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Effect of Organic, Biodynamic and Conventional Vineyard Management  
Inputs on Grapevine Growth and Susceptibility to Powdery Mildew and  
Botrytis Bunch Rot

By

Benjamin PA Pike

Thesis submitted to the School of Agriculture, Food and Wine  
of The University of Adelaide

in fulfilment of the requirements for the degree of

**Master of Agricultural Science**

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Effect of Organic, Biodynamic and Conventional Vineyard Management Inputs on Grapevine Growth and Susceptibility to Powdery Mildew and Botrytis Bunch Rot.

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## Abstract

Interest and uptake of organic agriculture (including biodynamics) has continued to increase with 37.5 million ha of agricultural land dedicated to these systems worldwide (IFOAM, 2014). Whilst the use of organic inputs and systems in vineyards is becoming increasingly better researched and reported, little reliable research exists for the same in biodynamic viticulture; especially in regard to disease control. It is claimed that using biodynamic inputs can control powdery mildew and Botrytis bunch rot, two of the more economically important diseases in Australian viticulture. This study investigates the efficacy of these inputs, compared with those used in organic and conventional approaches.

To this end three trials were established in 2010 in South Australia; two pot trials at the University of Adelaide Waite Campus, Urrbrae, Australia and the third in a commercial Cabernet Sauvignon vineyard in the McLaren Vale wine region, Australia. At the Waite trial site six treatments (2 organic, 2 biodynamic, 3 conventional and 1 control) were applied to a split plot design. Each treatment was replicated in three blocks, on three vines per treatment, to newly propagated cultivars of Chardonnay and Shiraz vines (*Vitis vinifera* L.). The same cultivars and a similar split plot design were used in a growth room trial in the winter of 2012. Four treatments of recommended herbal extract 'teas' from the biodynamic literature (Yarrow, Nettle, Equisetum and a combination of all three) and two controls (water and synthetic chemicals) replicated four times, were applied. In McLaren Vale an established trial site was utilised. Four treatments (organic, biodynamic, high input conventional and low input conventional) were applied to 20 year old Cabernet Sauvignon vines in a randomised split plot design replicated in four blocks. To assess the effect of compost, each management treatment was also separated to include both a plus and minus compost treatment.

Non-destructive assessment of powdery mildew severity was evaluated over three seasons at the Waite trial and Botrytis severity data via detached bunch assay were collected in the final season. Additionally selected growth data were also recorded; including nutritional status at flowering, harvest and mean bunch and berry weights, cane length and pruning weights to measure the effect on growth. Similar sets

of data were recorded in the McLaren Vale site. Severity of powdery mildew and Botrytis bunch rot data only were collected from the growth room trial.

In the wet and humid conditions of 2010-11, disease severity was high, in both field trials, across most treatments and the results were largely inconclusive. From the remaining two seasons in the field trials, the effects of the inputs on disease severity followed a consistent pattern in most situations. In the potted trial, plant extracts exhibited effective early season control of powdery mildew and reduced severity of Botrytis. In the McLaren Vale site, powdery mildew was found in only one of three years and the study of Botrytis was inconclusive. In both field trials, plant growth parameters suggested that conventionally grown vines were generally larger and more productive than those grown organically or biodynamically. The growth room trial suggested an acceptable level of powdery mildew control in response to a combined plant extract application when compared with conventional inputs.

Encouraging results from this trial would suggest benefits in the use of some BD extracts, but further field testing will be required. This study is the first study to compare biodynamic disease control inputs with the well-established conventional and increasingly accepted organic options. As some sections of the Australian Winegrape industry seek alternative disease control inputs, the biodynamic preparations examined here may be a viable option to augment established practices.

## Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any University or any other tertiary institution and, to the best of my knowledge and belief contains no material previously published or written by another person, except where due reference has been made in the text.

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.....

Benjamin PA Pike

Date



## **Journal of Papers Published as part of this Research**

**B.P.A. PIKE, E.S. SCOTT, C. PENFOLD and C. COLLINS (2014)**

Assessment and comparison of biodynamic inputs in the control of powdery mildew

Presented in Chapter 3

**B.P.A. PIKE, E.S. SCOTT, C. PENFOLD and C. COLLINS (2014)**

Biodynamic and organic vineyard management, a comparison of the control of powdery mildew and effects on vine growth measures in Cabernet Sauvignon

Presented in Chapter 4

*Each of these manuscripts is displayed in the thesis in either published or submitted form according to the instructions to author of the specific journal*

*This Thesis has been prepared according to the University of Adelaide's specifications for "Masters by publication" format*

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## **Conference Proceedings**

### **Pike B.P.A., Scott, E.S., Penfold C. and Collins C.**

The effect of organic, biodynamic and conventional vineyard management inputs on growth and susceptibility of grapevines to powdery mildew. In poster proceedings; 15th Australian Wine Industry Technical Conference, 14-18th July, 2015, Sydney, Australia

### **Pike B.P.A., Scott, E.S., Penfold C. and Collins C.**

The effect of organic, biodynamic and conventional vineyard management inputs on growth and susceptibility of grapevines to powdery mildew. Fresh Research Presenter; 15th Australian Wine Industry Technical Conference, 14-18<sup>th</sup>, July, 2015, Sydney, Australia

### **Pike B.P.A., Scott, E.S., Penfold C. and Collins C.**

Effect of Organic, Biodynamic and Conventional Vineyard Management Inputs on Grapevine Growth and Susceptibility to Botrytis Bunch Rot and Powdery Mildew. Organic Viticulture workshop presenter; 15th Australian Wine Industry Technical Conference, 14-18th July, 2015, Sydney, Australia

### **Pike B.P.A., Scott, E.S., Penfold C. and Collins C.**

Effect of organic, biodynamic and conventional vineyard management inputs on grapevine powdery mildew. In Diaz-Navajas, AM, Ortiz-Barredo, A, Menendez, C, Emmett R, Gadoury D, Gubler D, Kassemeyer H-H, Magarey P, Seem R (eds). Proceedings of the 7th International Workshop on Grapevine Downy and Powdery Mildew. Vitoria-Gasteiz, Spain, 30 June - 4 July 2014 (ISBN 978-84-7821-827-1).

## Abbreviations

AGWA	Australian Grape and Wine Authority
AUD	Australian Dollars
BAA	Biodynamic Agriculture Australia Pty. Ltd.
BD	Biodynamic
BOM	Bureau of Meteorology
CV	Conventional
DA	Department of Agriculture (Australian Quarantine Inspection Service)
DPI:NSW	Department of Primary Industries: New South Wales
HIC	High Input Conventional
IPM	Integrated Pest Management
IFOAM	International Federation of Organic Agriculture Movements
LIC	Low Input Conventional
OG	Organic
PR	Pathogenesis Related
NASAA	National Association of Sustainable Agriculture in Australia
SAR	Systemic Acquired Resistance
SARDI	South Australian Research and Development Institute
US	United States of America

## Chapter 1: Introduction

Powdery mildew and Botrytis bunch rot are ubiquitous fungal diseases, which consistently present challenges to vineyard management. Given favourable environmental conditions, crop loss (Gadoury, et al. 2001) and/or tainted wine are possible (Darriet, et al. 2002, Stummer, et al. 2005, Girbau, et al. 2004). In the temperate zones of south eastern Australia, powdery mildew and Botrytis bunch rot are generally considered to be the most important threats and costs associated with disease control and/or loss of revenue can be considerable (Scholefield and Morison 2010). Consequently in the wine grape industry there is a general reliance on effective canopy management and protectant fungicide inputs (sulfur, copper and synthetic fungicides) to prevent infection in vines (Emmett, et al. 1992). These management processes are well understood and are mostly influenced by cultivar, site selection and appropriate implementation. Adoption of these practices can also be a function of desired grape quality and cost of production. Arguably the use of fungicides may have negative social, environmental (Goldman and Clancy, 1991, Barber et al. 2009) and health impacts (Dich et al. 1997, Colosio, et al. 2003) and some fungal pathogens exhibit fungicide resistance (Tripathi and Schlosser, 1982 Lerroux and Clerjeux 1985, Gubler, et al. 1994, Savocchia, et al. 2004). Detrimental effects of phytotoxicity on beneficial insect populations and plant tissue have been reported (Calvert and Huffaker 1974, Gubler, et al. 1996).

As a consequence, there is an increasing interest in products that are environmentally and economically sustainable and socially acceptable (Dlott, et al. 2006). Organic agriculture is practised in 164 countries, and more than 35 million hectares of agricultural land are managed organically by 1.9 million farmers. The global sales of organic food and drink reached almost \$64 billion (US) in 2012 (IFOAM 2014) .

Organic and biodynamic management systems reportedly offer alternative inputs to conventional practices for disease control. Although sulfur and copper are acceptable inputs in both systems (Department of Agriculture 2013), their use maybe augmented or reduced by the inclusion of one or more of alternatives. These include, but are not restricted to; plant extracts and compost teas as foliar sprays or in the case of biodynamic inputs, specific natural preparations (Department of Agriculture

2010). Furthermore, proponents of both alternative systems claim that organic soil management with composts, mulches and green manures, with no synthetic inputs holds the key to promoting healthy soils which in turn will foster healthier, stronger plants that possess a higher resistance to fungal diseases (Steiner 1924, Balfour 1943, Howard 1940, IFOAM 2014).

However, in the absence of clear scientific evidence scepticism remains amongst many growers as to the potential benefits that the alternative inputs may offer in terms of disease control.

Some organic inputs have been well researched and in some cases are increasingly being adopted by growers; potassium bicarbonate and some milk by-products for example, have been shown to be effective in well managed canopies (Crisp, et al. 2006b, Godfrey, et al. 2010). Research into biological controls and compost teas has been conducted (Madge 2007), as they are seen as a way of introducing beneficial bacteria and fungi that out compete pathogens for space and nutrients or effect subtle changes in leaf surface pH. However, consistency and hygiene of the products seem to be of concern (Yohahem, et al. 1994, Scheuerell and Mahaffe, 2002). Extracts from seaweed, various terrestrial plants and fish emulsions continue to attract attention and some research has been conducted with varying results, as reported by Gladstones (2011).

The specific biodynamic inputs for disease control traditionally include a solution of silicon dioxide, from ground quartz silica and a plant extract of 'Horsetail' (*Equisetum arvense*) (Steiner 1924). These are applied as an aerial or foliar/soil spray respectively. Ideally and controversially, it is recommended they should be applied in conjunction with specific lunar and planetary movements based on the astrological calendar (Steiner 1924). Various biodynamic practitioners also recommend the inclusion of extracts/teas made from various other plants. These include, amongst many, willow, stinging nettle, camomile and oak bark (Proctor and Cole 1997, Klett 2006, Masson 2007). It is suggested that these, used with specific compost preparations for soil health, can improve plant protection against fungal disease; but very little robust research exists.

As the Australian Winegrape industry seeks to improve disease management through improved financial returns, environmental enhancement, and worker and consumer protection, alternatives to sulfur and having to manage for resistance to synthetic fungicides are increasingly being sought. Biodynamic inputs may provide some of these alternatives.

### **1.1 Objectives of the Research**

The research objectives of this thesis were to: i) compare biodynamic inputs with established conventional and organic practices for fungal disease control and severity; ii) examine the effect biodynamic inputs might have on vine productivity compared with conventional and organic inputs.

### **1.2 Linking Statement**

The research in this thesis is presented in chapters, including two research chapters presented as manuscripts prepared for publication, as follows.

- Chapter 1 comprises the introduction to the thesis
- Chapter 2 is a review of the literature. Accumulated and accepted knowledge as to the nature of the pathogens (history and significance, disease epidemiology, cultivar susceptibility) is presented, a comparison of management systems and practices is examined and potential avenues of research suggested.
- Chapter 3 presents the findings related to powdery mildew from a three year potted field trial at the Waite Campus, University of Adelaide, Australia. Two cultivars (Chardonnay and Shiraz) were used to compare foliar management strategies and inputs of conventional, organic and biodynamic systems. A growth room trial was run concurrently to test plant extracts and disease control efficacy.

- In Chapter 4 the results related to powdery from an established, commercial Cabernet Sauvignon vineyard in McLaren Vale wine region, Australia are examined. Systems similar in nature to the research in Chapter 3 were tested.
- Chapter 5 presents Botrytis bunch rot results and discussion from the Waite campus potted trial and the growth room trial. Significance, epidemiology and cultural control of the disease are presented in Chapter 2 - Literature review. General materials and methods are presented in the published material, extra details specific to the inoculation and assessment of Botrytis bunch rot in both trials are presented.

In Chapter 6 a general discussion is offered, general results will be discussed as required around the central themes. Biodynamic inputs and their efficacy in controlling powdery mildew and Botrytis and the effect on vine productivity in the field trial and the findings in the growth room trial will be discussed in conjunction with the results of the McLaren Vale field trial.



## Chapter 2: Literature Review

There is an already prodigious amount of accumulated research and accepted knowledge on the epidemiology of powdery mildew and Botrytis bunch rot and the management thereof. This literature review will therefore focus on a condensed examination of the history and significance of the diseases, epidemiology and cultivar susceptibility. Current accepted management systems and inputs are reported and the terms and practices of conventional, organic and biodynamic viticulture are defined. Inputs and systems utilised in each of the management methods are examined and thus potential avenues of research suggested.

### 2.1 Significance of powdery mildew, Botrytis bunch rot and Organic Agriculture

Grapevines were introduced to Australia in the early years of European settlement. Through a lack of understanding of plant disease and quarantine procedures, exotic pathogens, including *Erysiphe necator* (powdery mildew) and *Plasmopara viticola* (downy mildew), quickly made their way from America to Europe and from there to Australia (Emmett et al. 1992). *Botrytis cinerea* (Botrytis bunch rot) is ubiquitous. In the face of susceptibility of European *Vitis vinifera* cultivars to these fungal pathogens, growers then and now need to protect their crops from infection (Emmett et al. 1992).

Powdery mildew and Botrytis rots present the potential for significant crop loss and reduction in wine quality (Gadoury et al. 2001). Generally, in Australia, a threshold of 3-5% severity in the total harvested crop is considered acceptable (Viti-Notes 2005). Although this is an accepted industry guideline standards may vary from company to company and in response to supply and demand (Wicks, et al. 1997). As such, loss of revenue and profitability to growers can result (Scholefield and Morison 2010). Fungicide applications represent a significant annual cost and are an integral part of most disease management programs. Fungicides with protective and or eradicant capabilities give growers flexible control options. However resistance to some of the synthetic fungicides now exists and makes management programs more complex (Emmett et al. 1992). Savocchia et al. (2004) reported resistance

of *E. necator* to DMI (Demethylation Inhibiting) fungicides in Australia as did Gubler et al. (1994) and Erikson and Wilcox (1997) in the US. Resistance of *B. cinerea* to the MBC (methyl benzimidazole) fungicides was first reported in Germany in 1972, only 4 years after their introduction (Tripathi and Schlosser, 1982). Dicarboximides were released in 1976 and resistance to these occurred in the early 1980s (Leroux and Clerjeux, 1985). Growers are more and more faced with the dilemma of using intense routine fungicide programs or taking the risk of reducing spray applications, in the face of mounting social pressure to find alternative inputs and managing their vineyards to delay development of fungicide resistance.

Wine consumers are increasingly demanding pesticide-free products for two reasons. Consumers believe that chemical free products are healthier and taste better and secondly that these products are better for the environment (Goldman and Clancy, 1991). The health concerns are generally centred on the use of pesticides and the potential for residues in food. In the US, HealthFocus [sic] (1997) conducted a poll and found that 69% of US consumers are “extremely or very concerned” about these residues. To this end, organic and biodynamic growers claim to have adopted alternative practices that can reduce or eliminate the use of synthetic fungicides. Social acceptance of “organic” and demand of products is increasing. In 2001 approximately 16 million ha of land worldwide was certified as organic with the global value of the organic market worth \$20 billion (US) (Willer, 2001). By 2008 35 million hectares of agricultural land were managed organically and global sales reached \$50.9 billion US, doubling in value from \$25 billion US in 2003 (IFOAM 2014).

*Botrytis cinerea* causes grey mould, bunch rot and crop loss. Losses can be substantial in tight bunched varieties especially in areas with rainfall and humidity in the growing season such as the Hunter Valley, New South Wales (Emmett et al. 1992). Losses can also occur in many other Australian wine growing regions such as the Coonawarra, South Australia and Great Western, Victoria. The costs associated with control programs in Australia are summarised in Table 2-1.

**Table 2-1:** Average industry-wide economic impact of Botrytis and other bunch rots by climatic zone.

(From: Schofield and Morison 2010)

Climatic zone	Average industry-wide impact (\$m/annum) a, b		
	Reduced income	Increased costs	Reduced profit
Hot-Dry	7	7	14
Hot-Wet	6	4	10
Warm-Dry	6	5	11
Warm-Wet	2	3	5
Cool	7	4	11
All zones	29	23	52

a Over a period of 15 years in constant 2009 AUD.

b Costs incurred at the winery or Government levels are not included.

Source: EconSearch analysis.

The destructive nature of powdery mildew gives rise to many serious problems in vineyards. If poorly managed, a reduction in vine and crop size, a reduction in winter bud survival, severe defoliation after mechanical harvesting and high pH in the fruit can result (Pool et al. 1984). The most significant effects of powdery mildew in Concord grapes were on berry sugar levels and juice colour and acidity, which on unsprayed vines were above acceptable levels for wine production. Vineyards with high cropping levels were the worst affected (Gadoury et al. 2001). The costs associated with control programs in Australia are summarised in Table 2-2.

**Table 2-2:** Average industry-wide economic impact of powdery mildew by climatic zone.

(From: Schofield and Morison 2010)

Climatic zone	Average industry-wide impact (\$m/annum) a,b		
	Reduced income	Increased costs	Reduced profit
Hot-Dry	6	15	22
Hot-Wet	5	4	9
Warm-Dry	14	8	23
Warm-Wet	5	4	9
Cool	8	6	14
All zones	38	38	76

a Over a period of 15 years in constant 2009 AUD.

b Costs incurred at the winery or Government levels are not included.

Source: EconSearch analysis.

In the winery, grapes with Botrytis and powdery mildew can negatively affect wine quality. Powdery mildew on berries leads to the development of a characteristically intense mushroom-type odour considered a fault in wine grape quality (Darriet, et al. 2002, Stummer, et al. 2005) and wines may be difficult to process (Girbau et al. 2004, Gadoury et al. 2007). An oversupply of Botrytis-infected grapes in the winery can be disastrous for making deep coloured red wines. Botrytis produces oxidising enzymes which destroy the desirable anthocyanins (Ribereau-Gayon, et al. 1980; Steele, et al. 2013). Infected grapes can also be a host to vinegar flies (*Drosophila melanogaster*) and acetic acid bacteria. Secondary rots in bunches produce a bitterness or 'off flavours'. These include rots due to *Aspergillus* spp. or *Penicillium* spp (Hewitt and Jensen, 1974). Botrytis infected white grapes can lose their fruity flavours and may age quickly after fermentation (Nair and Hill, 1992).

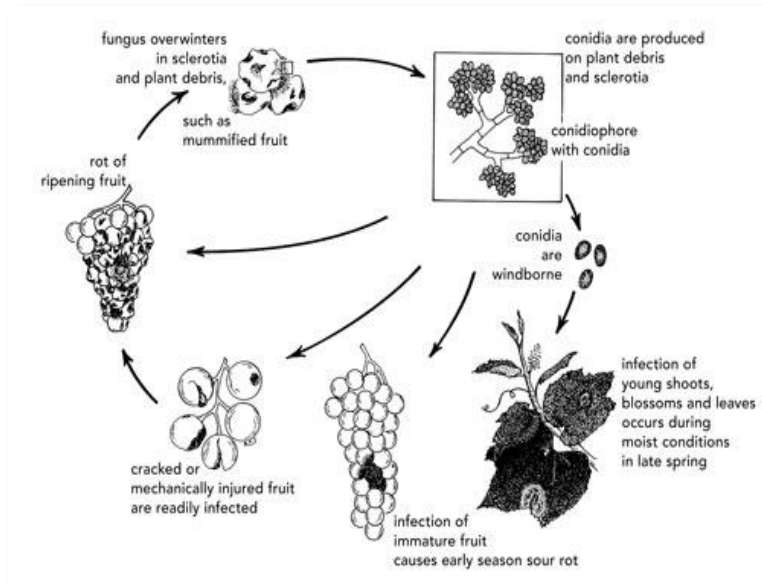
## 2.2 Disease epidemiology

The epidemiology of both diseases is well documented.

### 2.2.1 Botrytis bunch rot

Bulit and Dubos (1988) described bunch rot as being caused by the pathogen *Botrytis cinerea*, the asexual form of the Ascomycete, *Botryotinia fuckeliana*. The asexual spores are clusters of conidia on conidiophores, visible as a grey mass. *B. cinerea* overwinters in canes and vine debris, especially in grape mummies as sclerotia, a compact mass of hardened mycelium (Sall et al. 1981). Conidia can germinate from spring onwards (Pearson and Goheen, 1988). *B. cinerea* is ubiquitous to many fruits and plants and can infect various vine tissues at different stages over the growing season (Keller, 2010). Flowers and berries are infected from flowering to fruit set. Occasionally a latent infection of the berries can occur before fruit set when the stigma and style are colonised by conidia (McLellan and Hewitt, 1973, Viret et al. 2004). In this case, symptoms are not usually visible until after veraison. As berries enlarge and mature, the growth of *B. cinerea* also increases. When conditions are wet, the fungus sporulates and conidia are spread by wind and rain to potential infection sites. Soft brown rot around parts of, or whole shoots, weakens the plant tissues causing wilting and shoot death. This can occur after shoot trimming or on damaged shoots (Emmett et al. 1994).

Inflorescences can rot or dry out and abscise (Nair and Hill, 1992). From veraison onwards whole bunches or portions of them may become infected and show soft brown rot. Berries are infected via the berry epidermis or open wounds. After veraison, *B. cinerea* spreads through each bunch from berry to berry, with the inoculum stemming from infected parts of the bunch (Emmett et al. 1992). As summer and autumn advance, the fungus spreads over the bunch, berries rot and turn brown and skins become slippery; in tight bunched varieties it will spread quickly. If the weather becomes berries will desiccate and usually remain attached to the bunch. If the infection is persistent in the presence of free water, further complications can occur through infection by bacteria, other fungi and vinegar flies (Bulit and Dubos, 1988; Emmett et al. 1992). The disease cycle of Botrytis bunch rot (*Botrytis cinerea*) is illustrated in Figure 2-1.



**Figure 2-1:** Botrytis bunch rot disease cycle (from New York State Agricultural Experiment Station, 2010).

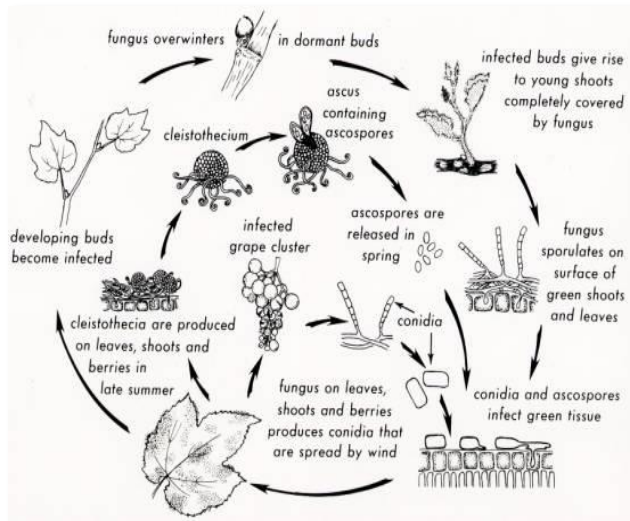
As noted above, sporulation occurs in early spring when conditions are wet. Wicks, et al. (1988) found that in order for leaf and shoot infection to occur with subsequent damage to the vine tissues, 12-24 hours of free moisture is ideal. Continuing presence of free water from flowering to fruit set will also create the ideal environment for the colonisation of aborted and developing berries and flower parts. Infection of developed berries will occur usually when the sugar content has reached 6° Brix or higher (Nair 1985). Essentially temperatures between 1-30°C and relative humidity greater than 90% will favour development (Keller 2010). However, ideal conditions for infection are 15-20°C in the presence of free water (Bulit and Dubos 1988).

Various other factors can lead to conditions that favour the development of Botrytis, especially damage to vine tissue sustained from wind, hail and rain, insects, birds and mechanical means in general (Emmett et al. 1994). Large, vigorous canopies can result in increased humidity around the fruit zone; reduce sunlight and fungicide penetration (Nair and Hill 1992). An excess of water and nitrogen can also lead to weak epidermal cell walls, creating an opportunity for colonisation by hyphae (Mengel and Kirby 2001).

### 2.2.2. Powdery mildew

Powdery mildew is caused by the Ascomycete fungus, *Erysiphe necator*; part of the *Erysiphaceae* family, *E. necator* is an obligate parasite of the genera in the *Vitaceae*, including *Vitis vinifera* and species of *Parthenocissus*, *Cissus* and *Ampelopsis* (Pearson 1988). Appressoria are formed to penetrate the epidermal cells of the host plant only. Asexual spores, colourless conidia, form singularly at the end of conidiophores. Ascospores are the sexually reproductive spores, and are formed inside asci inside chasmothecia which turn yellow and darken as they mature (Pearson and Gadoury 1992).

Powdery mildew was first observed in Australia in the 1860s, and is now endemic (Emmett et al. 1992). The powdery/dusty and whitish/grey appearance of powdery mildew occurs in the presence of mycelia with conidiophores and conidia on the surface of infected plant tissue (Pearson and Gadoury 1992). Leaves can become infected on either surface; leaf susceptibility however declines with age (Doster and Schnathorst 1985). Petioles are susceptible to infection throughout the growing season (Hewitt and Jensen 1974). Infection on green shoots is visible as feathery patches, dark brown to black in colour. Once the canes become dormant the infected tissue appears reddish-brown. New shoots at budburst occasionally appear as “flag shoots” and grow from infected buds from the previous season (Rumbolz and Gubler 2005). These shoots are covered with conidia of *E. necator*, which can be a source of primary inoculum in the growing season (Bulit and Lafon 1978; Hewitt and Jensen 1974; Pearson and Gadoury 1992). Infection of bunches is possible, but declines after veraison (Keller 2010). Infection of bunches around flowering can result in poor fruit set and crop loss. Both leaves and berries however, can express a level of ontogenic resistance as plant tissues age (Doster and Schnathorst 1985; Ficke et al. 2003) this means that there is potential for a decline in new infections as the growing season progresses. Scarring can occur if the berries are infected before full size as the epidermal cells are killed and where subsequent berry growth is restricted, splitting and secondary bunch rots can ensue, further complicating management and negative effects on fruit quality (Emmett et al 1992).



**Figure 2-2:** Powdery mildew disease cycle (from Pearson, 1988).

Powdery mildew overwinters in two forms; as hyphae in the bud scales of dormant buds and as chasmothecia in bark crevices (Built and Lafon 1978) as noted above. Primary infection can occur in one of two ways; (a) after bud burst the shoots resulting from infected buds are covered with mycelium (“flag shoots”), conidia are formed and dispersed by the wind to infect new green tissue (Emmett et al. 1992); (b) ascospores are released from chasmothecia after rain and infect newly emerged leaves when in close proximity to the bark and disperse ascospores. Ascospores can be distributed by free water or wind to infect new green tissue (Pearson and Gadoury 1992). New generations of conidia can be produced every 5-12 days in optimal conditions, resulting in secondary infection (Emmett et al. 1992). The risk of mutation in the pathogen population can give rise to resistant individuals, thereby complicating management strategies, as these individuals begin to demonstrate inherited resistance to selected fungicides (Brent 1995).

