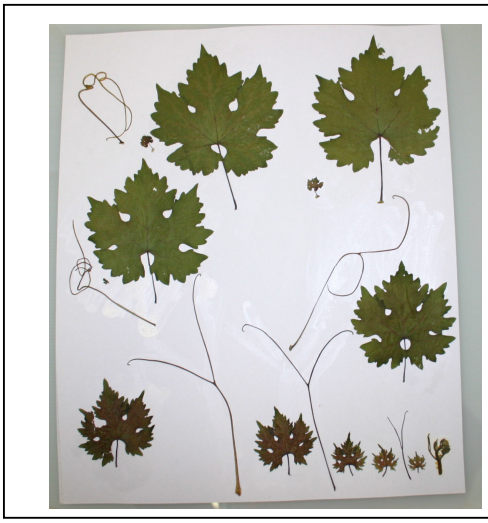
Ontogenic resistance in grape leaves to powdery mildew and models of shoot development

(original title: Response of grapevine canopies to chemical elicitors that induce disease resistance)





FINAL REPORT to

GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: **UT 04/01**

Principal Investigator: **Katherine J. Evans**

Research Organisation: **University of Tasmania**

Date: **31 December, 2008.**

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Project Title:

Ontogenic resistance in grape leaves to powdery mildew and models of shoot development

Original title: Response of grapevine canopies to chemical elicitors that induce disease resistance.

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Richard Richardson for Delamere Vineyard, near Pipers Brook.

Disclaimer: The reader is referred to the publication, in due course, of the PhD thesis of Ms Angela Smith. This thesis is likely to be available electronically through the library of the University of Tasmania. This GWRDC final report may be of assistance to you but the authors and their employer do not guarantee that the publication is without flaw of any kind or is wholly appropriate for your particular purposes and therefore disclaim all liability for any error, loss or other consequence which may arise from you relying on any information in this publication.

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Abstract
This report presents preliminary outcomes of the PhD project of Ms Angela Smith, supervised by Drs Kathy Evans and Steve Wilson at the Tasmanian Institute of Agricultural Research, University of Tasmania. Bayesian modelling was used to describe the area of colonisation by *Erysiphe necator*, the cause of powdery mildew, on all leaves of shoots of *Vitis vinifera* cv. Cabernet Sauvignon with two different rates of leaf emergence. Shoots with a higher rate of leaf emergence prior to inoculation with *E. necator* developed powdery mildew more severely. At both rates of leaf emergence, there was a strong association between leaf position for maximum severity of powdery mildew and the position of the leaf in the sink to source transition for carbohydrate, immediately after it had ceased importing photosynthates. Thermal time (degree days) was used to predict the rate of leaf emergence and leaf area development for Chardonnay and Pinot Noir vines grown commercially in southern Tasmania with shoots positioned vertically or by the Scott Henry method. This report describes how these empirical models, derived from the glasshouse and field data, can be applied for timing disease management strategically and for quantifying dynamic changes in the grapevine canopy structure for susceptibility to powdery mildew and hence disease risk. As susceptibility of leaves to powdery mildew represents the inoculum load for grape berry infection, small changes in management practices may have profound effects on disease expression on the berries. The report concludes with communication outcomes and recommendations for future research and development.

Executive Summary

The key drivers for reducing pesticide use in Australian viticulture are the need to reduce non-target, negative impacts and demands of some export markets for nil residues. Powdery mildew, caused by *Erysiphe necator*, is managed every year in most vineyards by multiple applications of fungicides. The severity of leaf infection at flowering represents the inoculum load for berry infection, which can alter wine composition and sensory characters. A sound knowledge of disease epidemiology is required in order to time fungicides for when they are really needed and to minimise their use. Past research has focused on how the environment affects the pathogen, not how the environment affects the host's susceptibility to the pathogen.

Both leaves and berries of *Vitis vinifera* express ontogenic resistance to infection by *E. necator*, which means that infection efficiency declines as the plant organ ages (Doster and Schnathorst 1985; Ficke *et al.* 2003). Potential, novel measures for disease control target the plant host, rather than the pathogen directly, such as benign chemical elicitors that stimulate the plant's natural defence to pathogen attack. What is not known is the relationship between the level of induced resistance and leaf physiology, with the latter varying significantly as leaves age. A basic understanding of inherent disease susceptibility in relation to leaf physiology is required before superimposing factors that induce disease resistance in the grapevine.

This report presents preliminary outcomes of the PhD project of Ms Angela Smith, who received an Australian Postgraduate Award from the University of Tasmania (UTAS) and support from GWRDC to conduct PhD research at the Tasmanian Institute of Agricultural Science (TIAR) and in commercial vineyards in southern Tasmania.

Bayesian modelling was used to describe the area of colonisation of *E. necator* on all leaves of shoots of *Vitis vinifera* cv. Cabernet Sauvignon with two different rates of leaf emergence under glasshouse conditions. The mean modal leaf position for maximum disease severity was estimated to be 3.7 and 4.7 for shoots developing at a mean temperature of 18 and 25^{o}C, respectively. The carbohydrate sink to source transition of leaves also occurred on average at leaf positions 3.8 and 4.7 for shoots developing at each temperature regime. This is the first report of the association between leaf position for maximum severity of powdery mildew and the position of the leaf in the sink to source transition, immediately after it had ceased importing carbohydrates. Furthermore, the rate of leaf emergence pre-inoculation affected the incidence and severity of powdery mildew for the entire shoot, with a higher rate of leaf emergence leading to more disease per shoot.

Models of the strong linear relationship between the rate of leaf emergence and thermal time (degree days above 10^{o}C) were developed for cane-pruned Chardonnay and Pinot Noir vines with vertical shoot positioning (VSP) or trellising by the Scott Henry method. In Chardonnay, shoots positioned close to or far from the trunk had a higher rate of leaf emergence than shoots positioned mid-way along the cane. Downward orientated shoots had a higher rate of leaf emergence than upward orientated shoots on Pinot Noir vines. Models for leaf area development have also been formulated. These variety-specific models can now be used to estimate the amount of leaf area or new leaves that have developed since the previous fungicide application and that are susceptible to new infection by *E. necator*. Moreover, these models, when combined with models of ontogenic resistance developed in glasshouse studies, can be used to simulate the proportion of leaves on primary grapevine shoots that are susceptible to powdery mildew at any time during the growing season. This research provides 'on ground' data and builds on the simulation of powdery mildew epidemics by Calonnec *et al.* (2006, 2008), whereby the timing and location of the first sporulation events by *E. necator* on leaves and shoots was correlated to the severity of powdery mildew on grape bunches.

The significance of the PhD research is that dynamic changes in the grapevine canopy structure for susceptibility to powdery mildew should be considered a key risk factor, along with environmental conditions, in explaining variation in disease severity among growing seasons. Further research is required to validate and apply models of shoot development for timing fungicide applications and to identify opportunities to prolong spray intervals for chemicals such as sulfur that have a very slow rate of decay (Emmett *et al.* 2002). More work is also required to predict canopy susceptibility to infection by *E. necator* over time, using models of ontogenic resistance that account for the temperature at which shoots develop and models of secondary shoot (lateral) growth. Basic research to explain why grapevine leaves are most susceptible to infection by *E. necator* during the carbohydrate sink to source transition might reveal novel options for disease management.

The research has been communicated to a broad cross-section of industry and scientific audiences, ranging from workshops organised by the Vineyards Association of Tasmania through to the International Congress on Molecular Plant Microbe Interactions in Italy in 2007. Ms Smith and her supervisor Dr Kathy Evans received an award for the best pest and disease poster and for the best student poster at the Thirteenth Australian Wine Industry Technical Conference, 2007. Subsequently, Ms Smith was invited to present a paper on her PhD research at the Australian Society of Viticulture and Oenology seminar on 'Breaking the mould: a pest and disease update' in 2008. Details of communication outcomes and recommendations for future research and development are provided.

Background

Powdery mildew, caused by *Erisyphe necator*, must be managed every year in most vineyards growing *Vitis vinifera* grapevines. Wine made from Chardonnay grapes with as little as 1-5% of bunches affected by powdery mildew can have altered composition and sensory characteristics (Stummer *et al.* 2005). In general, there is a low tolerance of infected grapes in the winery meaning that a high level of disease control is required, often by multiple applications of protective fungicides. Increased market demand for no or low fungicide residues, the threat of pathogen resistance to fungicides (eg. Erickson and Wilcox 1997) and negative, non-target impacts of sulfur (summarised by Scott *et al.* 2007) provide incentives to minimise fungicide inputs. A sound knowledge of disease epidemiology, in relation to vine development and environmental conditions, is required in order to time fungicides strategically.

There are two separate but closely related epidemics of powdery mildew occurring on grapevines: one occurs on leaves and the other on bunches. The frequency and spatial distribution of diseased leaves at flowering has been related to the severity and distribution of powdery mildew on bunches at bunch closure (Calonnec *et al.* 2006). *E. necator* can colonise green floral pedicels and caps (calyptrae) and will infect the developing fruit at a high frequency during early fruit set (Ficke *et al.* 2003). The leaf epidemic influences the amount of inoculum available for infection of highly susceptible flowers and immature fruit (Calonnec *et al.* 2008). The status of the leaf epidemic at flowering depends on when primary infection occurred and the proportion of the leaves in the canopy that were highly susceptible to *E. necator* during each infection event.

