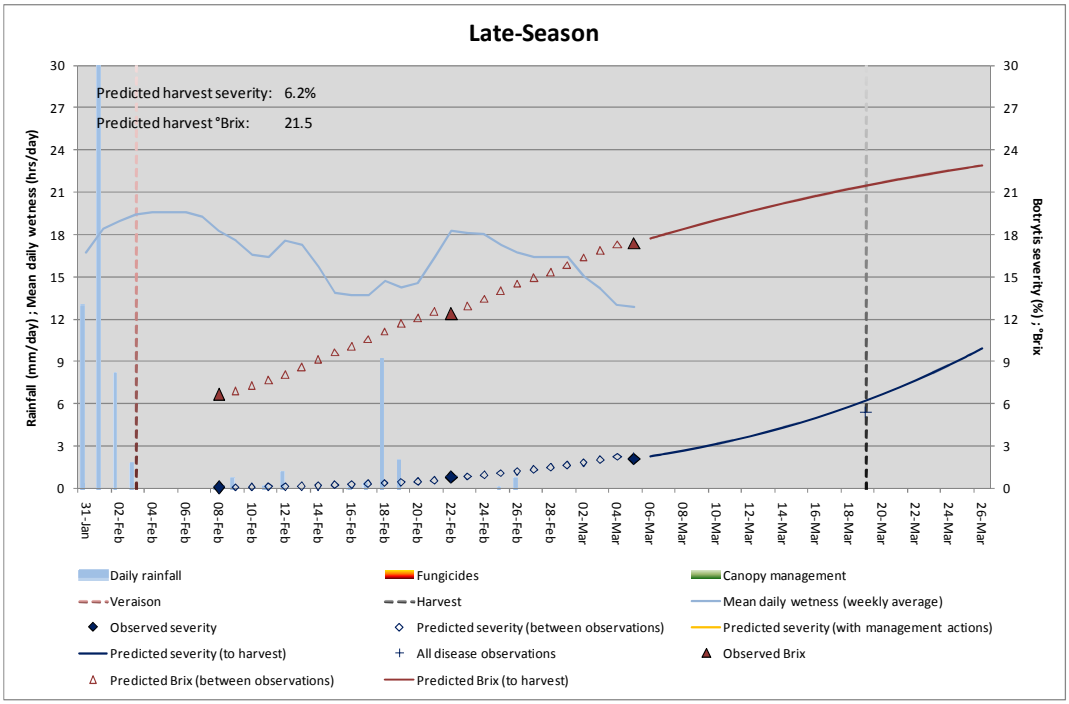


Figure 2. (Courtesy of P&FR). Late-season output (Excel® spreadsheet version) showing three disease observations, three °Brix observations as well and weather data up to the date of the second observation. All observations of actual disease in the vineyard are also shown for comparison.

Chapter 7 outlined the equipment and data needed to run the prototype BDSM, including a validated sampling strategy (derived from Chapter 6 for monitoring botrytis development). This information plus the weather, pathogen and vine variables identified during development of the quantitative methods addressed the sub-project B objective of providing instructions for measurement of selected variables for a 'prototype bunch rot database'. This database is now effectively any future publication in the public domain that details results from application of the standard quantitative methods, including inputs to the BDSM.

The benefits of implementing the Botrytis Decision Support Model (BDSM) will be:

- Reduced seasonal variability in grape production and wine quality due to better management of botrytis
- Improved efficiency of botrytis control programs, leading to reduced fungicide, diesel and labour inputs in low risk situations
- Improved awareness of the effectiveness of various botrytis management options and how and when they should be applied
    - Improved ability to manage fungicide residues and non-target impacts while maintaining disease control
- Reduced vineyard manager stress when botrytis risk is known well ahead of disease onset so that proactive measures can be taken, including planning harvest operations to minimise crop loss.
- Retrospective analysis of the performance of botrytis control programs
- A contribution to environmental credentials for accreditation systems

In achieving this outcome, the replicated, small-plot trials provided new, region-specific information about the effectiveness of registered fungicides applied at specific crop stages and other control measures tested, such as bunch trash removal, shoot thinning and leaf removal.

Ms Katie Dunne's PhD project achieved two major outcomes. The first outcome was a quantitative real-time polymerase chain reaction (qPCR) technique for quantifying the amount of *Botrytis cinerea* DNA in grape berries. Two potential opportunities for this laboratory-based research tool are (i) further research to provide insight on the timing and extent of colonisation of grape berries after latency for different cultivars and environmental conditions, and (ii) the use of qPCR in the development (calibration) of a more rapid, cost-effective tool for assessing botrytis risk or level in the vineyard. The second major outcome of the PhD project was a new application of the whole-of-block experimentation in which spatial variability in botrytis severity was used as an experimental tool. This trial, conducted in a 2.4 ha block of Chardonnay, revealed valuable information about interactions between spray effectiveness and topography. The conclusions about fungicide timing also related directly to the capability of the commercial spray equipment and the results suggested that secondary spread of botrytis at the site and season studied may have been of minor importance. The full outcome of this PhD project will become evident when the thesis has been submitted and examined.