Temperature, moisture and light are important environmental factors affecting the spread of powdery mildew. Gadoury and Pearson (1992) found that the release of ascospores is most likely to occur after rain or overhead irrigation events that delivered more than 2.5 mm between budburst and flowering. Germination declines as relative humidity decreases. However, free water can remove conidia from leaves or disrupt mycelium (Delp, 1954). Delp (1954) also reported that infection and disease



development occur in the optimal temperature range of 20-27°C, although fungal growth was possible between 6-32°C and 40-100% relative humidity was found to be sufficient for germination of conidia. Diffuse light favours powdery mildew development (Emmett et al. 1992). Large dense canopies can create such an environment (Pearson 1988).

### **2.3 Susceptibility to diseases**

*V. vinifera* in general is susceptible to many fungal diseases (Galet and Morton 1988). However cultivars vary in susceptibility to Botrytis bunch rot (Bulit and Dubos 1988) and powdery mildew (Emmett et al. 1992). The different physical characteristics of *V. vinifera* cultivars are important when considering varietal susceptibility. Emmett, et al. (1992) point out that all cultivars are susceptible to *B. cinerea*, although those with thin skinned berries and tight bunches such as Riesling, Chardonnay, Pinot noir and Grenache are more susceptible. Pearson and Gadoury (1992), citing Galet (1997), suggest that among *V. vinifera* cultivars considerable variation in susceptibility to *E. necator* exists. Pinot noir, Chardonnay and Colombard are listed as susceptible, whereas Grenache and Shiraz are weakly susceptible. Once *E. necator* inoculum comes into contact with grape vines, direct penetration of the plant surface occur through the epidermis (Agrios 2005). In contrast *B. cinerea* infects senescent or wounded tissue and as such bypasses the protective cuticle layer to infect the intracellular spaces of the vine tissue through the use of an appressorium and infection peg (Nair and Hill 1992). Direct penetration of the epidermal cell wall by *E. necator* is achieved by the generation of penetration pegs from appressoria (Pearson and Gadoury 1992). However most plants, including *V. vinifera* can resist fungal attack via two means, a) physical characteristics of the vine, e.g. thick cuticles and loose bunches as discussed above and b) natural or induced resistance through biochemical reactions (Agrios 2005).

## 2.4 Resistance

### 2.4.1 Natural resistance

Roberts et al. (2002) point out that plants can respond to damage by healing wounds, thus resisting infection, through the deposition of callose, lignin, glycoprotein and phenolics, and the production of chitinases and glucanases described as pathogenesis related (PR) proteins. PR proteins are able to degrade chitins and glucans in fungal cell walls (Selitrennikoff, 2001). Phytoalexins, some of which are known as stilbenes, are also produced in response to pathogen attack or wounding (Kuc 1995), and can inhibit spore germination and mycelium growth (Darvil and Albershien 1984). Hill (1985) describes suberisation as also part of the normal healing process in response to wounding. In forming a corky barrier suberisation protects tissue from fungal enzymes, toxins and physiological protrusions i.e., infection pegs.

In leaves, according to Keller (2010), resistance to *B. cinerea* differs between *V. vinifera* cultivars, for example Cabernet Sauvignon leaves produce twice the amount of stilbenes as Chardonnay which makes Cabernet less susceptible. Elad (1997) suggests that berries in tight bunches rub the cuticular wax off each other leaving them exposed to infection. In *V. vinifera*, leaf susceptibility to *B. cinerea* decreases as the leaves age (Langcake 1981). Goetz, et al. (1999) suggests that this is due to the accumulation of lignin and tannin in the leaves which can inhibit the destructive enzymes produced by *B. cinerea*.

Growth of *B. cinerea* in green grape berries post-infection can be slowed through the rapid accumulation of stilbenes (Hoos and Blaich 1990). However the ability to produce stilbenes declines as the berry ripens (Bais, et al. 2000), which occurs around 6-8°Brix (Nair, 1985). Keller, et al. (2003) also discovered that infection does not always initiate production of stilbenes.

Variation in resistance among *V. vinifera* cultivars to powdery mildew occurs through various chemical and physical processes (Staudt, 1997). Thin young leaves with thin cuticles are more susceptible to powdery mildew than older thick leaves (Doster and Schnathorst 1985a). Flavanols could play an

important role in resisting powdery mildew; these accumulate in the epidermis and cuticle wax when irradiated with UV light and in particular UVB, therefore providing an unfavourable environment for colonisation (Keller, et al. 2003). However, flavonol production is restricted by high N availability in the soil and high N in plants make them more susceptible to powdery mildew through providing lush green growth in the canopy (Bavaresco and Eibach 1987, Keller, et al. 2003).

As berries ripen, resistance to powdery mildew increases, most likely as a response to PR accumulation in the cell walls which can hinder hyphal growth and spore germination (Monteiro et al 2003). Robinson et al. (1997) and Salzman et al. (1998) suggest that chitinases and other anti-fungal proteins increase during ripening, thereby protecting the fruit from fungal attack. However Jacobs et al (1999) found that the preservation of grapevine in response to pathogenic attack does not always elicit the expression of PR genes but that the induction of these genes does not guarantee protection against *E. necator*.

Given the variability in resistance between cultivars to *E. necator*, breeding for resistance is difficult given the variability in resistance between cultivars and usually involves cross breeding with the naturally resistant American species (Pearson 1988). In addition to traditional breeding programs, research is being conducted at the CSIRO into the possibility of inactivating MLO (Mildew resistance locus o) in grapevines to develop resistance to powdery mildew. This involves the identification of the gene resistant to *Erysiphe necator 1* (*Run1*) from a wild American grape species, *Muscadinia rotundifolia*, and transferring it to a European *V. vinifera* cultivar (Dry et al. 2010).

#### **2.4.2 Induced Resistance**

Plants can also develop a general immune response to attack by pathogens and insects and, alternatively, via foliar applications of plant extracts and natural substances (Agrios 2005; Walters, et al. 2005). This is termed "induced resistance" and can lead to a reduction in the amount and incidence of the disease, but not always to complete control (Kuc 1982). According to Reglinski and Walters (2009), induced resistance is divided into two categories: Systemic Acquired Resistance (SAR) and Induced

Systemic Resistance (ISR). Where the plant develops a broad systemic resistance to a pathogen after a localised infection or treatment with an activator, this is defined as SAR. According to Van Loon, et al. (2006), SAR is associated with elevated levels of salicylic acid (SA) throughout the plant; although SA is not regarded as the mediator for the response. ISR on the other hand results generally from colonisation of plant roots by plant growth promoting rhizobacteria or other bacteria (Reglinski and Walters 2009).

Both forms of induced resistance can be the result of the direct activation of the plant's defence genes or by priming; where the plant's genetic defences are not directly activated, but have the potential for enhanced expression in response to a pathogen attack (Benhamou, et al. 2000). It is therefore a complex interaction between wound response or substance application, signal transduction and genetic expression.

Following infection of the vine by a biotrophic pathogen there may be a hypersensitive response (HR) involving an – 'oxidative burst' and the subsequent plant cell death prevents the pathogen from spreading, however this does not always occur (Heath 1998). After this response, signal transduction begins, causing complex changes in genetic expression throughout the plant, including the expression of PR proteins, culminating in the production salicylic acid and phenolics, as defence compounds throughout the unaffected parts of the plant (Taiz and Zeiger 2006). The salicylic acid pathway involved in SAR originates 'upstream' in the shikimic acid pathway (Walters 2009). It is thought that a build-up of salicylic acid around the wound site establishes SAR in other parts of the plant (Taiz and Zeiger 2006).

## 2.5 Management systems

### 2.5.1 Conventional viticulture

The term "conventional agriculture" is commonly used to define agricultural practices that are generally concerned with "Capital-intensive, large-scale, highly mechanized agriculture with monocultures of crops and extensive use of artificial fertilizers, herbicides and pesticides..." (Knorr and Watkins 1984). This approach to agriculture originated from the science and technology developed through both world wars being adapted for agriculture. Draft animals and human labour were steadily replaced with increasingly more efficient and powerful machinery and the development of mineral NPK fertilisers, pesticides and ever more productive varieties of crops made it possible to increase productivity and reduce costs (National Research Council, 1989). As the world's population began to expand rapidly in the 1950's and 1960's these technologies gave rise to the Green Revolution; the aim of which was to meet the challenge of high food production at a low cost (Parayil 2003). In the context of this study this is referred to as High Input Conventional agriculture.

Within only a few years of this approach gaining momentum, conventional agriculture began to raise social concerns. Increasingly it was felt that it could not be sustained into the future and that the true environmental cost of production was not being taken into consideration, especially the changes in soil chemistry in response to the use of synthetic fertilisers and chemical pesticides (Rodale 1973; Francis and Youngberg 1990) and ongoing issues which included soil degradation, habitat destruction and air, water and ground contamination (Ohmart 2011). In response to these perceived issues, it was thought by some that a more integrated and sustainable approach was required. With the development of Integrated Pest Management (IPM) (Stern, et al. 1959) and the publication of *Silent Spring* (Carson 1962) the concepts of environmental sustainability began to gain momentum, culminating in Dlott, et al. (2006) defining viticultural sustainability as using practices that are "environmentally sound, socially equitable and economically feasible". Sustainable viticulture has not yet been codified and its interpretation is subject to each grower's definition, with continued debate on how best to define it. In

simplest terms, sustainable agriculture is the production of food, fibre, or other plant or animal products using farming techniques that protect the environment, public health, human communities, and animal welfare (GRACE 2014). For the purposes of this paper this management approach will be referred to as Low Input Conventional agriculture.

### **2.5.2 Organic Viticulture**

Sustainable viticulture, as outlined above, takes many of its management cues from the concepts encapsulated in the principles of organic agriculture. This began to emerge in response to what was perceived as the destructive nature of intensive conventional agriculture. The current understanding of organic agriculture has its origin in the research of various scientists around the world. In particular, the publications *An Agricultural Testament* (Howard, 1943) and *The Living Soil* (Balfour, 1943) are seen as seminal works from which the modern understanding of organic agriculture stems. It was postulated by these and subsequent researchers, that good agricultural practice begins with soil fertility and the development of humus through the use of compost and that farming systems need to focus on the maintenance of a dynamic, living, balanced, organic, whole organism (Northborne 1940). From this idea many definitions of organic agriculture have been put forward. One example is a “holistic production management system which promotes and enhances agro-ecosystem health, including biodiversity, biological cycles and soil biological activity. It emphasises the use of management practices in preference to the use of off farm inputs, taking into account that regional conditions require locally adapted systems” (IFOAM, 2014).

In Australia the standards for organic and biodynamic agricultural practices in general are governed by the Department of Agriculture, formerly by the Australian Quarantine and Inspection Service, specifically in relation to the export of any products labelled as such. Any organic and/or biodynamic product destined for export must be certified by an approved certifying organisation, verifying that it has been prepared in accordance with the National Standard for Organic and Bio-Dynamic Produce (DA 2013).

To this end, organic growers must use alternative management standards to control diseases and the emphasis is on management rather than substances (Appendix 1). In the case of viticulture, several substances that control fungal pathogens are permitted provided they are registered as certified compliant source (Appendix 2) or that an exemption can be implied (DA 2013). Some of these products have been researched for efficacy; many have not.

### **2.5.3 Biodynamic viticulture**

Controversy surrounds the concepts and theories of biodynamic agriculture. At its heart, biodynamic tenets are very closely aligned with organic principles especially in regard to the judicious use of compost and no artificial inputs. The difference lies in the further supposition that there is an irrevocable and intrinsic relationship between plants and the cosmos which can be influenced by the spiritual connection between human, animals and their connection to the land. In effect, biodynamic means “an agricultural system that introduces specific additional requirements to an organic system. These are based on the application of preparations indicated by Steiner (1924) and subsequent developments for management derived from practical application, experience and research based on these preparations” (DA 2013).

In 1924, a series of eight lectures on agriculture was given by the well-respected, if somewhat controversial, philosopher, Rudolf Steiner at Koberwitz Castle in Silesia, Germany (now in southwest Poland), in which he sought to allay farmers’ fears of what they observed in their farms as declining fertility, seed viability and animal health, and reduced yields (Koepf, et al. 1976). He proposed a holistic/spiritual farming system based on his philosophies, his understanding of Goethean biological science, observations of historical farming practices and nature (Proctor and Cole 1997). Perhaps the most controversial aspect of biodynamic practices is that plant physiology and biological processes in particular are influenced by ‘earthly’ and ‘cosmic’ forces stemming from the earths interaction with the planets, astrological star signs and the moon (Steiner 1924). Steiner emphasised building soil health, a

high diversity of crops, the incorporation of animals and the provision of wildlife habitats, and the use of specific preparations to stimulate the natural process of nutrient and energy cycling but not to add nutrients or fertilisers *per se* (Koepef et al. 1976). To help achieve this he prescribed the use of a set of specific 'composted' preparations (Table 2.3). These were to be produced from particular plant, mineral and animal organ combinations, to be stored or buried over prescribed seasons for carefully defined lengths of time and to be used at precise times for particular functions.

Steiner (1924) and Masson (2007) described the uses of the preparations. Preparation 500 is made from lactating cow manure, ideally of organic or biodynamic origin, and packed into cow horns, buried over winter at the autumn equinox and raised again in the following spring equinox then stored for later use. It is mixed with rain water and stirred ('dynamised') for an hour by hand, or stirring machines, by the reverse vortex technique, where once a vortex is created by stirring in one direction it is stopped then stirred immediately in the other and so on (Mackay 2010). Before application the solution is strained and then sprayed to the soil surface in large droplets after 3 pm and before sunset; up to twice in spring and again in autumn ideally when the moon is opposite Saturn. Amongst other things, 500, allegedly, will improve soil structure, regulate soil pH, stimulate microbial activity, promote deeper rooting of plants and improve nodule development on legumes (Ohmart 2011).

Preparation 501 is used in the opposite way, in that it is sprayed between sunrise and 8 am, usually on mornings after a 500 application. 501 is finely ground quartz silica (silicon dioxide) and, like 500, is packed into cow horns. However 501 is connected to summer and light, thus needing to be buried over summer between the spring and autumnal equinoxes and stored after retrieval. When ready for use it is dynamised in the same way as 500 and sprayed into the air as a fine mist over the vineyard. It is claimed 501 aids in photosynthesis and sugar accumulation, a more upright canopy and carbohydrate production for stronger growth (Mackay 2010). This in turn aids in strengthening plants against attack from fungal diseases and insects. Both preparations should be applied in conjunction with specific planetary and lunar alignments.



**Table 2.3:** Biodynamic preparations for use in agriculture. Adapted from (Steiner, 1924), (Proctor and Cole 1997)

Preparation #	Constituent	Purpose	Application Method	Application
500	Cow horn manure	Improves soil structure	Soil spray	Mid-autumn/mid spring
501	Cow horn silica (Ground Quartz: silicon dioxide)	Strengthens plants	Aerial spray	Spring/Summer-full moon
502	<i>Achillea millifolium</i> (Yarrow)	Provide compost heap structure	Placed in to the compost -Spring	Broadcast - Autumn
503	<i>Matricaria chamomilla</i> (Chamomile)			
504	<i>Urtica dioica</i> (Stinging nettle)			
505	<i>Quercus robur</i> (Oak bark)			
506	<i>Taraxacum officinale</i> (Dandelion)			
507	<i>Valeriana officinalis</i> (Valerian)			
508	<i>Equisetum arvense</i> (Horsetail plant) Silica	Anti-fungal activity	Foliar spray	Spring: 4 consecutive days, early am, before each 501

The preparations 502-507 are made from specific plant tissues and are processed with particular animal organs. When added to the newly made compost pile in spring they are claimed to stimulate biological processes to improve the compost which, once mature, is broadcast into the vineyard in autumn. For example, chamomile (*Matricaria chamomilla*) flowers are packed in the small intestines of the cow and buried in rich soil during the winter. This preparation allegedly retains nitrogen and calcium in the compost heap. It is also claimed to stimulate plant processes that involve potassium, manganese and boron, as well as azotobacteria activity (Steiner 1924, Ohmart 2011). The other preparations play similar but distinctly different roles (Appendix 3).

The use of properly made compost is fundamental to BD agriculture (Masson 2007) and is an important source of nutrients and organic matter. There is a strong focus on sourcing the right materials especially a balanced carbon: nitrogen ratio, size of the compost heap and warmth, blending, moisture and oxygen control and nutrition status (Proctor and Cole 1997, Masson 2007).

Of particular interest is the preparation 508 made from dried horsetail plant (*Equisetum arvense*). Unlike the other preparations, 508 is made as a plant extract in the form of tea which can be used in this form or even left to ferment. Biodynamic literature varies in production methodologies. Regardless, it is used

is as a foliar or soil spray to mitigate fungal disease conditions. In the fermented form it encourages beneficial fungi when sprayed on the soil and as plant extract sprayed on the canopy, is supposedly detrimental to fungal diseases (Mackay 2010). Preparation 508 is used in conjunction with 501, usually being sprayed out on four consecutive mornings prior to an application of 501. Sourcing plant materials can be done ideally by harvesting local populations or purchasing from dried herb wholesalers, provided the plants have at least been grown organically.

## **2.6 Nutrition**

In grapevines and plants in general, there seems to be little disagreement as to the role nutrition plays in disease susceptibility. Agrios (2005) makes it quite clear that “nutrition affects the rate of growth and the state of readiness of plants to defend themselves against pathogenic attack”. Thus the interactions between soil type, depth, structure, moisture, pH and fertility play a complex role in making nutrients available to vines. Likewise the same relations are just as complex when considering the interaction between the vines, their environment and fungal diseases. Cell structure and function are directly affected by nutrition and, as a consequence, vine growth. An undersupply of nutrients can limit plant growth; conversely a luxuriant supply can cause rank growth and even be toxic (Robinson 1992).

In terms of disease management, an oversupply of nitrogen (N) causes high vine vigour and succulent growth, a prolonged vegetative state and may delay fruit maturity (Mullins et al. 1992). A high rate of growth only occurs in the presence of abundant N and optimum growth is achieved when there are balanced rates of photosynthate production and N assimilation, and the presence of other nutrients in adequate amounts (Mengel and Kirby 2001). Alternatively, plants lower in N are generally weaker, slower growing and can reach senescence sooner (Agrios 2005). Vines and fruit on weakened or young tissues are also prone to fungal diseases caused by obligate parasites (Bravesco 1989, Marschner 1995). Edmeades (2000) suggests that timing of application and optimal climatic conditions and availability of soil moisture are important factors. If either is not achieved appropriately, the plants' initial

demands for N may not be met and conversely, excess nitrate N to the plants' needs can accumulate and be lost to leaching. In the management of young vines, Boehm and Coombe (1992) point out that a high level of mineral nutrition, particularly in respect to nitrogen, is essential for the establishment of strong shoot growth. Vines deficient in potassium amongst other things, can have low yields and can show a reduction in carotenoid and anthocyanin pigments (Champagnol 1995). Susceptibility to fungal and bacterial diseases is also increased where potassium is deficient (Bravesco 1989, Marschner 1995). Phosphorus is also essential for plant growth and deficiencies can negatively impact yield. Phosphorus seems to improve the balance of nutrients in the plant, thereby accelerating the growth and maturity of the crop. This provides the plant with an opportunity to escape infection from pathogens that prefer younger tissue (Agrios 2005).

Of particular interest is the role of the micronutrient silicon, in plant defences. Silicon dioxide, generally found in the form of quartz silica is also present in the plant *Equisetum* where it is found in high concentrations. Both are used in the biodynamic preparations 501 and 508, respectively. Silicon has been found to assist in the formation of a physical barrier to pathogens in the epidermal cells of plants which can accumulate it (Marschner 1995). However it also appears that silicon can accumulate around the infection site of fungal pathogens in various plants, where the supply is continuous through foliar applications. This has been demonstrated in grapevines (Bowen, et al. 1992).

The role of vine nutrition in disease resistance or susceptibility is complex and is also affected by an even larger set of variables in management systems and the environment. Regardless of the system chosen to grow vines, a balanced supply of nutrients to ensure vine health appears essential.

## **2.7 Management options**

In any system growers seek the same outcome; a balanced canopy that minimises the predisposing factors for both diseases. These practices aim to increase light interception and air movement, reduce the retention of free water, reduce fruit zone relative humidity and temperature, and allow for better

penetration of fungicides, as it is under these conditions that the diseases are best controlled (Smart, 1985). Varietal susceptibility and vigour, nutrition, disease epidemiology, soil, trellis design and irrigation are important contributing factors when deciding which management tool will best suit an individual situation.

### **2.7.1 Canopy**

Chellemi and Marois (1992) found that powdery mildew can be controlled effectively with basal leaf removal and reduced application of fungicides. Leaf removal around grape clusters in *V. vinifera* reduced botrytis bunch rot in Zinfandel and Chenin Blanc (English, et al. 1989), although the use of fungicides is not mentioned; Volschenk and Hunter (2001) reported similar results. Shoot positioning assists in effective control of botrytis (Volschenk and Hunter 2001), which is supported by the report by Smart (1985) that manipulating shoot positioning and density helped to increase radiation, wind speed and evaporation. The effect of trellis system on disease pressure is well understood. These systems include but are not limited to VSP (Vertical Shoot Positioning), Non-Shoot Positioned (sprawl) and vertically divided canopies such as Smart-Dyson, Scott Henry and Geneva Double Curtain (Smart 1992, Magarey, et al. 1994). Selection of vineyard sites with good sun exposure and air flow is advantageous for disease control (Built and Lafon, 1978, Smart, 1985). Magarey, et al. (1994) noted that row orientation, varietal susceptibility and source of planting material should also be considered.

### **2.7.2 Fungicides**

Fungicides are by far the most widely used form of disease control and give growers excellent consistent results when applied correctly. Sulfur for control of powdery mildew is commonly used in many vineyards and synthetic fungicides are also available (Magarey, et al. 1994). As a multi-site fungicide, sulfur inhibits enzyme production and activity in fungi; it has been in use since 1857 and no

resistance has yet been reported (Magarey, et al. 1997). However the activity of sulfur can be poor at temperatures below 18°C due to poor volatilisation (Wicks, et al. 1997) and in reaction to being exposed to sulfur, vineyard workers' health can be affected (Dich, et al. 1997, Colosio, et al. 2003). While sulfur can be toxic to plants and beneficial insects (Calvert and Huffaker 1974, Gubler, et al. 1996), it remains an allowable input in both organic and biodynamic systems (DA 2013).

Synthetic fungicides can indirectly reduce the problems associated with the use of sulfur, as each has been developed with a specific mode of action to target a specific activity site within the cellular processes of the pathogens to protect crops (Walters 2009). However, resistance to synthetic fungicides can occur and is a potential problem for industry (Savocchia, et al. 2004, Emmett, et al. 1992). Export markets can reject wines if the residual chemicals used for disease control exceed the MRL of the importing country; consequently, fungicide use is tightly regulated and controlled by wineries, grape purchasers and government legislation (Bell and Essling 2010).

### **2.7.3 Fertilisers, compost and mulches**

The use of mineral fertilisers in High Input Viticulture provides readily available plant nutrients. These can be broadcast or applied through fertigation; but are usually added only when testing suggests that a deficiency in one or more essential nutrients exists.

Organic fertilisers, manures and composts generally have lower concentrations of nutrients than mineral fertilisers (Treeby, et al. 2004) and the slower rate of mineralisation of the organic forms of nutrients in the former regulates availability to the vine (Mengel and Kirby 2001). In OG/BD systems even certified organic fertilisers are rarely used in vineyards, as the relatively low nutrient requirements are considered satisfied by natural mineral cycling (Madge 2007, Procter and Cole 1997). Central to this cycle is the inclusion of manures, various composts, mulches and inter row green manuring to build soil structure and health and, indirectly, the vine (Steiner 1924, Howard 1943). These processes are reportedly enhanced by the use of BD preparations included in, and subsequent to compost additions (Steiner

1924). Some of the advantages of organic soil management are well known and integrated into management plans by many growers (Ohmart 2011). The benefits to soil health and structure are well researched and reported (Buckerfield and Webster 2001a, Cass 2002, Buckerfield and Webster 2003, Webster 2007) and with the exception of addition of the preparations, in general terms, manufacturing BD composts differs little from normal compost.

A flourishing population of soil micro and macro organisms is integral to general plant health through the decomposition of general plant waste, the cycling of trace elements and chelating agents (Hoitnick, et al. 1986). Webster (2007) pointed out that compost is a stable and immediate source of nutrients for the crop. It also conditions the soil by adding beneficial bacteria and fungi and essential micronutrients to aid in the development of humus. Hoitnick, et al. (1986) and Weckert (2002) suggest also that the promotion of soil health can potentially evoke in plants a systemic resistance to leaf and root diseases. Buckerfield and Webster (2001a, 2001b, 2003) examined the application of organic matter into the vineyard under vine zone using composts and mulches. They found benefits that included stabilised top soils that reduced erosion by wind and rain, providing a buffer against changes in soil moisture and chemistry.

#### **2.7.4 Foliar options- salts, bacterial, extracts and teas**

Crisp, et al. (2006) investigated the effects of several alternative products for the control of powdery mildew. Their conclusions were that milk, whey and potassium bicarbonate provided comparable control to sulfur and synthetic fungicides. Efficacy however appeared to be related to disease pressure, canopy size and vigour, consistent coverage and the concentration of the product. Biological controls are acceptable inputs in organic systems. Madge (2007) reports that a bio fungicide derived from the soil-borne fungus *Trichoderma harzianum*, has been demonstrated to outcompete *B. cinerea* for nutrients and space (Elad, 2000). *Bacillus*, *Pseudomonas* and *Trichoderma* sp. have been shown to stimulate systemic resistance in plants (Reglinski and Walters 2009). Products known specifically as 'resistance

activators' are being researched and are being considered as possible replacements for, or supplements to, fungicides for conventional and organic viticulture (Reglinski and Walters, 2009). In the US, Schilder, et al. (2002) and Gorbatenko, et al. (1996) reported success in using a chitosan product (the structural element of arthropod exoskeletons and fungal cell walls) to reduce the severity of powdery mildew via induced resistance. Success with some of these products has seen them used routinely in 'organic' and LIC vineyards.

Naturally made products such as fish emulsions and plant extracts (sea weed and terrestrial plants) used as foliar sprays are seen as being integral to developing plant health and disease resistance (Gladstones 2011). Compost teas are claimed to improve disease control by complementing already established practices in a system that incorporates optimal nutrition, sanitation, and disease forecasting and minimised plant stress. Although research continues into the efficacy of compost tea, results have been highly variable (Madge 2007). Modes of action are unclear. Nutrient status, manufacturing processes, variability in source materials and the subsequent effect on the target crop are all factors that need to be considered (Scheuerell and Mahaffee, 2002). Yohahem, et al. (1994) raised concerns that poor quality compost teas, made below Australian standards, have the potential to be contaminated with faecal matter infected with *E. coli* and other enteric pathogens which can persist after application.