Both leaves and berries of *Vitis vinifera* express ontogenic resistance to infection by *E. necator*, which means that infection efficiency declines as the plant organ ages (Doster and Schnathorst 1985; Ficke *et al.* 2003). This type of resistance has been reported for many biotrophic fungal pathogens that infect healthy, green tissue of woody, perennial plants. The mechanism of ontogenic resistance in leaves is also unknown. During development, each leaf passes through an initial stage of carbohydrate sink before gradual transition to carbohydrate source (Turgeon and Webb 1973). Along with the associated physiological changes, obvious morphological changes also occur. Relative to young leaves, older leaves are more robust with heavier wall lignification, have lower concentrations of nitrogen, water and other nutrients (Coleman 1986) and generally have an increase in chemical and physical defences to pathogen invasion. The consequence is that the proportion of all leaves on a grapevine that are highly susceptible to infection by *E. necator* varies during the growing season (Calonnec *et al.* 2008).

Plant organs, such as leaves and fruit, can be induced to resist fungal invasion by chemical elicitors that switch on genes involved in defence. Chemical elicitors of induced resistance (Kuć 1982), which are safe to humans and the environment, are potentially a novel means of disease control and an acceptable, 'reduced risk' input. The phenomenon of induced resistance in grapevine has been quantified mainly *in vitro*, either at the cellular level or using detached plant parts. What is not known is the relationship between the level of induced resistance and leaf physiology, with the latter varying significantly as leaves age. A basic understanding of inherent disease susceptibility in relation to leaf physiology is required before superimposing factors, and novel control measures, that induce disease resistance.

Another area which requires a better understanding is the susceptibility of leaves to *E. necator* during canopy development in relation to the timing of crop protectants. Fungicides are often applied at regular intervals, not taking into account temporal variation in the appearance of new, mildew susceptible leaves. In the interval after a fungicide application, rapid leaf emergence can result in new leaves that are unprotected by fungicide residue.

Conversely, application of the subsequent spray might occur when there has been no increase in susceptible leaf area and existing fungicide residues continue to provide an effective dose. In Germany, protective fungicides for downy mildew are timed according to the amount of unprotected leaf area emerged since the last spray, based on models that describe shoot development as a function of thermal time (degree days; Schulz 1992). These models are variety specific and are likely to work best in the region in which they are developed. Such models have not been developed for or tested under growing conditions in Australia.

Project Aims and Performance Targets

Ms Angela Smith commenced her PhD project on March 15, 2005. The major output for this project was completion of the PhD thesis. All experimental work and communication of research progress, specified as outputs in the original application and approved variations, have been completed and a significant proportion of the thesis has been prepared. The earliest, expected date for thesis submission is March 15, 2009.

Originally, it was proposed that the nature of induced disease resistance as a function of leaf age be characterised. However, it is the nature of basic research to discover new information that suggests a particular aspect of the research be explored in greater depth before proceeding to the next step. Indeed, it was discovered that very little was known about the interaction of *E. necator* and the host plant in relation to leaf physiology, and it was deemed necessary to improve our understanding of this topic before commencing work on induced disease resistance, especially for a PhD project. As such, the research objectives were modified to focus on components of leaf physiology that were found to be correlated with susceptibility to powdery mildew, in relation to leaf age. The extent of the work was dictated by the experimental system, which required a high level of technical precision and input to maintain dual cultures of the pathogen with its perennial grapevine host.

Ultimately, the following questions were addressed by scientific methods (hypothesis testing):

1. How does leaf age and position affect disease expression of powdery mildew? When in the infection process of *Erysiphe necator* is the pathogen stopped?
2. Does maximum susceptibility to powdery mildew in grape leaves relate to the carbohydrate sink to source transition in leaves?
3. Can mathematical models of leaf expansion at different nodal positions predict the final leaf area of a given leaf?
4. Do shoots of different ages show similar models of ontogenic resistance? When do basal leaves become resistant?
5. Do shoots arising from three distinct nodal positions on cane pruned vines of Chardonnay or Pinot noir vines show different rates of leaf appearance? Do shoots of different orientation have different rates of leaf appearance?
6. Can a “susceptible leaf area” model be developed using knowledge of leaf susceptiblity to powdery mildew according to leaf ontogeny and leaf area development? Can this model assist timing of fungicides or canopy management for powdery mildew control?

Method

The research questions were addressed by two types of studies: (1) field experiments in commercial vineyards to gather data on rates of leaf emergence and shoot development, and (2) glasshouse experiments where potted vines were inoculated with *E. necator* to study the host-pathogen interaction in relation to leaf physiology.

Shoot development in the vineyard

In 2005-06, six blocks of Chardonnay or Pinot Noir vines were selected at four vineyards in Tasmania. Vines had been cane-pruned and were trained according to vertical shoot positioning (VSP) or by the Scott Henry method (Smart and Robinson 1991). At each site, six vines were selected on a diagonal transect through the block. For each vine, three shoot positions on both sides of the vine trunk were designated distal, medial or proximal to the trunk. Six shoots were sampled per vine, three on either side of the vine trunk, and the shoot orientation, upwards or downwards, was noted. Commencing at bud burst, lamina lengths were measured every 5-21 days on leaves of shoots emerging from basal, medial or distal buds of canes. Measurements continued until shoots were trimmed. A data logger with a thermocouple (electric thermometer) was used to measure temperature within the grapevine canopy and thermal time was calculated as degree days above 10^{o}C. The rate of leaf emergence for each shoot was calculated from linear regressions of plastochron index (Erickson and Michelini 1957) against thermal time. The reference lamina length for calculation of the plastochron index was 30 mm. Excel Macro programs were developed to aid computation of large data sets.

In order to validate models developed in 2005-06, the same procedures were conducted at two sites measured 2006-07: Chardonnay with shoots positioned vertically (VSP) and Pinot Noir on Scott Henry trellis.

Models for leaf area development have also been developed and will be presented in the PhD thesis.

Effect of leaf physiology on development of powdery mildew

The method for the glasshouse experiment is described by Smith *et al.* (2008), which is attached as the file Breaking the Mould - Smith.pdf. In short, Cabernet Sauvignon vines were propagated in 15 cm-diameter pots from cuttings to generate a single vine with one shoot. Up to 20 vines were incubated from bud burst at an average temperature of 18 or 25^{o}C in a glasshouse to create different rates of leaf emergence. When shoots had developed leaves that were 50 days old, the batch of plants was divided in half for application of two treatments. Half the vines were used to inoculate all leaves on a shoot with *E. necator* to quantify the severity of powdery mildew (area of colonisation) as a function of leaf position or age. The remaining plants were used to characterise the status of each leaf (position) for one of three possible states: carbohydrate sink, transitioning from carbohydrate sink to source, or a source leaf for carbohydrate.

A Bayesian approach was used to model the colonisation of grape leaves by the powdery mildew fungus. The first model, called the pathogen growth model, described the colonisation of leaves by the powdery mildew fungus in the absence of any host plant reaction to suppress the fungal invasion. The second model, called the resistance model, described the reaction of the host plant to suppress infection by *E. necator*. The overall model described the area of colonisation of powdery mildew on leaves of *Vitis vinifera* variety Cabernet Sauvignon, as a function of leaf position.

Effect of leaf position on infection by E. necator

An additional ten vines grown at 25°C were used to study infection of *E. necator* on all inoculated leaves on a grapevine shoot. Conidia (spores) of *E. necator* were spread on leaves using an artists' paint brush. After 72 h, the leaves were cut into rectangles of approximately 2 x 4 cm, cleared in 3:1 ethanol:glacial acetic acid for 48 h, softened in lactoglycerol for 24 h and stained with lactoglycerol with 0.1% trypan blue for 48 h. Cleared and stained sections were examined by light microscopy. Forty conidia of *E. necator* per section were examined and each conidium assigned to one of three stages of infection: ungerminated, germinated with a primary germ tube or germinated with secondary hyphae. The latter phase of infection, termed secondary germination, is likely to be correlated positively with penetration and the formation of the fungal haustorium, which is the fungal structure that absorbs nutrients from the invaded plant cell. As such, observation of secondary germination was considered a successful infection event, even though a proportion of secondary hyphae may not have continued to colonise the plant tissue. The percentage of conidia achieving secondary germination was plotted against leaf position and a natural spline curve fitted.

Powdery mildew development in relation to shoot development in the vineyard

The results of the glasshouse experiments and the data for shoot development at each assessment date in the vineyard was used to model the temporal progression of the proportion of leaves on each primary shoot in a grapevine canopy that had the potential to develop powdery mildew.

Results

Shoot development in the vineyard

In both 2005-06 and 2006-07, there was a strong linear relationship between thermal time and plastochron index for all shoots at each of the three positions in both trellis types (for example, Fig. 1).