Another outcome flowing from the trans-Tasman collaboration is establishment of a new PhD project on botrytis epidemiology. Mr Gareth Hill, of P&FR, has accepted an 'Elite' scholarship

from the University of Tasmania with PhD supervisors Dr Kathy Evans and Dr Bob Dambergs (TIAR and Australian Wine Research Institute). Mr Hill will work primarily at P&FR with on-site supervisor Dr Rob Beresford and will make regular trips to Tasmania for collaborative research.

Finally, implementation of the BDSM, as outlined in Chapters 7 and 8, is not simply a matter of providing the prototype Excel® spreadsheet to a software developer and then distributing the final product. The algorithms underlying the BDSM are complex and appropriate programming is needed so that the models interface easily with weather and spray diary data, especially when these may be downloaded in different formats. This work involves building the website framework (for web-based delivery), designing the interface between the source code of the model and databases (weather and other information) as well as working directly with end users to refine the model and its delivery. In short, implementation requires a final stage of product development that involves end-users directly in product testing so that model accuracy is confirmed beyond sites used to develop the algorithms and so that the monitoring protocols and user-interface can be integrated readily to existing vineyard operations. Moreover, appropriate (weather station) infrastructure and suitably trained service providers or technical staff are needed to ensure that the system is implemented effectively.

Recommendations

The key recommendations flowing from the outcomes of GWRDC project UT0601 are:

- Develop the BDSM from its current prototype (Excel® spreadsheet) to a 'ready to adopt' web-based or stand-alone version suitable for supporting advice from industry service providers and vineyard technical managers to vineyard staff.
    - test model accuracy beyond the sites used to develop the BDSM
    - further refinement of the BDSM according to feedback during implementation
    - tailor the user-interface (software) to regional needs
    - validate or modify protocols for botrytis, Brix, vine canopy and fungicide data so that BDSM can be adopted readily at varying scales of commercial viticulture.
    - provide relevant training and support to BDSM end-users.
- P&FR New Zealand is working towards a web-enabled version of the BDSM, with support from New Zealand Winegrowers (NZW). There is potential for NZW to license the web-version to the GWRDC. If so, the user interface will need to be tailored to viticultural practices in Australia and the BDSM trialed and refined as described previously.
- Continue research towards rapid and cost-effective tools for quantifying non-visible and visible botrytis (total disease) for studies of disease epidemiology and for assessing botrytis risk.
- Promote adoption of consistent, quantitative methods among botrytis researchers for generating compatible data and comparison of results across multiple sites and seasons, and
- Promote and extend 'whole-of-block' experimentation for revealing the commercial efficacy and spatial variation in the response to disease or other viticultural management. Applications include
    - Investigating secondary spread of botrytis at other site-years
    - Adaptive research (on-farm trials, site-specific information)
    - Precision viticulture to improve grape and wine quality

Appendix 1: Communication

Publications

Evans KJ (2008) 'Overview of R&D for managing botrytis bunch rot in Australia', Proceedings of the Australian Society of Viticulture and Oenology Seminar on "Breaking the mould - a pest and disease update", 22-25 July 2008, Mildura, Victoria, pp. 4-15. ISBN 0 9775256 4 3

Beresford RM, Hill GN (2008) 'Predicting in-season risk of botrytis bunch rot in Australian and New Zealand vineyards', Proceedings of the Australian Society of Viticulture and Oenology Seminar on "Breaking the mould - a pest and disease update", 22-25 July 2008, Mildura, Victoria, pp. 24-28. ISBN 0 9775256 4 3

Hill GN, Beresford RM, Evans KJ (2010) 'Tools for accurate assessment of botrytis bunch rot (*Botrytis cinerea*) on wine grapes' *New Zealand Plant Protection*, **63**:174-181. Available online from http://www.nzpps.org/journal/contents.php?vol=63

Emerging Presenter Award at the 2010 New Zealand Plant Protection Society conference.

Bramley RGV, Evans KJ, Dunne KJ, Gobbett DL (2010) 'Spatial variation in response to 'reduced input' spray programs for powdery mildew and botrytis identified through whole of block experimentation', undergoing internal review prior to submission.

Articles in industry journals

Evans, KJ, Beresford RM, Edwards, J, (2007) 'Taking a more strategic approach to Botrytis bunch rot', *The Australian & New Zealand Grapegrower & Winemaker*, December 2007, pp 36-37.