In many cases recommended extracts have not been researched thoroughly. Most research focuses on extracts of *Reynoutria sachalinensis* and powdery mildew on cucumbers (Daayf, et al, 1997, Fofana et al. 2002). The studies, which do exist, extend over a wide range of crops and with an equally wide range of inputs being tested against a myriad of pathogens. Research into the control of powdery mildew in grape vines using plant extracts and teas, is scant. Various substances have been tested in other fruiting crops with some success. For example in pepper (*Capsicum annum* L.), powdery mildew was controlled by leaf extracts of papaya (*Carica papaya* L.) (Amadioha 1998). Extracts made with cold water successfully controlled powdery mildew (*Erysiphe cichoracearum* DC) by reducing the growth of the fungi *in vitro* and in reducing the spread on pepper plants. Kim et al. (2004) demonstrated 33 plant extracts that showed disease-control efficacy of more than 90% against at least one of six pathogens

including *Botrytis cinerea* on cucumbers and *Erysiphe graminis* on cereals in glasshouse trials. These studies suggest that control of powdery mildew with plant extracts should be possible and needs to be examined further.

Control of *Botrytis cinerea* in grapevines has been the subject of many more studies. Jacometti et al. (2010) published an extensive review of alternatives to conventional fungicides in vineyards. Wilson, et al. (1997) showed plant extracts from capsicum (*Capsicum annum*) and society garlic (*Tulbaghia violacea*), amongst others, successfully suppressed *B. cinerea*. Likewise Abou-Jawdah, et al. (2004) demonstrated relative success with extracts of emperor's mint (*Micromeria nervosa*) and wild marjoram (*Origanum syiacum*).

### **2.7.5 Biodynamic perspective**

In biodynamic farming, fungal infection is considered to be a result of 'diseased conditions'; the aim therefore is to improve the plant's strength against fungal attack and to relieve it of these environmental stresses (Steiner 1924). This is reportedly achieved by using preparations 501: quartz silica and 508: *Equisetum arvense* at least and ideally in conjunction with all preparations and methodologies prescribed by Steiner (Proctor and Cole, 1997) (Appendix 3). The modes of action were not described scientifically by Steiner. However Epstein (1999) and Bowen, et al. (1992) suggest that the application of silicon in proprietary products may induce a resistance response to fungal pathogens.

Research into the efficacy of silicon or *Equisetum* in the form of biodynamic preparations 501 and 508 is rarely published, although Radulović, et al. (2006) examined the effect of oil of *Equisetum arvense* stems against several microorganisms including the fungi *Aspergillus niger* and *Candida albicans*. A 1:10 dilution was shown to possess strong antimicrobial activity. A recent project in India is one of the few studies where 501 has been examined directly. Trivedi, et al. (2014), trialled various applications of Neem oil (a plant extract of *Azadirachta indica*) and foliar applications of 501 (1g/13 L) at different frequencies; higher concentrations of Neem oil and more frequent applications of 501 significantly



reduced the incidence of viral and leaf spot disease and increased the yield of black-gram (*Vigna mungo*).

Silica (Si) has been implicated in initiating an immune response in grapevines when used in proprietary products. In a study of the novel materials (Schmitt, et al. 2002), Milsana<sup>®</sup>, a formulated extract of the plant *Reynoutria sachalinensis* (high in silica; KHH Bio-Science Inc, USA), and Myco-Sin (diatomaceous earth, sulfuric basalt, silicic acid and *Equisetum* extract) reduced the incidence of powdery mildew and Botrytis bunch rot in grapes. Control was equal to or better than a mixed sulfur and copper spray. Silicon, the main component of preparation 501, could potentially induce SAR. Bowen, et al. (1992) reported that on grapevine leaves sprayed with a silica/water solution, fungal hyphae did not grow from conidia where the droplets of solution had fallen. Many BD practitioners believe that the preparations must only be made from plants specified by Steiner (1924). The contradiction lies in that ideally BD practitioners will use plants endemic to their area. All plants used as traditional preparations have their origins in the Northern Hemisphere, some of which are seen as weeds in Australia. A case in point is *Equisetum arvense* (Horsetail), while native to Europe, its cultivation is highly discouraged elsewhere. In an Australian context it is seen as a noxious weed. Through its high silica content, herbicide penetration into the stems is limited. The extensive root rhizome system limits herbicide effectiveness making it difficult to control, thus out-competing native species for space, water and nutrients (DPI: NSW, 2013). The needles of *Casuarina equisetifolia*, 'She-oak', native to Australia and much of South-east Asia (Orwa, et al. 2009), are also high in silica and is recommended as a suitable alternative to horsetail in Australia (BAA, 2013). However, dried plants of *Equisetum* and all other BD plant requirements are available through dried herb wholesalers.

### **2.7.6 Considerations**

An important consideration to note is that many of these studies report findings based on the oil of the plants being extracted and subsequently diluted as opposed to extracts created as 'tea', where the plant

is simply steeped in water simmered, soaked or fermented. The volatility of the oils is not always reported and the subsequent possibility of wine contamination from any malodourous nature of the extracts has not been investigated. Any phytotoxic effect these extracts as foliar sprays may have on insect populations and vines is only sometimes reported. Furthermore, any effect the lunar and astrological cycles may have is simply unsubstantiated.

## **2.8 Summary**

The life general cycles of both pathogens and their reactions to tested conventional fungicide programs and canopy management systems are understood. However, grape growers in the developed world are facing sustained and ever increasing pressure to use environmentally, economically and socially acceptable fungicide inputs as they attempt to satisfy key markets, limit chemical residues in their products, delay pathogen resistance and maintain worker safety. The efficacy of alternative inputs limits their inclusion in many established spray programs. However there are promising results in the use of milk, whey and potassium bicarbonate in the control of powdery mildew and there is evidence to suggest that plant extracts could be included.

Research continues into the action and efficacy of novel foliar inputs such as compost tea, plant extracts and activators of resistance as additional or stand-alone tools in fungal control. Some comparative studies have been done to date, however the inconsistent efficacy of the products presents some challenges. The efficacy of the biodynamic preparations 501 and 508 has not been studied in depth and very few comparative studies of organic, conventional and biodynamic inputs have been undertaken. While some research does exist on the efficacy and potential of silicon, in several forms, as interest in organic and biodynamic inputs increases, it is apparent that the shortcomings in relation to this research need to be addressed.

It should be possible to include BD products in conventional and organic systems. The use of equisetum and silicon dioxide is particularly intriguing. Their inclusion in a disease management strategy could

provide growers with a low cost option that can be produced locally and reliably, with minimal inputs, and included in conventional and organic systems, while remaining benign to the environment. Sustainable agriculture systems require practitioners to adopt management inputs that are socially acceptable, environmentally sound and financially viable. Growers of all persuasions face increasingly complicated disease management strategies as they balance conflicting demands concerning fungicide use, pathogen resistance, worker and consumer health concerns and the potential negative environmental impacts of chemicals. Natural and induced resistance has the potential to be exploited through the use of exogenous elicitors, such as silicon. In particular BD inputs and the claims concerning their use require investigation. The aim of this project was to lay the foundation for a vigorous scientific comparison of biodynamic, organic and conventional systems in terms of disease management and effects on yield.

## **Chapter 3. Prepared Manuscript: Assessment and comparison of biodynamic inputs in the control of powdery mildew**

Prepared manuscript for Australian Journal of Grape and Wine Research



# Assessment and comparison of biodynamic inputs in the control of powdery mildew

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## Abstract

**Background and Aims:** Little research on biodynamic viticulture, particularly disease management, has been documented. This study compared selected biodynamic inputs with well-established conventional management strategies and organic inputs in terms of control of powdery mildew and the effects on vine growth.

**Methods and Results:** Two experimental trials using Chardonnay and Shiraz vines (*Vitis vinifera* L.) in pots were established at the Waite Campus using split-plot designs. In trial one, two organic, two biodynamic, three conventional and a water control treatment were replicated in three blocks, on three vines per treatment. In trial two, three plant extracts were compared with conventional systemic chemicals and a water control. Powdery mildew severity was assessed. In trial one vegetative and reproductive growth measures were recorded. Powdery mildew was controlled by some biodynamic inputs, with efficacy similar to both organic inputs and conventional treatments. In addition, vines from biodynamic and organic treatments were significantly smaller and less productive than those managed conventionally.

**Conclusion:** While not always as effective as conventional programs, some organic and biodynamic treatments used in this research successfully controlled powdery mildew. Grapevine vegetative growth and reproduction were also affected by management practices.

**Significance of study:** To the best of our knowledge this is the first study to compare the efficacy of biodynamic inputs with conventional and organic inputs. Some biodynamic treatments may have potential for inclusion in disease management.

**Abbreviations:** **CV** conventional; **OG** organic; **BD** biodynamic; **MJT** Mean January Temperature

**Keywords:** *biodynamic management systems, disease management, vine physiology, plant extracts*

## **Introduction**

Powdery mildew has potential to cause significant crop loss and reduction in wine quality (Gadoury et al. 2001). Severity levels above 3-5% (Viti-Notes 2005) as a general industry standard are not acceptable, ‘off’ flavours in wine may occur and wines may be difficult to process (Girbau et al. 2004), as such, loss of revenue and profitability to growers can result (Scholefield and Morison 2010). Management decisions that best control disease are those that consider varietal susceptibility, site selection and environmental pressures (Emmett et al. 1992). Practices that reduce the risk of disease severity include light interception and air movement, reduce the retention of free water, moderate fruit zone relative humidity and temperature, and allow for better penetration of fungicides, are ideal (Pearson 1988, Smart 1992).

Conventional (CV) fungicides are routinely used to control powdery mildew and other fungal diseases (Pearson et al. 1988). However resistance to synthetic fungicides is an increasing problem (Savocchia et al. 2004). Sulfur and synthetic inputs can also have a negative effect on predatory insect populations (Prischmann et al. 2005). Wine consumers are increasingly demanding pesticide-free products, believing such products are healthier, taste better and are better for the environment (Barber et al. 2009). Additionally, concerns are being

raised about the handling of agricultural chemicals and their effect on vineyard worker health and safety (Colosio et al. 2003). These factors complicate management strategies and have led growers to seek alternative inputs.

Certified organic (OG) and biodynamic (BD) growers must adopt practices that emphasise environmental maintenance and enhancement (sustainability), without the use of prohibited synthetic chemicals (AQIS 2010). Sulfur is an allowable input in both OG and BD systems (AQIS 2010) for the control of powdery mildew, however many OG growers also seek alternative inputs to manage diseases.

Crisp et al. (2006) investigated the effects of alternative products for the control of powdery mildew and concluded that several limited severity to a level comparable to sulfur and synthetic fungicides, provided they were used in a well-managed canopy. Plant extracts have generated some interest, for example, an extract from Giant Knot Weed (*Reynoutria sachalinensis*) has been reported to control powdery mildew on grapes (Schilder et al. 2002). Schmitt et al. (2002) found 'Myco-Sin<sup>®</sup>' (diatomaceous earth, sulfuric basalt, silicic acid and *Equisetum* extract) controlled powdery mildew in grapevines. Proctor and Cole (1997) report that BD inputs such as extracts of *Urtica dioica* (stinging nettle), *Equisetum arvense* (horsetail) and *Achillea millifolium* (yarrow) (Masson 2007) are effective in the control of fungal diseases, although these reports of efficacy are anecdotal.

Two BD preparations have been reported to enhance plant "strength" against pathogenic infection; preparation 501 (ground quartz silica-aerial spray) and preparation 508 (plant extract from *Equisetum arvense*-foliar spray, also high in silica) (Proctor and Cole 1997). The modes of action of these preparations were not described scientifically by Steiner (1924); however Bowen et al. (1992) and Epstein (1999) suggested that the application of silica may provide some resistance to fungal pathogens. Little science based evidence exists to support these claims. Rupela et al. (2003) investigated all BD compost preparations and



suggested that the microorganisms in these products may suppress several plant pathogens, including *Rhizoctonia bataicola* (syn *Macrophomina phaseolina*) and *Aspergillus flavus*.

Baravesco (1989) reported that plant nutrient imbalances (both deficiencies and toxicities) can increase the risk of pathogenic attack. Central to BD and OG management systems is the incorporation of compost to the vineyard soil. This practice is generally regarded as essential in the development of a stable and healthy soil structure in which plants develop disease less severely than in poor quality soils (Steiner 1924, Howard 1943). Webster (2003) reported that compost conditions the soil, by adding beneficial bacteria and fungi and essential micronutrients to aid in the development of healthy soils. In BD systems this process is reportedly enhanced by the incorporation of six BD preparations 502-507 (Steiner 1924). These preparations are the products of specific plant and animal organ combinations which, when added to the compost, are thought to promote nutrient cycling and improve soil biology (Koepf et al. 1976, Proctor and Cole 1997). It is also claimed that the addition of preparation 500 (cow horn manures and/or derivations thereof) further enhances this process and improves soil structure, decreases bulk density and encourages an increase in root depth and mycorrhizal colonisation (Steiner 1924, Proctor and Cole 1997, Shepherd et al 2002). In OG/BD systems the supply of nutrients is provided through the natural cycling of a range of organic matter and manures with no addition of synthetic nutrients (Madge 2008).

The aim of this study was to assess and compare BD inputs in the control of powdery mildew in field conditions and a controlled environment. Compare these inputs with OG and conventional (CV) inputs and establish, if possible, scientific evidence for the BD control of fungal diseases.

## **Materials and methods**

### *Experimental trials, plant material and establishment*

Two trials were established at the Waite Campus, University of Adelaide, Urrbrae, South Australia. The Waite Campus is at an elevation of 123 m and, according to Gladstones (1992) has an MJT of 21.5°C, warm summers, moderate winters, a mean annual rainfall of 627 mm *per annum* (winter dominant), low relative humidity, cool afternoon breezes and 1765 sunshine hours. A field trial was established in 2010 and conducted over three growing seasons. The second trial was performed in a controlled temperature room in the winter of 2012. All trials were arranged as randomised split-plot designs.

In both trials Chardonnay was selected as a susceptible cultivar and Shiraz as moderately resistant to powdery mildew (Doster and Schnathorst 1985). For the field trial, 100 canes each of Chardonnay (clone 76) and Shiraz (clone 1654), 30-40 cm long, were provided by Yalumba Nursery, Tanunda, South Australia in May 2010. For the growth room trial, 80 30-40 cm cuttings each of Chardonnay (clone I10V1) and Shiraz (clone BVRC17) were sourced from the Waite Campus vineyards, South Australia.

All cuttings were processed according to the methods described by Nicholas et al. (1992). Cuttings were placed in a heated sand bed in a dark 4°C cool room and once a callous was formed (typically 35-40 days) the cuttings were transferred to pots and subsequently planted out as described below.

**Field trial.** In late September/early October the vines growing in pots were transferred to a greenhouse for 2 weeks to promote consistent growth in a controlled environment, then moved to a shade house to acclimatise. Each cutting received 10 g all-purpose controlled-release plant food (Scott's Osmocote<sup>®</sup>, Australia). Vines were sprayed with 2 g/10 L wettable sulfur to the point of runoff every 14 days. Vines were then moved to the field site in the Birksgate Orchard as late as December 16, 2010 which delayed the beginning of the treatment

cycle. Each vine was transplanted into a commercial potting mix (Jeffries<sup>®</sup> 'Potting-Mix', Australia) in 100-L white woven polyethylene planter bags (Ezi-lift<sup>®</sup>, Australia) with drainage holes. Seventy g of a National Association of Sustainable Agriculture in Australia (NASAA)-approved OG 3:2:2 NPK ratio fertiliser (Neutrog<sup>®</sup> 'Bounce Back'<sup>®</sup>, Australia) were placed below the expected root zone of each plant in anticipation of root growth. For the consistent and rapid establishment of all vines, a foliar application of liquid fertiliser (Amgrow<sup>®</sup> 'Nitrosol'<sup>®</sup>, Australia) was applied. This foliar spray was repeated on January 5, 2011.

**Growth room trial.** Calloused cuttings were potted directly into the growing medium of perlite and vermiculite (50:50), then placed in a growth room with no natural light and watered until fully moistened every 2 days. Once vines reached E-L 9 (2-3 leaves separated; Coombe 1995), they were watered with 150 mL three times per week. Hoagland's solution was prepared without the addition of magnesium chloride, as vermiculite is known to provide enough accessible magnesium to the vines (Baby et al. 2014). Once the plants reached 80% budburst, they were hand-watered with Hoagland's solution at 100 mL per plant three times per week. Nutrient solutions were made as required and the pH was adjusted to 5.6 with sodium hydroxide. The vines were grown and managed as described by Mullins and Rajasekaran (1981) and Baby et al. (2014). The day/night cycle was 16/8 hours, 27°C/22°C, light intensity was 400  $\mu\text{mol photons/m}^2/\text{s}$ , from metal halide lights and relative humidity fluctuated between 73% and 84%.

#### *Experimental design and treatments*

**Field trial.** The randomised split-plot design consisted of seven treatments and the two cultivars. All treatments were replicated in three blocks, each replicate containing three vines. Each of the seven treatments was randomly allocated along the rows of each cultivar. The six rows were aligned North/South, with 3 m row spacing and 1.5 m vine spacing (Appendix 4).

Once planted, each vine received 96 L per week of Adelaide mains water via 2x2 L/h drippers (Atelco<sup>®</sup>, Australia) for 3 weeks. Once established (E-L 9; Coombe 1995) the vines were irrigated twice daily for one hour, 3 days a week, increasing to 4-5 days in hot weather (Table S1). Mid-rows consisted mainly of volunteer growth and were slashed as required. Weeds in vine rows were brush-cut by hand and those in pots were removed by hand at various stages of weed growth and returned to the pots as mulch. Herbicides were not used in any treatment. The trial area was generally free from shading by trees with the exception of the northwest corner which was always clear by mid-morning.

After the uniform fertiliser applications during propagation and establishment, vines in each treatment received fertiliser applications appropriate to the system (Table S2). The OG and CV fertilisers were applied annually in spring. The timing of application of all BD soil preparations (500, barrel compost) was generally in accordance with the instructions from Mackay (2010) (Table S2), and changed yearly in relation to the BD calendar recommendations (Keats 2011, 2012, 2013). All pots were supplemented with NASAA certified OG compost (Jeffries<sup>®</sup> Compost, Australia), 1 kg/pot from fresh deliveries each season, spread evenly over the surface of the potting mix. For the BD treatments, the compost was first amended with all BD compost preparations (502, 503, 504, 505, 506, 507: 1 g each/3 tonnes compost, Appendix 3) and allowed to stand in ambient conditions for 6 weeks and mixed thoroughly for aeration on two occasions before application. The chemicals selected for the CV treatments are industry standards for control of powdery mildew (Bell and Essling 2010, 2011, Essling and Francis 2012). Once established to cordon height, the vines were trained to a dual bi-lateral cordon. Treatments were applied at 14-20 day intervals from 2 weeks after budburst, except in 2010/2011, when treatment did not begin until January (Table S3a, b & c). Copper hydroxide for the prevention of downy mildew was applied to all treatments.

The application of preparation 501 (silicon dioxide solution) in the BD treatments posed a unique challenge for a small trial setting; in normal field situations, 501 is applied as an aerial spray from a vehicle and allowed to drift over the canopy. Accordingly, preparation 501 was applied for two seconds per replicate at full pressure from a 2-L pressure spray bottle (Hills<sup>®</sup>, Australia). Preparation 508 (*Equisetum* extract) was applied as a foliar spray.

To prevent drift from one treatment to the next, all foliar treatments were applied within a moveable plastic tent. Water sensitive papers were attached randomly to the upper and lower surfaces of leaves throughout the canopy to assess spray coverage. These indicated that coverage of foliar treatments was more effective on exposed surfaces, and that adaxial leaf surfaces generally received better coverage than the abaxial. Treatments for disease control were applied using 5-L pressure sprayers (Stanley<sup>®</sup>, USA), except for preparation 501, as noted above, and sulfur, which was applied using a 50-L sprayer with 12 volt diaphragm pump (Selecta<sup>®</sup>, Australia). Each device was used specifically for its treatment category to prevent cross-contamination.

**Growth room trial.** To test the efficacy of herbal extracts, extracts of yarrow (*Achillea millifolium*), nettle (*Urtica dioica*) and equisetum (*Equisetum arvense*) (terminology as per Proctor and Cole 1997) and a combination of all three were prepared using the methods and application rates recommended by Masson (2007) (Table S4). A CV treatment of only synthetic fungicides and a water control were also used. Once 80% of plants had inflorescences visible (~E-L 12; Coombe 1995), the vines were divided into four blocks of six treatments in a split-plot design. Where three Shiraz and three Chardonnay vines within each treatment were separate from each other but in the same one line. Treatments were randomly allocated to each row of vines within each of four blocks. Treatments were applied on August 2, 2012 and fortnightly thereafter until pre-veraison (E-L 34; Coombe 1995) (Appendix 5). Vines were removed from the growth room for treatment to avoid cross-contamination. One

litre hand-held spray bottles were used for each treatment and leaves were sprayed to the point of fine droplets on both surfaces.

#### *Powdery mildew inoculation and assessment*

The field trial was exposed to wind-blown inoculum from nearby vineyards. Vines in the growth room were inoculated by shaking severely diseased Cabernet Sauvignon vines above the test plants. Powdery mildew severity was assessed non-destructively, on five leaves per vine in the field trial and three leaves per vine in the growth room. Leaves were randomly selected from each vine per treatment per block and tagged for observation. Once active colonies were observed, assessment was undertaken at 4-week intervals in the field trial and fortnightly in the growth room. The disease mean severity score on adaxial and abaxial surfaces was assessed independently using a 0-10 scale where 0=no obvious disease; 1=1-10% of leaf area covered by sporulating *E. necator*; 2=11-20%; 3=21-30%; 4=31-40%; 5=41-50%; 6=51-60%; 7=61-70%; 8=71-80%; 9=81-90% and 10=91-100% (Crisp et al. 2006). Given that the industry accepted maximum standard is a 5% level of severity (Viti-notes 2005) a mean severity score under 0.5 would indicate successful control. Control of powdery mildew was considered acceptable when measured at this level. In the absence of bunches this method was extended to leaves. Bunches, when available, were assessed for powdery mildew using a similar scale (Crisp et al. 2006).

#### *Vine measures: field trial*

Each year petiole samples were collected from all treatments in the field trial at E-L 23 (50% caps off, full flowering; Coombe 1995). Approximately 60-80 petioles were selected from the node opposite the basal inflorescence, detached from the leaves and processed by Waite Analytical Services (University of Adelaide, South Australia). The concentration of potassium, phosphorus, iron, manganese, boron, copper, magnesium and sodium was determined using

nitric acid and hydrogen peroxide digestion (Wheal et al. 2011). Total nitrogen and nitrate were determined via the Complete Combustion Gas Chromatography method (Searle 1984).

Vegetative components were measured in the winters of 2012 and 2013, comprising pruning weights, shoot length, count and non-count shoots, mean cane mass and length (Smart 1992). Reproductive components were measured in the 2012-13 growing season only and comprised total fruit mass/vine, mean bunch and berry mass, mean berries per bunch (Smart & Robinson 1991).

### *Statistical analysis*

Analysis of variance (ANOVA) using a split-plot design was used to determine significant differences among treatments for variables measured. This was performed using GenStat (Version 14.2; Lawes Agricultural Trust, UK).

## **Results and Discussion**

### *Field trial disease assessments*

In this trial, with the exception of 2010/11, all treatments were effective for early-season disease control (prior to E-L 35 50% caps off-flowering). This may be due to the new season canopies being less conducive to disease and early treatment coverage being effective and uniform. As an exclusion tent was used, this may have assisted in ensuring uniform coverage. In contrast, Savocchia et al. (2011) found that sulfur provided better early season control on the susceptible cultivar Chardonnay than alternative inputs in dry growing conditions. Savocchia et al. (2011) also found that in a wet humid season neither sulfur nor the alternative inputs provided acceptable control; similar to the results in the humid conditions experienced in 2010-2011 in this experiment.

**Powdery mildew severity 2010/2011 growing season.** Powdery mildew colonies were first observed on leaves on March 3, 2011 (E-L 41). After a February/March period that was wetter and more humid than average (Table S1), the mean severity score of powdery mildew on Chardonnay was 5.6 and on Shiraz 2.3 but did not differ significantly between cultivars ( $P=0.060$ ) nor were there significant differences among treatments (data not presented). Powdery mildew was not controlled effectively by any treatment, which was attributed to the wetter and more humid conditions than average. This was most likely also affected by the delay of the trial set up and subsequent treatment applications.

**Powdery mildew severity 2011/2012 growing season.** Prior to E-L 35, observations indicated that all treatments provided effective control of powdery mildew. Control was probably assisted by the dry climatic conditions in 2011-12 (Table S1), and effective spray coverage of the developing canopies. Sporulating colonies of powdery mildew were first observed on December 8, 2011 (E-L 31) (Table1). As the trial progressed a clear pattern of disease severity distribution began to emerge. Management of powdery mildew relative to the water treated control was generally achieved by treatments that included sulfur or conventional chemicals.

The combined mean severity scores of powdery mildew on both the leaf surfaces (Table 2) of Chardonnay for all treatments exceeded 1; suggesting that no treatment maintained effective disease control beyond E-L 31. At E-L 31 mean severity score in the water treated control was 2.68, at which stage disease in only CV2 and CV3 had the lowest mean severity score compared with the treatments and the control but was not significantly different.

At E-L 35 mean severity score in the Chardonnay water treated control dropped to 2.22 and the least effective treatment was OG2, 1.92. Crisp et al. (2006) reported that in well managed canopies, potassium bicarbonate can control powdery mildew as effectively as



sulfur which is supported in these results. There were no differences amongst the remaining treatments relative to each other and the control. That BD2 resulted in a mean severity score of 1.46 suggested that some level of efficacy might be attributed to the plant extracts utilised in BD treatments when compared with the water treated control.

By the last assessment date (E-L 41) mean severity score in the control had increased to 3.19. Treatments had ceased 4 weeks before in accordance with industry practice. Severity did not differ between treatments, but all treatments, except OG2 and BD1, were significantly lower than the control which possibly indicates some residual effect of the treatments.

There were no significant differences in mean severity scores in the combined scores of Shiraz (Table 2). However, the severity of powdery mildew on OG2 vines was greater than all treatments at E-L 31(1.39), but was not significantly different from the remaining treatments or the control. The mean disease severity score of BD2 was higher at E-L 35 than CV treatments or the control but not the remaining treatments. By E-L 41, disease severity decreased with no significant differences amongst treatments.

However, these results may have been skewed by the differences in severity between leaf surfaces. On both cultivars in 2011-12, disease was consistently less severe on the adaxial than the abaxial leaf surface (Table 1). Conditions on the adaxial surface, such as exposure to sunlight and airflow and better treatment coverage, are typically less conducive for disease (Emmett et al. 1992). On the adaxial surface of Chardonnay, powdery mildew severity remained below 0.5 at E-L 35 in most treatments except the control and OG2 (Table 1). Crisp et al. (2006) suggested that due to the action of milk products and potassium bicarbonate as a contact treatment, unless consistent coverage is achieved, control may not be effective as there is no residual effect. From veraison to harvest, on the abaxial surface, severity increased in OG1, decreased or remained relatively unchanged in the remaining treatments and the water treated control. This would also seem to contradict what is known about ontogenic resistance (Ficke et al. 2002). Despite some significant differences amongst treatments after

E-L 41, disease severity on the abaxial surface was greater than 1.5 at each assessment, suggesting that none of the treatments provided adequate late season control.