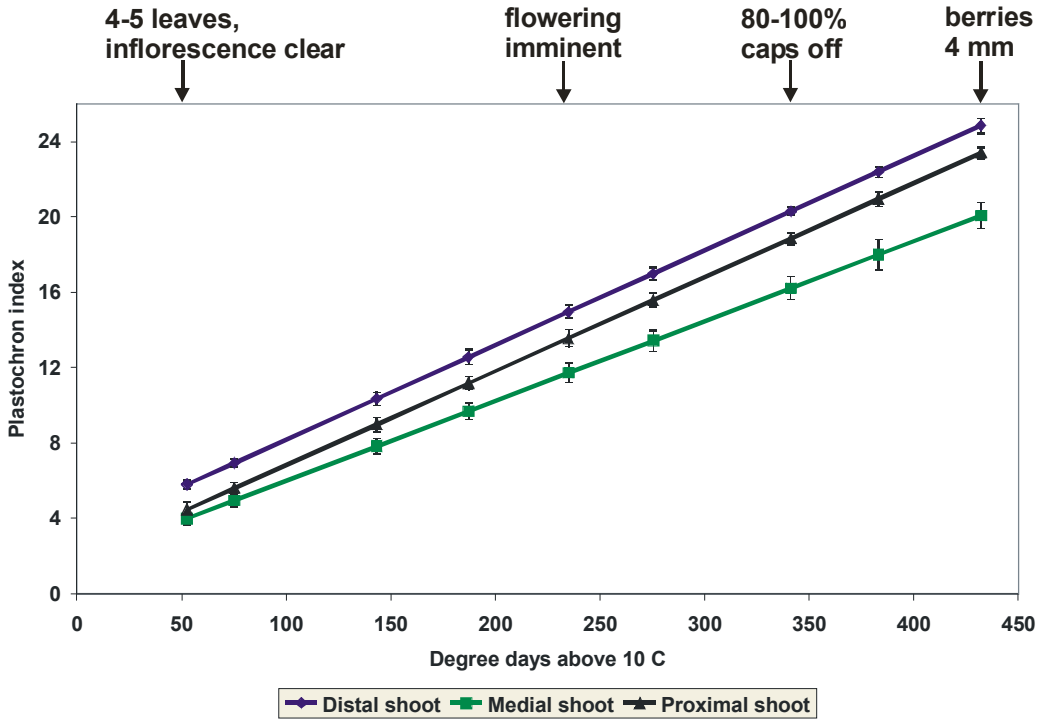


Figure 1 An example of linear models for leaf emergence (plastochron index) for 12 shoots trained vertically at each position on cane-pruned Chardonnay in the Coal River Valley, southern Tasmania, 2005-06.

In Chardonnay, rates of leaf emergence for distal and proximal shoots were not significantly different, but medial shoots had significantly lower rates of leaf emergence (Table 1). In contrast, there was no significant effect of shoot position on the rate of leaf appearance in Pinot Noir, but downward orientated shoots, from the lower arms of the Scott-Henry trellis had a higher rate of leaf emergence than the upwardly-trained shoots (Table 2).

Table 1 Mean plastochron index per degree day for Chardonnay with shoots positioned vertically (VSP). Means with the same letter, within each growing season (table column), are not significantly different at $P = 0.05$.

Shoot position	2005-06	2006-07
Distal	0.050 a	0.050 a
Medial	0.042 b	0.044 b
Proximal	0.049 a	0.049 a

Table 2 Mean plastochron index per degree day for Pinot Noir on Scott Henry trellis for shoots orientated upwards or downwards. Means with the same letter, within each growing season (table column), are not significantly different at $P = 0.05$.

Shoot Orientation	2005-06	2006-07
Upwards	0.037 b	0.032 b
Downwards	0.040 a	0.039 a

Effect of leaf physiology on development of powdery mildew

Under glasshouse conditions, the rate of leaf emergence on shoots of Cabernet Sauvignon varied for each pre-conditioning temperature; on average, 0.45 leaves emerged per day at 25°C, while only 0.18 leaves emerged per day at 18°C. There was, however, no difference between temperature regimes in the mean rate of leaf emergence per unit of thermal time, which was 0.028 leaves emerged per degree (°C) day.

At both preconditioning temperatures, disease expression was more severe on expanding leaves (positions 3-5) than on immature (positions 0-2) or mature leaves (position 6 or higher; Figure 2). Disease severity decreased as leaf maturity increased beyond leaf position 3-4 (Figure 2). The mean modal leaf position for maximum disease severity was estimated to be 3.7 and 4.7 for pre-conditioning temperatures of 18 and 25^{o}C, respectively (Table 3). Disease was expressed on a greater number of leaves on shoots preconditioned at the higher temperature (data not presented). No powdery mildew was observed at leaf positions ≥ 17 on shoots preconditioned at 25^{o}C nor at leaf positions ≥ 11 for shoots preconditioned at 18^{o}C.

The development of the Bayesian model describing the area of colonisation of powdery mildew on leaves of *Vitis vinifera* variety Cabernet Sauvignon, as a function of leaf position, will be presented in the PhD thesis. Figure A5.1 in Appendix 5 illustrates the output of the Bayesian approach to modelling.

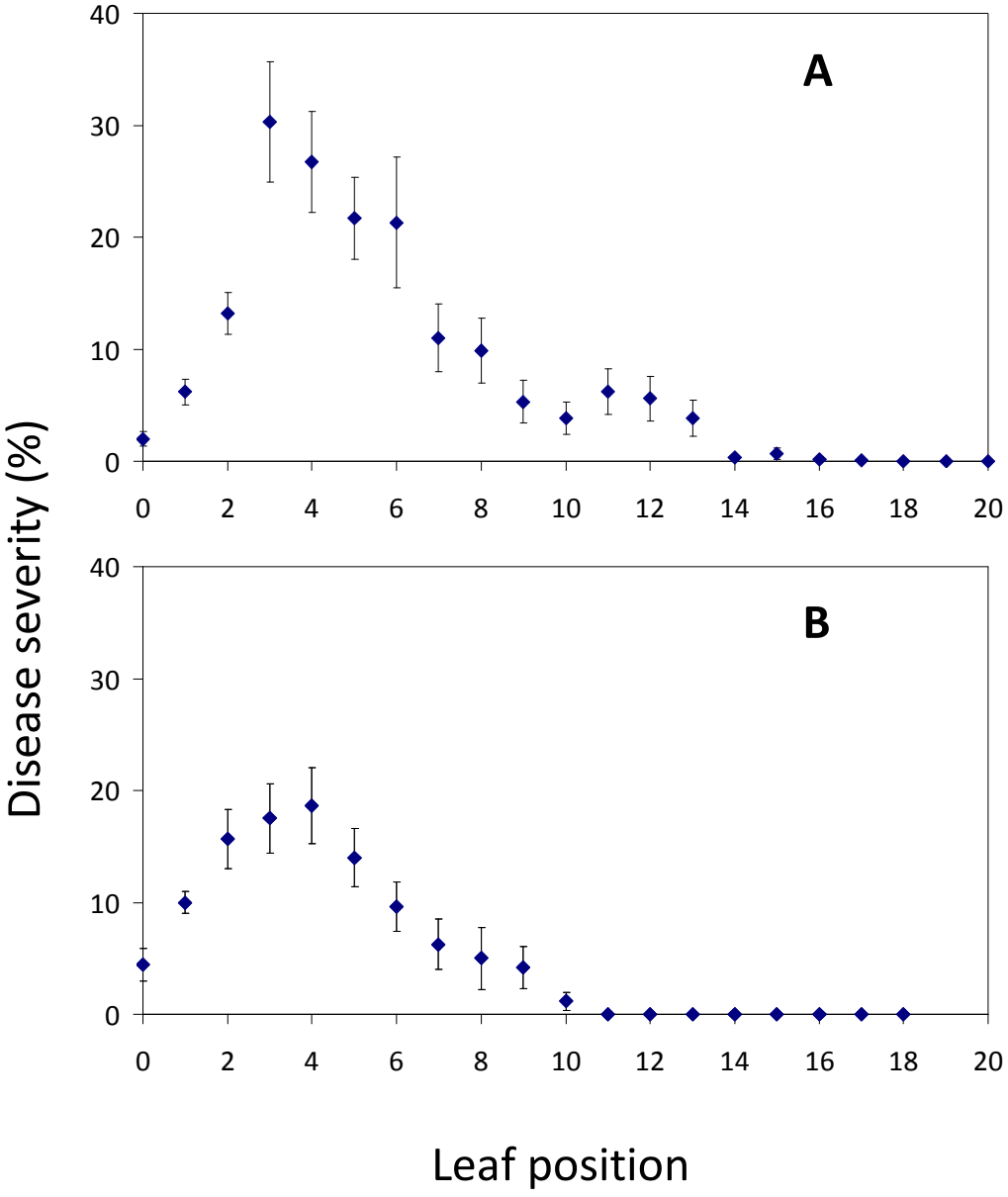


Figure 2 The effect of leaf position on shoots of glasshouse-grown Cabernet Sauvignon vines on mean severity of powdery mildew (± standard error) 14 days after the determination of leaf position and inoculation of the adaxial surface of each leaf with 10^5 *E. necator* conidia per ml. Values for leaf position increase with greater leaf maturity. Plants grown at **(A)** 25°C or **(B)** 18°C prior to inoculation.

Figure 3 illustrates the sink to source transition in a grapevine shoot. The sink to source transition occurred on average at leaf positions 3.8 and 4.7 for pre-conditioning temperatures of 18 and 25°C, respectively (Table 3). There was no difference between the mean modal leaf position at maximum disease expression and the mean leaf position when leaves were in the transition from a sink to a source leaf for either temperature regime (Table 3).

