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IMPROVED MANAGEMENT OF GRAPEVINE POWDERY MILDEW

FINAL REPORT to: GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

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GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

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Improved management of grapevine powdery mildew

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GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Improved management of grapevine powdery mildew

FINAL REPORT

Contents

Improved management of grapevine powdery mildew

1 Abstract

Research has produced new tactics for improving management of powdery mildew (*Erysiphe necator*) in vineyards by reducing primary infection from diseased 'flag' shoots produced by over-wintered infected buds. Spray programs aligned with vineyard disease potential and pruning system were developed to prevent bud infection and flag shoot formation, and reduce initial spore loads and the cost of controls. Bud infection studies confirmed that bud susceptibility declined with increasing bud age. Flag shoot formation differed between varieties and was related to disease severity on the surface of buds in the previous season. Hot dry weather in spring appeared to reduce bud infection and flag shoot formation. Further research is recommended to determine the effects of other fungicide treatments and environmental conditions on bud infection and flag shoot formation and to forecast seasonal powdery mildew activity in vineyards.

2 Executive Summary

Improved management of powdery mildew (*Erysiphe necator*) in vineyards is important for the sustainability of the Australian wine industry. The annual cost of powdery mildew in Australian viticulture is estimated to be around 4-5% of the value of the crop. Flag shoots arising from over-wintered infected buds are the main source of primary infection in most vineyards.

The principal objective of this project was to improve the management of powdery mildew in vineyards by reducing primary infection from over-wintered infected buds. Key sub-objectives of the project were to (1) determine bud susceptibility to infection by the powdery mildew pathogen in selected grapevine varieties grown in Australian vineyards (ie. Verdelho, Chardonnay and Sultana); (2) develop treatment programs to reduce bud infection, flag shoot expression and subsequent primary infection in vineyards, and (3) communicate results of the research and development (R&D) to project stakeholders and the Australian viticultural industry. The project was part of a larger internationally collaborative research project investigating the biology and management of powdery mildew perennation in grapevine buds in Australia and the United States.

Research in the project included the evaluation of inoculum preparation and bud inoculation techniques, studies of the susceptibility of buds of selected grapevine varieties to infection and the evaluation of fungicide treatment programs to reduce bud infection and flag shoot formation.

Spray programs specifically designed to prevent bud infection and flag shoot formation were developed in this project for the first time in Australia. The timing and type of fungicide treatments used in each program were aligned with vineyard disease potential, vine pruning system, periods of bud susceptibility and expected exposure to powdery mildew inoculum and infection.

Preliminary recommendations for spur-pruned vineyards with a high potential for powdery mildew include the application of a demethylation inhibiting (DMI) fungicide spray when shoots have 5 leaves, followed by a further spray of either a DMI fungicide or a morpholine and DMI fungicide tank mixture when shoots have 10 leaves. Further fortnightly sprays, firstly with a morpholine fungicide, then a DMI fungicide and finally another fungicide with different chemistry and systemic activity are suggested for cane-pruned or minimally pruned vineyards with a high risk of disease to prevent infection of buds to be retained on vines for the following season. Other spray programs for vineyards with lower disease potential are also presented.

Thorough application of these programs in at least three consecutive seasons should reduce primary infection from spores produced on flag shoots, the risk of perpetuating bud infection, vine disease severity, cleistothecium formation later in the season and the potential for disease in vineyards. When the vineyard disease potential is very low, fewer sprays will be required to maintain control. In the absence of primary infection from flag shoots, the annual cost of powdery mildew control could be reduced by up to 50%.

Studies of bud susceptibility to infection confirmed that grapevine shoots have a stage-specific susceptibility and the surfaces of buds, stems and leaves develop age-related (ontogenic) resistance.

Young, green buds (aged up to 16 days) on shoots with up to 6 separated leaves were most susceptible to infection. The risk of bud infection declined as buds aged and as their outer scales hardened. After exposure to severe powdery mildew epidemics, buds aged 1-24 days on shoots of very susceptible varieties (eg. Verdelho) produced flag shoots in the following season.

Susceptibility to bud infection and flag shoot formation differed between varieties. After inoculation with *E. necator*, more flag shoots were produced on Verdelho than on Chardonnay, and these varieties produced more flag shoots than Sultana. In unprotected vineyards of varieties with a high potential for flag shoot production, crop loss is more likely to occur because of the higher likelihood of initiation of powdery mildew epidemics early in the season.

Infection of the interior of buds occurred within 3 weeks of inoculation. Hyphae of *E. necator* appeared to penetrate young buds through gaps between the overlapping bud scales. As buds aged, physiological changes to the outer bud scales appeared to prevent entry of the pathogen and infection of the inner bud tissues. In infected buds, all parts of the bud interior were colonised by hyphae with haustoria, except for the meristems. Conidiophores and conidia also developed in colonised buds.

Only a small proportion of infected buds produced flag shoots in the following season. High disease severity on the surface of buds (external infection) was positively correlated with high flag shoot production. Extensive internal infection of buds and low bud fitness appeared to be the main causes of low flag shoot incidence. When shoots were severely diseased during epidemics, higher numbers of buds at the apical end of shoots than at the base of shoots died during winter, apparently because of the debilitating effects of powdery mildew on shoot growth. After severe epidemics on susceptible varieties, some infected buds remained latent and survived for more than one season to produce flag shoots.

In this project, temperatures of 22-28^{o}C and relative humidity of 65-85% from just before inoculation to at least 10 days after inoculation were most favourable for disease development on vine shoots and for bud infection. Hot dry weather (days with maximum temperatures exceeding 35^{o}C and a minimum daytime relative humidity of 10-25%) in the 10 days following inoculation of young shoots appeared to substantially reduce disease establishment on the surface of shoots and buds and subsequent bud infection. Hot dry weather (days with maximum temperatures of 30-40^{o}C and daytime humidity as low as 10%), especially in the first 9 weeks after bud burst, appeared to reduce flag shoot formation and survival.

Further research and development is recommended to (1) determine the effects of other fungicide treatments on bud infection and flag shoot formation so that more cost-effective and robust recommendations can be developed; (2) define the effects of weather and environmental conditions on bud infection, flag shoot formation and the initiation of disease epidemics from flag shoot inoculum in vineyards; (3) develop a robust simulation model to forecast seasonal powdery mildew activity in vineyards and indicate the need for selected fungicide treatments, and (4) determine the influence of biotypes of *E. necator* on biology and management of bud infection and flag shoot formation.

Information about grapevine bud infection and the management of powdery mildew in vineyards was communicated to scientific and industry audiences through more than 30 presentations given to project stakeholders and industry representatives at industry forums and seminars, scientific conferences, workshops, meetings and field days in Victoria, South Australia, New South Wales, the USA and Italy. Publications relating to work on the project included more than 30 project reports, articles in industry and scientific journals, papers in proceedings of scientific and industry conferences and workshops, and communications delivered through grape industry information services.

3 Background

3.1 Industry context

Powdery mildew is a widespread and persistent disease of grapevines. In most seasons, entire crops can be lost if control measures are inadequate. Consignments of grapes with as little as 3-5% bunch disease can be rejected by wineries because infected bunches can have a detrimental effect on grape juice and wine quality causing off-flavours and aromas (Calonnec *et al.* 2004, Gadoury *et al.* 2002b and Stummer *et al.* 2002a, 2002b, 2003). The annual cost of powdery mildew in Australian viticulture has been estimated to be around 4.7% of the value of the crop. In 1996, for example, the cost of grapevine powdery to Australian industry was estimated to be more than $17 million (Magarey and Emmett 1997, Emmett *et al.* 2005a).

Improved management of powdery mildew in vineyards was, and still is, important for the sustainability of the Australian wine industry. Depending on their approach to disease management, most grape growers apply 4-8 protectant fungicide sprays to control powdery mildew each season (Emmett 2005). Improved management will potentially reduce losses caused by the disease and the cost of disease control by increasing the efficiency of spray programs.

3.2 Research and development

Over the last 10-15 years, research in Australia and overseas has increased understanding of the behaviour of powdery mildew in vineyards and this knowledge has been used to develop more effective treatment programs. Studies of the spread of powdery mildew in vineyards (Emmett 2005) have shown that losses caused by the disease are more likely to occur when levels of over-wintering inoculum are high and disease spread occurs early in the growing season (especially before flowering). Inoculum of powdery mildew in vineyards each season consists of (1) conidiospores from diseased shoots (flag shoots) produced from over-wintering infected buds, (2) ascospores from over-wintering cleistothecia (fungal fruiting bodies), and later, (3) conidiospores from other diseased vineyards. While (1) and (2) are likely to cause infection and initiate disease epidemics early in the season, flag shoots are the most important source of disease in many seasons and in most vineyards.

When research in this project commenced, some of the factors influencing cleistothecium development and maturation, ascospore release and primary infection from ascospores had been defined (Gadoury and Pearson, 1990). However, little was known about the survival of the powdery mildew pathogen (*Erysiphe necator*) in grapevine buds (bud perennation) and the factors influencing expression of the disease on shoots arising from infected buds (flag shoots). Levels of over-wintering inoculum (and hence potential crop loss) varied from season to season. Knowledge of bud infection and flag shoot development on vines was important because the potential for crop loss and need for disease control was lower in seasons when flag shoot appearance was delayed.

A review of research on grape powdery mildew in 2001, sponsored by the Vinelink International Association (Emmett 2001), identified this knowledge gap as the most significant constraint on the development of more effective treatment programs for powdery mildew control.

Research in Germany (Bleyer *et al.* 2000 and Rügner *et al.* 2002) and in California, USA (Ypema and Gubler 2000; Rademacher and Gubler 2001; Gubler and Rademacher 2002 and Rumbolz *et al*. 2002) investigated the incidence and physiology of bud infection and its correlation to disease expression in the pre-flowering stages of grapevine development. The results suggested that early bud infection could play an important role in powdery mildew epidemics in the following season.