On Shiraz, powdery mildew severity on the adaxial surface decreased between E-L 31 and E-L 35, irrespective of treatment (Table 1). However, mean disease severity on the abaxial surface at E-L 35 ranged from 1.1 to 3, with CV2 and 3, OG1 and BD1 performing better than OG2 and BD2. Again OG2 was similar to the water treated control and disease in BD2 (without sulfur) was more severe than the control. There was no evidence that BD preparations were able to provide protection without the aid of sulfur. Like milk, potassium bicarbonate lacks a residual effect and successful control is also a function of coverage (Crisp et al. 2006). With the exception of OG2 (1.29) on the adaxial surface, by E-L 41 there was little powdery mildew on either surface, irrespective of treatment, which is likely to reflect the mature age of the leaves assessed (Evans et al. 2008).

**Powdery mildew 2012/13 growing season.** In the 2012/13 growing season, temperatures and relative humidity were largely consistent with the average; however rainfall was lower (Table S1). Sporulating colonies of powdery mildew were first observed on leaves on December 13, 2012 at approximately E-L 32 (Table 4). Powdery mildew was observed occasionally on bunches early in the season in all treatments; these colonies did not persist. The growing season remained warm and dry, conditions which were not favourable for the development of powdery mildew.

When examining the data for individual leaf surfaces, the pattern of infection and increases in severity in Chardonnay, follow a pattern similar to the year before (Table 3). On the more protected abaxial surface of the leaves, disease was more severe.. However there were a few clear trends. Generally BD1 performed better than both the OG and BD2 treatments and the control. Of particular interest was that BD1, which included sulfur, generally performed as well as the conventional treatments that also contained sulfur and

conventional fungicides. BD2 was no better than the water treated control so there was no evidence the herbal extract of Equisetum and the solution of silicon dioxide played a role in controlling disease. Shiraz remained largely free of powdery mildew throughout the season; even on the abaxial surface, mean severity score remained below 0.5 in all treatments up to and including E-L 35. In both cultivars it is likely that the dry conditions and possibly the smaller canopies in the OG/BD treatments contributed to minimal disease development

In Chardonnay the combined means (Table 4) showed a level of control in the early season clearly in favour of the conventional treatments CV and BD1 (with sulfur), where severity was below 1% OG2 continued to perform poorly throughout the season. There were no significant differences among the remaining treatments throughout the whole of the season, even relative to the water treated control; however, it is possible to infer that the smaller, more exposed canopies of the BD treatments relative to the control may have assisted in regulating disease severity. Additionally it could be argued that the drier than average growing conditions of 2012/2013 would also affect the results. The dry conditions coupled with the moderately susceptible nature of Shiraz almost certainly had an effect on the results for this cultivar as powdery mildew was minimal regardless of treatment.

#### *Growth room trial disease assessments*

**Powdery mildew severity on leaves.** In contrast to the field trial, disease in the growth room developed more quickly on the adaxial than abaxial leaf surface. In considering the surfaces separately, this may be explained by inoculation with conidia from above in a still, humid environment. As data from throughout the trial did not reveal any significant differences amongst treatments on Shiraz, the results for this cultivar are therefore not discussed in detail.

Sporulating colonies of powdery mildew were first observed on leaves at E-L 12 on both Chardonnay and Shiraz. At E-L 23, disease severity was below 6% on both surfaces in

all treatments except the control and yarrow (Table 5). On the abaxial surface, there was a significant difference between treatments in Chardonnay at E-L 35 (Table 5). The mean severity score in was significantly lower in the CV compared with yarrow and nettle, but not the combined or Equisetum treatments, but no significant difference between the individual extracts and the control. By E-L 38, severity on the abaxial surface of Chardonnay was similar to that on the adaxial, where the CV was significantly lower than the individual extracts and the control.

At all the assessment points of the trial, the combined plant extracts generally controlled powdery mildew on leaves as well as the conventional fungicide. In the high humidity environment (73-84%) of the growth room disease severity in the conventional treatments was consistently lower than that observed on plants treated with the plant extracts. From E-L 12, severity steadily increased in all treatments except CV where control was complete (Tables 5 and 6). Significant differences between the treatments occurred at E-L 35 and 38 where in Chardonnay disease severity in CV was significantly lower than observed on plants treated with individual extracts but not significantly different from the combined treatment (Table 5). At E-L 35 in particular all treatments performed significantly less well than the control and it was noted that disease severity followed a consistent pattern of distribution among the treatments. The combined solution of Equisetum, Nettle and Yarrow generally maintained mean severity score at or below 0.5 (5%), whereas, with the exception of Equisetum up to E-L 27, the individual treatments exhibited consistent and increasingly poor control as the trial progressed. Masson (2007) cautioned that plant extracts in BD programs should always be used in conjunction with other plant extracts and with the BD preparations themselves. No scientific explanation was offered, however Proctor and Cole (1997) and Klett (2006) reiterate the idea that each of these herbs contains nutrient elements that when combined, work synergistically. For example, Yarrow is reportedly rich in sulfur and potassium, likewise Nettle contains a high concentration of silica, iron and magnesium

and Equisetum is also rich in silica. Perhaps these, in combination, have an antagonistic effect or change the pH of the leaf surface to make a hostile environment for fungal infection.

The results for Equisetum demonstrated acceptable control in both cultivars until E-L 31, at which point severity continued to increase in Chardonnay, whereas it peaked at 0.6 (6%) in Shiraz, then declined to the end of the trial (Table 6). However, when comparing the combined treatment with the control and conventional treatment, the results indicate an acceptable level of control of powdery mildew until E-L31 in Chardonnay and throughout the trial in Shiraz where the mean severity score remained below 0.5. This reinforces the idea that control by the individual extracts is not as effective as the combined extracts.

**Powdery mildew severity on bunches.** Colonies of powdery mildew were first observed on bunches at E-L 31 (Table 7). In Chardonnay and Shiraz the CV treatment prevented powdery mildew throughout the trial. Severity was lower too in the combined treatments when compared with the control and the individual plant extracts, but no significant differences were found and in Chardonnay, the combined treatment mean, 0.58 was higher than acceptable (5%). Among the treatment means for the two cvs combined, only Nettle was significantly lower in performance than the combined treatment. At E-L 35 disease in the CV treatments was less severe than all other treatments. In Shiraz there were no significant differences amongst treatments or between cultivars (Table 7). In Shiraz by E-L 35 disease severity in the combined was 0.5 (5%), which is at the upper end of an acceptable disease severity, but could be considered an acceptable result. It is possible that colonies were missed on bunches during previous inspections for there to be such a large increase in severity between E-L 31 and 35.

The combined means for both cultivars (Table 7) do show some significant differences. Again control of disease severity in the CV treatment was total and was always significantly lower than the single plant extracts and the water control. However CV was not always

significantly different from the combined treatment, which in turn was also not significantly different from the single extracts, except for nettle at E-L 31. At E-L 31 the treatment mean of 0.292, while not significantly different from the single plant extracts exhibited a level of severity below 0.5 (5%). However by E-L 35, whilst not significantly different from the CV treatment, severity was unacceptably high where the industry accepted standard of 5% severity was exceeded.

In this case the combination of the three plant extracts provided an acceptable level of control at an earlier growth stage than individual extracts but not later in the growing season unlike the CV treatments. Perhaps the inclusion of sulfur at lower concentrations may support Masson (2007), who claimed that efficacy of combined plant extracts may make it possible to reduce the concentration of sulfur requirements by up to half to control powdery mildew. It has been suggested that Equisetum, which contains a high concentration of silicon, may protect plants against a diverse range of biotic and abiotic stresses and that the silica content of equisetum and silicon dioxide may induce an immune response in plants (Epstein 1999, Fauteux et al. 2005). Epstein (1999) and Bowen et al. (1992) suggest that the application of silicon in proprietary products may induce an immune response to fungal pathogens. Steiner (1924) hypothesised that the incorporation of both equisetum and silicon dioxide would have a “strengthening” effect and thus protects plants against “fungal conditions”. Despite some evidence that supports the claims that BD plant extracts may provide some control over powdery mildew, this trial would need to be repeated in the field and tested with varying concentrations of sulfur.

#### *Field trial physiological responses*

Some vegetative measures differed between treatments in both growing seasons. CV treated vines were larger and more productive with higher pruning mass and cane mass than the vines grown in the OG and BD systems (Table 8a). Pruning mass, cane mass and shoot length in

Chardonnay CV vines was two to three times greater than three of the four OG/BD treatments. This trend was also observed in Shiraz but not as pronounced. This may be a function of the vigorous nature of Shiraz when conditions are not limiting (Dry and Loveys 1998). No significant differences in the amount of count and non-count shoots were found between treatments for both cultivars. In this case nutrient availability may have limited vine size and, by extension, created more open canopies of the OG/ BD vines. This may have influenced the canopy microclimate and disease severity (Keller et al. 2003).

Reproductive measures were collected in 2012/2013 (Table 8b). In Chardonnay, yield (total harvest mass) was greatest in the CV treatments due to more berries per bunch and a higher mean bunch mass than OG and BD treatments. Berry mass, did not significantly differ between treatments in either cultivar. For Shiraz, yields of the CV2 and CV3 treatments were significantly higher ( $P < .001$ ) than both OG and BD treatments. Results in the OG treatments were similar to the BD. These differences point to the OG/BD treatments being less productive. Growers using either CV or OG/BD systems understand that lower vigour is advantageous in reducing relative humidity and shading which promote conditions favourable for disease and improve bud fruitfulness by better light interception (Smart 1985). However the results here indicate a difference in fruitfulness but possibly also fruit set (fewer berries per bunch) which may not be financially acceptable to the grower. This may point to a limited supply of carbohydrates and nutrients between seasons which can be detrimental to fruitfulness and fruit set (May 2004).

Reeve et al (2005) also reported that when comparing BD and OG systems used in a commercial Merlot vineyard, California, there were no differences in yield, bunches per vine, bunch and berry weight. Whilst average BD pruning weights were higher than the OG, both values fell within optimal range of 0.3 to 0.6kg/m (Kliewer and Casteel 2003) however no comparisons with CV grown vines were made. In the context of this trial, a similar lack of differences was exhibited. It could also be argued that the lower trend in productivity in the

BD vines could be detrimental to the long-term financial viability of the vines and future production. In this case however, the vines were newly established and would need extended monitoring to allow inferences to be made.

### *Nutrition*

As the growing medium, compost additions and weed management system were the same for all treatments; nutrition regimes may have influenced productivity. It is well understood that mineral fertiliser provides readily available nutrients, immediately accessible to the plant and this may explain the elevated productivity in the CV treatments. Organic fertilisers generally have lower concentrations of nutrients than mineral fertilisers (Treeby et al. 2004) and the slower rate of mineralisation of the organic forms of nutrients in the former regulates availability to the vine (Mengel and Kirby 2001). Edmeades (2000) suggests that timing of application and optimal climatic conditions are important factors. If either is not achieved appropriately, the plant's initial demands for N may not be met and conversely, nitrate N, excess to the plant's needs, can accumulate and be lost to leaching. As a result we would expect to see a reduced rate of growth in OG/BD systems if timing of application is misjudged and/or climatic conditions are not optimal. A high rate of growth only occurs in the presence of abundant N and optimum growth is achieved when there is balanced rate of photosynthate production, N assimilation and the presence of other nutrients in adequate amounts (Mengel and Kirby 2001). In BD systems no true fertilisers are added at all, instead there is a reliance on natural mineral cycling, reportedly enhanced by the use of BD preparations included in, and subsequent, to compost additions (Steiner 1924). When managing young vines, Boehm and Coombe (1992) point out that a high level of mineral nutrition particularly in respect to nitrogen is essential for the establishment of strong shoot growth. In this study it would suggest that the lower capacity for the OG/BD treatments to



store these nutrients in the woody parts of the vine affected productivity of shoot and leaf growth in both seasons (Löhnertz et al. 1989).

All other things being equal, plant capacity is limited by nutrient availability. While the concentrations of N, P and K were similar among treatments in this trial, it could be argued that the concentration is a measure of the nutrients to dry weight of sample petioles and not the size of the vines themselves. Consequently the concentration did not reflect the fact that the vine will only grow to the size that nutrient availability will allow. It should also be remembered that the values for adequate nutrient levels as suggested by Robinson et al. (1997) and Kristic et al. (2003) are loosely defined and may not reflect the true nature of nutrition in all cultivars.

Apart from Fe, Cu, K and N all elements were at acceptable levels for vine growth (Kristic et al. 2003, Robinson et al. 1997) in 2012/13 (Table 9b). Even though the concentration of N was below adequate in all treatments, signs of N deficiency such as pale green or yellow leaves or shoots were not observed in any treatment in either cultivar. However the lower rate of vine productivity in the OG/BD treatments than CV, despite a similar concentration of N amongst all treatments, could suggest a deficiency. Vines deficient in N exhibit low bud fruitfulness and bunches can also be smaller with fewer berries (May 2004), as was observed in this trial, which suggests that N concentration in petioles was a limiting factor in OG/BD productivity.

High K levels in all treatments can be attributed to the inclusion of grape-marc in the compost that was added to all treatments. Elevated K supply is not known to be detrimental to vine health but can result in high grape juice pH. Fe was below adequate for all treatments and did not significantly differ between treatments. Fe deficiency as inter-veinal chlorosis or yellow shoots was not observed. However Fe is also involved in converting nitrate into forms of plant accessible nitrogen (Treeby et al. 2004). In the BD treatments where N was not added in an organic or inorganic form, it is possible there was insufficient N for strong growth. Cu

was well above adequate in both years, most likely as a result of copper hydroxide foliar applications to all treatments before flowering (Table S3 a, b &c). The symptoms of Cu toxicity may include a reduction in concentration of P, Fe and Zn present in plant tissue (Treeby et al. 2004); this may have contributed to the lower than adequate measures of Fe in all treatments.

## **Conclusion**

As far as can be determined, this study is the first to scrutinise claims that selected BD inputs can control powdery mildew. These findings demonstrate that there is potential for the inclusion of plant extracts as alternative inputs in vineyard disease management. However, these appear to have potential in seasons where disease pressure is not excessive. In regard to the BD inputs, it is clear from the field trial that sulfur is more likely to control powdery mildew than the BD preparations alone. The smaller, less dense canopies of the OG and BD treatments may also play a role in mitigating favourable conditions for disease development. Efficacy of BD treatments will depend on coverage, cultivar susceptibility, disease pressure and treatment frequency.

The results of the growth room trial suggest that plant extracts when used in combination have the potential for early season disease control when used as prescribed. However, when disease pressure was high or environmental conditions were favourable for the rapid spread of powdery mildew, bunches and leaves were not protected.

Further research is needed to examine the efficacy of plant extracts in field conditions and, if proved effective, to determine the mode of action. A field trial could also be used to investigate whether sulfur concentration can be reduced when BD plant extracts are used (Masson 2007).

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**Table S4.** Treatment program applied to *Vitis vinifera* cvs Chardonnay and Shiraz, Waite Campus growth room trial: winter 2012.

**Table 1.** Effect of biodynamic (BD) and organic (OG) foliar treatments on the mean severity score of powdery mildew on adaxial and abaxial grapevine leaf surfaces, compared with water (CON) control and synthetic inputs (CV). Waite Campus trial 2011/2012.

Variable	Surface	E-L Stage*	Cultivar	Treatment						Cultivar		P-Value	
				CON	CV2	CV3	OG1	OG2	BD1	BD2	Mean		5% LSD
Adaxial	31	Chardonnay	<b>1.62b</b>	<b>0.47a</b>	<b>0.55a</b>	<b>0.62a</b>	<b>0.78a</b>	<b>0.82a</b>	<b>0.78a</b>	0.76	<b>0.37 (CxT)</b>	<b>&lt;0.001 (CxT)</b>	
		Shiraz	<b>0.40a</b>	<b>0.51a</b>	<b>0.42a</b>	<b>0.36a</b>	<b>0.49a</b>	<b>0.60a</b>	<b>0.36a</b>	0.48	ns (C)	ns (C)	
		Treatment Mean	1.01	0.49	0.47	0.49	0.63	0.71	0.57		0.25 (T)	0.003 (T)	
	35	Chardonnay	0.76	0.38	0.18	0.24	0.67	0.27	0.16	0.38	ns (CxT)	ns (CxT)	
		Shiraz	0.04	0.00	0.00	0.00	0.00	0.00	0.07	0.02	ns (C)	ns (C)	
		Treatment Mean	0.40	0.19	0.09	0.12	0.33	0.13	0.11		ns (T)	ns (T)	
	41	Chardonnay	2.71	2.70	2.04	3.04	2.25	2.13	1.84	<b>2.280b</b>	ns (CxT)	ns (CxT)	
		Shiraz	0.44	0.00	0.10	0.24	0.47	0.54	0.54	<b>0.291a</b>	<b>1.603 (C)</b>	<b>0.033 (C)</b>	
		Treatment Mean	1.58	1.35	1.07	1.64	1.36	1.33	1.19		ns (T)	ns (T)	
	Abaxial	31	Chardonnay	3.73	1.76	1.58	2.20	3.38	2.76	2.76	2.57	ns (CxT)	ns (CxT)
			Shiraz	2.40	1.04	1.36	1.69	2.89	2.00	1.73	1.84	ns (C)	ns (C)
			Treatment Mean	<b>3.07d</b>	<b>1.40a</b>	<b>1.47a</b>	<b>1.94ab</b>	<b>2.83cd</b>	<b>0.06bc</b>	<b>2.24bc</b>		<b>0.669 (T)</b>	<b>&lt;0.001 (T)</b>
35		Chardonnay	<b>3.67e</b>	<b>2.42bc</b>	<b>2.07ab</b>	<b>1.93a</b>	<b>3.18d</b>	<b>2.24ab</b>	<b>2.76c</b>	2.58	<b>0.83 (CxT)</b>	<b>0.019 (CxT)</b>	
		Shiraz	<b>2.56c</b>	<b>1.11a</b>	<b>1.27ab</b>	<b>1.84b</b>	<b>2.53c</b>	<b>1.51b</b>	<b>3.00d</b>	1.91	ns (C)	ns (C)	
		Treatment Mean	3.11	1.77	1.67	1.89	2.86	1.88	2.88		0.440 (T)	<0.001 (T)	
41		Chardonnay	3.67	1.86	1.51	3.25	3.08	1.83	2.22	<b>2.40b</b>	ns (CxT)	ns (CxT)	
		Shiraz	0.91	0.22	0.22	0.46	1.29	0.87	0.33	<b>0.57a</b>	<b>1.14 (C)</b>	<b>0.020 (C)</b>	
		Treatment Mean	2.29	1.04	0.89	1.86	2.19	1.35	1.28		ns (T)	ns (T)	

\*Grapevine growth stage based on the Modified E-L system (Coombe 1995)

CON: Water only; CV2: S + Systemic; CV3: S Only; OG1: potassium bicarbonate; OG2: Full cream bovine milk + Seaweed extract; BD1: Biodynamic inputs + S; BD2: Biodynamic inputs only.

For all treatments and cultivars, each value represents the mean of three replicates (nine vines total) from each treatment. The 5% LSD values listed are for comparison of treatments (T), cultivars (C) and their interaction (CxT). Means within a row sharing a letter are not significantly different at P=0.05 differences. ns, not significantly different at Severity score for visual assessment of leaf area affected by colonies of powdery mildew; 0; 0, 1-10%=1; 11-20%=2; 21-30%=3; 31-40%=4; 41-50%=5; 51-60%=6; 61-70%; 71-80%=8; 81-90%= 9; 91-100%=10

**Table 2.** Effect of biodynamic (BD) and organic (OG) foliar treatments on the mean severity score of powdery mildew on combined grapevine leaf surfaces, compared with water (CON) control and synthetic inputs (CV). Waite Campus trial 2011/2012.

E-L Stage*	Cultivar	Treatment							Cultivar		P-Value
		CON	CV2	CV3	OG1	OG2	BD1	BD2	Mean	5% LSD	
31	Chardonnay	2.68	1.11	1.04	1.41	2.08	1.79	1.77	1.52	ns (CxT)	ns (CxT)
	Shiraz	1.40	0.78	0.89	1.02	1.39	1.30	1.04	1.13	ns (C)	ns (C)
	<b>Treatment Mean</b>	<b>2.04c</b>	<b>0.94a</b>	<b>0.96a</b>	<b>1.21ab</b>	<b>1.73bc</b>	<b>1.54bc</b>	<b>1.40bc</b>		<b>0.41 (T)</b>	<b>0.004 (T)</b>
35	Chardonnay	2.22	1.40	1.12	1.09	1.92	1.26	1.46	1.37	ns (CxT)	ns (CxT)
	Shiraz	1.30	0.56	0.63	0.92	1.27	0.76	1.53	0.91	ns (C)	ns (C)
	<b>Treatment Mean</b>	<b>1.76b</b>	<b>0.98a</b>	<b>0.88a</b>	<b>1.01a</b>	<b>1.59b</b>	<b>1.00a</b>	<b>1.49b</b>		<b>0.32 (T)</b>	<b>&lt;.001 (T)</b>
41	Chardonnay	3.19	2.21	1.47	2.58	2.53	1.81	1.78	1.96	ns (CxT)	ns (CxT)
	Shiraz	0.68	0.10	0.14	0.32	0.83	0.68	0.37	0.37	ns (C)	ns (C)
	<b>Treatment Mean</b>	<b>1.94</b>	<b>1.16</b>	<b>0.81</b>	<b>1.45</b>	<b>1.68</b>	<b>1.24</b>	<b>1.07</b>		ns (T)	ns (T)

\*Grapevine growth stage based on the Modified E-L system (Coombe 1995)

**CON:** Water only; **CV2:** S + Systemic; **CV3:** S Only; **OG1:** potassium bicarbonate; **OG2:** Full cream bovine milk + Seaweed extract; **BD1:** Biodynamic inputs + S; **BD2:** Biodynamic inputs only.

For all treatments and cultivars, each value represents the mean of three replicates (nine vines total) from each treatment. The 5% LSD values listed are for comparison of treatments (T), cultivars (C) and their interaction (CxT). Means within a row sharing a letter are not significantly different at P=0.05 differences. ns, not significantly different at P=0.05 level.

Severity score for visual assessment of leaf area affected by colonies of powdery mildew; 0; 0, 1-10%=1; 11-20%=2; 21-30%=3; 31-40%=4; 41-50%=5; 51-60%=6; 61-70%; 71-80%=8; 81-90%= 9; 91-100%=10

**Table 3.** Effect of biodynamic (BD) and organic (OG) foliar treatments on the mean severity score of powdery mildew on adaxial and abaxial grapevine leaf surfaces, compared with water (CON) control and synthetic inputs (CV). Waite Campus trial 2012/13.

Variable	Surface	E-L Stage*	Cultivar	Treatment						Cultivar Mean	5% LSD	P-Value	
				CON	CV1	CV2	OG1	OG2	BD1				BD2
Adaxial	32		Chardonnay	0.07	0.00	0.70	0.02	0.31	0.00	0.10	0.07	ns (CxT)	ns (CxT)
			Shiraz	0.00	0.00	0.00	0.08	0.00	0.00	0.02	0.01	ns (C)	ns (C)
			Treatment Mean	0.03	0.00	0.03	0.06	0.16	0.00	0.07		ns (T)	ns (T)
	35		Chardonnay	0.27	0.00	0.00	0.20	0.29	0.00	0.20	0.12	ns (CxT)	ns (CxT)
			Shiraz	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	ns (C)	ns (C)
			Treatment Mean	0.14	0.00	0.00	0.10	0.14	0.00	0.10		ns (T)	ns (T)
	38		Chardonnay	1.02	0.48	0.45	1.31	0.95	0.07	0.45	0.71	ns (CxT)	ns (CxT)
			Shiraz	0.00	0.00	0.04	0.11	0.00	0.00	0.00	0.02	ns (C)	ns (C)
			Treatment Mean	0.51	0.24	0.25	0.71	0.47	0.03	0.23		ns (T)	ns (T)
	41		Chardonnay	1.70	0.94	0.80	2.00	2.24	0.10	0.95	1.50	ns (CxT)	ns (CxT)
			Shiraz	0.00	0.00	0.00	0.87	0.13	0.03	0.00	0.14	ns (C)	ns (C)
			Treatment Mean	0.85	0.47	0.40	1.44	1.90	0.03	0.48		ns (T)	ns (T)
Abaxial	32		Chardonnay	0.47	0.00	0.10	0.29	0.64	0.15	0.44	0.27	ns (CxT)	ns (CxT)
			Shiraz	0.00	0.00	0.00	0.04	0.02	0.00	0.00	0.01	ns (C)	ns (C)
			Treatment Mean	0.17	0.00	0.06	0.17	0.33	0.08	0.22		ns (T)	ns (T)
	35		Chardonnay	1.13	0.24	0.90	1.00	1.30	0.00	1.10	0.64	ns (CxT)	ns (CxT)
			Shiraz	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	ns (C)	ns (C)
			Treatment Mean	0.57	0.12	0.04	0.50	0.67	0.00	0.55		ns (T)	ns (T)
	38		Chardonnay	0.89	0.36	0.28	1.00	1.28	0.09	0.67	0.65	ns (CxT)	ns (CxT)
			Shiraz	0.00	0.03	0.04	0.22	0.05	0.03	0.00	0.05	ns (C)	ns (C)
			Treatment Mean	0.45	0.19	0.16	0.60	0.66	0.06	0.34		ns (T)	ns (T)
	41		Chardonnay	0.86	0.64	0.17	1.31	1.80	0.11	1.33	0.08	ns (CxT)	ns (CxT)
			Shiraz	0.00	0.03	0.00	0.52	0.05	0.00	0.00		ns (C)	ns (C)
			Treatment Mean	0.43	0.34	0.09	0.91	0.93	0.04	0.67		ns (T)	ns (T)

\*Grapevine growth stage based on the Modified E-L system (Coombe 1995)

CON: Water only; CV2: S + Systemic; CV3: S Only; OG1: potassium bicarbonate; OG2: Full cream bovine milk + Seaweed extract; BD1: Biodynamic inputs + S; BD2: Biodynamic inputs only.

For all treatments and cultivars, each value represents the mean of three replicates (nine vines total) from each treatment. The 5% LSD values listed are for comparison of treatments (T), cultivars (C) and their interaction (CxT). Means within a row sharing a letter are not significantly different at P=0.05 differences. ns, not significantly. Severity score for visual assessment of leaf area affected by colonies of powdery mildew; 0; 0, 1-10%=1; 11-20%=2; 21-30%=3; 31-40%=4; 41-50%=5; 51-60%=6; 61-70%; 71-80%=8; 81-90%= 9; 91-100%=10



**Table 4.** Effect of biodynamic (BD) and organic (OG) foliar treatments on the mean severity score of powdery mildew on combined grapevine leaf surfaces, compared with water (CON) control and synthetic inputs (CV). Waite Campus trial 2012/13.