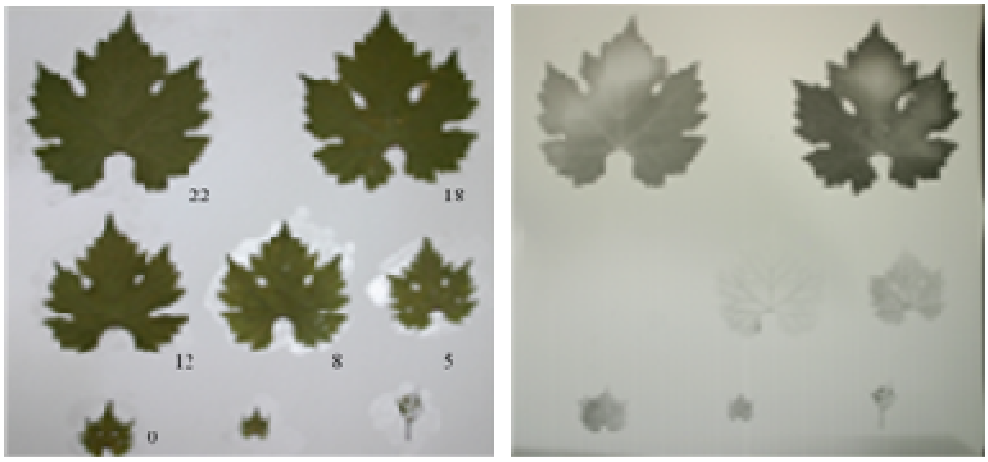


Figure 3 Autoradiograph (right) illustrating leaves aged 8 days and younger importing carbohydrate in a shoot of Cabernet Sauvignon (left). Leaves treated with $^{14}CO_2$ were 18 and 22 days old.

Table 3 The mean and range for leaf position of maximum severity of powdery mildew and of the leaf in the sink to source transition as determined by cessation of import. Data presented for shoots pre-conditioned at two temperature regimes.

	18°C		**25°C**	
	Mean	**Range**	**Mean**	**Range**
Leaf position for maximum disease severity	3.7	2.1–4.8	4.7	3.0–6.6
Leaf position for sink to source leaf	3.8	3.0–5.0	4.7	3.0–7.0

Effect of leaf position on infection by E. necator

Pathogen infection, characterized by the percentage of secondary hyphae, appeared to be higher at leaf position 3 than on immature (positions 0-2) or mature leaves (position 6 or higher; Figure 4). The percentage of secondary hyphae appeared to decline as leaf maturity increased beyond leaf position 3 (Figure 4).

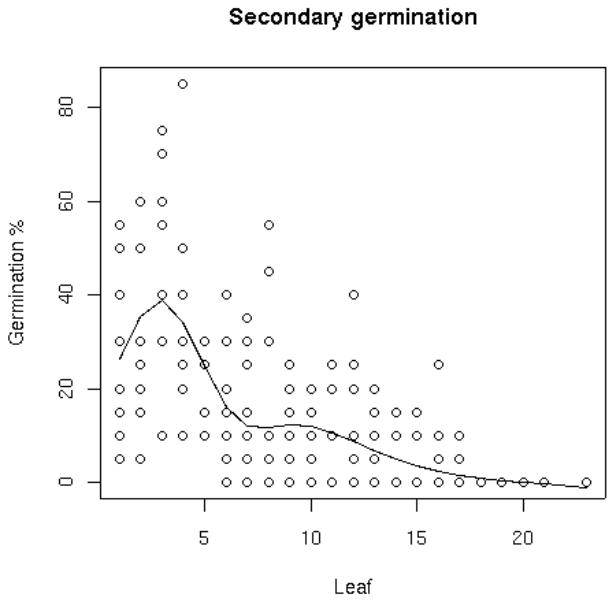


Figure 4 The effect of leaf position on shoots of glasshouse-grown Cabernet Sauvignon vines on percentage of germinated conidia of *E. necator* with secondary hyphae 72 h after inoculation. Values for leaf position increase with greater leaf maturity.

Powdery mildew development in relation to shoot development in the vineyard
Based on the results of the glasshouse experiment, it was assumed that all leaves on a grapevine shoot that were 30–90% expanded were able to support colonization by the powdery mildew fungus. In a preliminary analysis, leaves that were 0–100% expanded on each shoot at each assessment date in the vineyard were identified. An example of the proportion of leaves on primary grapevine shoots that had the potential to develop powdery mildew, as a function of the number of days after budburst, is presented for one vineyard site (Figure 5). This preliminary model indicated that the proportion of leaves on primary grapevine shoots that are susceptible to powdery mildew declines as the growing season progresses. The complete set of models predicting the proportion of leaves on primary grapevine shoots that are susceptible to infection by *E. necator* will be presented in the PhD thesis. Thermal time (degree days) is being used as the explanatory variable to improve prediction of grapevine canopy status, in terms of susceptibility to powdery mildew, across growing seasons.

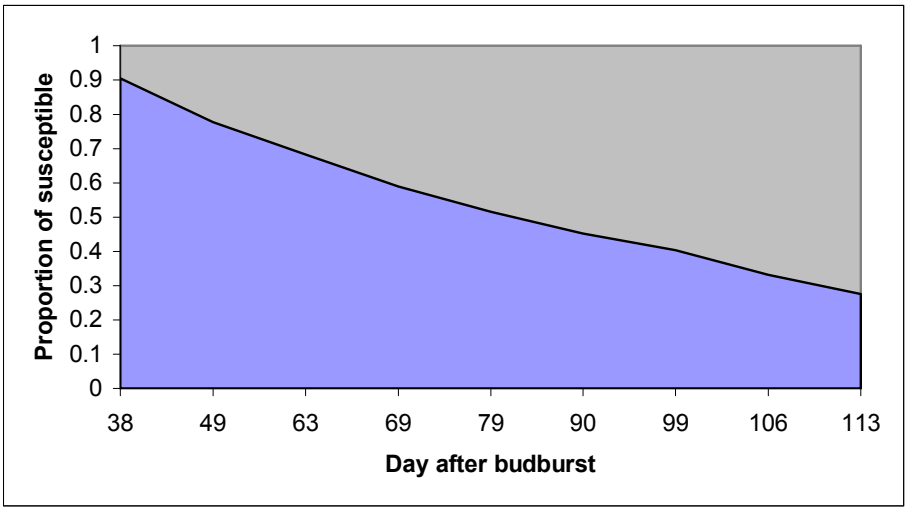


Figure 5 A preliminary model that predicts the proportion of leaves on primary grapevine shoots that are susceptible to powdery mildew, as a function of days after budburst in Chardonnay vines grown in southern Tasmania in 2005-06.

Discussion

Effect of leaf physiology on development of powdery mildew

A grapevine leaf becomes increasingly resistant to colonisation by the powdery mildew fungus as it ages, as reported previously (Doster and Schnathorst 1985, Singh and Munshi 1993). Unlike previous studies, the non-linear change in disease severity for immature leaves was quantified precisely so that the leaf position for maximum disease severity could be identified. This is the first report of the association between leaf position for maximum severity of powdery mildew and the position of the leaf in the sink to source transition, immediately after it had ceased importing carbohydrates. Moreover, this correlation was maintained when the rate of leaf emergence varied, as influenced by ambient temperature. Furthermore, the rate of leaf emergence pre-inoculation affected the incidence and severity of powdery mildew for the entire shoot, with a higher rate of leaf emergence leading to more disease per shoot.

Although the results clearly indicate a peak in leaf susceptibility to disease, the severity of powdery mildew observed on immature and expanding leaves may be influenced by the rate of leaf expansion after inoculation. When compared with the use of detached leaves for inoculation studies, rapid leaf expansion after inoculation may have resulted in an apparently lower severity per area of leaf when compared with leaves with slower or completed expansion. However, Reuveni (1998) reported a similar response curve using a detached grapevine leaf disc assay, where there was no expansion in leaf area between inoculation with the downy mildew pathogen, another biotroph, and assessment of disease severity.

The decline in susceptibility to powdery mildew, with increasing leaf position, beyond the position of maximum disease severity, suggests a concurrent increase in the defence response of leaves. Physical and biochemical defences that might contribute to ontogenic resistance include cell wall and cuticle thickness, the amount of cell wall lignification and the activity of antifungal enzymes. With regard to cuticle thickness, Heintz and Blaich (1989) found a negative linear relationship between cuticle thickness of young grape leaves and the intensity of *E. necator* sporulation on these leaves. Leaves taking a longer time to develop, for example at the 18°C pre-conditioning regime, may have formed a thicker cuticle than leaves at an equivalent position on shoots developing at a higher temperature. Changes in

cuticle thickness and cell wall lignification as leaves mature needs to be quantified as a function of the temperature at which shoots develop.