In the absence of knowledge about bud perennation and the development of flag shoots, Australian grape growers have been encouraged to apply fungicide sprays early in the season to prevent infection from over-wintering inoculum (Emmett 2005). Fungicide sprays are also applied fortnightly up to 2-3 weeks after flowering to prevent infection of young susceptible vine foliage and bunches. It is likely that some of these sprays also prevent infection of buds. In Germany, for example, flag shoots were eliminated over a five-year period in vineyards where pre-flowering sprays were applied. Furthermore, there are also indications that spraying at a specific time may substantially reduce bud infection and flag shoot development. In Germany, sprays applied up to when shoots had eight separated leaves reduced the incidence of flag shoots as much as a full early-season spray program on vines with canes that had up to 10 buds (Hill and Breth 1995, Bleyer *et al.* 2000; Georg Hill, unpublished data).

In Australia, fungicides with different chemistry are used to control grapevine powdery mildew. These include sulphur, demethylation inhibiting (DMI), strobilurin, quinoline and morpholine

fungicides. Some of these fungicides (eg. DMI and morpholine fungicides) have some systemic activity in young vine tissues. Little was known about the effects of these fungicides on infected buds. When correctly timed, treatments with these fungicides may reduce or eradicate infections in buds.

Recent studies of the development of resistance in grape berries to powdery mildew infection have indicated that berries are highly susceptible to infection one week after flowering, but acquire ontogenic (age-related) resistance 2-3 weeks later (Gadoury *et al.* 2003; Ficke *et al.* 2003 and Ficke *et al.* 2004). These observations have implications for the timing of fungicide treatments in trials in this project because treatments applied just before or around flowering to reduce disease development on bunches may also prevent or eradicate bud infection.

With the above in mind, research on the biology and management of the perennation of powdery mildew in grapevine buds in Australian vineyards was the focus of this project. Furthermore, because of the importance of powdery mildew in international viticulture and the significance of research on bud perennation, work in this project was linked to research and development (R&D) in a larger international project. The latter project was established with the assistance of the Vinelink International Association (Vinelink), an independent association of 285 public and private wine-related organisations (including GWRDC) from 25 countries, including Australia. This link was significant because it gave Australian researchers, and industry, immediate access to the results of R&D conducted by overseas researchers.

In the international project, collaborative research on the biology and management of powdery mildew perennation in buds of grapevines was conducted in Australia by researchers in this project and in the United States by researchers at the University of California, Davis CA (UCD). Bud inoculation, histology and other techniques developed in the US research were used in this project. Knowledge produced from the R&D at UCD was also used along with knowledge from this project to develop recommendations for the management of bud perennation of powdery mildew in Australian vineyards.

3.3 Project report

This report covers R&D conducted in Australia on the biology and management of powdery mildew perennation in buds of grapevines from July 2002 to October 2006 in GWRDC Project DNR 02/06 'Improved management of grapevine powdery mildew'. A summary of studies conducted in the United States at UCD on the 'Susceptibility of grapevine buds to infection by powdery mildew, *Erysiphe necator*' (Rumbolz and Gubler 2005) is also included because this research was closely linked with the Australian studies in the international research project. A report on R&D conducted in the United States was also a required output for GWRDC Project DNR 02/06 (see Table 1, Section 5. 'Project outputs and performance targets').

4 Project objectives

4.1 R&D in the United States

The main objective of the R&D project conducted in the United States was to examine bud colonisation and the perennation of the powdery mildew pathogen (*E. necator*) in the highly susceptible grapevine variety Carignane.

Sub-objectives of the project were to:
(1) Determine the period of bud susceptibility to infection by *E. necator*;
(2) Examine the correlation between bud infection and grapevine phenological stage;
(3) Examine the susceptibility of dormant bud tissue to infection to increase understanding of the process of reactivation of *E. necator* perennating in dormant buds.

4.2 R&D in Australia

The main objective of this project was to improve the management of powdery mildew in vineyards by reducing primary infection from over-wintering infected buds.

Sub-objectives of the project were to:
(1) Determine bud susceptibility to infection by the powdery mildew pathogen in selected grapevine varieties grown in Australian vineyards (ie. Verdelho, Chardonnay and Sultana);

(2) Develop treatment programs to reduce bud infection, the expression of disease and/or primary infection in vineyards;
(3) Communicate results of the R&D to project stakeholders and the Australian viticultural industry.

A further sub-objective 'to examine the effects of environmental factors (eg. temperature and moisture) on bud infection and the expression of disease (flag shoots)' was included in the original project proposal. Subsequently however, this sub-objective and the associated R&D were deleted from the project R&D plan because of funding constraints (see Section 5 'Project outputs and performance targets').

5 Project outputs and performance targets

Some project outputs and time-lines for performance targets were revised in agreement with GWRDC from 2003/04 onwards (Table 1) because the project budget allocation for 2003/04 was reduced. Alteration of the project plan was required to allow some project staff to complete outstanding final reports on other projects. Following negotiations with the Department of Primary Industries in Victoria (DPI) in early June 2004, GWRDC agreed to continue financial support for the project provided that the project plan was revised and an updated draft of the final project report was submitted in July each year (ie. in July 2004 and July 2005).

Options for variation of the project plan, outputs, performance targets and budget were presented to GWRDC in August 2004. GWRDC decided to support the project budget for 2004/05 and 2005/06. Completion of all outputs in the original project plan, however, required an extension of the project into 2006/07 but this was not approved by GWRDC. Hence, in the revised project plan supported by GWRDC, studies of the effects of temperature and moisture on bud infection and the expression of disease on shoots arising from infected buds were omitted. Field trials fully evaluating treatments for the control of bud infection and/or disease expression were also to be conducted in one season only, instead of being replicated over two seasons. In September 2006, GWRDC also agreed to extend the time-line for submission of the final project report to 31 October 2006.

Table 1. Revised outputs and performance targets for GWRDC Project DNR 02/06.

Outputs	Performance Targets
1. Report on the progress of R&D in the United States[1]	1. Overseas research on bud infection reviewed and reported by July 2003.
2. Report on the period of susceptibility of grapevine buds to infection by *E. necator*	2. Results of inoculation experiments and field assessments reported by January 2005 (previously July 2004).
3. Report on the effects of temperature and moisture on bud infection (Output deleted in project revision, August 2004)	3. Results of growth room experiments reported by January 2005. Target deleted in project revision, August 2004.
4. Report on the effects of temperature and moisture on the expression of disease on shoots arising from infected buds (Output deleted in project revision, August 2004)	4. Results of growth room experiments reported by July 2005. Target deleted in project revision, August 2004.
5. Treatment programs to reduce bud infection in vineyards developed and reported	5. Results of at least two field evaluations reported by June 2006. Target modified to 'Results of at least one field evaluation reported' in project revision, August 2004.
6. R&D results communicated to industry	6. At least three presentations at industry conferences, seminars, workshops or field days and publications in industry journals by June 2006.
7. Project final report	7.1 Updated versions of project final report submitted to GWRDC by 31 July 2004 and 31 July 2005. Target added in project revision, August 2004. 7.2 Project final report written and submitted to GWRDC by 30 September 2006 (extended to 31 October 2006).

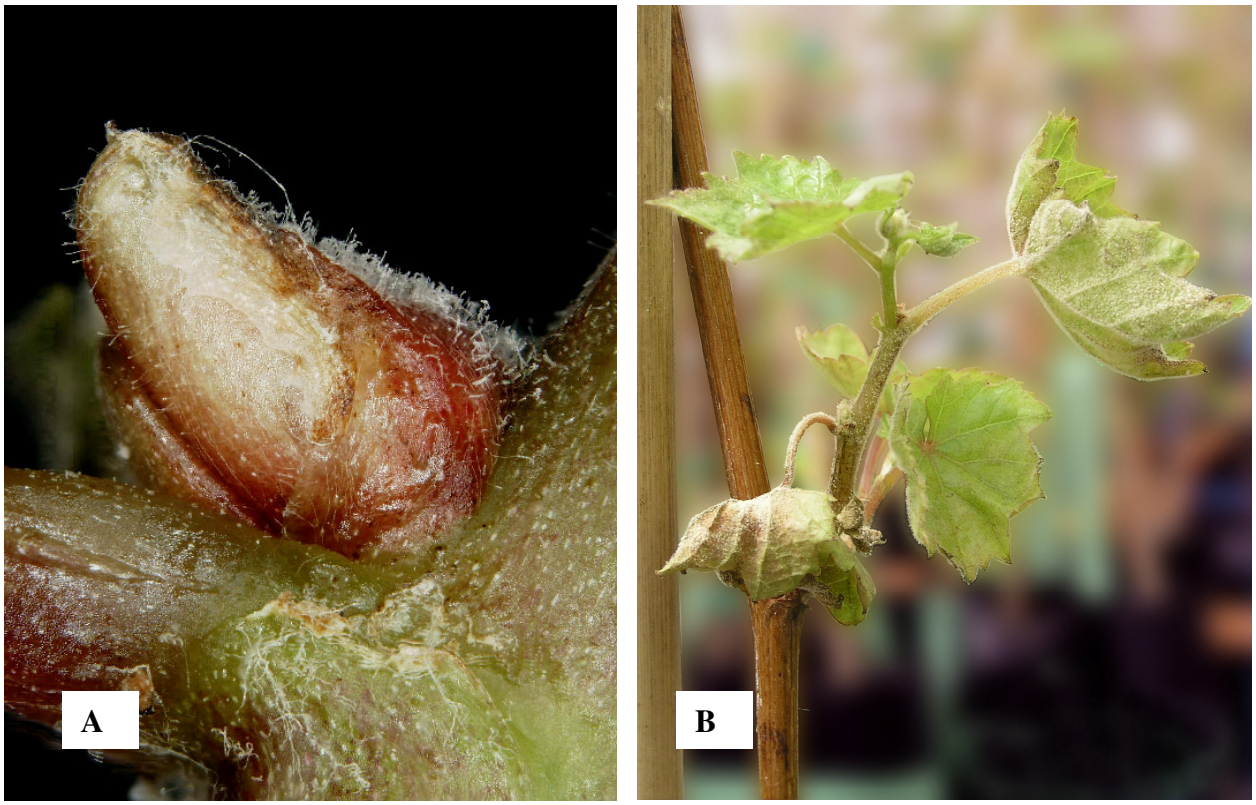
[1] The report on R&D in the United States is summarised in Sections 3.2 (background R&D), 7.1 (methods) and 8.1 (results) of this report.