Edwards J, Riches D, Evans K, Beresford R, Hill G, Wood P, Mundy D (2009) The need for a risk-based approach to botrytis management. *The Australian & New Zealand Grapegrower & Winemaker*. Annual Technical Issue 2009, pp. 6-9.

Conference Proceedings

Beresford, RM, Edwards, J, Evans, KJ (2007), 'Predicting the risk of *Botrytis* bunch rot in cool climates', *Proceedings of the 13th Australian Wine Industry Technical Conference*, Adelaide, South Australia. Poster presentation.

Beresford, RM, Evans KJ, Edwards J (2007), 'Predicting the seasonal risk of Botrytis bunch rot in wine grapes', *Proceedings of the14th International Botrytis Symposium*, Cape Town, South Africa. Oral presentation.

Evans, KJ, Beresford, RM, Edwards, J (2007), 'Predicting the risk of botrytis bunch rot in cool climate viticulture', *Proceedings of the 16th Biennial Australasian Plant Pathology Society Conference Back to Basics: Managing Plant Disease*, Adelaide, South Australia, p 151. Poster presentation.

Evans, KJ and Beresford, RM (2009) 'Latest advances in bunch rot management and epidemiology : Standard and novel methods for evaluating botrytis bunch rot in wine grapes', Australasian Plant Pathology Society Pre-conference Workshop on Biology and Management of Organisms Associated with Bunch Rot Diseases of Grapes, 28th September 2009, GWRDC and Charles Sturt Univeristy, Pokolbin, NSW.

Edwards J, Riches D, Evans KJ, Beresford RM, Hill GN, Wood PN, Mundy DC (2009) Botrytis bunch rot control strategies in cool climate viticultural regions of Australia and New Zealand. , *Proceedings of the 17th Biennial Australasian Plant Pathology Society Conference, Plant Health Management: An Integrated Approach*, Newcastle, New South Wales, p 24. Oral presentation.

Dunne KJ, Evans KJ, Bramley RGV (2010) Secondary spread may not be the main driver of within-season increase in the severity of botrytis bunch rot. *Proceedings of the 14th Australian Wine Industry Technical Conference*, Adelaide, South Australia. Poster presentation. **Winner of the Wine Innovation Cluster - Best Student Poster prize**.

Workshops

Dunne KJ (2010) Botrytis epidemiology and management in Tasmania. Presented at workshop WO7 Botrytis bunch rot in cool climate regions, convened by D. Mundy. Australian Wine Industry Technical Conference, Adelaide, July, 2010.

Riches D (2010) Botrytis bunch rot control: cool climate viticultural trials in Aus. & NZ. Presented at workshop WO7 Botrytis bunch rot in cool climate regions, convened by D. Mundy. Australian Wine Industry Technical Conference, Adelaide, July, 2010.

Other (oral) presentations (Australia only)

- Evans KJ - Wine Industry of Tasmania, grower field day, Craigow Vineyard, November 16, 2007.
- Evans KJ - Wine Industry of Tasmania, grower field day, Moorilla Estate, December 7, 2007.
- Evans KJ – 'Management of botrytis bunch rot' at the Tasmanian Pinot Noir forum, Swansea, Tasmania, July 5, 2008.
- Evans KJ – 'Management of botrytis bunch rot' at the AWRI Roadshow Seminar held in Hobart, Tasmania, February 13, 2009.
- Evans KJ – 'Collecting data in practice: grape botrytis example' 'Disease Prediction Workshop', Plant & Food Research in Christchurch, NZ, April 3, 2009 (in conjunction with project planning/review meeting).
- Evans, KJ – 'Mouldy wine grapes: the good, the bad and the ugly", presentation to Year 10-12 students from Hobart College as part of the PICSE program (Primary Industry Centre for Science Education), Hobart, Tas., May 14, 2010.
- Evans KJ – 'Predicting botrytis risk' at an information day hosted by Wine Industry Tasmania, Coal Valley Vineyard, Tasmania, July 2, 2009.
- Evans, KJ - 'Managing Botrytis Bunch Rot', presentation to the NSW wine and grape industry. Spring Vine Health Workshop on "Promoting Vineyard Health". Funded by DAFF and hosted by the NSW Government and the Hunter Valley Wine Industry Association, Pokolbin, NSW, October 2, 2009.
- Evans, KJ – 'Understanding the risk of botrytis', presentation to the Orange Vignerons Association. Spring Vine Health Field Day coordinated by the National Grape & Wine Industry Centre, Orange, NSW, September 7, 2010.
- Evans, KJ – 'Understanding the risk of botrytis', presentation to the Tumbarumba Vignerons Association. Spring Vine Health Field Day coordinated by the National Grape & Wine Industry Centre, Tumbarumba, NSW, September 9, 2010.
- Evans, KJ – 'Supporting decisions about botrytis management', presentation to the Yarra Valley Winegrowers Association. Spring Vine Health Field Day coordinated by the National Grape & Wine Industry Centre, Lilydale, Vic., October 21, 2010.