In the downy mildew-grapevine pathosystem, leaf ontogenic resistance was correlated positively with increases in peroxidase and β-1,3-glucanase activities (Reuveni 1998). Enhanced peroxidase activity is a marker for leaf senescence (Thomas and Stoddart 1980; Takahama *et al.* 1999) and enzymes associated with leaf senescence may also be involved in plant defence mechanisms against pathogens (Lamb and Dixon 1997). Separating biochemical effects relating to leaf senescence and pathogen defence is likely to be a challenging research problem. Nevertheless, the activation of secondary biochemical pathways required for induced defence could be constrained in immature leaves that are a strong sink for carbohydrate. These leaves rely on other plant parts for their carbohydrate supply and concentrations of free sugars are low as they get used up immediately to build the new lamina. The severity of disease observed on immature leaves may be the result of a number of factors including rapid expansion of leaf area, a lack of secondary metabolism for defence and possibly a pathogen that is less able to compete with the sink strength of the host (Cole 1966).

Coleman (1986) suggested that leaves that are making the transition from sink to source are more susceptible to abiotic or biotic stress. This study provides evidence that just after leaves have lost the ability to import carbohydrate the leaf is most susceptible to infection by the powdery mildew fungus. At this stage of physiological development, export begins as import ceases (Turgeon and Webb 1973) and there are many structural and metabolic changes associated with accumulation and export of photosynthate in the transition to a source organ (Dickson and Larson 1981). When leaves are expanding and changing from sink to source they have two sources of photosynthate, *in situ* and imported. An ideal ecological niche for infection by *E. necator* might be created at this time, considering that the powdery mildew fungi are classed as high-sugar pathogens (Horsfall and Dimond 1957). The powdery mildew fungi co-evolved intimately with their host plants and appear to have found a mechanism to acquire available photosynthate for obligate parasitism. Additionally, leaves in the sink-source transition are not yet physiologically mature, resulting in weak defence response to pathogen attack if the leaf is allocating more resources into growth, rather than defence. Once the mildew pathogen has established its feeding structures called haustoria, then the fungus itself becomes a strong sink for carbohydrate and redirects the carbohydrate metabolism of the plant (Brem *et al.* 1986).

Powdery mildew development in relation to shoot development in the vineyard

Modelling of shoot development in the vineyard for Chardonnay and Pinot Noir vines confirmed that thermal time can be used to reliably predict variety-specific rates of leaf emergence. Differences in the rate of leaf emergence among shoots of different position or orientation suggest a more complex model may be needed to describe leaf emergence for the entire grapevine canopy. The models also need to be validated further at other cool climate sites, and also in warm climates for Chardonnay. Nevertheless, the models can be applied immediately in the region in which they have been developed (southern Tasmania), where they can be used to make predictions about the number of leaves that have emerged since the last fungicide application, and hence the number of leaves, as a proportion of the entire primary shoot, that are unprotected by fungicide. Models of leaf area development since the last fungicide application will allow the use of thermal time to aid decisions about the interval between fungicide applications that protect leaves from infection by *E. necator*. Sulfur is known to persist on leaves for more than 50 days in the absence of significant rainfall (Emmett *et al.* 2002) and it is likely that applications of sulfur are being made too frequently at many sites. Therefore, a second application of sulfur, for protecting grapevine leaves, should only be needed when a significant area of new leaves has developed.

Powdery mildew has often been classed as a 'compound interest' or 'polycyclic' disease (Vanderplanck, 1963), whereby severe disease develops after multiple cycles of infection, with an infection rate that is proportional to the amount of available plant tissue. The development of ontogenic resistance and the dynamics of shoot growth, however, alter the proportion of susceptible plant tissue available for infection (eg. Figure 5) and the nature of the disease progression. Calonnec *et al.* (2008) simulated the growth of grapevines and used environmental and pathogen input variables to model powdery mildew dynamics in relation to the timing of the first sporulation event. The model of leaf ontogenic resistance used by Calonnec *et al.* (2008) was based on an 'educated guess' about the mathematical relationship, rather than the quantitative model developed here. These authors also assumed that the model for ontogenic resistance was constant with variation in shoot age and/or the temperature at which leaves developed. Nevertheless, the modelling identified hypotheses for further testing. In particular, the percentage of infected leaves during the first sporulation event of a powdery mildew epidemic was correlated positively with the percentage of susceptible leaves during this period. This conclusion is consistent with the results reported here, although our findings suggest that the estimation of the percentage of susceptible leaves can be improved, given knowledge of the temperature at which leaves develop.

Calonnec *et al.* (2008) also identified that the number of leaves infected was correlated negatively with the length of the shoot when the first sporulation event occurred. The simulations showed that the severity of the leaf epidemic at the time when grape bunches are most susceptible to infection by *E. necator* was conditional on (1) the time of the first sporulation event and (2) the number and location of susceptible leaves during this period. The distance between the first colonies of powdery mildew on leaves and the grape bunch appears to influence the severity of mildew on grape bunches (Calonnec *et al.* 2006). In short, the timing and location of the first generation of *E. necator* on leaves determines the severity of powdery mildew on grape bunches. The work of Calonnec *et al.* (2006, 2008) and the results reported here demonstrate the potential to use canopy status (leaf development), among other factors, as an indicator of disease risk. Further research is required to model the temporal progression of the proportion of leaves in a grapevine canopy that are susceptible to powdery mildew, accounting for variation in the temperature at which leaves develop. Any differences among varieties of *V. vinifera* for the development of ontogenic resistance should also be quantified.

Outcome/Conclusion

This study has clearly demonstrated the effect ontogeny of leaves has on the ability of the powdery mildew fungus to express disease in *V. vinifera* variety Cabernet Sauvignon. New knowledge on leaf ontogenic resistance and the physiological state of leaves as ontogenic resistance develops provides a sound basis to explore whether or not benign chemical elicitors can induce resistance to powdery mildew in grape leaves.

In addition to weather conditions that favour development of powdery mildew, dynamic changes in crop structure for susceptibility to powdery mildew should be considered a key factor in explaining variation in the severity of disease epidemics from one growing season to the next. Past research has focused on how the environment affects the pathogen, not how the environment affects the host's susceptibility to the pathogen. Consequently, grape growers may have greater control over the disease from cultural management than previously thought. The results of this study suggest that growers might be able to manipulate vigour or leaf physiology, through vineyard floor or canopy management, to create more robust leaves and a smaller proportion of the canopy that is susceptible to powdery (or downy mildew) at any given time. As susceptibility of leaves to powdery mildew represents the inoculum load for grape infection (Calonnec *et al.* 2008), small changes in management practices may have profound effects on disease expression on the berries.

Knowledge about the interaction of *E. necator* and its host in relation to leaf physiology was related to new models of shoot development in the vineyard. Development of the research should provide support for making decisions about disease risk and the timing of integrated management (chemical, cultural, biological) according to that risk.

Recommendations

1. Repeat the glasshouse assays reported here for the downy mildew pathogen, *Plasmopara viticola*, to determine if the expression of leaf ontogenic resistance is similar to or different from that observed for the powdery mildew fungus.
2. Determine if the leaves susceptible to powdery mildew, which physiologically are conducting primary rather than secondary metabolism, are capable of inducing resistance to infection by *E. necator* after application of a chemical elicitor. (Can chemical elicitors of induced resistance control powdery mildew?)
3. Quantify leaf cuticle thickness and cell wall lignifications as leaves mature, as a function of the temperature at which leaves develop.
4. Quantify ontogenic resistance in other varieties of *V. vinifera*. (Are there differences among grapevine varieties in the expression of ontogenic resistance?)
5. Apply the shoot growth models in field trials to determine the optimum interval between fungicide applications, using thermal time to predict the number and area of unprotected leaves emerging since the previous fungicide application.
6. Validate the shoot growth models for Chardonnay and Pinot Noir in other cool climate regions, and in warm climate regions for Chardonnay.
7. Generate shoot development models for secondary grapevine shoots (laterals) and model data for primary grapevine shoots further to develop a robust model for the entire grapevine canopy.
8. Conduct further research and variety-specific modelling to aid prediction of the temporal progression of the proportion of leaves in a grapevine canopy susceptible to infection by *E. necator*, accounting for the temperature at which leaves develop.
9. Conduct fundamental research to explain why grapevine leaves are most susceptible to infection by *E. necator* when they are emerging from the carbohydrate sink-to-source transition. This research may reveal novel mechanisms for preventing infection of grape leaves and lead to new disease management options for many biotrophic plant pathogens infecting woody, perennial plants.

Appendix 1: Communication
Conference Proceedings

Evans KJ, Smith AM (2006) Towards integrated and sustainable management of grapevine powdery mildew in Tasmania. A poster for the Proceedings of the Sixth International Cool Climate Symposium for Viticulture & Oenology, Christchurch, New Zealand, 5-10 February, 2006.

Smith, AM, Evans, KJ, (2007) 'Effect of grapevine leaf physiology on development of pathogens that cause powdery and downy mildew', Proceedings of the 13th Australian Wine Industry Technical Conference, Adelaide, South Australia. **AWARD for best pest and disease AND best student poster.**

Smith, AM, Wilson, SJ, Evans, KJ, (2007) 'Models of shoot development for Chardonnay and Pinot noir vines in Tasmania', Proceedings of the 13th Australian Wine Industry Technical Conference, Adelaide, South Australia

Smith, AM, Evans, KJ, Wilson, SJ, (2007) 'Leaf physiology associated with ontogenic resistance to a biotrophic pathogen', Proceedings of the XII International Congress on Molecular Plant Microbe Interactions 2007, Sorrento, Italy

Smith, AM, Wilson, SJ, Evans, KJ, (2007) 'Ontogenic resistance in grapevine leaves to powdery mildew and the sink to source transition', Proceedings of the 16th Biennial Australasian Plant Pathology Society Conference Back to Basics: Managing Plant Disease, Adelaide, South Australia, p 184

Smith, AM, Wilson, SJ, Evans, KJ, (2008) 'The effect of grapevine leaf physiology on development of powdery mildew', *Proceedings of the Australian Society of Viticulture and Oenology Seminar on Grapevine Pests and Disease*, 22-25 July 2008, Mildura, Victoria, pp. 46-49. ISBN 0 9775256 4 3

Other (oral) presentations

Smith, AM (2005) Preliminary PhD Seminar to the Tasmanian Institute of Agricultural Research, University of Tasmania.