6 Review of research in USA

In the initial year of the project, Australian researchers reviewed progress of the latest research on the perennation of *E. necator* in grapevine buds. This research was being conducted by staff of the Department of Plant Pathology at the University of California, Davis CA and was coordinated by Dr Doug Gubler. In early October 2002, Dr Bob Emmett and Kathy Clarke attended the Fourth International Workshop on Powdery and Downy Mildew in Grapevines at Napa CA, USA. The workshop provided an opportunity to review the latest knowledge on grapevine powdery mildew and discuss work on this project with international researchers. After the workshop, meetings were held with Dr Gubler, Dr Joachim Rumbolz and Ernesto La Red at UC Davis to discuss research techniques and the results of their R&D.

Further discussions on bud inoculation methodology and timing of control strategies were held at Loxton in November 2003 with Dr David Gadoury, a visiting scientist from Cornell University in New York State, USA with an extensive background in powdery mildew research. In February 2004, Dr Gubler also visited research centres at Loxton and Mildura to review and discuss the progress of research in the Australian project, in relation to previous and current collaborative research on grapevine bud infection conducted in the United States.

A brief report on the research conducted in the USA was included in the GWRDC Annual Industry Report on the project in February 2003. More detailed summaries of the methods and results of the research at UCD are presented in the methods and results sections of this report.



Cover Figures. A. An inoculated bud on a Verdelho vine infected with the powdery mildew pathogen, *E. necator*. **B.** A diseased 'flag' shoot produced from a Verdelho bud that was inoculated in the previous season. (Photos by Terry Hunt, DPI).

7 Methods

7.1 R&D in the United States (Summary)

[See Rumbolz and Gubler (2005) for more detailed descriptions of these methods]

Inoculum preparation

E. necator originating from a natural infection in a vineyard near Lodi CA, USA was maintained on seedlings and rootlings of Carignane grapevines in a growth chamber under 16 hours (h) light and 8 h darkness at 22°C and 75% relative humidity. The pathogen was re-propagated at 14-21 day (d) intervals by inoculating uninfected seedlings or rootlings with conidia. Conidia harvested from new colonies on foliage aged 14-21 d were used as inoculum for each experiment. Spore suspensions were prepared from heavily infested leaves taken from inoculum source vines. The leaves were sub-sectioned, placed in 200 mL deionised water with 100 µL Tween 20 stored on ice and shaken vigorously. The leaf material was removed with tweezers and the spore suspension was adjusted to a final concentration of 4×10^4conidia/mL.

Colonisation of the surface and interior of buds in vitro

Four separate experiments were conducted to develop a method for bud infection. In each experiment, 3-6 developing shoots were taken from Carignane grapevines maintained in a glasshouse with a maximum temperature of 30°C. Four to six nodes with buds were excised from the shoots, surface sterilised by vigorous shaking for 2 minutes (min) in 50 mL of 5% sodium hypochlorite solution containing 50 µL Tween 20, rinsed five times in sterile deionised water, and dried thoroughly on sterilised paper towels inside a laminar flow cabinet. After drying, the nodes with buds were placed on 0.8% w/v water agar in small Petri dishes and kept in the airflow cabinet until inoculation.

Each of the surface sterilised green buds was inoculated with a single chain of conidia picked from colonies of *E. necator* aged 14-21 d. The inoculated buds were maintained at 24°C under natural photo-period. Colonisation of the bud surface and the surrounding tissue was monitored daily by light microscopy.

Susceptibility of the bud surface was determined by assessing the latent period of *E. necator*, ie. the time from inoculation to sporulation. Infection success was expressed as the ratio between the number of buds with sporulation and the total number of inoculated buds. Three weeks after inoculation, bud tissue with sporulating mildew colonies was fixed and processed for histological studies (see below). Thin sections were screened under the light microscope for fungal structures inside each bud. The colonisation rate was expressed as the ratio between the number of buds with mycelia inside the bud and the total number of inoculated buds.

Susceptibility of developing shoots and bud surfaces to infection (Season 1)

Fifty potted Carignane grapevines each with a single shoot with 0-1, 3, 6 or 9 unfolded (separated) leaves were inoculated with conidia of *E. necator*. Shoots with 0-1, 3, 6 and 9 separated leaves were at phenological stages E-L 5, 10, 13 and 15-16, respectively, in the modified Eichhorn and Lorenz system and at stages BBCH 09, 13, 16 and 19, respectively, in the extended BBCH system (Coombe 1995). Each inoculation at each shoot stage represented one treatment. Entire shoots (50 per phenological stage/treatment) were sprayed with inoculum. To check the efficiency of inoculation, 10 microscope slides were sprayed in parallel. After the slides had been kept on a wet paper towel in a polyethylene chamber at 24°C for 24 h, the germination rate was assessed under a light microscope. Infection on vines was allowed to establish unhindered during an infection window for 3 weeks in the glasshouse at 20-25°C. The plants were then transferred to an open glasshouse with laths for shading. Three weeks after the last inoculation, the experimental plot (consisting of 200 vines) was exposed to natural field conditions. The vines were then treated with 2% (w/v) JSM Stylet Oil® (JMS Flower Farms Inc., Pendleton OR, USA) at 10-d intervals to prevent further bud colonisation from the exterior.

Plants were drip-irrigated once a day until the end of the experiment in summer of the following season, ie. after flag shoot assessment. Disease incidence and severity on shoot stems and leaves were assessed on individual vines on days 10, 17, 21, 28 and 35 after inoculation. Disease severity on leaves was rated in classes as follows: 0, no disease; 1, diseased area 1-5%; 2, diseased area 6-20%; 3, diseased area >20%. On stems, severity was rated in the following classes: 0, no disease; 1, one colony; 2, two to nine colonies; 3, = 10 or aggregated colonies >10 cm^2. Seven weeks after infection, buds from 20 shoots (vines) of each phenological stage were collected (40% of all inoculated shoots).

The presence of powdery mildew infection on the surface of the bud scales was checked microscopically and identified either by the presence of hyaline hyphae characteristic for *E. necator*, or by the occurrence of necrotic epidermal cells. Subsequently, entire buds were processed for histological analyses (see below). The remaining 30 shoots per vine and stage were kept until spring of the second season to assess flag shoot incidence (see below). Disease severity data and the percentage of buds with external infection between treatments (= different dates of inoculation) were compared by χ^2 analyses using STATVIEW (version 5.01, SAS Institute, Cary NC, USA).

Histological studies of internal infection in developing buds (Season 1)

Dormant buds that were exposed to high inoculum loads during their development for 7 weeks after inoculation were removed from shoots. Bud tissue was fixed in FAA (67% v/v ethanol, 5% v/v glacial acetic acid, 2·5% v/v formalin, 25·5% v/v deionised water) and embedded in paraffin according to the method of Rademacher (1999). The embedded buds were sectioned at a thickness of 10µm with a rotary microtome, and placed onto glass slides after floating the sections in a water bath at 42°C. The slides were then placed on a slide dryer at 42°C for at least 24 h.

The staining procedure was initiated by removing the paraffin from sections by immersion in two washes of 100% clearing agent and one wash in 50% clearing agent and 50% absolute ethanol. Sections were hydrated in a four-step ethanol series from 100 to 50% ethanol. Hydrated sections were stained with Pianeze IIIb consisting of 0·25% Malachite Green, 0·05% Acid Fuchsin and 0·005% Martius Yellow dissolved in 150 mL deionised water and 50 mL 95% ethanol (all dyes from Sigma Aldrich, St Louis, MO, USA). The sections were then screened under the light microscope for fungal structures inside the bud. The number of infected buds from each treatment (date of inoculation in the first year) was determined, and the percentage of buds with internal infections between treatments was compared by χ^2 analysis.

Incidence of flag shoot formation (Season 2)

Buds from the inoculated shoots that remained on vines during winter (60% of all inoculated shoots) were monitored in spring of the second season for the occurrence of primary infection originating from over-wintered mycelium of *E. necator*. Starting from bud burst, shoots emerging from canes inoculated in the previous season were carefully examined at 2 d intervals for the appearance of typical flag shoots with dense colonisation of sections of shoots, petioles, leaves and/or inflorescences. After identification, the phenological stage of the vine at inoculation in the previous season and the position of the flag shoot on the cane were recorded. In parallel, disease incidence on 100 randomly chosen leaves in the experimental plot (four phenological stages × 30 vines) was assessed by visual examination of the lower leaf surface where the first secondary lesions were visible.

Relationships between external and internal bud infection and flag shoot formation

Correlations between levels of infection of the bud exterior (bud surface) and bud interior in Season 1 and flag shoot formation in Season 2 after inoculation of shoots at the four phenological stages in the first season were examined by linear regression analysis (STATVIEW version 5.01). Analyses were based on a total number of 523 buds assessed for external infections, 546 analysed for internal infections, and 1104 shoots assessed for flag shoot symptoms.

Susceptibility of internal tissues of dormant buds

At two dates before bud burst, 13 and 9 dormant buds, respectively, were removed from cuttings of Thompson Seedless and Carignane (clone BKS F6 VI) grapevines and surface sterilised as described for the nodes with buds in the bud colonisation studies above. Under a stereomicroscope, the outer scales of each bud were carefully removed and the primary bud was dissected using a scalpel. The green parts of each dissected bud (ie. inner bud scales, prophylls, leaf primordia and the apical meristem) were placed on 0·8% water agar in Petri dishes and inoculated with a single chain of conidia of *E. necator* in a laminar airflow cabinet. The dissected buds in the Petri dishes were then maintained at 24°C under a natural photo-period. A light microscope was used to monitor spore germination, growth and sporulation of the pathogen and determine susceptibility of the dormant tissue during the latent period (time from inoculation to sporulation) prior to bud burst. Data were compared using a single-factor ANOVA.

Effects of temperature on flag shoot formation
One hundred and eighty developing shoots with 3-6 unfolded leaves on Carignane rootlings (one shoot per rootling) were inoculated with a conidial suspension of *E. necator* (4 x 10^4 conidia/mL) and incubated for 5 weeks in the glasshouse. Thereafter, plants were transferred to two growth chambers providing equal conditions to induce dormancy (short-day conditions: 8 h light at 10°C/16 h darkness at 15°C; 90% relative humidity). Plants were maintained under these conditions for 9 weeks until dormancy was apparent by leaf discolouration and fall. Fourteen weeks after inoculation, 90 rootlings, each with two to seven buds, were randomly assigned to two growth chambers to initiate bud burst (long-day conditions: 16 h light/ 8 h darkness; 70–80 $\mu E \times m^2 \times s^{-1}$). Chamber conditions differed with respect to daylight temperatures (Chamber 1, 22°C for 16 h; Chamber 2, 30°C for 16 h), but provided similar night temperatures (15°C for 8 h) with constant 90% relative humidity. Developing shoots were examined at 2-3 d intervals for the appearance of primary infection, identified by dense, white mycelia on unfolded leaves and petioles.