- Evans, KJ – 'Botrytis Q&A, Botrytis Decision Support Model', presentation to the Victorian Wine Industry Association, VWIA Grassroots Spring Workshop. Wangaratta, Vic., November 25, 2010.
- Evans, KJ – 'Botrytis Q&A, Botrytis Decision Support Model', presentation to the Victorian Wine Industry Association, VWIA Grassroots Spring Workshop. Bendigo, Vic., November 26, 2010.

Related publications

Refer to the GWRDC's Innovators Network website for the Botrytis Fact Sheet, Powerpoint presentation and presentation notes on botrytis management prepared by Dr Kathy Evans.
http://www.gwrdc.com.au/webdata/resources/files/BotrytisFactSheet.pdf
http://www.gwrdc.com.au/webdata/resources/files/BotrytisTechnicalNotes.pdf
http://www.gwrdc.com.au/webdata/resources/files/Botrytis_Presentation.pdf

Appendix 2: Intellectual Property

According to the Project Agreement, any development arising from the project that may be subject to a form of Intellectual Property protection must be decided on "in the joint names of the GWRDC, the Research Organisation and all relevant Third Parties as tenants-in-common in the Project IP Shares" and "at the cost of the GWRDC, the Research Organisation and all relevant Third Parties in the same proportions as they hold the Project IP Shares" (clause 12.4 (b)).

This project delivered a prototype Botrytis Decision Support Model (BDSM) for wine industries in cool climates of Australia and New Zealand. The final industry implementation and the ongoing servicing of the BDSM were not part of this project. The New Zealand Institute for Plant and Food Research Limited (P&FR) owns the Botrytis Decision Support Model (algorithms underlying the BDSM) and database systems that were used in this project. Funding from GWRDC project UT0601 was used to gather Australian data to calibrate the BDSM and create a prototype suitable for implementing in Australia. P&FR is expected to license the use of the BDSM, as a royalty free licence, to GWRDC and New Zealand Winegrowers (NZW). NZW is expected to take up the licence (see next paragraph) and GWRDC has yet to do so. P&FR will retain the right to use and develop the BDSM further.

New Zealand Winegrowers (NZW) are supporting web programming of the BDSM, meaning that a web version of the BDSM could be available to the NZ industry in 2011. If so, then NZW will own the source code of the web version. If a web-version of the BDSM is to be implemented in Australia, then there is potential for NZW to licence the web-version to the GWRDC.

One model that has been proposed for industry implementation is that relevant organisations in Australia would enter into a support agreement with P&FR. This support agreement would cover training in the use of the system and some validation of data. Any agreements would be for a fixed period and offer defined benefit to the contracted organisations. P&FR believe that such a support agreement may be an effective way of ensuring uptake of the model by industry and ensuring correct use of the model.

Appendix 3: References

References are cited at the conclusion of each chapter in the report.

Appendix 4: Staff

PhD project	
Ms Katie Dunne	PhD candidate, TIAR & School of Agricultural Science, UTAS
Dr Kathy Evans	Senior Research Fellow, TIAR, University of Tasmania (UTAS)
Dr Karen Barry	Lecturer, School of Agricultural Science, UTAS
Dr Lance Cadle-Davidson	Geneva Campus, Cornell University, New York, USA
Dr Jacky Edwards	Department of Primary Industries, Victoria
Trials in Tasmania	
Dr Kathy Evans	TIAR, University of Tasmania (UTAS)
Mr Justin Direen	TIAR, University of Tasmania (UTAS)
Trials in Victoria	
Dr Jacky Edwards	Department of Primary Industries, Victoria
Mr David Riches	Department of Primary Industries, Victoria
Collaborators/intellectual input/project feedback for research conducted in Australia	
Dr Robert Beresford, Mr Gareth Hill & colleagues	The New Zealand Institute for Plant and Food Research Limited.
Mr Greg Lee	Biometrician, TIAR, UTAS
Dr Rob Bramley & Mr David Gobbett	CSIRO Ecosystem Sciences, Adelaide
Dr Bob Emmett	Department of Primary Industries, Victoria
Dr Chris Steel	NWGIC, Charles Sturt University, Wagga Wagga, NSW
Ms Karen McGuire	EnviroLogix Inc., Portland, ME, USA.

Appendix 5: Other relevant material

The following report is attached:

Bereford RM, Hill GN, Wood PN, Mundy D. (2009) Inoculum load prediction for improved botrytis risk management: Final report for year 3, 2008-2009. A report for New Zealand Winegrowers, Project NZW 07-201, The New Zealand Institute for Plant & Food Research Limited, Contract number 22230.

Appendix 6: Budget reconciliation

Refer to attached file UT0601_End_of_Project_ Financial_Statement_TIARGSTExc.doc