Smith, AM (2006) Presentation to Tasmanian grape growers about progress on PhD research at a workshop on bud dissection (convenor: J. Jones nee Healzewood), University of Tasmania.

Smith, AM (2008) The effect of grapevine leaf physiology on development of powdery mildew. Seminar to the School of Agricultural Science, University of Tasmania.

Smith AM, Evans KJ (2007) Presentation of PhD research to a meeting of grape pathologists at SARDI, Loxton, South Australia, during the visit of Dr David Gadoury, Cornell University.

Evans, KJ (2006) Progress on A. Smith's PhD project reported at the Grapevine Powdery Mildew R&D Workshop (GWRDC), University of Adelaide (convenor: Bob Emmett, DPI Vic.).

Evans, KJ (2007) Progress on A. Smith's PhD project reported to grape pathologists at a meeting held in conjunction with the Biennial Conference of the Australasian Plant Pathology Society, Adelaide.

Evans, KJ (2006-2008) Outline of A. Smith's PhD project presented within other presentations to various audiences: (1) 'Reducing pesticide use in viticulture' workshop at

the AWITC in Adelaide, 2007 (2) various grower field days in Tasmania and (3) the Tasmanian Grape and Wine Research Group.

Participation by PhD student Angela Smith in viticulture-related activities

- ASVO Seminar 'Transforming Flowers to Fruit', Mildura, Victoria, July 29, 2005
- ASVO 60th Anniversary Roadshow, Moorilla Winery, Hobart Tasmania, 2005
- Vineyards Association of Tasmania (VAT), Vineyard of the Year, Field Day, Lebrina, Tasmania, May 25, 2005
- VAT/DPIWE/TIAR, Interactive seminar on the Botrytis Checklist, July 5, 2005
- VAT Field Day, Spray application technology, Richmond, Tasmania, November 10, 2005
- TIAR (GWRDC supported) workshop on 'Planning research toward sustainable management of bunch rot in grapes', Hobart, November 2-3, 2005.
- Attended a publishing workshop chaired by Prof. Paul Kriedemann, then Editor of *Australian Journal of Grape and Wine Research*. Prof. Kriedemann also reviewed A. Smith's PhD project
- Interacted with visiting scientists including Prof. Mike Trought from the Marlborough Wine Research Centre, New Zealand, and Dr David Gadoury, Cornell University.
- Active contributor at regular meetings of the TIAR Grape and Wine Research Group
- Tasmanian Pinot Noir forum
- Hobart Wine Show

Appendix 2: Intellectual Property

No intellectual property has been identified.

Appendix 3: References

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Appendix 4: Staff

PhD supervisors

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Dr Kathy Evans	Senior Research Fellow, TIAR, University of Tasmania (UTAS)
Dr Stephen Wilson	Lecturer, School of Agricultural Science, UTAS

Collaborators/intellectual input/project feedback

Mr Adrian Hallam	Grape grower co-operator, Meadowbank Wines
Mr Tony Scherer	Grape grower co-operator, Frogmore Creek vineyard
Mr Matt Barwick	Grape grower co-operator, Clarence House vineyard
Mr Richard Richardson	Grape grower co-operator, Delamere vineyards
Dr Phil Brown	Senior Lecturer, School of Agricultural Science, UTAS
Dr Ross Corkrey	Senior Biometrician, TIAR, UTAS
Dr Paul Kriedemann	Former Editor, Australian Journal of Grape and Wine Research
Dr Ian Dry	CSIRO, Adelaide.
Dr David Gadoury	Senior Researcher, Cornell University
Dr Bob Emmett	Department of Primary Industries, Victoria
Dr Joanna Jones & TIAR Grape and Wine Group	Other UTAS PhD students, post-doctoral researchers, UTAS staff

Appendix 5: Other relevant material
The seminar paper presented to the Australian Society of Viticulture and Oenology, Inc. is attached as the file Breaking the Mould - Smith.pdf

The citation of this paper is:

Smith, AM, Wilson, SJ, Evans, KJ, (2008) 'The effect of grapevine leaf physiology on development of powdery mildew', *Proceedings of the Australian Society of Viticulture and Oenology Seminar on Grapevine Pests and Disease*, 22-25 July 2008, Mildura, Victoria, pp. 46-49. ISBN 0 9775256 4 3

Bayesian models
Figure A5.1 illustrates the shape of the models derived using Bayesian approach.

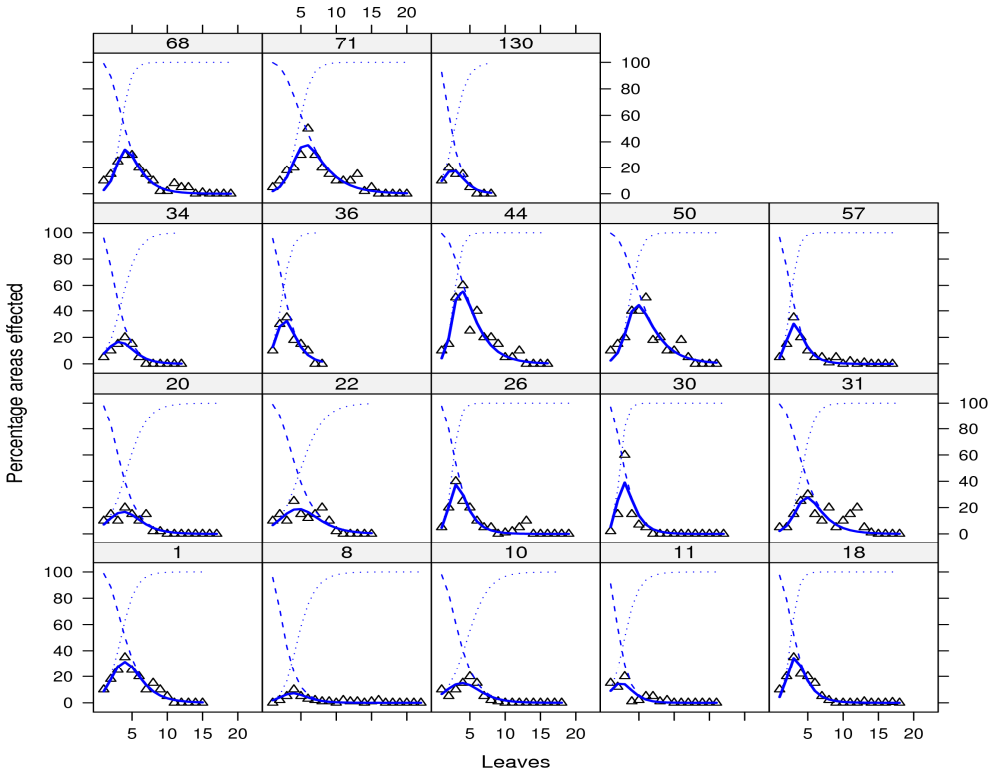


Figure A5.1. The effect of leaf position on shoots of glasshouse-grown Cabernet Sauvignon vines on severity of powdery mildew 14 days after the determination of leaf position and inoculation of the adaxial surface of each leaf with 10^5 *E. necator* conidia per ml. The higher the value for leaf position the older the leaf. Each inset represents a single shoot and the observed data are shown as triangles, the fitted curves are shown for the overall model (–), resistance model (- - - -) and pathogen growth model (····).

Appendix 6: Budget reconciliation
Refer to attached file UT0401_End of Project Financial Statement.doc

The effect of grapevine leaf physiology on development of powdery mildew

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Introduction

Powdery mildew, caused by *Erisyphe necator*, is an important and widespread fungal disease of *Vitis vinifera* grapevines. The pathogen attacks leaves as well as fruit and can result in serious losses in grape yield and quality in most years if uncontrolled. Wine made from Chardonnay grapes with as little as 1–5% of bunches affected by powdery mildew can have altered composition and sensory characteristics (Stummer et al. 2005). In general, there is a low tolerance of infected grapes in the winery meaning that a high level of disease control is required, often by multiple applications of protective fungicides. With current economical and environmental pressures, there is increased grower and consumer interest in reducing inputs of synthetic pesticides. The threat of pathogen resistance to fungicides, for example the DMI fungicides (Erickson and Wilcox 1997), is another incentive to minimise their use. In order to time fungicides strategically, a sound knowledge of disease epidemiology, in relation to vine development and environmental conditions, is required.