7.2 R&D in Australia

7.2.1 Evaluation of inoculum preparation and inoculation techniques

The success of the bud susceptibility and chemical treatment trials in this project relied on the use of effective plant inoculation methods that could be applied in glasshouse and field environments. Pilot studies were conducted to validate the inoculation techniques used by project collaborators at UCD and to measure their success under Australian conditions. The main aim of these studies was to develop an effective inoculation method that used minimal quantities of inoculum.

Effects of wetting agent concentration on inoculum viability
The first series of laboratory tests was conducted to determine the viability of conidia of *E. necator* in aqueous suspensions with or without a wetting agent (Tween 20®, polyoxyethylene sorbitan monolaurate, Chem-Supply, Gillman SA) at different concentration rates; 25µL/100mL, 50µL/100mL and 100µL/100mL. Researchers at UCD (J Rumbolz, pers. comm. 2002) used Tween 20® at 50µL/100mL to maintain even dispersal of spores in the aqueous suspensions (see 7.1 R&D in the United States, Inoculum preparation). This rate was among those tested in this study.

In each laboratory test, young leaves with colonies of powdery mildew aged 14-21 d were collected from Chardonnay vines in the glasshouse, segmented and evenly distributed between two sterile 25mL McCartney bottles. Ten mL of chilled (6°C) deionised water solution containing Tween 20® at 25µL/100mL, 50µL/100mL or 100µL/100mL was added to the first set of bottles. The same amount of chilled deionised water without the wetting agent was added to the second set of bottles, for comparison. After the bottles were sealed and gently agitated for 1 min, conidia in the suspensions were examined microscopically after 10 min to determine their viability. Conidia that were turgid with intact cell walls and were not misshapen were classed as viable. The number of viable spores in each suspension was counted using a haemocytometer.

Effects of storage time on inoculum viability
A second series of laboratory tests was conducted to investigate the effects of inoculum storage time on conidium viability. Aqueous suspensions of conidia of *E. necator* with Tween 20® at 25µL/100mL were prepared as described above and stored for 60 min at 5-6°C, an optimal temperature range for the maintenance of conidia in aqueous solutions (D. Gadoury, pers. com.). Starting from immediately after preparation, sub samples of the suspensions were collected at 10 min intervals and immediately examined microscopically to determine conidium viability. The number of viable conidia in each suspension was counted using a haemocytometer.

Effects of inoculum storage time on conidium germination, leaf infection and disease development
A third series of laboratory tests was conducted to investigate the effects of inoculum storage time on conidium germination and on infection of grapevine leaves.

Samples of the spore suspensions prepared as described above (with Tween 20® at 25µL/100mL) were applied to glass slides with a fine sable-haired brush (Number '0') after storage at 5-6°C for 0, 30 or 60 min. The inoculated slides were placed in disposable Petri dishes and incubated at 21-25°C with up to 85% relative humidity. After 6 h, the slides were examined microscopically to determine the

number of viable and germinated conidia. Conidial survival and germination rates were calculated for each suspension to compare the effects of the three different inoculum storage times.

Young grapevine leaves of similar age were collected from Chardonnay, Verdelho and Sultana vines in the glasshouse and examined microscopically to ensure that they were not infected with powdery mildew or other pathogens. Each leaf was placed in a joined double Petri dish. The petiole was supplied with water from the bottom dish to maintain the living leaf enclosed and suspended in the top dish. Three circles of 1 cm diameter were marked on the upper surface of each leaf with a fine, black permanent marker pen, to indicate sites of inoculation with inoculum stored for 0, 30 and 60 min (Figure 1A). After inoculation, Petri dishes containing the leaves were incubated at 21-25°C with 85% relative humidity. After 11 d, the inoculation sites on each leaf were examined microscopically to determine if colonies of powdery mildew had developed (as shown in Figure 1B).

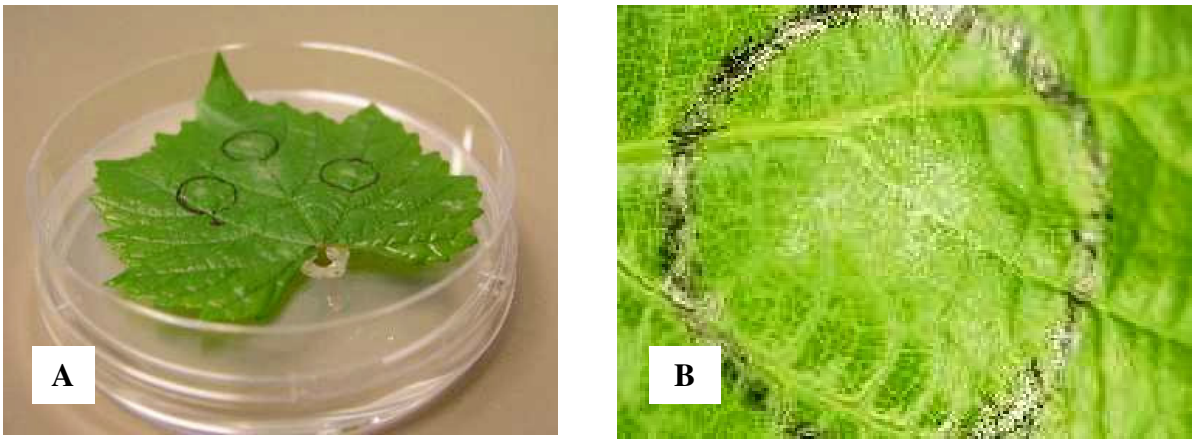


Figure 1. A. Powdery mildew growth on a young Sultana leaf incubated in a joined double Petri dish inoculated 11 days earlier at three sites (circled) with spore suspensions of *E. necator* stored at 5-6°C for 0, 30 or 60 min. **B.** A closer view of sporulating colonies of powdery mildew on a circled leaf inoculation site after inoculation with a spore suspension stored for 30 min. (Photos by Terry Hunt, DPI)

Effects of leaf age and condition on infection and disease development

Leaves of similar age were collected from Chardonnay, Verdelho and Sultana vines in the glasshouse for inoculation. The leaves included young healthy leaves with a glossy appearance and slightly older leaves with little or no shine on the upper leaf surface. Each leaf was placed in a joined double Petri dish and inoculated as described above. After 11 d, the inoculation sites on each leaf were examined microscopically to determine the extent of powdery mildew colony development.

Effects of inoculum type and application technique on conidium survival and germination and on bud infection

Two 'wet' inoculum application techniques were tested, the first using a micro-pipette and the second using a sable hair paint brush (Number '0'). Both techniques required only small amounts of inoculum and could be used to inoculate only the buds on young shoots. In comparison, spraying entire shoots (Rumbolz and Gubler 2005) required large quantities of inoculum. Samples of the spore suspensions prepared as described above (with Tween 20® at 25µL/100mL) were applied to glass slides using each technique. The inoculated slides were placed in disposable Petri dishes and incubated at 21-25°C with up to 85% relative humidity. After 6 h, the slides were examined microscopically to determine the number of viable and germinated conidia.

Field inoculation techniques using 'wet' or 'dry' inoculum were also tested. Young grapevine leaves with sporulating powdery mildew lesions were collected from Sultana vines in the glasshouse and placed in individual Petri dishes lined with pre-moistened 90 mm filter paper and incubated at 21-25°C with up to 85% relative humidity for 24 h. Sets of clean glass slides were placed in an identical set of dishes. The sets of glass slides and the sets of Petri dishes with incubated leaves enclosed in press-seal polyethylene bags were taken to the field in an insulated container. From 30-50 shoots on

the eastern side of a row of unsprayed Sultana vines were selected, numbered and tagged. A dry, fine sable paint brush was brushed across the surface of one of the incubated, infected leaves to collect conidia and the inoculum was 'dabbed' onto a slide in one of the Petri dishes. The lid was replaced and the dish returned to the insulated container. A second brush-load of inoculum was used to 'dry' inoculate the three youngest buds on the first tagged shoot. The shoot was marked horizontally below the node of the lowest or third bud with indelible marker to identify the inoculated buds. If there was sufficient inoculum on the surface of the incubated leaf, a second brush was dipped in a deionised water solution containing Tween 20® at 25µL/100mL. This dampened brush was then swept across the infected leaf surface again to collect conidia and used to 'wet' inoculate the youngest three buds on the second tagged shoot. Again, the shoot stem was marked to identify the inoculated buds. The dual inoculation process was repeated on the three youngest buds of the remaining 48 shoots. Each infected grapevine leaf provided sufficient powdery mildew inoculum for inoculating buds on at least two shoots.

After the slides with inoculum were incubated in the laboratory at 21-25°C with up to 85% relative humidity for 24-48 h, the percentage of germinating conidia on each slide was assessed. After 11-15 d at 4-31°C, the surface of each inoculated bud on the shoots in the field was examined with a hand lens (x10) to determine the extent of powdery mildew colony development.

7.2.2 Studies of the period of susceptibility of grapevine buds to infection by powdery mildew

Inoculum preparation

Isolates of *E. necator* originating from a natural infections in vineyards near Loxton SA and Irymple Vic. were maintained on rootlings of Chardonnay and/or Sultana grapevines in the glasshouse under 16 hours (h) light and 8 h darkness at 20-25°C and 50-75% relative humidity. The pathogen was re-propagated regularly by inoculating uninfected rootlings with conidia. Conidia harvested from new colonies of the isolates on foliage aged 14-21 d were mixed and used as inoculum for each experiment. Spore suspensions were prepared from heavily infested leaves taken from inoculum source vines. The leaves were sub-sectioned, placed in deionised water with Tween 20® at 25µL/100mL, stored on ice, and shaken vigorously. The leaf material was removed with tweezers and the spore suspension was adjusted to the final concentrations for each trial shown in Table 2.