E-L Stage*	Cultivar	Treatment						Cultivar		P-Value	
		CON	CV2	CV3	OG1	OG2	BD1	BD2	Mean		5% LSD
32	Chardonnay	0.27	0.00	0.09	0.16	0.48	0.08	0.28	0.17	ns (CxT)	ns (CxT)
	Shiraz	0.00	0.00	0.00	0.16	0.48	0.08	0.28	0.01	ns (C)	ns (C)
	Treatment Mean	0.13	0.00	0.04	0.11	0.24	0.39	0.14		ns (T)	ns (T)
35	Chardonnay	0.70	0.12	0.04	0.60	0.81	0.00	0.66	0.38	ns (CxT)	ns (CxT)
	Shiraz	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	ns (C)	ns (C)
	Treatment Mean	0.35	0.06	0.02	0.30	0.40	0.00	0.33		ns (T)	ns (T)
38	Chardonnay	0.95	0.42	0.36	1.15	1.11	0.08	0.56	0.08	ns (CxT)	ns (CxT)
	Shiraz	0.00	0.01	0.04	0.16	0.02	0.01	0.00	0.03	ns (C)	ns (C)
	Treatment Mean	0.47	0.22	0.20	0.66	0.57	0.05	0.28		ns (T)	ns (T)
41	Chardonnay	1.28	0.79	0.48	1.65	2.02	0.16	1.14	1.11	ns (CxT)	ns (CxT)
	Shiraz	0.00	0.02	0.00	0.70	0.09	0.02	0.00	0.12	ns (C)	ns (C)
	Treatment Mean	1.28	0.40	0.24	1.65	1.06	0.09	0.57		ns (T)	ns (T)

\*Grapevine growth stage based on the Modified E-L system (Coombe 1995)

**CON:** Water only; **CV2:** S + Systemic; **CV3:** S Only; **OG1:** potassium bicarbonate; **OG2:** Full cream bovine milk + Seaweed extract; **BD1:** Biodynamic inputs + S; **BD2:** Biodynamic inputs only.

For all treatments and cultivars, each value represents the mean of three replicates (nine vines total) from each treatment. The 5% LSD values listed are for comparison of treatments (T), cultivars (C) and their interaction (CxT). Means within a row sharing a letter are not significantly different at P=0.05 differences. ns, not significantly different at P=0.05 level.

Severity score for visual assesment of leaf area affected by colonies of powdery mildew; 0; 0, 1-10%=1; 11-20%=2; 21-30%=3; 31-40%=4;41-50%=5; 51-60%=6; 61-70%; 71-80%=8; 81-90%= 9; 91-100%=10

**Table 5.** Effect of herbal extracts both individually and combined on the severity of powdery mildew on adaxial and abaxial grapevine leaf surfaces, cvs. Chardonnay and Shiraz compared with conventional fungicides and a water control. Growth room trial, winter 2012.

Variable	Surface	E-L Stage*	Cultivar	Treatment					Cultivar		P-Value	
				H <sub>2</sub> O	Yarrow	Nettle	Equisetum	Combined	Synthetic	Mean		5% LSD
Adaxial	12	Chardonnay	0.14	0.11	0.06	0.00	0.00	0.00	0.05	ns (CxT)	ns (CxT)	
		Shiraz	0.00	0.11	0.17	0.06	0.00	0.00	0.06	ns (C)	ns (C)	
		Treatment Mean	0.07	0.11	0.11	0.03	0.00	0.00		ns (T)	ns (T)	
	23	Chardonnay	0.56	0.56	0.36	0.17	0.06	0.00	0.28	ns (CxT)	ns (CxT)	
		Shiraz	0.19	0.11	0.04	0.11	0.06	0.00	0.15	ns (C)	ns (C)	
		Treatment Mean	<b>0.38b</b>	<b>0.33b</b>	<b>0.39b</b>	<b>0.14ab</b>	<b>0.06a</b>	<b>0.00a</b>		<b>0.268 (T)</b>	<b>0.014(T)</b>	
	27	Chardonnay	1.28	1.47	1.36	0.50	0.56	0.00	0.86	ns (CxT)	ns (CxT)	
		Shiraz	0.58	0.54	0.22	0.39	0.19	0.00	0.32	ns (C)	ns (C)	
		Treatment Mean	<b>0.93cd</b>	<b>1.01d</b>	<b>0.74bcd</b>	<b>0.45abc</b>	<b>0.38ab</b>	<b>0.000a</b>		<b>0.500 (T)</b>	<b>0.002 (T)</b>	
	31	Chardonnay	1.69	1.75	1.42	0.86	0.72	0.00	1.07	ns (CxT)	ns (CxT)	
		Shiraz	0.93	0.96	1.00	0.78	0.19	0.00	0.64	ns (C)	ns (C)	
		Treatment Mean	<b>1.31c</b>	<b>1.35c</b>	<b>1.21c</b>	<b>0.82bc</b>	<b>0.46ab</b>	<b>0.000a</b>		<b>0.709 (T)</b>	<b>0.002 (T)</b>	
	35	Chardonnay	1.56	1.36	1.35	0.75	0.61	0.00	0.94	ns (CxT)	ns (CxT)	
		Shiraz	1.25	0.96	0.83	0.56	0.25	0.00	0.64	ns (C)	ns (C)	
		Treatment Mean	<b>1.40d</b>	<b>1.16cd</b>	<b>1.09bcd</b>	<b>0.65abc</b>	<b>0.43ab</b>	<b>0.00a</b>		<b>0.675 (T)</b>	<b>0.002 (T)</b>	
	38	Chardonnay	2.68	2.09	1.60	0.93	0.43	0.36	1.35	ns (CxT)	ns (CxT)	
		Shiraz	1.75	1.25	1.49	0.37	0.33	0.07	0.88	ns (C)	ns (C)	
		Treatment Mean	<b>2.21d</b>	<b>1.67cd</b>	<b>1.54bcd</b>	<b>0.65abc</b>	<b>0.38ab</b>	<b>0.21a</b>		<b>1.168 (T)</b>	<b>0.007 (T)</b>	
	Abaxial	12	Chardonnay	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
			Shiraz	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
			Treatment Mean	0.00	0.00	0.00	0.00	0.00	0.00			
		23	Chardonnay	0.00	0.00	0.11	0.00	0.03	0.00	0.02	ns (CxT)	ns (CxT)
			Shiraz	0.00	0.00	0.00	0.00	0.00	0.00	0.00	ns (C)	ns (C)
			Treatment Mean	0.00	0.15	0.05	0.00	0.14	0.00		ns (T)	ns (T)
27		Chardonnay	0.25	0.22	0.25	0.17	0.17	0.00	0.18	ns (CxT)	ns (CxT)	
		Shiraz	0.25	0.07	0.22	0.56	0.03	0.00	0.10	ns (C)	ns (C)	
		Treatment Mean	0.25	0.15	0.24	0.11	0.97	0.00		ns (T)	ns (T)	
31		Chardonnay	0.86	0.39	0.56	0.44	0.31	0.00	0.43	ns (CxT)	ns (CxT)	
		Shiraz	0.31	0.38	0.51	0.42	0.08	0.00	0.28	ns (C)	ns (C)	
		Treatment Mean	0.58	0.38	0.54	0.43	0.94	0.00		ns (T)	ns (T)	
35		Chardonnay	<b>1.09c</b>	<b>0.75b</b>	<b>0.92b</b>	<b>0.69ab</b>	<b>0.31ab</b>	<b>0.000a</b>	<b>0.75</b>	<b>0.709 (CxT)</b>	<b>0.010 (CxT)</b>	
		Shiraz	0.220	0.640	0.290	0.140	0.060	0.000	<b>0.23</b>	ns (C)	ns (C)	
		Treatment Mean	1.014	0.694	0.604	0.417	0.181	0.00		0.434 (T)	<0.001 (T)	
38		Chardonnay	<b>3.42c</b>	<b>2.57c</b>	<b>1.47bc</b>	<b>1.29b</b>	<b>0.87ab</b>	<b>0.03a</b>	1.61	<b>1.253 (CxT)</b>	<b>0.043 (CxT)</b>	
		Shiraz	0.60	0.60	0.88	0.08	0.08	0.06	0.38	ns (C)	ns (C)	
		Treatment Mean	2.01	1.59	1.17	0.69	0.05	0.05		0.913 (T)	0.001 (T)	

¶ Plant extracts prepared in accordance with Masson (2007)

\*Grapevine growth stage based on the Modified E-L system (Coombe 1995)

**H<sub>2</sub>O:** Water control; **Yarrow:** Plant extract of *Achillea millefolium* only; **Nettle:** Plant extract of *Urtica dioica* only; **Equisetum:** Plant extract of *Equisetum arvense* only; **Combined:** Yarrow, Nettle and Equisetum; **Conventional:** Systemic fungicides only, used in rotation.

For all treatments and cultivars, each value represents the mean of four replicates (twelve vines total) from each treatment. The 5% LSD values listed are for comparison of treatments (T), cultivars (C) and their interaction (CxT). Means within a row sharing a letter are not significantly different at P=0.05 differences. ns, not significantly different at P=0.05 level.

Severity score for visual assessment of leaf area affected by colonies of powdery mildew; 0; 0, 1-10%=1; 11-20%=2; 21-30%=3; 31-40%=4; 41-50%=5; 51-60%=6; 61-70%; 71-80%=8; 81-90%= 9; 91-100%=10

**Table 6.** Effect of herbal extracts both individually and combined on the severity of powdery mildew on combined grapevine leaf surfaces, cvs. Chardonnay and Shiraz compared with conventional fungicides and a water control. Growth room trial, winter 2012.

E-L Stage*	Cultivar	Treatment						Cultivar	5% LSD	P-Value
		H2O	Yarrow	Nettle	Equisetum	Combined	Conventional			
12	Chardonnay	0.07	0.06	0.03	0.00	0.00	0.00	0.03	ns (CxT)	ns (CxT)
	Shiraz	0.00	0.06	0.08	0.03	0.00	0.00	0.03	ns (C)	ns (C)
	Treatment	0.03	0.02	0.06	0.01	0.00	0.00		ns (T)	ns (T)
23	Chardonnay	0.28	0.28	0.24	0.09	0.05	0.00	0.15	ns (CxT)	ns (CxT)
	Shiraz	0.10	0.06	0.21	0.06	0.03	0.00	0.07	ns (C)	ns (C)
	Treatment	<b>0.19cd</b>	<b>0.17bcd</b>	<b>0.22d</b>	<b>0.07abc</b>	<b>0.04ab</b>	<b>0.00a</b>		<b>0.14 (T)</b>	<b>0.016(T)</b>
27	Chardonnay	0.77	0.85	0.81	0.33	0.36	0.00	0.52	ns (CxT)	ns (CxT)
	Shiraz	0.42	0.30	0.22	0.48	0.11	0.00	0.21	ns (C)	ns (C)
	Treatment	<b>0.59c</b>	<b>0.58c</b>	<b>0.51bc</b>	<b>0.28abc</b>	<b>0.24ab</b>	<b>0.00a</b>		<b>0.32 (T)</b>	<b>0.004 (T)</b>
31	Chardonnay	1.28	1.07	0.99	0.65	0.52	0.00	0.75	ns (CxT)	ns (CxT)
	Shiraz	0.62	0.67	0.76	0.60	0.14	0.00	0.46	ns (C)	ns (C)
	Treatment	<b>0.95c</b>	<b>0.87bc</b>	<b>0.87bc</b>	<b>0.63bc</b>	<b>0.33ab</b>	<b>0.00a</b>		<b>0.55 (T)</b>	<b>0.008 (T)</b>
35	Chardonnay	1.33	1.06	1.13	0.72	0.46	0.00	0.84	ns (CxT)	ns (CxT)
	Shiraz	0.74	0.80	0.56	0.35	0.16	0.00	0.43	ns (C)	ns (C)
	Treatment	<b>1.21d</b>	<b>0.93cd</b>	<b>0.85cd</b>	<b>0.54bc</b>	<b>0.31ab</b>	<b>0.00a</b>		<b>&lt;.001 (T)</b>	<b>0.490 (T)</b>
38	Chardonnay	3.05	2.33	1.54	1.11	0.65	0.19	1.48	ns (CxT)	ns (CxT)
	Shiraz	1.17	0.93	1.18	0.23	0.21	0.08	0.63	ns (C)	ns (C)
	Treatment	<b>2.11c</b>	<b>1.63c</b>	<b>1.36bc</b>	<b>0.67ab</b>	<b>0.43ab</b>	<b>0.14a</b>		<b>&lt;.001 (T)</b>	<b>0.936 (T)</b>

¶ Plant extracts prepared in accordance with Masson (2007)

\*Grapevine growth stage based on the Modified E-L system (Coombe 1995)

**H<sub>2</sub>O:** Water control; **Yarrow:** Plant extract of *Achillea millefolium* only; **Nettle:** Plant extract of *Urtica dioica* only; **Equisetum:** Plant extract of *Equisetum arvense* only; **Combined:** Yarrow, Nettle and Equisetum; **Conventional:** Systemic fungicides only, used in rotation.

For all treatments and cultivars, each value represents the mean of four replicates (twelve vines total) from each treatment. The 5% LSD values listed are for comparison of treatments (T), cultivars (C) and their interaction (CxT). Means within a row sharing a letter are not significantly different at P=0.00. ns, not significantly different at P=0.05 level.

Severity score for visual assessment of leaf area affected by colonies of powdery mildew; 0; 0, 1-10%=1; 11-20%=2; 21-30%=3; 31-40%=4; 41-50%=5; 51-60%=6; 61-70%=7; 71-80%=8; 81-90%=9; 91-100%=10

**Table 7.** Effect of herbal extracts both individually and combined on the severity of powdery mildew on grape vine bunches, cvs. Chardonnay and Shiraz compared with conventional fungicides and a water control. Growth room trial, winter 2012.

Variable	E-L Stage*	Cultivar	Treatment						Mean	5% LSD	P-Value
			H <sub>2</sub> O	Yarrow	Nettle	Equisetum	Combined	Conventional			
Severity	31	Chardonnay	1.08	1.58	2.12	1.04	0.58	0.00	1.069	ns (CxT)	ns (CxT)
		Shiraz	0.67	0.00	0.46	0.38	0.00	0.00	0.251	ns (C)	ns (C)
		Treatment Mean	<b>0.875bc</b>	<b>0.792bc</b>	<b>1.292c</b>	<b>0.709bc</b>	<b>0.292ab</b>	<b>0.000a</b>		<b>0.608 (T)</b>	<b>0.003 (T)</b>
	35	Chardonnay	3.96	2.88	3.12	1.67	1.21	0.00	2.140	ns (CxT)	ns (CxT)
		Shiraz	3.63	0.50	0.69	0.63	0.50	0.00	0.950	ns (C)	ns (C)
		Treatment Mean	<b>3.793c</b>	<b>1.688b</b>	<b>1.907b</b>	<b>1.146b</b>	<b>0.855ab</b>	<b>0.000a</b>		<b>1.148 (T)</b>	<b>&lt;0.001 (T)</b>

† Plant extracts prepared in accordance with Masson (2007)

\*Grapevine growth stage based on the Modified E-L system (Coombe 1995)

**H<sub>2</sub>O:** Water control; **Yarrow:** Plant extract of *Achillea millefolium* only; **Nettle:** Plant extract of *Urtica dioica* only; **Equisetum:** Plant extract of *Equisetum arvense* only; **Combined:** Yarrow, Nettle and Equisetum; **Conventional:** Systemic fungicides only, used in rotation.

For all treatments and cultivars, each value represents the mean of four replicates (twelve vines total) from each treatment. The 5% LSD values listed are for comparison of treatments (T), cultivars (C) and their interaction (CxT). Means within a row sharing a letter are not significantly different at P=0.05 differences. ns, not significantly different at P=0.05 level.

Severity score for visual assessment of leaf area affected by colonies of powdery mildew; 0; 0, 1-10%=1; 11-20%=2; 21-30%=3; 31-40%=4; 41-50%=5; 51-60%=6; 61-70%=7; 71-80%=8; 81-90%=9; 91-100%=10

**Table 8a.** The effect of biodynamic (BD) and organic (OG) inputs, compared with water control (CON) and conventional inputs (CV), on vine growth measures, cvs Chardonnay and Shiraz, Waite Campus Trial, South Australia, Australia; 2011-2012.

Variable	Cultivar	Treatment							Cultivar		
		CON	CV2	CV3	OG1	OG2	BD1	BD2	Mean	5% LSD	P-Value
<b>2011/2012 Season</b>											
<b>Count Shoot Number</b>	Chardonnay	12	14	13	11	10	8	11	11	ns (CxT)	ns (CxT)
	Shiraz	11	11	13	12	11	10	11	12	ns (C)	ns (C)
	Treatment Mean	12	13	13	12	11	9	11		ns (T)	ns (T)
<b>Non-Count Shoot Number</b>	Chardonnay	<b>7.3b</b>	<b>4.0a</b>	<b>8.0b</b>	<b>5.7ab</b>	<b>6.0ab</b>	<b>5.3ab</b>	<b>5.3ab</b>	6	<b>2.6 (CxT)</b>	<b>0.041 (CxT)</b>
	Shiraz	<b>4.3ab</b>	<b>5.0ab</b>	<b>6.7b</b>	<b>6.3b</b>	<b>3.6a</b>	<b>4.3ab</b>	<b>8.3b</b>	6	ns (C)	ns (C)
	Treatment Mean	6	5	7.3	6	5	5	7		ns (T)	ns (T)
<b>Laterals</b>	Chardonnay	3	5	10	4	4	1	0	5	ns (CxT)	ns (CxT)
	Shiraz	7	9	12	3	1	7	2	7	ns (C)	ns (C)
	Treatment Mean	<b>5.0ab</b>	<b>6.7bc</b>	<b>10.8c</b>	<b>3.5ab</b>	<b>2.5ab</b>	<b>4.3ab</b>	<b>1.7a</b>		<b>4.37 (T)</b>	<b>&lt;0.001 (T)</b>
<b>Pruning Mass (g)</b>	Chardonnay	400	767	617	217	350	300	267	442	ns (CxT)	ns (CxT)
	Shiraz	617	733	867	417	433	383	283	608	ns (C)	ns (C)
	Treatment Mean	<b>508.3b</b>	<b>750.0c</b>	<b>741.7c</b>	<b>316.7ab</b>	<b>391.7ab</b>	<b>341.70ab</b>	<b>275.0a</b>		<b>225.4 (T)</b>	<b>&lt;0.001 (T)</b>
<b>Mean Cane Mass (g)</b>	Chardonnay	42	62	55	25	41	42	30	45	ns (CxT)	ns (CxT)
	Shiraz	58	68	72	41	45	44	35	55	ns (C)	ns (C)
	Treatment Mean	<b>49.80b</b>	<b>64.90bc</b>	<b>63.75bc</b>	<b>33.03a</b>	<b>42.56ab</b>	<b>43.11ab</b>	<b>32.46a</b>		<b>16.38 (T)</b>	<b>&lt;0.001 (T)</b>
<b>Mean Cane Length (cm)</b>	Chardonnay	82	114	86	62	76	75	68	82	ns (CxT)	ns (CxT)
	Shiraz	110	88	98	101	94	81	74	93	ns (C)	ns (C)
	Treatment Mean	<b>96.13b</b>	<b>100.83b</b>	<b>92.10b</b>	<b>81.17a</b>	<b>84.87a</b>	<b>77.67a</b>	<b>70.87a</b>		<b>20.09 (T)</b>	<b>0.045 (T)</b>

CON: Water only; CV2: S + Systemic; CV3: S Only; OG1: potassium bicarbonate; OG2: Full cream bovine milk + Seaweed extract; BD1: Biodynamic inputs + S; BD2: Biodynamic inputs only.

For all treatments and cultivars, each value represents the mean of three replicates (nine vines total) from each treatment. The 5% LSD values listed are for comparison of treatments (T), cultivars (C) and their interaction (CxT). Means within a row sharing a letter are not significantly different at P=0.05 differences. ns, not significantly different at P=0.05 level.

**Table 8b.** The effect of biodynamic (BD) and organic (OG) inputs, compared with water control (CON) and conventional inputs (CV), on vine growth measures, cvs Chardonnay and Shiraz, Waite Campus Trial, South Australia, Australia; 2012-2013.

Variable	Cultivar	Treatment							Cultivar Mean	5% LSD	P-Value
		CON	CV2	CV3	OG1	OG2	BD1	BD2			
<b>2012/2013 Season</b>											
Count Shoot Number	Chardonnay	28	26	31	26	26	24	20	27	ns (CxT)	ns (CxT)
	Shiraz	27	27	31	25	25	24	22	27	ns (C)	ns (C)
	Treatment Mean	28	27	31	26	26	24	21		ns (T)	ns (T)
Non-Count Shoot Number	Chardonnay	10	12	9	6	6	8	7	<b>8.7a</b>	ns (CxT)	ns (CxT)
	Shiraz	11	12	10	10	11	8	15	<b>10.8b</b>	<b>1.71 (C)</b>	<b>0.031 (C)</b>
	Treatment Mean	11	12	10	8	9	8	11		ns (T)	ns (T)
Laterals	Chardonnay	0	1	0.7	0	0	0.7	0	0.3	ns (CxT)	ns (CxT)
	Shiraz	0	0	0	0	0	0	1.3	0.2	ns (C)	ns (C)
	Treatment Mean	0	0.5	0.3	0	0	0.3	0.7		ns (T)	ns (T)
Pruning Mass (g)	Chardonnay	800	1300	1267	467	733	600	267	887	ns (CxT)	ns (CxT)
	Shiraz	833	867	933	733	800	567	667	846	ns (C)	ns (C)
	Treatment Mean	<b>817ab</b>	<b>1083bc</b>	<b>1100bc</b>	<b>600a</b>	<b>767a</b>	<b>583a</b>	<b>467a</b>		<b>433.8 (T)</b>	<b>&lt;0.001 (T)</b>
Mean Cane Mass (g)	Chardonnay	21	34	31	14	23	17	11	24	ns (CxT)	ns (CxT)
	Shiraz	22	23	22	21	23	16	17	22	ns (C)	ns (C)
	Treatment Mean	<b>21.4abc</b>	<b>28.3cd</b>	<b>26.7bcd</b>	<b>17.5ab</b>	<b>22.7abc</b>	<b>16.6ab</b>	<b>13.9a</b>		<b>10.69 (T)</b>	<b>0.003 (T)</b>
Mean Cane Length (cm)	Chardonnay	94	152	115	98	144	85	92	119	ns (CxT)	ns (CxT)
	Shiraz	121	106	100	113	130	95	87	114	ns (C)	ns (C)
	Treatment Mean	108	129	107	106	137	90	90		ns (T)	ns (T)
Total Harvest Mass (g)	Chard	2343	3543	2753	1350	1627	1667	797	1633	2514 (CxT)	NS (CxT)
	Shiraz	4917	5483	9917	4733	4200	2100	3367	4066	1326 (C)	0.010 (C)
	Treatment Mean	<b>3630ab</b>	<b>4513bc</b>	<b>6335c</b>	<b>3042ab</b>	<b>2913ab</b>	<b>1883a</b>	<b>2082a</b>		<b>1845 (T)</b>	<b>&lt;0.001 (T)</b>
Mean Bunch Mass (g)	Chard	57.6	64.6	62.3	49.9	45.4	63.6	57.8	62.2	NS (CxT)	NS (CxT)
	Shiraz	88.8	107.9	113.4	84.6	90.0	58.2	75.5	91.3	NS (C)	NS (C)
	Treatment Mean	<b>73.2ab</b>	<b>86.2abc</b>	<b>87.8bc</b>	<b>67.3ab</b>	<b>67.7ab</b>	<b>60.88a</b>	<b>66.7ab</b>		<b>26.2 (T)</b>	<b>0.040 (T)</b>
Mean Berry Mass (g)	Chard	1.2	1.2	1.2	1.2	1.2	1.5	1.2	1.2	NS (CxT)	NS (CxT)
	Shiraz	1.2	1.1	1.2	1.3	1.3	1.1	1.1	1.2	NS (C)	NS (C)
	Treatment Mean	1.2	1.2	1.2	1.3	1.3	1.3	1.1		NS (T)	NS (T)
Mean Berries per bunch	Chard	50	53	51	41	37	45	48	51	NS (CxT)	NS (CxT)
	Shiraz	77	95	94	64	67	53	70	76	NS (C)	NS (C)
	Treatment Mean	<b>63ab</b>	<b>73bc</b>	<b>72bc</b>	<b>52a</b>	<b>52a</b>	<b>49a</b>	<b>59.ab</b>		<b>18.8 (T)</b>	<b>0.007 (T)</b>
Mean Rachis Mass (g)	Chard	3.3	4.1	3.8	2.3	2.7	2.1	1.6	3.01	NS (CxT)	NS (CxT)
	Shiraz	5.3	7.1	7.2	5.1	5.1	3.8	4.6	5.60	NS (C)	NS (C)
	Treatment Mean	<b>4.2b</b>	<b>5.6c</b>	<b>5.5c</b>	<b>3.7ab</b>	<b>3.9ab</b>	<b>2.9a</b>	<b>3.1a</b>		<b>1.08 (T)</b>	<b>&lt;0.001 (T)</b>

CON: Water only; CV2: S + Systemic; CV3: S Only; OG1: potassium bicarbonate; OG2: Full cream bovine milk + Seaweed extract; BD1: Biodynamic inputs + S; BD2: Biodynamic inputs only.

For all treatments and cultivars, each value represents the mean of three replicates (nine vines total) from each treatment. The 5% LSD values listed are for comparison of treatments (T), cultivars (C) and their interaction (CxT). Means within a row sharing a letter are not significantly different at P=0.05 differences. ns, not significantly different at P=0.05 level.

**Table 9a.** Effect of biodynamic (BD), organic (OG) and conventional (CV) soil amendments on petiole nutrient status; cvs Chardonnay and Shiraz, at 80% flowering from the Waite Campus Trial, South Australia, Australia; 2011/12.

Variable	Cultivar	Adequate	Treatment							Cultivar Mean	5% LSD	P-Value
			CON	CV1	CV2	OG1	OG2	BD1	BD2			
<b>2011/2012 Season</b>												
Cu mg/kg	Chardonnay		79	59	57	103	76	70	81	55		
	Shiraz	>6 mg/kg	86	77	77	104	122	96	87	68	ns (C)	ns (C)
	Treatment Mean		<b>82ab</b>	<b>68a</b>	<b>67.0a</b>	<b>104c</b>	<b>99bc</b>	<b>83ab</b>	<b>84.0ab</b>		<b>18.9 (T)</b>	<b>0.002 (T)</b>
Mg %	Chardonnay		<b>0.45b</b>	<b>0.50b</b>	<b>0.44ab</b>	<b>0.44ab</b>	<b>0.41ab</b>	<b>0.44ab</b>	<b>0.48b</b>	0.5	<b>0.084 (CxT)</b>	<b>0.048 (CxT)</b>
	Shiraz	>0.4%	<b>0.37ab</b>	<b>0.31a</b>	<b>0.34a</b>	<b>0.38ab</b>	<b>0.41b</b>	<b>0.45b</b>	<b>0.35a</b>	0.4	ns (V)	ns (V)
	Treatment Mean		0.4	0.4	0.4	0.4	0.4	0.4	0.4		ns (T)	ns (T)
Na %	Chardonnay		0.1	0.1	0.1	0.1	0.1	0.1	0.1	<b>0.07a</b>	ns (CxT)	ns (CxT)
	Shiraz		0.2	0.2	0.2	0.1	0.2	0.2	0.2	<b>0.16b</b>	<b>0.138 (C)</b>	<b>0.001 (C)</b>
	Treatment Mean	0.1-0.3 %	0.1	0.1	0.1	0.1	0.1	0.1	0.1		ns (T)	ns (T)
N %	Chardonnay		0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	ns (CxT)	ns (CxT)
	Shiraz	0.8-1.1 %	0.6	0.6	0.6	0.7	0.6	0.6	0.6	0.6	ns (C)	ns (C)
	Treatment Mean		0.5	0.6	0.6	0.6	0.5	0.5	0.5		ns (T)	ns (T)
P %	Chardonnay		<b>0.55b</b>	<b>0.33a</b>	<b>0.31a</b>	<b>0.62b</b>	<b>0.55b</b>	<b>0.55b</b>	<b>0.64b</b>	0.5	<b>0.151 (CxT)</b>	<b>0.002 (CxT)</b>
	Shiraz	0.50%	<b>0.76b</b>	<b>0.23a</b>	<b>0.27a</b>	<b>0.80b</b>	<b>0.78b</b>	<b>0.73b</b>	<b>1.00c</b>	0.6	ns (C)	ns (C)
	Treatment Mean		0.7	0.3	0.3	0.7	0.7	0.6	0.8		0.111 (T)	<0.001 (T)
K %	Chardonnay		3.4	2.9	3.1	3.7	3.6	3.3	3.2	3.3	ns (CxT)	ns (CxT)
	Shiraz	0.25%	4.9	4.7	4.4	5.2	4.9	4.9	5.1	3.6	ns (C)	ns (C)
	Treatment Mean		<b>4.17b</b>	<b>3.82a</b>	<b>3.78a</b>	<b>4.42b</b>	<b>4.23b</b>	<b>4.08ab</b>	<b>4.17b</b>		<b>0.2934 (T)</b>	<b>0.001 (T)</b>

CON: Water only; CV1: S + Systemic; CV2: S Only; OG1: potassium bicarbonate; OG2: Full cream bovine milk + Seaweed extract; BD1: Biodynamic inputs + S; BD2: Biodynamic inputs only.