There are two separate but closely related epidemics of powdery mildew occurring on grapevines: one occurs on leaves and the other on bunches. The frequency and spatial distribution of diseased leaves at flowering has been related to the severity and distribution of powdery mildew on bunches at bunch closure (Calonnec et al. 2006). *E. necator* can colonise green floral pedicels and caps (calyptrae) and will infect the developing fruit at a high frequency during early fruit set (Gadoury et al. 2003). The leaf epidemic influences the amount of inoculum available for infection of highly susceptible flowers and immature fruit (Calonnec et al. 2008). The status of the leaf epidemic at flowering depends on when primary infection occurred and the proportion of the leaves in the canopy that were highly susceptible to *E. necator* during each infection event.

Erysiphe necator is a biotrophic fungus that infects green tissue and obtains its nutrition from living plant cells. Both leaves and berries of *Vitis vinifera* express ontogenic resistance to infection by *E. necator*, which means that infection efficiency declines as the plant organ ages (Doster and Schnathorst 1985; Gadoury et al. 2003). This type of resistance is recognised among many biotrophic pathogens that infect healthy, green tissue of woody, perennial plants. The consequence is that the proportion of all leaves on a grapevine that are highly susceptible to infection by *E. necator* varies during the growing season (Calonnec et al. 2008).

Fungicides are often applied at regular intervals, not taking into account temporal variation in the appearance of new, mildew susceptible leaves. In the interval after a fungicide application, rapid leaf emergence can result in new leaves that are unprotected by fungicide residue. Conversely, application of the subsequent spray might occur when there has been no increase in susceptible leaf area and existing fungicide residues continue to provide an effective dose. In Germany, protective fungicides for downy mildew are timed according to the amount of unprotected leaf area emerged since the last spray, based on models that describe shoot development as a function of thermal time (degree days; Schulz 1992). Smith *et al.* (2007) determined rates of leaf appearance and leaf area development in Tasmania for cane pruned Chardonnay vines trellised by vertical shoot positioning (VSP) and for Pinot Noir vines on Scott Henry trellis. There is potential to use of this information to time fungicides or novel approaches to crop protection, such as chemical elicitors of induced disease resistance (Kuć 1982), when the grapevine canopy is at greatest risk of infection. However, application of shoot development models depends on a better understanding of the susceptibility of individual leaves to *E. necator* as they develop.

Doster and Schnathorst (1985) noted that older grapevine leaves had fewer, smaller powdery mildew colonies when compared with younger leaves, but the relationship between disease expression and leaf maturity was not quantified precisely. The mechanism of ontogenic resistance in leaves is also unknown. During development, each leaf passes through an initial stage of carbohydrate sink before gradual transition to carbohydrate source (Turgeon and Webb 1973). Along with the associated physiological changes, obvious morphological changes also occur. Relative to young leaves, older leaves are more robust with heavier wall lignification, have lower concentrations of nitrogen, water and other nutrients (Coleman 1986) and generally have an increase in chemical and physical defences to pathogen invasion. In a preliminary report, Smith and Evans (2007) described the development of powdery mildew on maturing grape leaves. Expanding leaves that were approximately 50–90% of their full leaf area expressed more disease than younger or older leaves.

The objective of this study was to quantify the rate of development of leaf ontogenic resistance to *E. necator*, in relation to two different rates of leaf emergence. In addition, the hypothesis that maximum expression of powdery mildew occurs when leaves are infected during the photosynthetic sink to source transition was tested.

Materials and methods

Plant material and determination of leaf position

Own rooted grapevines, *Vitis vinifera* L. cv Cabernet Sauvignon, were grown in 15 cm-diameter pots in a glasshouse with a 16 h day length supplemented by 400 W mercury halide lights. Vines were pruned to one bud at the time of planting and grown until there were approximately 20 leaves. Twenty plants were grown at 25°C (±5°C) and 16 plants were grown at a later date at approximately 18°C (±10°C) in the glasshouse. Ambient temperature was monitored from budburst for calculation of daily thermal time above a base temperature of 10°C (Schultz 1992). Shoots were maintained free from powdery mildew using vapours of penconazole (Topas®, Syngenta Crop Protection Pty Limited, Szkolnik 1983). Topas® was removed from the glasshouse 7 days prior to treatment.

Immediately before treatment, leaves were numbered according to position from the apex using an ordinal measure starting at 0 for immature leaves, 1 for the first leaf with a lamina length of at least 30 mm, and then 2, 3, etc, for older leaves. Leaves at leaf position 0–2, 3–5 or ≥6 were referred to as immature, expanding or mature to indicate that leaves in each category had expanded to approximately <50%, 50% to 90% and >90%, respectively, of their mature size.

To determine the rate of leaf appearance for each temperature regime, lamina lengths on all leaves of each shoot were measured every 3–4 days until treatment, for the calculation of the plastochron index (PI, Erickson and Michelini 1957). A reference length of 30 mm was chosen based on earlier results in grapevine (Freeman and Kliewer, 1984; Schultz 1992). The PI denotes the number of leaves on a shoot. The rate of leaf emergence for each shoot was calculated from linear regressions of PI against calendar day or cumulative thermal time.

Treatment 1: Inoculation with *E. necator* and disease assessment

Half of the batch of plants per pre-conditioning temperature for shoot development were used to test if the rate of leaf appearance, before inoculation of all leaves per shoot with *E. necator*, influenced disease development and hence the expression of ontogenic resistance.

Erysiphe necator was collected, as a bulk isolate, from vineyards in southern Tasmania and cultured on detached grape leaves as described by Evans et al. (1996). Twelve-day old cultures were used to prepare a suspension of 10^5 conidia per mL water according to the method of Gadoury et al. (2001). The conidial suspension was applied to the adaxial side of all leaves using a hand held atomizer. Leaves were dried of moisture with fans immediately after inoculation. Plants were then maintained at 25°C to provide optimum conditions for fungal infection and colonisation of leaves (Chellemi and Marois 1992). After 14 days, disease severity per leaf, defined as the percentage of leaf area colonised by *E. necator*, was determined with the aid of a standard area diagram (B. Emmett, Department of Primary Industries, Victoria, pers. comm.). The incidence of powdery mildew was defined as the presence or absence of powdery mildew at each leaf position. To describe ontogenic resistance, mean disease severity or percentage incidence was calculated for each leaf position across shoots and plotted as a function of leaf position for each pre-conditioning temperature.

Treatment 2: $^{14}CO_2$ labeling and autoradiography

The other half of the batch of plants per pre-conditioning temperature for shoot development were used to identify the position of leaves on each shoot undergoing the transition from carbohydrate sink to source.

Two fully expanded leaves on opposite sides of each shoot were enclosed within a polyethylene bag and $^{14}CO_2$ released by the addition of lactic acid to ^{14}C-labeled sodium carbonate. Treatments were done at 08:00 and photosynthesis allowed to continue for 2 h before the polyethylene bag was removed. Twenty four hours later, exposed leaves and leaves apical to the exposed leaves were cut from the shoot and dried for 7 days in a plant press at room temperature. Dried leaves of each shoot were then enclosed within a cassette on X-ray film (Agfa) for 3 weeks prior to film development. The leaf position for the sink/source transition at the cessation of carbohydrate import, as determined by lack of ^{14}C accumulation, was estimated visually from the autoradiographs.

Data analyses

The modal leaf position at maximum disease severity for each shoot was identified by a bootstrap approach (Efron and Tibshirani 1993) that estimates the mode. The mean of modal leaf positions for maximum disease severity was then calculated for each temperature regime. This mean was then compared for similarity with the mean leaf position for the transition from photosynthetic source to sink for each pre-conditioning temperature.

Results

The rate of leaf emergence varied for each pre-conditioning temperature; on average, 0.45 leaves emerged per day at 25°C, while only 0.18 leaves emerged per day at 18°C. There was, however, no difference between temperature regimes in the mean rate of leaf emergence per unit of thermal time, which was 0.028 leaves emerged per degree (°C) day.

At both preconditioning temperatures, disease expression was more severe on expanding leaves (positions 3–5) than on immature (positions 0–2) or mature leaves (position 6 or higher; Figure 1). Disease severity decreased as leaf maturity increased beyond leaf position 3–4 (Figure 1). The mean modal leaf position for maximum disease severity was estimated to be 3.7 and 4.7 for pre-conditioning temperatures of 18 and 25°C, respectively (Table 1).

Overall, disease expression of powdery mildew was more severe on shoots preconditioned at 25°C (Figure 1a) than on shoots preconditioned at 18°C (Figure 1b), with a greater mean leaf area per shoot infected. Disease was expressed on a greater number of leaves on shoots preconditioned at the higher temperature (Figure 2). No powdery mildew was observed at leaf positions ≥17 on shoots preconditioned at 25°C (Figure 2a), nor at leaf positions ≥11 for shoots preconditioned at 18°C (Figure 2b).

The sink to source transition occurred on average at leaf positions 3.8 and 4.7 for pre-conditioning temperatures of 18 and 25°C, respectively (Table 1). There was no difference between the mean modal leaf position at maximum disease expression and the mean leaf position when leaves were in the transition from a sink to a source leaf for either temperature regime (Table 1).