Trial design and treatments

Replicated glasshouse or polyhouse trials were conducted over three seasons (Trials 1A-1F, Table 2) on potted rootlings of grapevines, *Vitis vinifera* cv. Verdelho (Figure 2), Chardonnay or Sultana. Previous observations of flag shoot incidence in vineyards indicated that the varieties Verdelho, Chardonnay and Sultana had high, moderate and moderate to low susceptibility to bud infection, respectively. Selected buds of different age on shoots with 0-1, 3, 6 and 9 separated leaves [growth stages E-L 5, 10, 13 and 15-16, or BBCH 09, 13, 16 and 19, respectively (Coombe, 1995)] were inoculated with conidial suspensions of *E. necator* (2-7 x 10^4 or 2.3 x 10^5 conidia/mL). One shoot on each vine was inoculated. In Trials 1A and 1D-F (Table 2), buds on shoots with 12 separated leaves (growth stage E-L 17) were also exposed to infection.

Vines with different treatments (ie. shoots at different growth stages) were arranged in a randomised block design and replicated 140 times. Each block contained five vines, each with a shoot at one of the four growth stages. Four of the vines had a shoot at a different growth stage that was inoculated. An exception was Trial 1B, where each block contained four vines and only three of the vines had a shoot at a different growth stage that was inoculated. The remaining vine in each block was not inoculated and was a random choice from groups of 35 vines with shoots at one of the four growth stages.

After inoculation, the plants were incubated in the glasshouse or polyhouse at 20-30°C and 40-75% relative humidity to allow unhindered establishment of *E. necator* on the surface of shoots and inside buds. After five weeks, when powdery mildew epidemics had developed, the plants were thoroughly sprayed to run-off with mineral oil (JMS Stylet Oil®, JSM Flower Farms Inc., Vero Beach, Florida USA) to prevent further external disease development. At least two oil sprays were applied at 7-day intervals in most experiments. If required, sprays of wettable sulphur (Thiovit®, 800g/kg sulphur, Syngenta Crop Protection, Adelaide SA) at 200g/100L were applied subsequently at 2-week intervals to prevent disease development. In all trials, the inoculated plants were kept in the glasshouse for at least 8 weeks after inoculation to encourage growth of the pathogen within infected buds. The

plants were then moved to a shade house where they were kept for the rest of the season and allowed to enter dormancy and over-winter.

Assessment of shoot development and bud age

At the start of each experiment, the potted trial vines were cut back to two dormant buds. After bud burst, one new shoot on each vine was grown in the glasshouse at a temperature range of either 12-15^{o}C or 20-30^{o}C, and at 40-75% relative humidity. The rate of leaf separation was recorded every week on 35 shoots that produced 9-12 separated leaves between bud burst and the time of inoculation. The rate of leaf separation was used to calculate the average age of buds at each node on the shoots at the time of inoculation. After inoculation, changes in the colour (suberisation) and length of buds at each node on 15 randomly selected shoots were recorded weekly.

Assessment of disease development after inoculation

In the glasshouse, disease epidemics developed on shoots after the time of inoculation. These epidemics were generated from primary inoculum applied during inoculations that caused primary infection and from secondary inoculum (produced on diseased foliage after inoculation) that caused secondary infection.

The incidence and severity of powdery mildew on the buds, stems (and in some cases, the leaves) of 35 uninoculated shoots were assessed using standardised powdery mildew disease assessment keys (Emmett, unpublished). Assessments to indicate the increase in disease on shoots caused by secondary infection after the time of inoculation were conducted weekly from just before the time of inoculation until the oil spray(s) were applied to prevent further external disease development. After the first oil sprays were applied, 2-3 further weekly assessments were conducted to indicate the effects of the oil treatments on disease development.

Histological studies of infected buds

At 8-10 weeks after inoculation, buds from 40 inoculated and 10 uninoculated shoots at each growth stage were selected at random, dissected from the stems and fixed in FAA solution. This ensured that samples could be examined later histologically, using techniques described by Rademacher and Gubler (2001), to determine the number of buds of different age that had been colonised by *E. necator*.

Assessment of flag shoots produced from infected buds

In the following spring, the number of flag shoots produced from over-wintered buds at each node position on each shoot was recorded for each vine variety. The number of buds that died was also recorded in most experiments. The percentage of inoculated and uninoculated buds that burst or died and the percentage of buds that burst and produced flag shoots were determined in relation to growth stage and bud age at the time of inoculation.

Statistical analyses

MS Access (Microsoft Access 97.2. Microsoft Corporation, 1996) was used to tabulate the data. StatXact 7 (StatXact 7.0 with Cytel Studio, Cytel Software Corporation, 2005) was used to carry out the statistical analyses.

With the data from Trial 1C (Sultana, Irymple 2004/2005), the Fisher Exact test was used to test for differences between inoculated and uninoculated buds. The Cochran-Armitage test for trend was used to test data on bud surface and/or sheath infection and bud death for trends related to bud age on shoots inoculated at the four different phenological stages. To measure associations between pairs of outcomes for buds (eg. bud surface and/or sheath infection and infection of adjacent tissues (stems, leaf petioles and/or lateral shoots), the Phi contingency coefficient was calculated and tested to determine if it was significantly different from zero.

With the data from Trials 1D and 1E (Verdelho and Chardonnay, Loxton 2004/2005), conditional logistic regression was carried out to test whether the incidence of flag shoots was related to bud number (position) on shoots or inoculation, when stratified on a 'by vine' basis.

Table 2. Summary of glasshouse (G/H) trials conducted to determine the period of susceptibility of grapevine buds to infection by powdery mildew from 2003 to 2006.

Trial number	Location (Year)	Vine variety	Parts of shoot inoculated	Inoculum (conidia/mL)	Vine growth stages[1]	Shoots at each growth stage inoculated (number)	Buds exposed to primary[2] and secondary[3] infection assessed for flag shoots (total numbers)
1A	Irymple G/H (2003-2004)	Verdelho	Buds	3.8×10^4	A, B, C, D, E	140	1120[2], 2638[3]
1B	Loxton G/H (2003-2004)	Chardonnay	Buds	4.0×10^4	B, C, D	140	840[2], 2520[3]
1C	Irymple G/H (2004-2005)	Sultana	Buds	7.2×10^4	A, B, C, D	140	1120[2], 1120[3]
1D	Loxton G/H (2004-2005)	Verdelho	Buds, stems, leaves	2.3×10^4	A, B, C, D, E	639	5112[2], 7029[3]
1E	Loxton G/H (2004-2005)	Chardonnay	Buds, stems, leaves	2.3×10^4	A, B, C, D, E	561	4488[2], 6171[3]
1F	Loxton G/H (2005-2006)	Chardonnay	Buds	2.3×10^5	A, B, C, D, E	128	1536[2], 2043[3]

[1] Vine growth stages: A = 0-1 leaves; B = 3 leaves; C = 6 leaves; D = 9 leaves; E = 12 leaves.
[2] Buds exposed to primary infection = buds inoculated
[3] Buds exposed to secondary infection = buds exposed to infection from conidia produced on diseased foliage after the time of inoculation. This includes inoculated buds and buds that were not inoculated (including those produced during disease epidemics that developed after the time of inoculation).

Figure 2. Verdelho grapevines with shoots at different phenological stages inoculated with *E. necator* in Trial 1A in the glasshouse at DPI Irymple Vic. (Photo by Terry Hunt, DPI).

7.2.3 Evaluation of treatment programs to reduce bud infection and expression of flag shoots

Inoculum preparation

Suspensions of conidia of *E. necator* prepared as described in Section 7.2.2 above and adjusted to the final concentration for each trial shown in Table 3 were used as inoculum.

Trial designs and vine inoculations

A glasshouse trial and three field trials were conducted over three seasons from spring in 2004/05 to early summer in 2006/07 (Table 3). The glasshouse trial was conducted on spur pruned potted Verdelho (clone SA168) vines at Irymple (Trial 2A). The field trials were conducted on spur pruned Chardonnay vines (clone I10V5, Trial 2B at Loxton and Trial 2D at Irymple) and on cane pruned Sultana vines (clone M12, Trial 2C at Irymple).

Plots of vines with inoculated shoots in trials 2A, 2B, 2C and 2D were arranged in a randomised complete block design and replicated 10, 3, 4 and 8 times, respectively. The number of vines in each plot was 20, 23, 13 and 8, respectively and the average number of shoots inoculated on each vine in the first season was 1, 17, 12 and 10 respectively. Other information on each trial, including the number of shoots inoculated in each plot, is summarised in Table 3.

Buds on shoots with 5-6 separated leaves (growth stages E-L 12-13) were inoculated with conidial suspensions of *E. necator* (1-6 x 10^4 conidia/mL). In Trial 2C, the same Sultana shoots were re-inoculated again one week and then two weeks later, when they had 9-10 separated leaves (growth stage E-L 15-16 or BBCH 19) and 12-15 separated leaves (growth stage E-L 17-18). These additional inoculations were required to ensure that canes with up to 16 inoculated buds could be retained on vines over winter.

After inoculation, the vines in Trial 2A were incubated in the glasshouse for six weeks at 24-30^{o}C and 65-85% relative humidity to promote the growth of *E. necator* on the surface of shoots and inside buds. Subsequently vines were kept in the glasshouse for 8 weeks at 18-22^{o}C and 40-65% relative humidity before they were exposed to climatic conditions that induced dormancy to allow them to over-winter. In Trials 2B, 2C and 2D, the vines were exposed to seasonal weather conditions after inoculation. Environmental conditions during the trials were recorded using data loggers or automatic weather stations located within or adjacent to the trial plots.