For all treatments and cultivars, each value represents the mean of three replicates (nine vines total) from each treatment. The 5% LSD values listed are for comparison of treatments (T), cultivars (C) and their interaction (CxT). Means within a row sharing a letter are not significantly different at P=0.05 differences. ns, not significantly different at P=0.05 level.

**Table 9b.** Effect of biodynamic (BD), organic (OG) and conventional (CV) soil amendments on petiole nutrient status; cvs Chardonnay and Shiraz, at 80% flowering from the Waite Campus Trial, South Australia, Australia; 2012/13.

Variable	Nutrient	Cultivar	Treatment							Cultivar Mean	5% LSD	P-Value
			CON	CV1	CV2	OG1	OG2	BD1	BD2			
2012/2013 Season		Adequate										
Fe mg/kg	Chardonnay		<b>18a</b>	<b>19ab</b>	<b>18a</b>	<b>20ab</b>	<b>22b</b>	<b>20ab</b>	<b>21b</b>	20	<b>2.4 (VxT)</b>	<b>0.024 (VxT)</b>
	Shiraz	<b>70 mg/kg</b>	<b>17ab</b>	<b>19ab</b>	<b>18ab</b>	<b>21b</b>	<b>19ab</b>	<b>17a</b>	<b>17a</b>	18	ns (C)	ns (C)
	Treatment Mean		17.6	19.3	18.4	20.7	20.7	18.6	18.0		1.6 (T)	0.004 (T)
Cu mg/kg	Chardonnay		7	28	42	10	10	32	9	22	ns (CxT)	ns (CxT)
	Shiraz	<b>&gt;6 mg/kg</b>	9	23	56	12	10	37	10	25	ns (C)	ns (C)
	Treatment Mean		<b>8.4a</b>	<b>25.3b</b>	<b>49.2d</b>	<b>11.1a</b>	<b>10.1a</b>	<b>34.3bc</b>	<b>9.8a</b>		<b>12.6 (T)</b>	<b>&lt;0.001 (T)</b>
Na %	Chardonnay		0.06	0.05	0.06	0.07	0.06	0.07	0.11	<b>0.07a</b>	ns (CxT)	ns (CxT)
	Shiraz	<b>0.1-0.3 %</b>	0.13	0.14	0.12	0.10	0.12	0.13	0.15	<b>0.13b</b>	<b>0.029 (C)</b>	<b>0.012 (C)</b>
	Treatment Mean		0.09	0.10	0.09	0.09	0.09	0.10	0.11		ns (T)	ns (T)
N %	Chardonnay		<b>0.49ab</b>	<b>0.49ab</b>	<b>0.50ab</b>	<b>0.49ab</b>	<b>0.47a</b>	<b>0.47a</b>	<b>0.51ab</b>	0.49	<b>0.056 (VxT)</b>	<b>0.020 (VxT)</b>
	Shiraz	<b>0.8-1.1 %</b>	<b>0.59ab</b>	<b>0.64b</b>	<b>0.66b</b>	<b>0.59ab</b>	<b>0.550a</b>	<b>0.56a</b>	<b>0.55a</b>	0.59	ns (C)	ns (C)
	Treatment Mean		0.54	0.57	0.58	0.54	0.51	0.51	0.53		0.032 (T)	<0.001 (T)
P%	Chardonnay		0.6	0.3	0.3	0.3	0.6	0.6	0.6	0.6	ns (CxT)	ns (CxT)
	Shiraz	<b>0.50%</b>	0.8	0.2	0.2	0.3	0.8	0.8	0.7	1.0	ns (C)	ns (C)
	Treatment Mean		0.7	0.3	0.3	0.3	0.7	0.7	0.6		ns (T)	ns (T)
K %	Chardonnay		3.4	3.3	3.77	3.8	3.4	3.4	3.5	<b>3.5a</b>	ns (CxT)	ns (CxT)
	Shiraz	<b>0.25%</b>	4.3	4.0	4.37	4.9	4.5	4.1	4.3	<b>4.3b</b>	<b>0.530 (C)</b>	<b>0.023 (C)</b>
	Treatment Mean		3.8	3.7	4.07	4.4	3.9	3.7	3.9		ns (T)	ns (T)

CON: Water only; CV1: S + Systemic; CV2: S Only; OG1: potassium bicarbonate; OG2: Full cream bovine milk + Seaweed extract; BD1: Biodynamic inputs + S; BD2: Biodynamic inputs only.

For all treatments and cultivars, each value represents the mean of three replicates (nine vines total) from each treatment. The 5% LSD values listed are for comparison of treatments (T), cultivars (C) and their interaction (CxT). Means within a row sharing a letter are not significantly different at P=0.05 differences. ns, not significantly different at P=0.05 level.



**Table S1.** Summary of relative mean maximum and minimum temperature, rainfall and humidity, Waite Campus field trial, 2010-2013, South Australia (BOM, 2013).

Year	Variable†	Month						
		October	November	December	January	February	March	April
<b>2010/11</b>	Mean Maximum°C	21	24	27	31	28	26	23
	Mean Minimum°C	12	14	16	18	19	15	12
	Mean Rainfall (mm)	29	23	87	17	43	78	14
	Mean RH (3pm)	46	38	32	33	40	39	42
<b>2011/12</b>	Mean Maximum°C	22	27	28	31	28	26	24
	Mean Minimum°C	13	15	16	19	17	15	13
	Mean Rainfall (mm)	43	25	18	18	25	63	27
	Mean RH (3pm)	36	31	33	29	33	38	23
<b>2012/13</b>	Mean Maximum°C	23	28	28	30	31	28	24
	Mean Minimum°C	11	15	16	17	18	18	13
	Mean Rainfall (mm)	20	18	12	12	12	20	33
	Mean RH (3pm)	36	31	33	29	33	38	41
<b>Average</b>	Mean Maximum°C	22	25	27	29	29	26	23
	Mean Minimum°C	12	14	16	17	17	15	12
	Mean Rainfall (mm)	43	31	29	19	14	27	40
	Mean RH (3pm)	45	40	39	36	36	41	47

† Data collected at Bureau of Meteorology station: Kent Town 1977-2013

**Table S2.** Waite Campus trial fertiliser applications by treatment per year.

Treatment/product	Rate	Year		
		2010/2011	2011/2012	2012/2013
<b>Control</b>				
Scott's 'Osmocote'®	30g/100L soil	23-Dec	25-Oct	27-Oct
<b>Conventional</b>				
Complete-D'®	50g/100L soil	23-Dec	25-Oct	27-Oct
<b>Organic</b>				
Nutrog® Rapid Raiser'®	70g/100L soil	23-Dec	25-Oct	27-Oct
<b>Biodynamic</b>				
Barrel Compost†	4.4g/L water; 45mL/treatment	23-Dec	28-Oct	27-Sep; 24-Oct; 15-Mar; 12-Apr
Horn Manure: Preparation 500	2.4g/L water; 45mL/treatment	23-Dec	20-Sep; 25-Nov; 19-Mar; 17-Apr	24-Oct; 21-Nov; 15-Mar; 12-Apr
Combined Soil Spray¶	7.2b/L water; 45mL/treatment	N/A	20-Sep	27-Sep

† Cow manure, BD preparations 502-507, rock basalt, egg shells

¶ Horn manure, barrel compost, winter horn clay, fermented equisetum 508

**Table S3 a, b & c:** Treatment program applied to *Vitis vinifera* cvs Chardonnay and Shiraz, Waite Campus field trial; 2010/11a, 2011/12b, 2011/13c

**Table S3 a 2010/11**

System	Code	Treatments†	Treatment Date										
			7-Jan	9 to 11 Jan	12-Jan	21-Jan	4-Feb	9 to 13 Feb	7-Mar	5-Apr			
2010/11													
Control	CON	Water <sup>a</sup>	+				35	+					
Conventional	2	Sulfur <sup>b</sup>	2				4		6				
	3	Quinoxifen <sup>c</sup> Sulfur <sup>b</sup>	2				4		6				
Organic	1	Potassium bicarbonate	4				4		4				
	2	Botanical oil (wetter) <sup>e</sup> Full cream bovine milk (1:10) <sup>f</sup> Seaweed extract <sup>g</sup>	100				100		100				
Biodynamic	1	Sulfur <sup>b</sup>	2				4		6				
	2	Horn silica 501 <sup>h</sup> <i>Equisetum</i> extract 508 <sup>i</sup> Horn silica 501 <sup>h</sup> <i>Equisetum</i> extract 508 <sup>i</sup> Copper hydroxide <sup>n</sup>			0.2							0.2	0.2
All treatments			1.2								320	0.2	0.2

†All treatments received copper hydroxide for downy mildew

<sup>a</sup> Water applied to run off; + treatment applied

<sup>b</sup> Sulfur applied as a wettable powder; g/L

<sup>c</sup> mL/L

<sup>e</sup> Emulsified vegetable oil; mL/L

<sup>f</sup> Pura® full cream milk; mL/L

<sup>g</sup> Seasol®, mL/L

<sup>h</sup> Silicon dioxide solution prepared and applied according to Mackay (2010); g/L

<sup>i</sup> *Equisetum arvense* solution prepared according to Masson (2007) applied according to (Mackay 2010);  
d, j, k, l, n g/L

<sup>m</sup> Approx E-L stage (Coombe 1995) for Chardonnay

**Table S3 b 2011/12**

System 2011/12	Code	Treatments†	Treatment Date																			
			14-Sep	11 to 14-Sep	15-Sep	4-Oct	17-Oct	16 to 19-Oct	20-Oct	31-Oct	4 to 7-Nov	14-Nov	28-Nov	12-Dec	27-Dec	21-Mar	17-Apr					
Conventional	CON	Water <sup>a</sup>	+			+		+		19		+			31		+		35		+	
	CV2	Sulfur <sup>b</sup>	2			3		3				4			4		5			5		5
		Trifloxystrobin <sup>j</sup> Pyraclostrobin <sup>k</sup>				0.15			0.4				0.15									
Organic	CV3	Sulfur <sup>b</sup>	2			3		3				4			4		5			5		5
	OG1	Potassium bicarbonate <sup>d</sup> Botanical oil (wheat) <sup>e</sup>	4 2			4 2		4 2				4 2			4 2		4 2			4 2		4 2
Biodynamic	OG2	Full cream bovine milk (1.10) <sup>f</sup> Seaweed extract <sup>g</sup>	100 10			100 10		100 10				100 15			100 15		100 15			100 15		100 15
	BD1	Sulfur <sup>b</sup> Horn silica 501 <sup>h</sup> Equisetum extract 50g <sup>i</sup>	2			3		3				0.2			0.2		0.2			0.2		0.2
All treatments	BD2	Horn silica 501 <sup>h</sup> Equisetum extract 50g <sup>i</sup>		320						320				320					320			320
		Copper hydroxide <sup>a</sup>	1.2					1.2														

† All treatments received copper hydroxide for downy mildew  
a Water applied to run off, + treatment applied  
b Sulfur applied as a wettable powder; g/L  
c m/L/L  
d Emulsified vegetable oil, m/L/L  
e Full cream milk, m/L/L  
f Purified full cream milk, m/L/L  
g Seacol®; m/L/L  
h Silicon dioxide solution prepared and applied according to Mackay (2010); g/L  
i Equisetum crivense solution prepared according to Masson (2007) applied according to (Mackay 2010); m/L/L  
d, j, k, l, n g/L  
m Approx E-L stage (Coombe 1995) for Chardonnay

**Table S3 c 2012/13**

System	2012/13	Code	Treatments <sup>f</sup>	Treatment Date													
				11-Sep	27-Sep	10-Oct	22-Oct	21 to 24 Oct	25-Oct	2-Nov	3-Dec	3 to 6 Dec	7-Dec	18-Dec	18 to 20 Dec	31-Dec	16-Mar
Control		CON	Water <sup>a</sup>	6	+	+	+	+	+	19	+	+	+	+	35	38	38
Conventional																	
	2	CV2	Sulfur <sup>b</sup> Spiroxamine <sup>c</sup> Trifloxystrobin <sup>d</sup> Pyraostrobil <sup>e</sup> Sulfur <sup>b</sup>	2	2	0.6	1.5		1.5	1.5	3	2	0.4	2	4	3	4
	3	CV3		2	2	2	2	2	2	3	3	3	3	3	4	3	4
Organic																	
	1	OG1	Potassium bicarbonate <sup>d</sup> Botanical oil (vetiver) <sup>e</sup>	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	2	OG2	Full cream bovine milk (1:10) <sup>f</sup> Seaweed extracts <sup>e</sup>	100	100	100	100	100	100	100	100	100	100	100	100	100	15
Bio-dynamic																	
	1	BD1	Sulfur <sup>b</sup> Horn silica 501 <sup>h</sup> Equisetum extract 508 <sup>j</sup>	2	2	2	2	2	2	0.2	3	2	2	0.2	4	3	4
	2	BD2	Horn silica 501 <sup>h</sup> Equisetum extract 508 <sup>j</sup> Copper hydroxide <sup>k</sup>	1.2				320	320	0.2	320	320	320	0.2	320	320	0.2

<sup>†</sup> All treatments received copper hydroxide for downy mildew  
<sup>a</sup> Water applied to run off; + treatment applied  
<sup>b</sup> Sulfur applied as a wettable powder, g/L  
<sup>c</sup> mL/L  
<sup>d</sup> Emulsified vegetable oil, mL/L  
<sup>e</sup> Pure<sup>®</sup> full cream milk, mL/L  
<sup>f</sup> All treatments received copper hydroxide for downy mildew  
<sup>g</sup> Sasol<sup>®</sup>, mL/L  
<sup>h</sup> Silicon dioxide solution prepared and applied according to Mackay (2010), g/L  
<sup>i</sup> Equisetum arvense solution prepared according to Masson (2007) applied according to (Mackay 2010); mL/L  
<sup>j</sup> d, j, k, l, n g/L  
<sup>m</sup> Approx E-L stage (Coombe 1995) for Chardonnay



**Table S4.** Treatment program applied to *Vitis vinifera* cvs Chardonnay and Shiraz, Waite Campus growth room trial: winter 2012

System	Code	Treatments	Treatment date								
			(Shiraz only)								
			Bud burst								
			13-Jul	27-Jul	10-Aug	26-Aug	10-Sep	25-Sep	10-Oct	24-Oct	7-Nov
Control	H <sub>2</sub> O	Water		+	+	+	+	+	+	+	+
Conventional	CV	Spiroxamine <sup>b</sup>		6	6	6					
		Trifloxystrobin <sup>c</sup>					0.15	0.15			
		Quinoxifen <sup>d</sup>							0.2	0.2	
Plant Extract <sup>†</sup>	Yar	Yarrow <sup>e</sup>	100	100	100	100	100	100	100	100	100
	Net	Nettle <sup>f</sup>	142	142	142	142	142	142	142	142	142
	Equ	<i>Equisetum</i> <sup>g</sup>	313	313	313	313	313	313	313	313	313
	Com	Combined <sup>h</sup>	555	555	555	555	555	555	555	555	555

<sup>†</sup> Plant extracts prepared and applied according to Masson (2007)

a Water applied to run off; + treatment applied

b,d,e,f,g mL/L

c g/L

**Chapter 4. Prepared Manuscript: Biodynamic and Organic vineyard management, a comparison of the control of powdery mildew and effects on selected vine growth measures in Cabernet Sauvignon**

Prepared manuscript for Vitis



# **Biodynamic and Organic vineyard management control of powdery mildew in Cabernet Sauvignon**

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## **Summary**

**Little research on fungal disease management in biodynamic and organic viticulture has been documented. Although anecdotal claims suggest that these alternative management systems create healthier soils which in turn foster and promote plants that possess natural resistance to fungal diseases. However, in the absence of clear scientific evidence, scepticism remains amongst many growers as to the potential benefits that the alternative inputs may offer.**

**We compared biodynamic and organic with conventional management strategies in terms of their control of powdery mildew in Cabernet Sauvignon grown in McLaren Vale, Australia. Powdery mildew and vine physiological measures were assessed in 2011, 2012 and 2013. Powdery mildew was controlled by some biodynamic and organic inputs. Generally, biodynamic and organic vines were smaller and less productive than those managed conventionally. Results of this study suggest that biodynamic treatments may have potential for inclusion in disease management.**

Abbreviations: LIC - Low Input Conventional; HIC - High Input Conventional;

OG - Organic; BD - Biodynamic

Key words: biodynamic, organic, management systems, disease management, vine physiology, Cabernet Sauvignon

## **Introduction**

Powdery mildew in grapevines presents potential for significant crop loss and reduction in wine quality (GADOURY *et al.* 2001). To control fungal diseases, synthetic fungicides and sulfur are used routinely. However, several factors complicate management strategies and increasingly growers seek other options for the management of powdery mildew. The powdery mildew fungus has shown resistance to synthetic fungicides (SAVOCCHIA *et al.* 2004). These products can also have a negative effect on predatory insect populations (PRISCHMANN *et al.* 2005). Consumer demand for pesticide-free wines continues to grow (BARBER *et al.* 2009) and the effects of chemicals on vineyard worker safety continue to be researched as health concerns are raised (COLOSIO *et al.* 2003).

In Australia a maximum threshold of 3-5% severity of powdery mildew on fruit is considered acceptable for winemaking (VITI- NOTES 2005). The annual cost of all disease control to the Australian wine industry is approximately AUD \$128 million, which does not include loss of revenue and profitability for growers (SCHOLEFIELD and MORISON 2010). Practices that manipulate the canopy microclimate and allow for better penetration of fungicides are well understood. These include varietal susceptibility and vigour, site selection soil, trellis design, irrigation and understanding disease epidemiology.

Certified organic (OG) and biodynamic (BD) growers must use organically certified inputs, as synthetic chemicals are prohibited, although sulfur is allowed (DEPARTMENT OF AGRICULTURE 2013). OG/BD growers can use alternative inputs such as plant extracts or

compost teas (DEPARTMENT OF AGRICULTURE 2013). In BD systems it is suggested that disease control can be achieved by strengthening plants against disease through the use of two prescribed preparations. Preparation 501 (ground quartz silica) is applied as an aerial spray and preparation 508 (a plant extract of *Equisetum arvense*) and also high in silica is applied as a foliar spray (STEINER 1924, PROCTOR and COLE 1997).

A luxuriant supply of nutrients and water will result in rank vegetative growth (MENGEL AND KIRBY 2001), which is conducive to the development of fungal diseases (KELLER *et al.* 2003). Synthetic fertilisers which provide an ready supply of mineralised nutrients are banned in OG/BD systems. Instead, a strong emphasis is placed upon the nurturing of soil health, structure and fertility through the use of composts, plant cover, and inter-row and under vine crops and mulches (WEBSTER 2003). In organic systems the supply of nutrients is ideally provided through the natural cycling of a range of organic material (BUCKERFIELD AND WEBSTER, 2001). The supply of nutrients is therefore more regulated and as such can reduce vine productivity (MENGEL AND KIRBY 2001). In BD systems this process is reportedly enhanced by the incorporation of the preparation 500 (cow horn manure) and compost preparations 502-507. These are considered essential inputs for natural nutrient cycling and building soil biology (STEINER 1924,).

The aim of this study was to compare the effect of BD and OG with conventional management systems on the control of powdery mildew in relation to vine growth in a commercial vineyard.

## **Materials and Methods**

Experimental site: This study was conducted at a trial site established in 2008 in McLaren Vale, South Australia, in a 9.3 ha block of own rooted cv. Cabernet Sauvignon (*V. vinifera*) vines planted in 1989. Vines were trained to a two tiered bilateral cordon spaced at 2 m between vines and 3 m between rows (1,666 vines/ha). Vines were spur pruned using a

mechanical pre-pruner, followed by a manual clean up pass, leaving approximately 2-4 nodes/spur. Yields ranged between 10 t/ha and 15 t/ha in previous years (2006-2008). Bore water (EC 1200 to 1800 ppm) was used for irrigation.

Experimental design and treatments: Four treatments, organic; **OG**, biodynamic; **BD**, low input conventional; **LIC** (the control) and high input conventional; **HIC** were defined by a steering committee in September 2008<sup>1</sup> (Table 1). Due to the commercial status of the vineyard, it was not possible to establish a water treated control; instead the LIC treatment was considered as the treatment most likely to develop powdery mildew. All treatments also received applications of copper hydroxide for the control of downy mildew. Each treatment was sub-divided into 'with compost' or 'without compost' (Table 2). Four 0.4 ha blocks were established, containing each of the four treatment systems, one row 'with' and one row 'without' compost in each block, as a randomised split-plot design. To avoid cross contamination between treatments the sample vines were located in the middle two rows of each treatment block, separated by one row, thus creating a buffer zone (Appendix 6). Data were collected over a three year period from 2011 to 2013. All measures were collected from the 6 tagged sample vines from each replicate over all seasons.

Powdery mildew inoculation and assessment: The trial site was exposed to natural wind-dispersed inoculum. Powdery mildew severity was assessed non-destructively on 60 leaves vines per replicate. Leaves were tagged for repeated observation each season. Disease severity was assessed on both the abaxial and adaxial surfaces and combined to be averaged using a 0-10 scale where 0=no obvious disease; 1=1-10% of leaf area covered by sporulating *E. necator*; 2=11-20%; 3=21-30%; 4=31-40%; 5=41-50% (CRISP *et al.* 2006). Twenty five tagged bunches on the same sample vines, on the top cordon, were examined for powdery mildew using the same scale as for leaves.

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<sup>1</sup> Committee consisted of: Dr Cassandra Collins, Dr Petra Marschner, Mr Chris Penfold and Tim Marshall (Organic consultant), Anton Van Klopper (Biodynamic practitioner), David Hansen (Treasury Wine Estates) and Troy Elliker (Gemtree Vineyards).

Vine growth and nutrition measurements: Over three seasons (2011-2013) canopy density was determined prior to veraison by using the Mark IV Vineyard Scorecard (SMART and ROBINSON 1991). Pruning mass, cane length and mean cane mass were recorded at pruning. Harvest and mean bunch mass and mean bunch number were recorded at harvest. From this data, a pruning mass to fruit mass ratio was calculated as an indicator of vine balance (SMART and ROBINSON 1991). Petiole samples were collected from all treatments at E-L 23 (50% caps off; full flowering; COOMBE 1995). The concentration of potassium, phosphorus, iron, manganese, boron, copper, magnesium and sodium was determined using nitric acid and hydrogen peroxide digestion (WHEAL *et al.* 2011). Total nitrogen and nitrate were determined via the Complete Combustion Gas Chromatography method (SEARLE 1984).

Statistical analysis: Analysis of variance (ANOVA) using a split-plot design was used to determine significant differences among treatments for variables measured, using GenStat (Version 14.2; Lawes Agricultural Trust, UK).

## **Results and Discussion**

Powdery mildew severity: In 2010/11 early season severity was low amongst all treatments. Flag shoots were not observed in any of the treatments. Powdery mildew was first observed at E-L 31; berries pea size (Table 2) and severity did not increase at E-L 34. At both pre-veraison time points there were no differences amongst the treatments. At E-L 37 in the wetter than average February (37.8 mm) conditions (BOM 2013), although differences were not statistically significant, severity fluctuated between 0.11 to 0.7 amongst all treatments and was generally higher for compost treatments than compost-free treatments (Table 2). Relative to the LIC treatment, severity was higher in HIC and lower in both the OG and BD treatments. Severity in the LIC control was over twice that of OG and BD. In the treatments without compost, disease was most severe in BD, and HIC was more severe than the LIC control;



least disease occurred in OG. Severity on bunches, when disease was observed, was generally inconsistent amongst treatments throughout the season and was generally well below the 5% level (data not presented).

Cabernet Sauvignon is moderately susceptible to *E. necator* (DOSTER and SCHNATHORST 1985) and, with no significant differences being found amongst treatments, it is difficult to conclude that the treatments alone gave effective early season control. Certainly, where powdery mildew severity on leaves changed at E-L 37, it appeared that in both HIC and LIC with compost, a commercially acceptable level of control (below 5% severity) was not achieved (Table 2). However as severity on bunches was low it is possible that as berries matured powdery mildew further infection of pea-sized berries was most likely prevented because they are resistant to infection (DOSTER and SCHNATHORST 1985) as by the treatments themselves. In this case too the lower severity on bunches may not be of concern to the grower.

Disease severity on bunches in treatments without compost was similar to those with compost. So whilst severity on leaves was variable it was not reflective of how the disease affected bunches. It can be concluded therefore that there was no difference amongst treatments at controlling disease in 2010/11.

In the remaining two seasons, 2011/12 and 2012/13, when climatic conditions were warmer and drier than 2010/11, powdery mildew was not observed. SAVOCCHIA *et al.* (2011) found that in the warm-dry area of Wagga -Wagga, New South Wales, novel control of powdery mildew on Cabernet Sauvignon resulted in negligible to slight disease over two seasons. This contradicts findings by BOUBALS (1961) and DOSTER and SCHNATHORST (1985), who found Cabernet Sauvignon to be highly susceptible and moderately susceptible respectively. In regions with a warm, dry climate, powdery mildew on Cabernet Sauvignon might be controlled with a reduced concentration or frequency of sulfur applications, perhaps augmented with plant extracts. It is not possible to say, given these results, if the inclusion of

silicon dioxide helped to improve control of disease, as three treatments received the same amount of sulfur.

Vegetative measurements: The results (Table 3) suggest a tendency for OG and BD vines to have a more “open canopy with moderate vigour shoots” (Smart and Robinson 1991), with canopy density scores between 50 and 60, whereas the HIC and LIC canopies were between 40 and 50 suggesting that these vines have a “dense canopy, but with low to moderate vigour shoots” (Smart and Robinson 1992). Although it appears as though the OG/BD vines were smaller and less productive than the conventionally managed vines, the yield to pruning ratio (Y/P) suggests the vines in these treatments are generally better balanced, with Y/P ranging from 6 to 8 across the first two seasons. In 2011/12 HIC and LIC treatments the scores of 4 suggested excessive vigour, whereas the scores for 2010/11 indicated that vines were in balance. In 2012/13, Y/P was 2 for all treatments, suggesting that no treatment achieved a balanced canopy (Smart and Robinson 1992). The differences seem marginal; however with the exception of the results in 2012/13, the values achieved were at least above 3 suggesting that in reality all treatments were well balanced. Reeve *et al.* (2005) also found that OG and BD vines in their trial achieved optimal balance, but no comparison with conventionally grown vines was made.