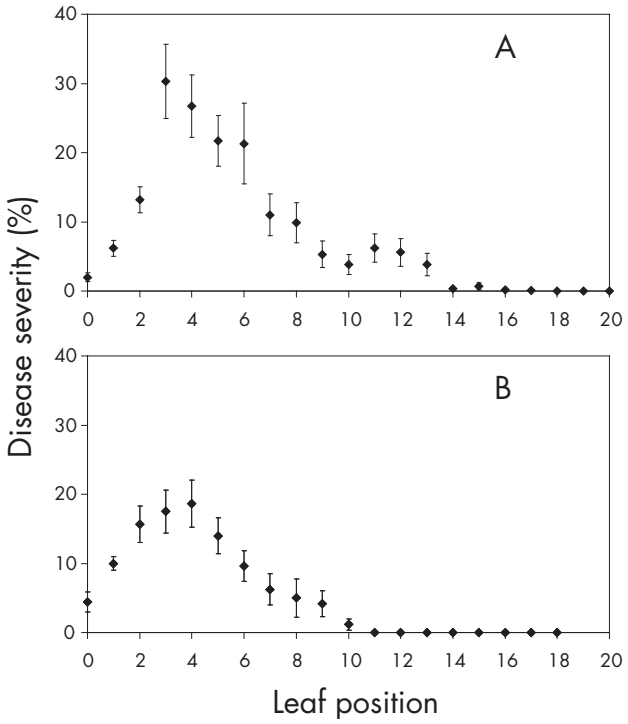


Figure 1. The effect of leaf position on shoots of glasshouse-grown Cabernet Sauvignon vines on mean severity of powdery mildew (± standard error) 14 days after the determination of leaf position and inoculation of the adaxial surface of each leaf with 10^5 *E. necator* conidia per ml. Values for leaf position increase with greater leaf maturity. Plants grown at **(A)** 25°C or **(B)** 18°C prior to inoculation.

Discussion

The results of this study demonstrate that a grapevine leaf becomes increasingly resistant to colonisation by the powdery mildew fungus as it ages, as reported previously (Doster and Schnathorst 1985, Singh and Munshi 1993). Unlike previous studies, the non-linear change in disease severity for immature leaves was quantified precisely so that the leaf position for maximum disease severity could be identified. This is the first report of the association between leaf position for maximum severity of powdery mildew and the position of the leaf in the sink to source transition, immediately after it had ceased importing carbohydrates. Moreover, this correlation was maintained when the rate of leaf emergence varied, as influenced by ambient temperature. Furthermore, the rate of leaf emergence pre-inoculation affected the incidence and severity of powdery mildew for the entire shoot, with a higher rate of leaf emergence leading to more disease per shoot.

Although the results clearly indicate a peak in leaf susceptibility to disease, the severity of powdery mildew observed on immature and expanding leaves may be influenced by the rate of leaf expansion after inoculation. When compared with the use of detached leaves for inoculation studies, rapid leaf expansion after inoculation may have resulted in an apparently lower severity per area of leaf when compared with leaves with slower or completed expansion.

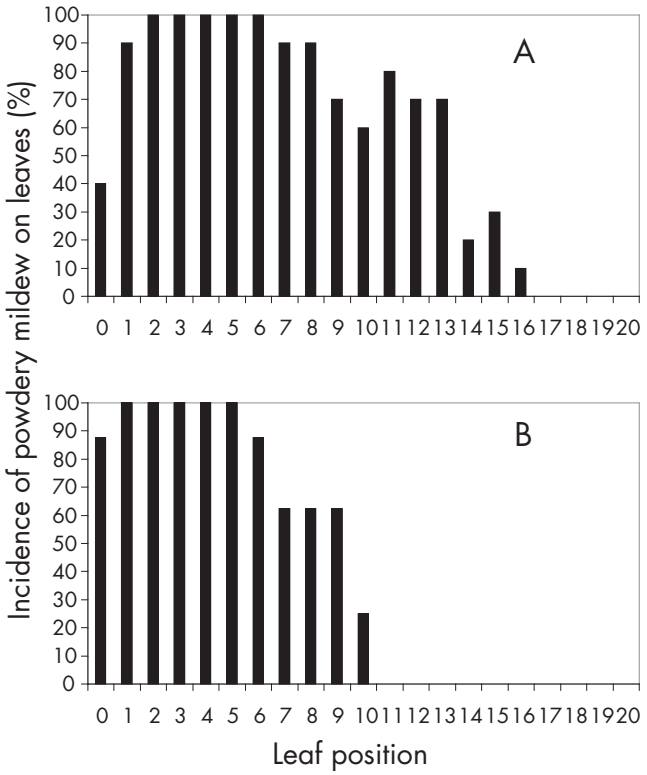


Figure 2. The effect of leaf position on shoots of glasshouse-grown Cabernet Sauvignon vines on mean severity of powdery mildew (± standard error) 14 days after the determination of leaf position and inoculation of the adaxial surface of each leaf with 10^5 *E. necator* conidia per ml. Values for leaf position increase with greater leaf maturity. Plants grown at **(A)** 25°C or **(B)** 18°C prior to inoculation.

Table 1. The mean and range for leaf position of maximum powdery mildew severity and of the leaf in the sink to source transition as determined by cessation of import. Data presented for shoots pre-conditioned at two temperature regimes.

	18°C		25°C	
	Mean	**Range**	**Mean**	**Range**
Leaf position for maximum disease severity	3.7	2.1–4.8	4.7	3.0–6.6
Leaf position for sink to source leaf	3.8	3.0–5.0	4.7	3.0–7.0

However, Reuveni (1998) reported a similar responset curve using a detached grapevine leaf disc assay, where there was no expansion in leaf area between inoculation with the downy mildew pathogen, another biotroph, and assessment of disease severity.

The decline in susceptibility to powdery mildew, with increasing leaf position, beyond the position of maximum disease severity, suggests a concurrent increase in the defence response of leaves. Physical and biochemical defences that might contribute to ontogenic resistance include cell wall and cuticle thickness, the amount of cell wall lignification and the activity of antifungal enzymes. With regard to cuticle thickness, Heintz and Blaich (1989) found a negative linear relationship between cuticle thickness of young grape leaves and the intensity of *E. necator* sporulation on these leaves. Leaves taking a longer time to develop, for example at the 18°C pre-conditioning regime, may have formed a thicker cuticle than leaves at an equivalent position on shoots developing at a higher temperature. Changes in cuticle thickness and cell wall lignification as leaves mature needs to be quantified as a function of the temperature at which shoots develop.

In the downy mildew-grapevine pathosystem, leaf ontogenic resistance was correlated positively with increases in peroxidase and β-1, 3-glucanase activities (Reuveni 1998). Enhanced peroxidase activity is a marker for leaf senescence (Thomas and Stoddart 1980; Takahama et al. 1999) and enzymes associated with leaf senescence may also be involved in plant defence mechanisms against pathogens (Lamb and Dixon 1997). Separating biochemical effects relating to leaf senescence and pathogen defence is likely to be a challenging research problem. Nevertheless, the activation of secondary biochemical pathways required for induced defence could be constrained in immature leaves that are a strong sink for carbohydrate. These leaves rely on other plant parts for their carbohydrate supply and concentrations of free sugars are low as they get used up immediately to build the new lamina. The severity of disease observed on immature leaves may be the result of a number of factors including rapid expansion of leaf area, a lack of secondary metabolism for defence and possibly a pathogen that is less able to compete with the sink strength of the host (Cole 1966).

Coleman (1986) suggested that leaves that are making the transition from sink to source are more susceptible to abiotic or biotic stress. This study provides further evidence that just after leaves have lost the ability to import carbohydrate the leaf is most susceptible to infection by the powdery mildew fungus. At this stage of physiological development, export begins as import ceases (Turgeon and Webb 1973) and there are many structural and metabolic changes associated with accumulation and export of photosynthate in the transition to a source organ (Dickson and Larson 1981). When leaves are expanding and changing from sink to source they have two sources of photosynthate, *in situ* and imported. An ideal ecological niche for infection by *E. necator* might be created at this time, considering that the powdery mildew fungi are classed as high-sugar pathogens (Horsfall and Dimond 1957). The powdery mildew fungi co-evolved intimately with their host plants and appear to have found a mechanism to acquire available photosynthate for obligate parasitism. Additionally, leaves in the sink-source transition are not yet physiologically mature, resulting in weak defence response to pathogen attack if the leaf is allocating more resources into growth, rather than defence. Once the mildew pathogen has established its feeding structures called haustoria, then the fungus itself becomes a strong sink for carbohydrate and redirects the carbohydrate metabolism of the plant (Brem et al. 1986).

This study has clearly demonstrated the effect ontogeny of leaves has on the ability of the powdery mildew fungus to express disease.

Past research has focused on how the environment affects the pathogen, not how the environment affects the host's susceptibility to the pathogen. Consequently grapegrowers may have greater control over the disease from cultural management than previously thought. The results of this study suggest that growers might be able to manipulate vigour to create more robust leaves and a smaller proportion of the canopy susceptible at any given time. As disease susceptibility of leaves represents the inoculum load for grape infection (Calonnec et al. 2008), small changes in management practices may have profound effects on disease expression on the berries.

This work is ongoing to examine which component of the infection and colonisation process by *E. necator* is inhibited as ontogenic resistance develops, including spore germination and hyphal development. A mathematical model describing the development of powdery mildew on maturing grapevine leaves is being developed to gain further insight into the nature and expression of ontogenic resistance in different environments. Ultimately, this knowledge will be related to shoot development in the vineyard towards developing cultural measures or better timing of crop protection for improved management of powdery mildew. .

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