Fungicide treatments

Fungicide treatment programs that were evaluated in Trials 2A-2D are summarised in Tables 4-7, respectively. Fungicides included in the spray programs were the demethylation inhibiting (DMI, triazole) fungicide, penconazole (Topas®, 100g/L penconazole, Syngenta Crop Protection, Adelaide SA) applied at 12.5mL/100L (the recommended application rate) and wettable sulphur (Thiovit®, 800g/kg sulphur, Syngenta Crop Protection, Adelaide SA) applied at 300g/100L (the recommended application rate). In Trial 2A, a tank mixture of the morpholine fungicide, spiroxamine (Prosper®, 500g/L spiroxamine, Bayer CropScience East Hawthorn Vic.) at 40mL/100L and the DMI (triazole) fungicide, tebuconazole (Folicur®, 430g/L tebuconazole, Bayer CropScience, East Hawthorn Vic.) at 11.6mL/100L was also applied to vines in two spray programs. The latter mixture of fungicides was equivalent to that in the product Milord® (400g/L spiroxamine and 100g/L tebuconazole, Bayer AG, Leverkusen, Germany) applied at 50mL/100L. Milord® has high synergistic systemic activity and, to date, it has been registered for powdery mildew control in Europe but not in Australia (S. Cross pers. com.). All vines were thoroughly sprayed to the point of run-off when treatments were applied. Hand held sprayers (Trial 2A), motorised backpack sprayers (Trials 2B and 2D) or trailer-mounted sprayers (Trials 2C) were used to apply the DMI fungicide sprays. Commercial air-assisted spray machines were used to apply the sulphur sprays where required.

Assessment of disease development on shoots after inoculation in Season 1

The incidence and severity of powdery mildew on the leaves, stems and buds of three randomly selected inoculated shoots in each plot were assessed just before the first fungicide sprays were applied in each trial, using standardised powdery mildew disease assessment keys (Emmett, unpublished). In Trials 2A, 2B and 2C, initial disease assessments were conducted at two, six and four weeks after inoculation, respectively. Five randomly selected inoculated shoots per plot were assessed in Trial 2A and three randomly selected inoculated shoots per plot were assessed in Trials 2B and 2C.

Table 3. Summary of glasshouse (G/H) and field trials (F) conducted to determine the effects of fungicide treatments on buds infected with powdery mildew and the expression of flag shoots at Irymple Vic. and Loxton SA in 2004-2006.

Trial number	Location (Year)	Vine variety (Pruning type)	Inoculum (conidia/mL) applied to buds, stems and leaves of shoots	Vine growth stages[1] when shoots were inoculated[1] (Season)	Vine growth stages[1] when shoots were treated with fungicides[2] (Season)	Shoots/plot at each growth stage[1] inoculated x plots per treatment (number)	Buds exposed to primary[3] and secondary[4] infection assessed for flag shoots in Season 2 per treatment[2] (total numbers)
2A	Irymple G/H (2004-2005)	Verdelho (spur pruned)	5.5×10^4	C (Season 1)	D or E (Season 1) or A (Season 2)	20 x 10	1200[3], 1600[4]
2B	Loxton F (2004-2005)	Chardonnay (spur pruned)	$1.3\text{-}2.4 \times 10^4$	C (Season 1)	F (Season 1) or A (Season 2)	400 x 3	7200[3], 7200[4]
2C	Irymple F (2004-2005)	Sultana (cane pruned	$2.3\text{-}2.8 \times 10^4$	C, D, E (Season 1)	F (Season 1) or A (Season 2)	156 x 4	9360[3], 9360[4]
2D	Irymple F (2005-2006)	Chardonnay (spur pruned)	$2.3\text{-}2.8 \times 10^4$	C (Season 1)	D or E (Season 1) or A (Season 2)	80 x 8	3200[3], 3200[4]

[1] Vine growth stages: A = 0-1 leaves; B = 3 leaves; C = 5-6 leaves; D = 9-10 leaves; E = 12-15 leaves; F = just before flowering, 16 leaves.
[2] See text and Tables 4-7 for details of the fungicide treatments applied.
[3] Buds exposed to primary infection = buds inoculated in Season 1 and retained on vines for assessment in Season 2.
[4] Buds exposed to secondary infection = buds exposed to infection from conidia produced on diseased foliage after the time of inoculation in Season 1 and retained on vines for assessment in Season 2. This includes inoculated buds and buds that were not inoculated (including those produced during disease epidemics that developed after the time of inoculation in Season 1).

The incidence and severity of powdery mildew induced scarring on the internodes of 5, 50, 24 and 20 randomly selected inoculated shoots/plot in Trials 2A, 2B, 2C and 2D, respectively, was also assessed during vine dormancy.

Assessment of flag shoots produced from over-wintered infected buds in Season 2

In the following spring, the number of flag shoots produced from over-wintered buds at each node position on each shoot was recorded for each vine variety. The number of buds that died was also recorded. The percentages of buds that burst or died and the percentage of buds that burst and produced flag shoots were determined for each plot and in relation to each fungicide treatment program.

Weather conditions

During the field experiments, weather data were collected using automated weather stations in vineyards adjacent to the trial sites.

Statistical analyses

Analysis of variance (ANOVA) and linear regression (GenStat for Windows 2006) were used to investigate differences between treatments in relation to flag shoot incidence and relationships between disease incidence or severity on internodes and buds and treatments, respectively.

Table 4. Timing of inoculations, fungicide treatments and assessments in Trial 2A conducted in the glasshouse at Irymple Vic. in 2004-2005 to determine the effects of fungicide treatments on buds infected with powdery mildew and the expression of flag shoots on spur pruned Verdelho grapevines.

Season	Season 1						Season 2	
Weeks after bud burst	2	4	6	8	9	10-16	0-1	2-8
Vine growth stage	5-6 leaves	9-10 leaves	12-15 leaves	16 leaves Pre-flowering	Flowering	Berries 2-9mm	0-2 leaves	5-16 leaves
E-L stage number (Coombe 1995)	12-13	15-16	17-18	19	20-26	27-32	5-8	12-19
Bud infection								
Primary infection (inoculation)[1]	+	-	-	-	-	-	-	-
Secondary infection[2]	-	+	+	+	+	+	-	-
Fungicide treatments:								
Program 1	-	DMI[3]	-	-	-	S[4]	-	-
Program 2	-	-	DMI[3]			S[4]		
Program 3	-	-	-	-	-	S[4]	DMI[3]	-
Program 4	-	M+DMI[5]	-	-	-	S[4]	-	-
Program 5	-	-	M+DMI[5]	-	-	S[4]	-	-
Program 6	-	-	-	-	-	S[4]	-	-
Disease assessments	-	+[6]	-	-	-	-	-	+[7]

[1] Primary infection = inoculation in Season 1 (see text).
[2] Secondary infection = infection from conidia produced on diseased foliage after the time of inoculation in Season 1.
[3] DMI = a demethylation inhibiting fungicide [penconazole (Topas®)].
[4] S = a sulphur fungicide [wettable sulphur (Thiovit®)]. Up to three sprays applied at two-week intervals.
[5] M+DMI = a morpholine fungicide + a demethylation inhibiting fungicide [spiroxamine (Prosper®) tank mixed with tebuconazole (Folicur®)].
[6] Assessment of disease on shoots (buds, stems and leaves). Disease on shoot internodes was also assessed during vine dormancy.
[7] Assessment of flag shoots produced from over-wintered infected buds.

Table 5. Timing of inoculations, fungicide treatments and assessments in Trial 2B conducted in a vineyard at Loxton SA in 2004-2005 to determine the effects of fungicide treatments on buds infected with powdery mildew and the expression of flag shoots on spur pruned Chardonnay grapevines.

Season	Season 1						Season 2	
Weeks after bud burst	2	4	6	8	9	10-16	0-1	2-8
Vine growth stage	5-6 leaves	9-10 leaves	12-15 leaves	16 leaves Pre-flowering	Flowering	Berries 2-9mm	0-2 leaves	5-16 leaves
E-L stage number (Coombe 1995)	12-13	15-16	17-18	19	20-26	27-32	5-8	12-19
Bud infection								
Primary infection (inoculation)[1]	+	-	-	-	-	-	-	-
Secondary infection[2]	-	+	+	+	+	+	-	-
Fungicide treatments								
Program 1	-	-	-	DMI[3]	-	S[4]	DMI[3]	-
Program 2	-	-	-	-	-	S[4]	-	-
Disease assessment	-	-	+[5]	-	-	-	-	+[6]

[1] Primary infection = inoculation in Season 1 (see text).
[2] Secondary infection = infection from conidia produced on diseased foliage after the time of inoculation in Season 1.
[3] DMI = a demethylation inhibiting fungicide [penconazole (Topas®)].
[4] S = a sulphur fungicide [wettable sulphur (Thiovit®)]. Up to three sprays applied at two-week intervals.
[5] Assessment of disease on shoots (buds, stems and leaves). Disease on shoot internodes was also assessed during vine dormancy.
[6] Assessment of flag shoots produced from over-wintered infected buds.

Table 6. Timing of inoculations, fungicide treatments and assessments in Trial 2C conducted in a vineyard at Irymple Vic. in 2004-2005 to determine the effects of fungicide treatments on buds infected with powdery mildew and the expression of flag shoots on cane pruned Sultana grapevines.

Season	Season 1						Season 2	
Weeks after bud burst	2	4	6	8	9	10-16	0-1	2-8
Vine growth stage	5-6 leaves	9-10 leaves	12-15 leaves	16 leaves Pre-flowering	Flowering	Berries 2-9mm	0-2 leaves	5-16 leaves
E-L stage number (Coombe 1995)	12-13	15-16	17-18	19	20-26	27-32	5-8	12-19
Bud infection								
Primary infection (inoculation)[1]	+	+	+	-	-	-	-	-
Secondary infection[2]	-	+	+	+	+	+	-	-
Fungicide treatments								
Program 1	-	-	-	DMI[3]	-	S[4]	-	-
Program 2	-	-	-	-	-	S[4]	DMI[3]	-
Program 3	-	-	-	-	-	S[4]	-	-
Disease assessment	-	-	+[5]	-	-	-	-	+[6]

[1] Primary infection = inoculation in Season 1 (see text).
[2] Secondary infection = infection from conidia produced on diseased foliage after the time of inoculation in Season 1.
[3] DMI = a demethylation inhibiting fungicide [penconazole (Topas®)].
[4] S = a sulphur fungicide [wettable sulphur (Thiovit®)]. Up to three sprays applied at two-week intervals.
[5] Assessment of disease on shoots (buds, stems and leaves). Disease on shoot internodes was also assessed during vine dormancy.
[6] Assessment of flag shoots produced from over-wintered infected buds.