Throughout the trial, canopies in the HIC and LIC control were generally larger and denser than the BD/OG treatments (Table 3) but with the exception of Canopy Density, there were no significant differences amongst the treatments in any measure of any year. Mean pruning mass and mean cane length were trended higher in both the HIC and LIC treatments with compost. In 2012-13 a similar trend was observed amongst all treatments with compost. Also in 2012-13, the canopy density measure for the BD treatment in particular was higher than the other treatments, indicating a less dense canopy. There were no differences in harvest mass at any point of the trial indicating no differences in fruitfulness. Double cordon canopies tend to have higher levels of vegetative and reproductive efficiency than single cordon

canopies (REYNOLDS *et al.* 2009). In this case, it is likely that in the established high capacity vines, the well exposed leaf area and the number of shoots per vine contributed to these results. Without any significant differences, no conclusions can be suggested.

Nutrition: Fertilisers were not applied during this trial. Results from petiole analysis revealed that while some significant differences existed amongst treatments in both years, all nutrients were at a concentration considered adequate or above (data not presented) (KRISTIC *et al.* 2003). There was no clear pattern and, given that nutrient concentrations were adequate, these differences may not be biologically significant. Worthy of note are the high concentrations of Cu (18 – 96 mg/kg; adequate: >6mg/kg) in all treatments in both years and the low levels of Fe (21-29 mg/kg; adequate: 70 mg/kg). For Cu this is most likely a result of copper sprays for protection against downy mildew (Table 1). Fe deficiency symptoms were not observed. Fe is involved in converting nitrate into forms of plant accessible nitrogen (TREEBY *et al.* 2004) but, given the adequate N (0.8-0.9%; adequate; 0.8-1.1%) status of all treatments, the low concentration of Fe did not seem to be a limiting factor. Differences in canopy density and structure may therefore be a result of increased under vine competition by weeds for soil moisture and nutrients in the OG and BD treatments, as observed by TESIC *et al.* (2007).

## **Conclusion**

This study is the first to compare the effect of BD and OG inputs with conventional management in the control of powdery mildew in a commercial vineyard. It would appear that when the season is dry and moderately warm, control of powdery mildew with OG and BD systems is possible in moderately susceptible cultivars such as Cabernet Sauvignon. When conditions were wet and humid all management systems were put to the test. A trial that compares powdery mildew in cultivars with varying susceptibility at several locations over a number of years is required to confirm the efficacy of BD and OG treatments. In terms of an

effect on vine growth the results were inconclusive. It is not possible to say that the differences were significant for the size of the canopy to influence disease control. In terms of productivity, OG/BD vines in this trial appeared more balanced than conventionally managed vines. A longer term trial would allow this trend in vine growth to be examined further. Given the same nutritional status of the vines amongst treatments with and without compost, it is possible to speculate that differences in vine canopy density may result from competition for water by weeds in the OG/BD treatments.

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Table 1  
Treatment program applied to *Vitis vinifera* cv Cabernet Sauvignon, McLaren Flat field trial; 2010 to 2013

Management component	Inputs	Treatments/Frequency			
		HIC	LIC	OG	BD <sup>x</sup>
Soil					
	Herbicide				
	Pre-emergent (Simazine ®)	1x September			
	Knock-down (Basta ®; Round-up®)	1x November 1x April	1x September 1x November 1x April		
	Under vine cultivation			1x October 1x November 1x March	1x October 1x November 1x March
	Compost additions				
	<sup>v</sup> Nitra-fines®	1x Spring	1x Spring	1x Spring	1x Spring
	BD <sup>w</sup> Soil Spray				
	Horn Manure '500'				2x Spring <sup>y</sup>
	Manure concentrate <sup>x</sup>				2x Autumn <sup>y</sup>
Canopy					
	Fungicides				
	Wettable Sulfur - in rotation	10-14 days			
	DMI - in rotation	10-14 days			
	Strobilurins	E-L 19			
	Wettable Sulphur		10-14 days	10-14 days	10-14 days
	Copper Hydroxide <sup>t</sup> - All treatments	E-L 12 and 19			
	Foliar additives				
	Seaweed extract <sup>u</sup>			1x October 1x November	
	BD <sup>w</sup> Aerial spray				
	Horn silica <sup>xz</sup> '501'				2x Spring <sup>y</sup> 2x Autumn <sup>y</sup>

t All treatments received copper hydroxide for the prevention of downy mildew

u Seaweed extract registered as Seasol® (Seasol International®)

v Compost is a registered (NASSA) organic product (predominantly green waste) applied to one row in each treatment

w All biodynamic additions were prepared and applied in accordance with instructions from Mackay (2010)

x Manure concentrate included BD preparation 502-507

y Timing of BD preparations application based on calendar dates as specified by Keats, (2011, 2012 and 2013)

z Ground silicon dioxide

Table 2

Effect of biodynamic (BD), high input conventional (HIC), low input conventional (LIC) and organic (OG) treatments, with (+) and without (-) compost, on severity of powdery mildew on leaves in Cabernet Sauvignon; McLaren Vale SA 2010-11

Assessment Date	Compost	Treatments				P value (TxC) <sup>z</sup>	5% LSD <sup>z</sup> (TxC)
		LIC <sup>y</sup>	BD <sup>y</sup>	OG <sup>y</sup>	HIC <sup>y</sup>		
18-Dec. E-L 31	+	0.02	0.02	0.02	0.00	ns	ns
	-	0.00	0.00	0.00	0.02		
19-Jan. E-L 34	+	0.02	0.03	0.03	0.01	ns	ns
	-	0.00	0.00	0.00	0.00		
21-Feb. E-L 37	+	0.55	0.11	0.26	0.70	ns	ns
	-	0.37	0.70	0.13	0.53		

<sup>z</sup> For all treatments and cultivars, each value represents the mean of four replicates (24 vines total) from each treatment. The 5% Least Significant Difference (LSD) values listed are for comparison of treatments (T), cultivars (C) and their interaction (CxT). Means within a row sharing a letter are not significantly different at P=0.05 differences. ns, not significantly different at P=0.05 level.

<sup>y</sup> **LIC**: S+Cu, occasional herbicide; **BD**: S+Cu, 500+501, no herbicide, with cultivation; **OG**: S+Cu, no herbicide, with cultivation; **HIC**: S+conventional fungicides and herbicides

Severity score for visual assessment of leaf area affected by colonies of powdery mildew; 0; 0, 1-10%=1; 11-20%=2; 21-30%=3; 31-51-60%=6; 61-70%; 71-80%=8; 81-90%= 9; 91-100%=10



Table 3

The effect of biodynamic (BD), high input conventional (HIC), low input conventional (LIC) and organic (OG) treatments, with (+) and without (-) compost, on physiological measures in Cabernet Sauvignon, McLaren Vale SA

Variable	Season	Compost	Treatments				Compost			
			LIC <sup>w</sup>	BD <sup>w</sup>	OG <sup>w</sup>	HIC <sup>w</sup>	Mean	P value (TxC)	5% LSD <sup>y</sup> (TxC)	
Canopy Density <sup>x</sup>	2010-11	+	49.75a	58.75b	61.50b	47.62a	54.41	0.45	6.38	
		-	50.94a	59.44b	57.50b	49.25a	54.28			
	2011-12	+	55.12b	54.12b	57.50b	45.75a	53.12	0.19	6.69	
		-	49.50ab	55.12b	54.62ab	48.38a	51.91			
	2012-13	+	42.62ab	52.25c	45.50b	38.25a	44.66	0.05	5.96	
		-	47.12	50.00	46.38	50.00	48.38			
	Mean Pruning Mass (kg)	2010-11	+	1.0	1.0	0.8	1.0	1.0	ns	ns
			-	0.9	0.9	0.8	0.9	0.9		
2011-12		+	2.0	1.3	1.2	2.1	1.6	ns	ns	
		-	1.7	1.2	1.3	1.9	1.5			
2012-13		+	2.0	1.5	1.5	2.1	1.7	ns	ns	
		-	1.6	1.4	1.4	1.7	1.5			
Mean Cane Length (cm)		2010-11	+	75	74	69	71	72.0	ns	ns
			-	69	68	67	68	68.0		
	2011-12	+	112	98	93	114	104.0	ns	ns	
		-	103	93	91	111	100.0			
	2012-13	+	35	31	35	38	34.0	ns	ns	
		-	34	32	32	34	33.0			
	Mean Harvest Mass (kg)	2010-11	+	6.29	6.14	5.32	7.17	6.23	ns	ns
			-	5.15	6.37	6.14	6.39	6.01		
2011-12		+	8	6.04	6.51	7.69	7.06	ns	ns	
		-	7.38	6.74	6.73	8.91	7.44			
2012-13		+	3.08	2.87	3.05	3.09	3.02	ns	ns	
		-	2.91	2.53	3.04	2.95	2.86			
Mean Y/P Ratio <sup>z</sup>		2010-11	+	6	7	7	7	6.9	ns	ns
			-	6	7	8	7	7.2		
	2011-12	+	4	6	6	4	4.8	ns	ns	
		-	5	6	6	5	5.2			
	2012-13	+	2	2	2	2	2	ns	ns	
		-	2	2	2	2	2			

w LIC: S+Cu, occasional herbicide; BD: S+Cu, 500+501, no herbicide, with cultivation; OG: S+Cu, no herbicide, with cultivation; HIC: S+conventional fungicides and herbicides

x Canopy density based on Mark IV Vineyard Scorecard by visual assessment. Each value represents the mean of the canopy density point score across four blocks where 20 points = dense canopy/high vigour, -50 points = dense canopy/low to moderate vigour, 75-80 points = open/moderate canopy (Smart and Robinson 1991)

y For all treatments and cultivars, each value represents the mean of four replicates (24 vines total) from each treatment. The 5% Least Significant Difference (LSD) values listed are for comparison of treatments (T), cultivars (C) and their interaction (CxT). Means within a row sharing a letter are not significantly different at P=0.05 differences. ns, not significantly different at P=0.05 level.

z Y/P Ratio: Values <3 indicate excessive vigour, values 5-10 indicate a balanced vine, values >12 indicate low vigour (Smart and Robinson 1991)

## **Chapter 5. Botrytis bunch rot**

### **5.1 Introduction**

The disease, Botrytis bunch rot, caused by *Botrytis cinerea*, is ubiquitous and has the potential to cause devastating crop losses in *Vitis vinifera* (Emmett, et al. 1992). Control of the disease is usually achieved through a combination of conventional cultural practices which are well understood (Jacometti, et al. 2010). The history, significance, epidemiology and control of Botrytis bunch rot are presented in Chapter 2; Literature Review. Please refer to 2.2.1 Botrytis Bunch Rot.

The aim of this study was to compare the efficacy of BD inputs with OG and CV inputs for the control of Botrytis bunch rot on three cultivars; Shiraz, Chardonnay and Cabernet Sauvignon (*Vitis vinifera* L.).

### **5.2 Materials and Methods**

A field and growth room trial (Appendix 4 and 5 respectively) at the Waite Campus were used to compare BD inputs with a water control and management systems utilising synthetic fungicides and OG alternatives for the management of Botrytis diseases (in combination with the materials and methods in Chapter 3). The efficacy of BD and OG inputs compared with HIC and the LIC control (Appendix 6) in limiting Botrytis rots was tested at the trial site at McLaren Vale (in combination with the materials and methods in Chapter 4).

In all trials, treatments were applied to grapevines in conjunction with treatments for powdery mildew. All protectant treatments against Botrytis were applied at E-L 23-25 (50-80% flowering) to prevent latent infection. All treatments were applied in accordance with the suggested protocols for each system as directed by the labels, for conventional and organic inputs and suggested methodologies for BD (Mackay, 2010). The results presented and discussed here, relate to findings from all three trial sites; Waite Campus field site and growth room trial, and the McLaren Vale site.

### **5.2.1 Waite Campus field trial**

No inputs were used in the BD treatments additional to those already referred to for use against general fungal disease; that is 501 (silicon dioxide) and 508 (*Equisetum* extract). In OG1 an accepted and registered commercial alternative fungicide, K salts of fatty acids, was applied (Purtich, et al. 1981, Calvo-Garrido et al. 2014). In OG2 no additional inputs were used beyond milk and seaweed extract. In CV1 and CV2 conventional systemic fungicides (Table 5.1) were used to control Botrytis bunch rot (Appendix 4); the difference between them being treatments for powdery mildew. The chemicals selected for the CV treatments were industry standards for control of each disease (Bell and Essling 2011, Essling and Francis 2012). Treatments were applied at 14-20 day intervals from 2 weeks after budburst, except in 2010/11, when treatment did not begin until January (Chapter 3).

Methods for application of treatments and assessment of spray coverage were as described in Chapter 3.

### **5.2.2 Waite Campus growth room trial**

No inputs were used in the BD treatments additional to those already referred to for use against powdery mildew; that is Yarrow, Nettle, and *Equisetum* individually and combined and were tested against a water control (Appendix 5). The CV fungicide cyprodinil+fludioxonil and iprodione for the control of Botrytis was applied in accordance with the recommended label instructions (Table 5.2). Treatments were randomly allocated to three vines within each of four blocks. Vines were removed from the growth room for treatment to avoid cross-contamination. One litre hand-held spray bottles were used for each treatment and leaves were sprayed to the point of fine droplets on both surfaces.

### **5.2.3 McLaren Vale trial**

In McLaren Vale, South Australia, a 9.3 ha block of own rooted cv. Cabernet Sauvignon (*V. vinifera*) vines planted in a 20 year old commercial vineyard was used as the experimental site (Chapter 4). As such, the program utilised in the LIC, OG and BD treatments reflected the company's approach to

disease management and satisfied their logistical requirements for application. The four treatments organic; OG, biodynamic; BD, low input conventional; LIC (the control) and high input conventional; HIC, were established in four 0.4 ha blocks; containing one replicate each of the four treatment systems, 'with' and 'without' compost as a randomised split-plot design (Appendix 6). Copper hydroxide and a strobilurin (Flint®:conventional fungicide) treatment was applied in the HIC program for protection against powdery mildew. No other conventional chemicals were used in the other programs (Table 5-3). Seasol® (a NASSA certified organic seaweed solution, Seasol International® Pty Ltd, Australia) was included in the OG program. To avoid cross contamination from other treatments the sample vines were located in the middle two rows of each treatment block with a middle row separating compost from no-compost treatments, thus creating a buffer zone.

#### **5.2.4 Botrytis inoculation and assessment**

Botrytis severity was assessed using a detached bunch assay system recommended by Trevor Wicks (2011 Pers Comm). Individually tagged inflorescences (E-L 25) were sprayed with a suspension of conidia of *B. cinerea* ( $3.6 \times 10^7$  conidia/mL sterile distilled water) using a 1-L pressure sprayer (Hills®, Australia). Clusters were covered overnight with plastic bags containing 2 mL of sterile reverse osmosis water. This methodology promotes establishment of latent infection by encouraging the germination of conidia. In the Waite Campus field trial a total of 30 bunches per treatment was inoculated, 10 bunches per treatment replicate. All tagged fruit in the field trial was harvested at 20 °Brix for Chardonnay and 21 °Brix for Shiraz. Brix levels above this for moist incubation can encourage secondary fungi, bacteria and yeast when exposed to artificially high humidity and may overpower the Botrytis, reducing the chance of an accurate indication of infection (Barbara Hall. Pers. Comm. 2013). In the growth room one bunch per vine, where present, was inoculated as above and all bunches were harvested from both cultivars at the same time due to severe powdery mildew. In the McLaren Vale trial, a total of 80 bunches were inoculated per treatment (20 bunches per treatment replicate) in 2011/12 and again in 2012/13. Treated bunches were harvested a day prior to the commercial harvest. In all trials, bunches were sealed in individual paper bags for transport. In both field trials, bunches were stored in cool boxes with ice for

transport then stored overnight in the Waite Campus winery cool room at approximately 0°C. All bunches were then moist-incubated for 7-10 days to induce sporulation. A scoring system similar to that used for powdery mildew on bunches was used to assess disease severity (Crisp, et al. 2006b). Given that the industry accepted maximum standard is a 5% level of severity (Viti-notes 2005) a mean severity score under 0.5 would indicate successful control.

### **5.2.5 Statistical analysis**

Analysis of variance (ANOVA) using a split-plot design was used to determine significant differences among treatments for variables measured. This was performed using GenStat (Version 14.2; Lawes Agricultural Trust, UK).

## **5.3 Results and discussion**

### **5.3.1 Botrytis bunch rot severity on bunches: Waite Campus Field Trial.**

In Chardonnay there were no significant differences in severity of Botrytis bunch rot in the fruit collected and moist-incubated in the 2012/13 growing season. As expected, mean disease severity, at  $\geq 5\%$ , was highest in the water control (Table 5.4). When compared with the water control however, all treatments resulted in disease under 5%, whereas in Shiraz disease severity was at 5.5%. This level of efficacy is consistent with results from trials with similar products in grapevines (Purtich, et al. 1981, Calvo-Garrido, et al. 2014) and the product used is an accepted industry input (Essling and Francis 2012). Severity in OG2 (0.16) and BD 1 (0.22) showed to be 50% more severe than CV1 and CV2. However, as there were no significant differences amongst treatments, it is difficult to draw any definitive conclusions.

Why this might be so is not clear. Both CV1 and CV2 received established Botrytis fungicide applications at flowering yet severity in Chardonnay was at the upper end of acceptable (5%). However, for the control of Botrytis both of the BD treatments received applications of 501 and 508 in addition to the BD preparations added to the compost, which in BD2 was not entirely sufficient to provide effective

control of powdery mildew in BD2 (Chapter 3: Results and Discussion), less so for the treatments applied to OG2. In Shiraz, the water control also exhibited unacceptably high level of disease severity following moist incubation (0.54). In contrast to the results in Chardonnay, OG1 exhibited poor disease control. Water sensitive paper in the canopy indicated poor coverage on the internal leaves, which may indicate reduced coverage over bunches. Amongst the remaining treatments mean potential severity of Botrytis bunch rot was kept below 0.3, indicating an acceptable level of control.

When compared with the water control, disease was limited by all treatments including the *Equisetum* plant extracts and silicon dioxide of both BD treatments in Chardonnay and Shiraz, while K salts of fatty acids have previously been shown to effectively control Botrytis bunch rot (Purtich, et al. 1981, Calvo-Garrido et al. 2014). Jacometti, et al. (2010) reviewed several plant extracts that exhibit antifungal properties, for example, research on the product Milsana® (a formulated extract of the plant *Reynoutria sachalinensis*, high in silica similar to *Equisetum*) demonstrated effective control of Botrytis in grapes (Schilder, et al. 2002), strawberries (Carlen, et al. 2004) and cucumber (Konstantini-dou Doltsnis, et al. 2002). Furthermore Jacometti, et al. (2010) reported varying levels of disease suppression by several investigators when targeting Botrytis with plant extracts in laboratory and field conditions. For example Gyung, et al. (2004) reported effective control of Botrytis germination and growth of mycelium on tomatoes by using extracts of *Chloranthus japonicas* (Chloranthus), *Helianthus tuberosus* (Jerusalem artichoke) and *Patrinia scabiosaefolia* (Golden Lace Patrinia) amongst others. However, it is just as likely that the less dense canopies of the OG/BD treatments allowed sufficient interception of light and air at flowering to inhibit the germination of conidia after inoculation. An adequate level of disease control with BD inputs, therefore, probably lies in an appropriate balance of nutrition and effective canopy management (Savage and Sall 1984, Gubler, et al. 1987) augmented with plant extracts. However where the treatments resulted in a disease severity score less than the threshold, we would expect the mean to be significantly lower than the water control. In this case it is not and can probably be explained by a large variation in the individual severity scores.

### **5.3.2 Botrytis bunch rot severity on bunches: Growth Room Trial.**

Chardonnay and Shiraz bunches were harvested on October 23 and November, 5 respectively. After moist incubation, mean severity score was > 1.5 across all treatments in both cultivars. Mean severity score of Botrytis bunch rot on Chardonnay and Shiraz fluctuated from 1.7 and 1.5 (conventional fungicides) to 4.8 and 6.7 (yarrow extract), respectively (Table 5.5). This finding indicates a failure of all treatments to control Botrytis bunch rot when compared with the water control. Also measurements of total soluble solids (Brix°) were not possible to predict the optimal harvest time. As such it could not be determined if the brix level was higher than optimal for moist incubation, which, if so, may have contributed to disease development. Some colonies of *Aspergillus* and *Penicillium* were observed, which also suggests sugar levels may have been too high. If the experiment were to be repeated, more vines would be required to produce sufficient bunches. It is also possible that the humid environment in the growth room was ideal for the establishment of latent infection at flowering. The fact that botrytis was present in treatments that included conventional fungicides, known to be effective against Botrytis, seems to suggest so. However this would need to be confirmed with further testing.

### **5.3.3 Botrytis bunch rot severity on bunches: McLaren Vale Trial**

The results for Botrytis bunch rot indicate that, with the exception of HIC and OG without compost in 2011/12 and ORG with compost in 2012/13, all treatments exceeded the threshold for acceptable botrytis severity (Table 5.6). Where botrytis was present, in 2011/12, both HIC and OG were not significantly different from LIC and BD. Research on effects of soil-applied compost on plant diseases is rare and what does exist (Jacometti, et al 2007) illustrates that the effects and mechanisms are not well understood (Webster, 2007). Webster (2007) reviewed much of the available literature and pointed out that results from tests of compost materials across a broad range of crops were highly variable. Indeed most literature focuses on the effect of compost on soil borne pathogens and arthropods (Hoitink and Fahy 1985, Powell, et al 2007), or on the effect compost teas when applied as foliar sprays (Elad and Shtienberg 1994, Scheuerell and Mahaffee 2006, Hargreaves, et al. 2009).

The results in this study suggest no discernible difference in efficacy, regardless of treatment, with or without compost, and that this portion of the trial was largely inconclusive. For example, it is not obvious if the bunches of Cabernet in the less dense canopies of the OG/BD treatments had greater exposure to sunlight thereby reducing infection resulting from the simulated latent infection. The results presented here would suggest not. Although the generally loose nature of bunch architecture in Cabernet Sauvignon does not normally predispose the cultivar to severe outbreaks of Botrytis in the field, berries in test conditions have been shown to be susceptible to Botrytis (Vail and Marois 1991). The artificial environment of moist incubation therefore may not be appropriate to test cultivars that in a natural setting would not normally come under such pressure. Also, in the normally dry and warm summers of McLaren Vale, Botrytis protectants may not be necessary for well-managed, weakly susceptible cultivars such as Cabernet Sauvignon.

#### **5.4 Summary**

In this study the additional protectant treatments on both OG and BD systems in the Waite field trial performed no differently to the CV treatments in controlling botrytis severity. In the growth room and McLaren Vale trials, the °Brix level may have been excessive for the moist incubation process, causing a higher than acceptable bunch rot severity. Cultivars with loose bunch structure and or thick skins are generally less susceptible to Botrytis, such as Shiraz (Emmett, et al 1992) and Cabernet Sauvignon (Vail and Marois 1991) in the field. However the methodologies described in both the growth room and McLaren Vale trials may have created an artificial environment not suitable for tests of this nature. Plant extracts have been shown to suppress Botrytis in grapes and other crops, and Equisetum may have a role to play in well managed vineyards. Application of compost to vines did not affect subsequent development of Botrytis bunch rot.



**Table 5-1:** Treatment program applied to *Vitis vinifera* cvs Chardonnay and Shiraz, Waite Campus field trial; 2010-2011.

System	Target	Treatment	Treatment dates									
			21 to 24 Oct	25-Oct	2-Nov	3 to 6 Dec	7-Dec	18-Dec	18 to 20 Dec	31-Dec	16-Mar	12-Apr
2012/13												
Control		Water <sup>a</sup>	+		+	+			+		+	
Conventional <sup>h</sup>												
1	Botrytis	Cyprodil+Fludioxonil <sup>b</sup>			0.8							
2	Botrytis	Cyprodil+Fludioxonil <sup>b</sup>			0.8							
Organic												
1	Botrytis	K salts of fatty acids <sup>c</sup>			40				40			
		Botanical oil (wetter) <sup>d</sup>			2				2			
2		Seaweed extract <sup>e</sup>			10				1		15	
Biodynamic												
1&2	General fungal conditions	Horn silica 501 <sup>f</sup>		0.2				0.2				0.2
		<i>Equisetum</i> extract 508 <sup>g</sup>	320				320			320	320	

a Water applied to run off; + treatment applied

b Switch®; mL/L

c Ecopoprector®; mL/L

d Emulsified vegetable oil; mL/L

e Seasol®; mL/L

f Silicon dioxide solution prepared and applied according to Mackay (2010); g/L

g *Equisetum arvense* solution prepared according to Masson (2007) applied according to (Mackay 2010)

h distinction between treatments: CV1 - sulfur only for powdery mildew; CV2 - sulfur and conventional chemicals in rotation for powdery mildew

**Table 5-2:** Treatment program applied to *Vitis vinifera* cvs Chardonnay and Shiraz, Waite Campus growth room trial: winter 2012.

System	Treatment	Treatment date										Harvest (Chardonnay)	Harvest (Shiraz)
		Bud burst					(Shiraz only)						
		13-Jul	27-Jul	10-Aug	26-Aug	10-Sep	25-Sep	10-Oct	24-Oct	7-Nov	1-Nov	12-Nov	
Control	Water <sup>a</sup>		+	+	+	+	+	+	+	+			
Synthetic	Cyprodil+Fludioxonil <sup>b</sup>				0.8								
	Iprodione <sup>c</sup>							15					
Plant Extract <sup>d</sup>	Yarrow	100	100	100	100	100	100	100	100	100			
	Nettle	142	142	142	142	142	142	142	142	142			
	<i>Equisetum</i>	313	313	313	313	313	313	313	313	313			
	Combined	555	555	555	555	555	555	555	555	555			

a Water applied to run off; + treatment applied

b Switch®; g/L

c Rovral Aquaflo®; mL/L

d Plant extracts Yarrow: *Achillea millefolium* only; Nettle: *Urtica dioica* only; Equisetum: *Equisetum arvense* only; Combined: Yarrow, Nettle and Equisetum; prepared and applied according to Masson (2007); mL/L

**Table 5-3:** Treatment program applied to *Vitis vinifera* cvs Cabernet Sauvignon, McLaren Vale trial 2010-2013.

Management component	Inputs	Treatments/Frequency			
		HIC	LIC	ORG	BD <sup>b</sup>
Soil	Herbicide				
	Pre-emergent (Simazine ®)	1x September			
	Knock-down (Basta ®; Round-up®)	1x November	1x September		
		1x April	1x November		
			1x April		
	Under vine cultivation			1x October	1x October
				1x November	1x November
				1x March	1x March
	Compost additions				
	<sup>a</sup> Nitra-fines®				
	BD <sup>b</sup> Soil Spray				
	Horn Manure '500'				2x Spring <sup>d</sup>
	Manure concentrate <sup>c</sup>				2x Autumn <sup>d</sup>
Canopy	Fungicides				
		Strobilurins	Flowering		
	Foliar additives				
	Compost tea			1x October	
				1x November	
	BD <sup>b</sup> Aerial spray				
	Horn silica <sup>e</sup> '501'				2x Spring <sup>d</sup>
					2x Autumn <sup>d</sup>

a Compost is a registered (NASSA) organic product (predominantly green waste)

b All biodynamic additions were prepared and applied in accordance with instructions from Mackay (2010)

c manure concentrate included BD preparation 502-507

d Timing of BD preparations application based on calendar dates as specified by Keats, (2011, 2012 and 2013)

e Ground silicon dioxide

**Table 5-4:** Effect of biodynamic (BD) and organic (OG) foliar treatments on the mean severity score of Botrytis bunch rot on grape bunches following moist incubation, compared with water (CON) control and conventional inputs (CV). Waite Campus trial 2012/13.