Table 7. Timing of inoculations, fungicide treatments and assessments in Trial 2D conducted in a vineyard at Irymple Vic. in 2005-2006 to determine the effects of fungicide treatments on buds infected with powdery mildew and the expression of flag shoots on spur pruned Chardonnay grapevines.

Season	Season 1						Season 2	
Weeks after bud burst	2	4	6	8	9	10-16	0-1	2-8
Vine growth stage	5-6 leaves	9-10 leaves	12-15 leaves	16 leaves Pre-flowering	Flowering	Berries 2-9mm	0-2 leaves	5-16 leaves
E-L stage number (Coombe 1995)	12-13	15-16	17-18	19	20-26	27-32	5-8	12-19
Bud infection								
Primary infection (inoculation)[1]	+	-	-	-	-	-	-	-
Secondary infection[2]	-	+	+	+	+	+	-	-
Fungicide treatments:								
Program 1	-	DMI[3]	-	-	-	S[4]	-	-
Program 2	-	-	DMI[3]			S[4]		
Program 3	-	-	-	-	-	S[4]	DMI[3]	-
Program 4	-	-	-	-	-	S[4]	-	-
Disease assessments	-	-	-	-	-	-	-	+[5]

[1] Primary infection = inoculation in Season 1 (see text).
[2] Secondary infection = infection from conidia produced on diseased foliage after the time of inoculation in Season 1.
[3] DMI = a demethylation inhibiting fungicide [penconazole (Topas®)].
[4] S = a sulphur fungicide [wettable sulphur (Thiovit®)]. Up to three sprays applied at two-week intervals.
[5] Assessment of flag shoots produced from over-wintered infected buds. (Disease on shoot internodes was also assessed during vine dormancy).

8 Results

8.1 R&D in the United States (Summary)

[See Rumbolz and Gubler (2005) for more details]

Colonisation of the surface of buds inoculated in vitro

Sporulation was observed on inoculated buds after 6 d. The average latent period of *E. necator* ranged from 7.4 to 11.1 d and infection success on the bud surface varied from 0.21 to 0.88 in four experiments. Histological studies of buds with surface growth of *E. necator* revealed infection of the bud interior 22 d after inoculation. From 13-65% of all inoculated buds were infected internally.

Colonisation of the interior of buds inoculated in vitro

All fungal structures of the anamorphic stage of *E. necator* were present in thin sections of infected buds. These included conidiophores with attached conidia, germinated conidia with primary appressoria and haustoria, either attached to hyphae or isolated. The inner bud scales, prophylls and leaf primordia were colonised. In the same samples, the lateral shoot that is located opposite the compound bud inside the leaf axil, was also often colonised. The mean latent period on the lateral shoot was comparable with that on the bud surface (9.5 and 11.2 d on average). Internal infection of *E. necator* was observed in thin sections of lateral shoots (13 and 30% of the total number of inoculated samples). At this developmental stage, the lateral shoot was enclosed in the axillary bud complex.

Susceptibility of developing shoots and bud surfaces (Season 1)

Ten d after inoculation disease incidence on leaves of emerging shoots was at least 75% in all inoculation treatments. Disease expression on shoot stems was delayed compared with leaves, but reached 80% and higher by 17 d after inoculation. Between 10 and 17 d after inoculation, disease incidence on shoot stems was slightly lower from inoculations at E-L stages 5 (0-1 leaves) and 15-16 (9 leaves) compared with E-L stages 10 (3 leaves) and 13 (6 leaves). Disease severity also increased with time ($P < 0.0001$). Most leaves reached more than 20% leaf area diseased by 17 d after inoculation at E-L stages 10, 13 and 15-16, whereas inoculation at E-L stage 5 showed lower severity. Disease severity increased more slowly on shoot stems than on leaves and as a result, at each stage, development of disease was more noticeable than on leaves. Severity on shoot stems was lower from inoculation at E-L 5 and 15-16 compared with E-L 10 and 13 on all assessment dates ($P < 0.0001$). Infection of powdery mildew on the bud surface was evident on 68 and 62% of all buds inoculated at E-L 10 and 13, respectively, but only 16 and 22% of those inoculated at E-L 5 and 15-16 showed infections on the exterior (Figure 6). Figure 3A shows a bud infected externally by *E. necator*. External growth of the pathogen covered the overlapping bud scales (Figure 3B).

Susceptibility of developing buds to internal infection (Season 1)

Thin sections of the buds from shoots inoculated at E-L stages 5, 10, 13 and 15-16 showed all anamorphic stages of *E. necator*. Except for the apical meristem and the meristem yielding the inflorescences of the next season, all green parts of the bud interior were colonised by hyphae with haustoria. This consisted of the inner side of the bud scales, the prophylls, leaf primordia and trichomes. A longitudinal section through a representative dormant bud is shown in Figure 4. Most of the entire space between leaf primordia and apex is filled with trichomes. Although prophylls and/or leaf primordia were often infected by *E. necator* (Figures 5A and 5B), the majority of infected buds contained haustoria in trichomes (Figure 5C). Because of their size, these organs appear to harbour the most haustoria. In turn, formation of haustoria in meristematic cells appeared to be impeded. The colonisation of buds originating from shoots inoculated during their development was also quantified. In total, 13.2% of 546 buds examined were infected by *E. necator*. The highest proportion (32.3%) of infected buds was found on canes inoculated as shoots at E-L 10 (3 leaves) in the previous season. In contrast, only 6.6-9.3% of buds from shoots inoculated at E-L stages 5, 13 or 15-16 was colonised (Figure 6).

Incidence of flag shoot formation (Season 2)

Buds from inoculated shoots that remained on vines during winter were monitored in spring of the second year for the occurrence of primary infections originating from over-wintering mycelium of *E. necator*. The first seven flag shoots (39% of the total number) were identified on Day 19 after bud burst in the plot. A further 10 flag shoots (56% of the total number) were found between Days 20 and

30 after bud burst. A single additional flag shoot developed about 9 weeks after bud burst from a dormant bud that grew after the removal of a primary shoot. Except for this late flag shoot, all others appeared when shoots on vines had 3-6 unfolded leaves. The flag shoots displayed typical features including colonised shoot sections, petioles and leaves while many had stunted growth. Later, some of the flag shoots died. Nine of the 18 flag shoots were found on canes from shoots inoculated in the previous year with three unfolded leaves and five were found on canes from shoots inoculated with six unfolded leaves. However, overall the incidence of flag shoots was low (1.6% of all shoots) and no significant difference was detected between treatments (different dates of inoculation in first year) with respect to the number of flag shoots (χ^2 test, $P = 0.07$). Twenty-eight days after the appearance of the first flag shoot, a severe epidemic (100% disease incidence) developed on vines in the field plot.

Relationships between external and internal bud infection and flag shoot formation
Infection of the bud exterior in the first year was positively correlated with flag shoot formation in the second year ($R^2 = 0.94$, $P < 0.03$). In contrast, no significant relationship was observed between internal bud infection and flag shoot formation or between external and internal bud infection. These results indicate that the incidence of flag shoots may be predicted by assessing the extent of external bud infection in the preceding year.

Susceptibility of internal tissues of dormant buds
Dissected tissues from dormant buds towards the end of dormancy and before to the onset of shoot development were highly susceptible to infection by *E. necator*. Sporulating mycelia developed on most tissue samples after inoculation. While colonies sporulated prolifically on Thompson Seedless leaf primordia, no infection was found on bud apices. However, leaf primordia adjacent to the apical meristem produced conidiophores 6 d after inoculation.

The mean latent periods for internal bud tissues of Thompson Seedless and Carignane were 6.0-6.5 and 5 d, respectively. Although there was no significant difference, there was a trend towards a longer latent period for internal bud tissues of Thompson Seedless than for Carignane.

Effects of temperature on flag shoot formation
Bud burst occurred within 5 d and 8 d after induction in Chamber 2 (daylight temperature 30°C for 16 h) and Chamber 1 (daylight temperature 22°C for 16 h), respectively. Five flag shoots were found on rootlings in Chamber 1, whereas no flag shoots were produced on rootlings in Chamber 2.

8.2 R&D in Australia

8.2.1 Evaluation of inoculum preparation and inoculation techniques

Effects of wetting agent concentration on inoculum viability
Mean numbers of viable conidia of *E. necator* in spore suspensions with deionised water and with Tween 20® at 25µL/100mL were 25,500 and 70,500, respectively. Addition of the wetting agent increased the spore count by 176%. Numbers of viable conidia in spore suspensions with Tween 20® at 50µL/100mL were lower than in suspensions with Tween 20® at 25µL/100mL. In these tests, use of Tween 20® at rates higher than 25µL/100mL appeared to have a toxic affect on conidium viability. Nearly all conidia were destroyed in suspensions with Tween 20® at 100µL/100mL.

Effects of storage time on inoculum viability
The number of viable conidia declined when spore suspensions with Tween 20® at 25µL/100mL were stored at 5-6°C for up to 60 min. After the conidial suspensions were stored for 30 and 60 min, the number of viable conidia was reduced by 37% and 66%, respectively, in relation to the number of viable conidia at 0 min (Table 8).

Effects of inoculum storage time on conidium germination, leaf infection and disease development
Storage of conidial suspensions up to 60 min had no effect on conidium germination rate. After the conidial suspensions with Tween 20® at 25µL/100mL were stored at 5-6°C for 30 and 60 min, the proportion of viable conidia that germinated on glass slides was 55% and 56%, respectively (Table 9).

Figure 3. A. A grapevine bud infected externally by *E. necator*. (Photo by Terry Hunt, DPI). **B.** External growth of *E. necator* covering the overlapping scales of a grapevine bud. (Photo courtesy of Joachim Rumbolz and Doug Gubler, UCD).