Variable	Cultivar	Treatment							Cultivar		P-Value
		CON	CV2	CV3	OG1	OG2	BD1	BD2	Mean	5% LSD	
2012/2013 Season											
Severity	Chardonnay	0.51	0.07	0.44	0.03	0.16	0.22	0.27	0.267	NS (CxT)	NS (CxT)
	Shiraz	0.54	0.00	0.14	0.55	0.17	0.03	0.28	0.238	NS (C)	NS (C)
	Treatment Mean	0.53	0.04	0.29	0.29	0.16	0.13	0.27		NS (T)	NS (T)

\*Grapevine growth stage based on the Modified E-L system (Coombe 1995)

CON: Water only; CV2: S + Systemic; CV3: S Only; OG1: potassium bicarbonate; OG2: Full cream bovine milk + Seaweed extract; BD1: Biodynamic inputs + S; BD2: Biodynamic inputs only.

For all treatments and cultivars, each value represents the mean of three replicates (nine vines total) from each treatment. The 5% LSD values listed are for comparison of treatments (T), cultivars (C) and their interaction (CxT). Means within a row sharing a letter are not significantly different at P=0.05 differences. ns, not significantly different at P=0.05 level.

Severity score for visual assesment of leaf area affected by colonies of powdery mildew; 0; 0, 1-10%=1; 11-20%=2; 21-30%=3; 31-40%=4; 41-50%=5; 51-60%=6; 61-70%; 71-80%=8; 81-90%= 9; 91-100%=10

**Table 5-5:** Effect of herbal extracts<sup>1</sup>, both individually and combined, on the severity of Botrytis bunch rot on grapevine bunches following moist incubation, cvs Chardonnay and Shiraz, compared with synthetic fungicides and a water control. Growth room trial 2012.

Variable	Variety	Treatment					Cultivar Mean	5% LSD	P-Value	
		H2O	Yarrow	Nettle	Equisetum	Combined				Conventional
<b>Severity</b>										
	Chard	3.16	4.75	3.39	3.04	2.42	1.66	3.07	NS (CxT)	NS (CxT)
	Shiraz	3.13	6.67	5.09	4.40	4.87	1.54	4.28	NS (C)	NS (C)
	Treatment Mean	3.15	5.71	4.24	3.72	3.65	1.60		NS (T)	NS (T)

**H2O:** Water control; **Yarrow:** Plant extract of *Achillea millefolium* only; **Nettle:** Plant extract of *Urtica dioica* only; **Equisetum:** Plant extract of *Equisetum arvense* only; **Combined:** Yarrow, Nettle and Equisetum; **Conventional:** Systemic fungicides only, used in rotation  
 For all treatments and cultivars, each value represents the mean of four replicates (12 vines total) from each treatment. The 5% LSD values listed are for comparison of treatments (T), cultivars (C) and their interaction (CxT). Letters following the mean indicate significant ( $P < 0.05$ ) differences.  
 Severity score for visual assesment of leaf area affected by colonies of powdery mildew; 0; 0, 1-10%=1; 11-20%=2; 21-30%=3; 31-40%=4; 41-50%=5; 51-60%=6; 61-70%; 71-80%=8; 81-90%= 9; 91-100%=10

**Table 5-6:** The effect of Biodynamic (BD), high input conventional (HIC), low input conventional (LIC) and organic (OG) treatments with (+) and without (-) compost, on the severity of Botrytis bunch rot (following moist incubation) in Cabernet Sauvignon, McLaren Vale, SA.

Year	Compost	Treatments				Compost Mean	5% LSD	P-Value
		LIC	BD	HIC	ORG			
2011-2012								
Severity	+	0.60	0.58	0.55	0.53	0.57	NS (TxC)	NS (TxC)
	-	0.55	0.60	0.43	0.48	0.52	NS (C)	NS (C)
	Treatment Mean	0.58	0.59	0.49	0.58		NS (T)	NS (T)
2012/2013								
Severity	+	0.56	0.52	0.74	0.47	0.57	NS (TxC)	NS (TxC)
	-	0.60	0.68	0.65	0.56	0.68	NS (C)	NS (C)
	Treatment Mean	0.58	0.60	0.70	0.52		NS (T)	NS (T)

**LIC:** S+Cu, occasional herbicide (control); **BD:** S+Cu, 500+501, no herbicide, with cultivation

**HIC:** S+Synthetic fungicides and herbicides; **ORG:** S+Cu, no herbicide, with cultivation

For all treatments, each value represents the mean four replicates (24 vines total) from each treatment. The 5% LSD values listed are for comparison of treatments (T), compost (C) and their interaction (CxT). Letters following the mean indicate significant ( $P < 0.05$ ) differences.

Severity score for visual assesment of leaf area affected by colonies of powdery mildew; 0; 0, 1-10%=1; 11-20%=2; 21-30%=3; 31-40%=4;41-50%=5; 51-60%=6; 61-70%; 71-80%=8; 81-90%= 9; 91-100%=10

## Chapter 6: General Discussion

This study is possibly the first to evaluate the potential of BD preparations to control fungal diseases in grapevines and their potential for inclusion in treatment programs. Control of powdery mildew was generally less successful in the susceptible cultivar Chardonnay than in Shiraz and Cabernet Sauvignon which are less susceptible. This response was also observed in the controlled environment of the growth room; where the moist, humid and warm environment presented ideal conditions for disease. Understanding the relationship between cultivar susceptibility and the environment in which it is grown is central to effective disease management in any viticultural enterprise. Regardless of the inputs used, there is no substitute for sensible management practices which are founded on a thorough understanding of disease epidemiology, monitoring, canopy microclimate and manipulation, cultivar susceptibility, environmental factors and the effect of foliar applications.

Fungicide efficacy is a function of coverage, appropriateness of the inputs, correct concentration and timing (Magarey, et al. 1994). Current conventional practices give growers flexible management options. In the cooler months of the early growing season at the Waite Campus, for example, when plant tissue is sensitive, systemic fungicides can be an effective alternative to sulfur (Wicks, et al. 1997). In this study, all treatments were effective for control of powdery mildew prior to flowering (E-L 23). As the season progressed response to inputs varied. Severity of powdery mildew in the CV treatments was generally lowest and the potassium bicarbonate in OG1 generally performed as effectively as the CV treatments. Despite the susceptibility of Chardonnay, in 2011/2012, severity in both BD1 and BD2 ranged between 1.5 and 4 times lower than the water treated control. The applications of *Equisetum* in the field trial may have had some effect on the development of powdery mildew. This concept is reinforced by the results in the growth room trial where *Equisetum*, within the BD treatments demonstrated acceptable levels of control in both cultivars until E-L 31 at which point severity in Chardonnay increased, whilst in Shiraz, severity declined after peaking at a mean of 0.6 (6%). Single plant extract application was not as effective as the combined plant extracts and, as a silicon dioxide solution was not used, any role this input might have cannot be reported.

The successful control of powdery mildew in Cabernet Sauvignon in the McLaren Vale trial indicates that a BD program in a commercial setting may be appropriate for powdery mildew management. However, in this trial there was little to differentiate between the treatments; indeed from a disease control perspective, the LIC, OG and BD treatments were essentially the same. The same rate of sulfur was applied with the same frequency; the only difference being that BD treatments included 500 and 501, but no 508 was used. Whilst in the HIC treatments, sulfur was used interchangeably with conventional fungicides. As there was no water treated control, it is difficult to substantiate claims that any of the management treatments provided superior disease control.

On bunches in the growth room trial only the CV treatment prevented powdery mildew in both cultivars. Severity was lower in the combined plant extract treatments when compared with the control and the individual plant extracts. As this was the only trial in which powdery mildew on bunches was observed, it is not possible to draw any definitive conclusions. However, Masson (2007) claims that it may be possible to reduce the concentration of sulfur requirements by half to control powdery mildew in vineyards when used in conjunction with some combined plant extracts. Findings in the growth room trial support this idea and testing them in the field against applications of only *Equisetum* and varying concentrations of sulfur is recommended. It has been suggested that *Equisetum*, which contains a high concentration of silicon, may protect plants against a diverse range of biotic and abiotic stresses and that the silica content of *Equisetum* and silicon dioxide may induce an immune response in plants (Epstein 1999, Fauteux, et al. 2005). Given the results in the growth room, the effect of *Equisetum* might suggest an immune response mode of action consistent with the observations of Epstein (1999) and Bowen, et al. (1992).

Savocchia, et al. (2011) found only sulfur provided better early season control on the susceptible cultivar Chardonnay compared with alternative inputs in dry growing conditions. This was not the case in this study. Savocchia, et al. (2011) also found that in a wet humid season neither sulfur nor the alternative inputs provided acceptable control; similar to the results in this trial where in 2010-2011 mean severity score of powdery mildew on Chardonnay was 5.6 and on Shiraz 2.27 and did not differ



significantly between the cultivars. Control in this study may have been assisted by climatic conditions in the second and third seasons and effective spray coverage of the developing canopies. Crisp, et al. (2006b) reported that in well managed canopies, potassium bicarbonate can provide control of powdery mildew as effectively as sulfur. However, like milk, potassium bicarbonate has a limited residual effect and successful control is also a function of coverage. This might explain why late season increase in severity was generally higher in the OG/BD treatments. However potassium bicarbonate can be used up until 7 days before harvest as an eradicant, unlike sulfur, which has a restriction of 30 days, and some conventional fungicides cannot be used beyond E-L 25 or E-L 32 (Essling and Francis 2012). Assuming BD plant extracts have no malodorous properties and no negative effect on the fermentation process, there may be potential for late season use if required, which would be useful if there was an effect in reducing the risk of severe botrytis.

As the season progressed, powdery mildew severity fluctuated, within and amongst treatments and cultivars in both field trials in response to climatic conditions. Overall in Chardonnay severity on the adaxial surface decreased between E-L31 and EL35 and increased again by EL 41, whereas on the abaxial surface severity remained relatively consistent at all growth stages. In Shiraz on the adaxial surface severity decreased between E-L 31 and 35 and only increased again at E-L 41 in OG2 and both BD treatments. However in both cases severity was  $\leq 0.5$ .

In 2012/2013 severity was much lower in all treatments in both cultivars, although severity was still higher by E-L 41 in Chardonnay but not in Shiraz. However, severity in both BD treatments was lower than both OG. There are evidently inconsistencies in the results but most of the treatments, with the exception of OG2, controlled disease. The changing dynamic of the canopy and leaf age may be having an effect. In particular the increase in severity later in the season seems to contradict evidence of Doster and Schnathorst (1985) and Ficke, et al. (2003) that infection decreases as plant tissue ages (ontogenic resistance). However the interactions between the environment and vine growth are complex. Turgeon and Webb (1973) describe that as cucurbit leaves age there is a gradual transition within the leaf moving from a carbohydrate sink to a source (Merry et al. 2013). This mechanism coupled with

foliar treatments, canopy size effects on microclimate, evidence that older leaves generally have thicker cell walls, lower concentrations of nutrients and water and increased chemical and physical defences (Coleman 1986), may also go some way towards explaining the fluctuations in severity through all the trials (Calonnec, et al. 2008).

Certainly the varying nature of vegetative measures between treatments in all growing seasons and both field trials supports the proposition that CV-treated vines had generally had canopies with higher density than vines grown in OG and BD systems. Despite the lack of significant differences between treatments for both cultivars in the Waite Campus trial, nutrient availability may have limited vine size and, by extension, created more open canopies of the OG/ BD vines, however most nutrients were in abundant supply. These differences in canopy size may have influenced the canopy microclimate and disease severity (Smart 1985, Keller, et al. 2003). However in McLaren Vale, the relationship between nutrient status and vine productivity is not as clear. Similar to the Waite trial, no consistent differences existed between nutrients and those that did could be deemed as not biologically significant as they were at or above adequate concentration levels. In both trials no physical evidence of a nutrient deficiency or toxicity was observed.

With the exception of the Waite Campus field trial, the results for effective control of Botrytis were inconclusive. However it appears that the *Equisetum* plant extracts and silicon dioxide of both BD treatments in Chardonnay and Shiraz were effective in affecting Botrytis development. In the growth room trial there was no evidence that *Equisetum* played a role in disease suppression at all. It has been noted already though that the interactions between pathogens, the host plant, their environment and the nutrient status are complex. Given that only one season's data was collected, it is not possible to draw a robust conclusion. The smaller canopies of the OG/BD treatments may have affected the microclimate and the adequate or above state of nutrient concentration suggests that the plant's health affected disease development as much as the BD preparations.

Reproductive measures collected in 2012/2013 in the Waite trial consistently showed yield was greatest in the CV treatments due to more bunches per vine, berries per bunch and a higher mean bunch mass than OG and BD treatments in both cultivars. CV treatments in Shiraz in particular had a yield significantly higher than both OG and BD treatments. When considered in conjunction with the results for vegetative growth, this may be a function of the vigorous nature of Shiraz when conditions are not limiting (Dry & Loveys 1998). OG/BD had a lower fruitfulness (fewer bunches per vine) and lower fruit set (fewer berries per bunch) than other treatments, which may not be financially acceptable to the grower. This may point to a limited supply of carbohydrates and nutrients between seasons which can be detrimental to fruitfulness and fruit set (May 2004). However in the context of the Waite trial the nutrient status was influenced by the deliberate addition of fertilisers. In vineyards planted in nutrient rich soils, the additions of fertilisers may not be required, if nutrient status is well managed. In the case of the McLaren Vale, no fertilisers were used during the trial. There were certainly differences in canopy density, where CV treatments consistently had larger denser canopies. However there were no yield component differences or differences in nutrient concentration. Competition from weeds may have been responsible for the smaller OG/BD vines (Tescic, et al. 2007). Small canopies may have been the cause of consistently low levels of powdery mildew throughout the trial. In areas where supplementary irrigation is available and easily managed, competition from other vegetation maybe a realistic canopy manipulation tool, for disease management, in high vigour situations; provided changes to vegetative growth do not adversely affect fruit production or vine balance. Given the low nutrient requirements of *Vitis vinifera*, it seems possible to manage an appropriate nutrient regime with properly prepared compost and the benefits to the soil are well reported (Buckerfield and Webster 2001b, Cass 2002, Weckert 2010) especially in areas where soils are high in nutrients and which are plant accessible.

It would appear therefore that more research needs to be undertaken; as there is evidence that plant extracts in general can exert some control over plant fungal diseases. In the context of these trials it would also appear that the BD inputs of *Equisetum* or combinations of the recommended plant extracts, offered potential for use in control programs. In some cases, whilst not to an acceptable level, results

were at least better than no control. Certainly, this was the case in a temperate environment. But so long as sulfur is affordable, available and continues as an allowable input in both OG and BD systems for the control of powdery mildew it would appear that the use of plant extracts may be limited. Their use, in conjunction with lower concentrations of sulfur, should however be tested. At present, little research examines the co-application of products that may allow for reduced concentrations of sulfur. Research into mineral oils (Dell, et al. 1998) or biological controls (Elad, 2000) for example are ongoing and these products will likely have a role to play. Broome and Warner (2008) report that in the politically charged environmental landscape of California, like Australia, premium winegrapes are grown in areas that are under increasing pressure for urban development. There, the winegrape sector is actively investing time, effort and money in developing enterprises that will enable the industry to work towards reducing pesticide use. The programs are progressing to a reduced risk pest management system (Campos and Zhang, 2004) that draws on organic and other alternative techniques. The ultimate aim of these programs is to satisfy a broad set of environmental goals as stipulated by the U.S. Department of Agriculture's National Organic Program. Biodynamic plant extracts may present a new alternative to conventional fungicides in this constantly evolving paradigm.

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## Appendices

**Appendix 1:** Plant protection standards for organic agriculture, from Department of Agriculture, (2013).

**Pest and disease control must include any combination of the following standards.**

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Choice of appropriate species and varieties.

Biological control.

Appropriate rotation programs.

Specific bio-dynamic measures.

Mechanical controls such as traps, barriers.

Light and sound.

Mechanical cultivation.

Mulching and mowing.

(mulching materials must not contain substances prohibited by this standard and their use must be documented)

Grazing of livestock.

Protection of natural enemies of pests through provision of favourable habitats (e.g. hedges, nesting sites).

Flame / steam weeding.

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**Appendix 2:** Permitted substances for disease control in organic agriculture, from Department of Agriculture, (2013).

<b>Plant disease control</b>	<b>Specific conditions/restrictions</b>
Ayurvedic preparations	None
Biological controls	Naturally occurring cultured organisms only
Copper e.g. Bordeaux and Burgundy mixture	Hydroxide is the preferred form, Bordeaux only on dormant tissue. Annual copper application must be less than 8Kg/Ha.
Essential oils, plant oils and extracts	None
Granulose virus preparations	Need recognised by certification organisation.
Homeopathic preparations	None
Light mineral oils (such as paraffin)	None
Lime	None
Lime-sulphur	None
Natural plant extracts excluding tobacco	Obtained by infusion and/or made by the farmer without additional concentration
Potassium permanganate	None
Potassium soap (soft soap)	None
Propolis	None
Seaweed, seaweed meal, seaweed extracts	None
Sea salts and salty water	None
Skim milk or skim milk powder	None
Sodium bicarbonate	None
Sodium silicate (water-glass)	None
Sulphur	In wettable or dry form only
Vegetable oils	None
Vinegar	None

### Appendix 3: Description of biodynamic preparations and their function, from Mackay, (2010).

<p>Cow Horn Manure</p> <p>500</p>	<p>Is made from cow manure, which is filled into cow horns and buried in the soil over winter. It brings in the earthly forces and helps the soil develop humus and structure. It also attracts earthworms and soil micro-organisms.</p>
<p>Horn Silica</p> <p>501</p>	<p>Is made from ground quartz crystals, brings in the silica activity. It is buried in the horns over summer. Only a tiny amount is used to take the light forces into the roots and to aid photosynthesis.</p>
<p>Yarrow</p> <p><i>Achillea millefolium</i></p> <p>502</p>	<p>Yarrow flowers placed in a stags bladder. Stimulates the potassium, silica and selenium activating bacteria and helps combine sulphur with other substances. Remedies weaknesses in flowering and fruiting and strengthens the plant against insect attack</p>
<p>Chamomile</p> <p><i>Chamomilla officinalis</i></p> <p>503</p>	<p>Chamomile flowers placed in the small intestines of cow. Helps retain nitrogen, calcium and sulphur. Also stimulates manganese and boron, as well as azotobacter activity- the best bacteria for making nitrogen in the soil.</p>
<p>Stinging Nettle</p> <p><i>Urtica dioica</i></p> <p>504</p>	<p>Nettle is buried without an animal sheath. Conveys intelligence to the soil, helps proper decomposition, aids chlorophyll formation and stimulates iron, potassium, calcium, magnesium and sulphur bacteria activity in the soil.</p>
<p>Oak Bark</p> <p><i>Quercus robur</i></p> <p>505</p>	<p>Oak bark placed in a cow skull and in water over winter. Helps restore balance when water activity is working too strongly, such as after lots of rain or at full moon. It also helps protect against fungal disease. Helps calcium and phosphorus work into the earth in a living form.</p>
<p>Dandelion</p> <p><i>Taraxacum officinale</i></p> <p>506</p>	<p>Dandelion placed in a cow's mesentery. Stimulates the potassium/silica bacteria and fungi in the soil to enable it to work more effectively. Silica makes the plant more inwardly sensitive. Can help increase flowering and filling of fruit out to tips. Also stimulates the magnesium, boron and selenium soil activity.</p>
<p>Valerian</p> <p><i>Valeriana officinalis</i></p> <p>507</p>	<p>A Tincture made of valerian flowers. Stimulates the phosphorus process and mobilises the phosphorus activating bacteria in the soil, as well as selenium and magnesium. Prevents the flowering process becoming excessive. Forms a warmth blanket around the compost heap. If sprayed onto blossoms in spring can provide protection against late frost.</p>
<p>Equisetum/Casuarina</p> <p><i>Equisetum arvense</i></p> <p>508</p>	<p>As Equisetum is seen as a noxious weed in Australia, we have found Casuarina to be a good substitute. Fresh Casuarina Preparation works with the water balance in the atmosphere as a fresh tea and is used to prevent and stop fungal growth, sooty mould and tightens plants against becoming soft and open to mildew infection. Fermented Casuarina tea works in the soil to stimulate the growth of beneficial fungi and large hyphae and is applied with the afternoon soil sprays. All Casuarina seems to be effective, especially the Casuarina equisetifolia from eastern Australia.</p>



#### Appendix 4: Waite Campus field trial - treatments and project design

**Field Design** – Randomly allocated over three blocks. Total of 9 vines/treatment

← North

Block	Row	Variety	Treatments/Panel (3 vines/treatment)							
			1	2	3	4	5	6	7	8
1	1	Chardonnay	CV2	BD2	CON	OG1	CV3	OG2	BD1	CV1
	2	Shiraz	CV2	BD2	CON	OG1	CV3	OG2	BD1	CV1
2	3	Chardonnay	BD2	CV3	OG1	CV1	OG2	CV2	CON	BD1
	4	Shiraz	BD2	CV3	OG1	CV1	OG2	CV2	CON	BD1
3	5	Chardonnay	BD2	OG1	CV1	CV3	CV2	CON	OG2	BD1
	6	Shiraz	BD2	OG1	CV1	CV3	CV2	CON	OG2	BD1

**Treatments** – Water control; BD treatments with and without S; to compare with CV and OG treatments based on accepted industry standards. CV2 and CV3 used in Chapter 3. CV1 and CV2 used in Chapter 5.

	Code	Powdery mildew	Botrytis
Control	CON	Water	Water
Biodynamic 1	BD1	508 ( <i>Equisetum</i> extract) + sulfur	501 (SiO <sub>2</sub> solution)
Biodynamic 2	BD2	508 ( <i>Equisetum</i> extract) - sulfur	501 (SiO <sub>2</sub> solution)
Conventional 1	CV1	Sulfur	Conventional
Conventional 2	CV2	Sulfur + Conventional	Conventional
Conventional 3	CV3	Sulfur	None
Organic 1	OG1	Potassium bicarbonate	K salts of fatty acids
Organic 2	OG2	Full cream bovine milk	Seaweed extract

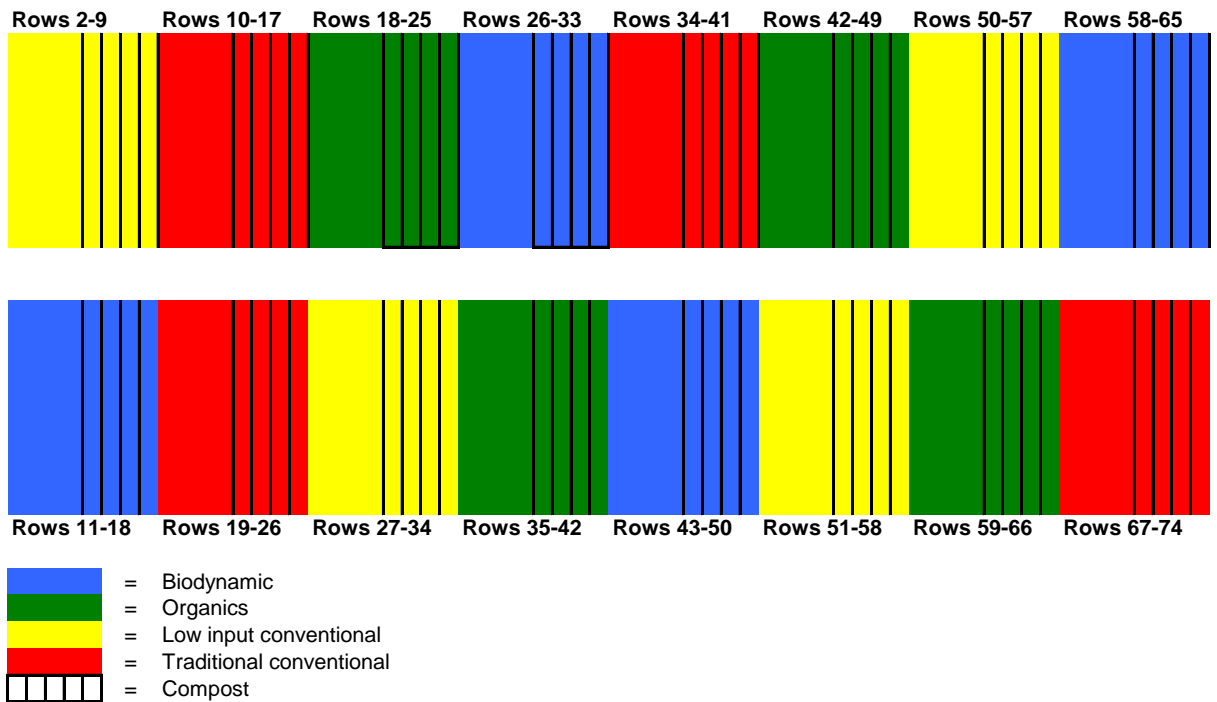
## Appendix 5: Waite Campus growth room trial – treatments and project design

Block 1			Block 2		
Variety	Treatment	# Vines	Variety	Treatment	# Vines
Chardonnay	H <sub>2</sub> O	3	Chardonnay	Yar	3
Shiraz		3	Shiraz		3
Chardonnay	CV	3	Chardonnay	H <sub>2</sub> O	3
Shiraz		3	Shiraz		3
Chardonnay	Yar	3	Chardonnay	Equ	3
Shiraz		3	Shiraz		3
Chardonnay	Equ	3	Chardonnay	Com	3
Shiraz		3	Shiraz		3
Chardonnay	Net	3	Chardonnay	Net	3
Shiraz		3	Shiraz		3
Chardonnay	Com	3	Chardonnay	CV	3
Shiraz		3	Shiraz		3
Block 3			Block 4		
Chardonnay	CV	3	Chardonnay	Com	3
Shiraz		3	Shiraz		3
Chardonnay	Com	3	Chardonnay	H <sub>2</sub> O	3
Shiraz		3	Shiraz		3
Chardonnay	Yar	3	Chardonnay	Net	3
Shiraz		3	Shiraz		3
Chardonnay	H <sub>2</sub> O	3	Chardonnay	CV	3
Shiraz		3	Shiraz		3
Chardonnay	Equ	3	Chardonnay	Equ	3
Shiraz		3	Shiraz		3
Chardonnay	Net	3	Chardonnay	Yar	3
Shiraz		3	Shiraz		3

### Treatments

Code	Treatment
H <sub>2</sub> O	Water (control)
CV	Conventional fungicides
Com	Combined mixture of Yarrow, Nettle and Equisetum extracts
Net	Nettle
Equ	Equisetum
Yar	Yarrow

## Appendix 6: McLaren Vale field trial – treatments and project design



### Treatments

**BD:** S+Cu, 500+501, no herbicide, with cultivation;  
**HIC:** S+Synthetic fungicides and herbicides;  
**LIC:** S+Cu, occasional herbicide;  
**ORG:** S+Cu, no herbicide, with cultivation