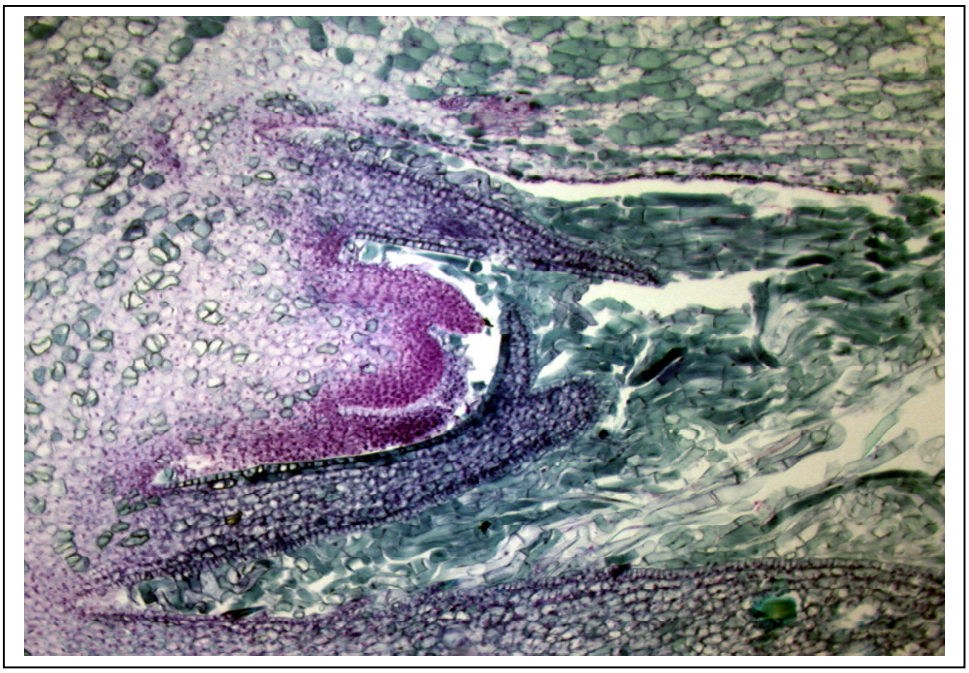


Figure 4. A longitudinal section through a grapevine bud showing the inner sides of the bud scales (top and bottom), the prophylls, leaf primordia (centre and left centre) and trichomes (right centre). (Photo courtesy of Joachim Rumbolz and Doug Gubler, UCD).

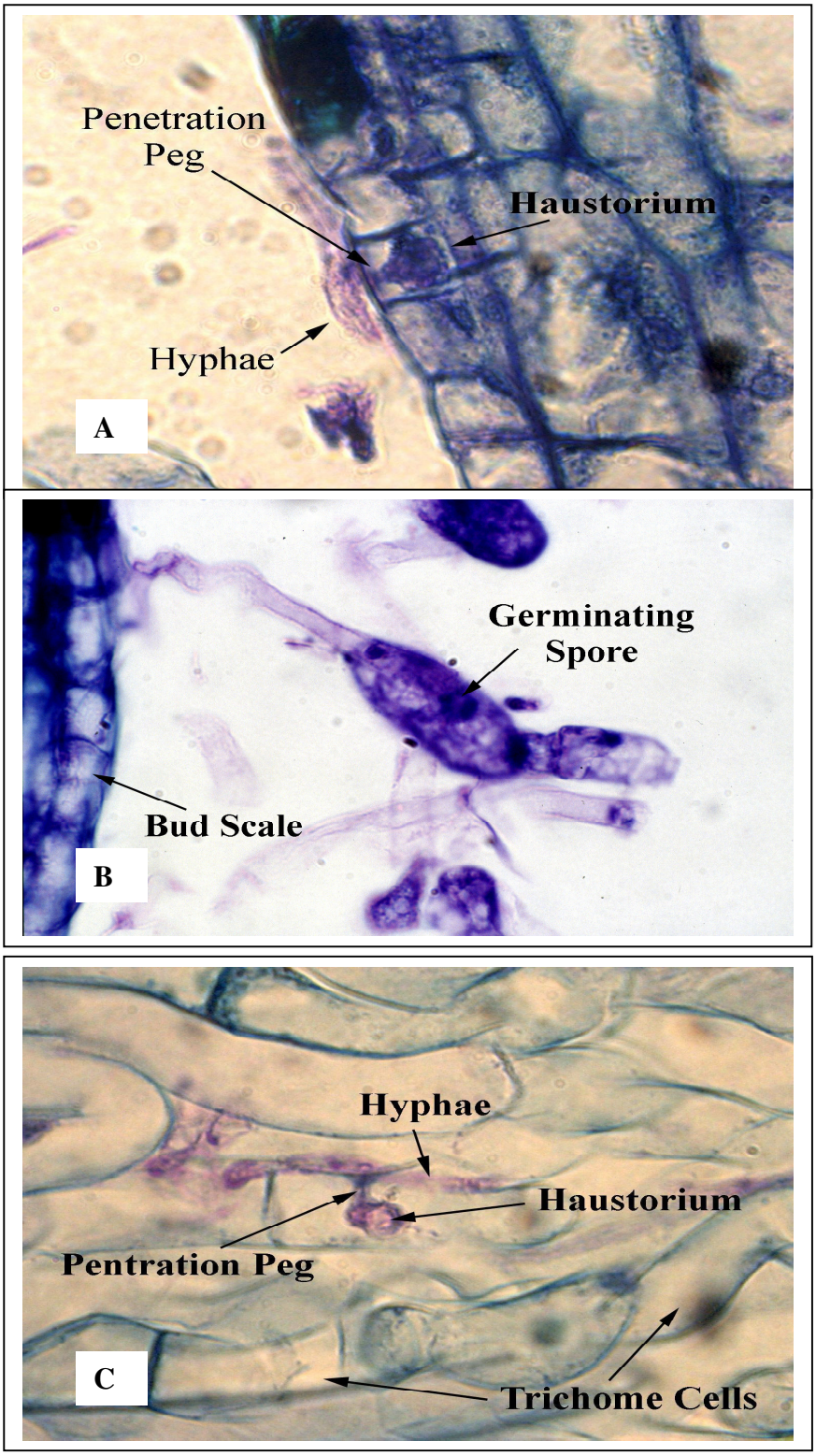


Figure 5. Sections of internal organs of grapevine bud infected by *E. necator*. **A.** A hypha, infection peg and haustorium of *E. necator* on/in a leaf primordium. **B.** A conidiophore and conidium of *E. necator* on the surface of a leaf primordium. **C.** Hyphae and an infection peg and haustorium on/in trichome cells of an infected bud. (Photos courtesy of Doug Gubler, UCD).

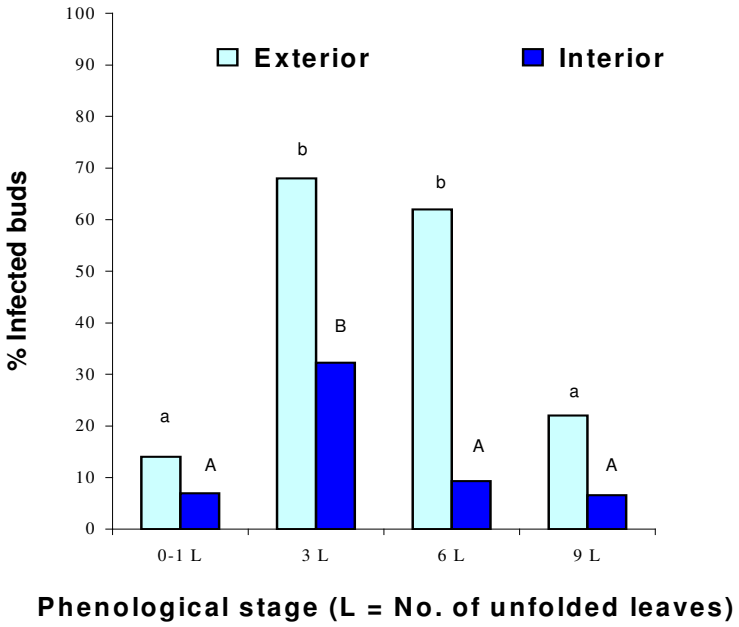


Figure 6. *E. necator* colonisation of the exterior and interior of buds on shoots of *Vitis vinifera* cv. Carignane inoculated at different phenological stages (0-1, 3, 6 and 9 unfolded leaves). (Adapted from Rumbolz and Gubler 2005). [0-1 unfolded leaves = E-L 5, 3 unfolded leaves = E-L 10, 6 unfolded leaves = E-L 13 and 9 unfolded leaves = E-L 15-16].

Table 8. Numbers and proportions of viable conidia of *E. necator* in spore suspensions with Tween 20® at 25µL/100mL stored for up to 60 min at 5-6°C.

Time (minutes)	Viable conidia (Number/mL)	Proportion of viable conidium count at 0 minutes (%)
0	40,000[1]	100
10	34,400	86
20	30,800	77
30	25,200	63
40	18,800	47
50	21,200	53
60	13,600	34

[1] Values are mean numbers of viable conidia in four separate tests for each time interval.

Table 9. Numbers and proportions of viable conidia of *E. necator* that germinated on glass slides for spore suspensions with Tween 20® at 25µL/100mL stored for up to 60 min at 5-6°C.

Suspension storage time (minutes)	Viable conidia [1] (Number)	Conidia germinated [1] (Number)	Proportion of viable conidia germinated [1] (%)
0	91	37	41
30	119	65	55
60	94	53	56

[1] Values are mean numbers of viable conidia and germinated conidia in four separate tests for each time interval.

Effects of leaf age and condition on infection and disease development

Leaf age and condition affected infection and disease development on inoculated leaves. Infection, colony development and sporulation were most extensive on young leaves that had a glossy appearance when inoculated. Less colony development and sporulation occurred on older leaves with little or no shine on the upper surface.

Effects of inoculum type and application technique on conidium survival and germination and on bud infection

In most tests, conidium survival and germination on glass slides were similar for the different inoculum types and application techniques. In some tests, conidium survival was slightly greater with the pipette method than the wet brush method, and with 'dry' inoculum than with 'wet' inoculum. Wetness and humidity after inoculation, however, had a greater effect on conidium survival and germination than inoculum type and application technique. Prolonged wetness after inoculation substantially reduced conidium survival because conidia trapped in water droplets or films eventually ruptured and died. Prolonged low humidity after inoculation also substantially reduced conidium survival because conidia were desiccated.

The field evaluation of bud inoculation techniques using 'wet' or 'dry' inoculum conducted in mid October 2003 was unsuccessful. After 15 d, little or no powdery mildew developed on inoculated buds, apparently because of the unusually hot dry windy weather with daytime temperatures of more than 30°C that occurred during the 7 d after inoculation. This result highlighted some of the factors (eg. adverse weather) that were likely to prevent the successful artificial inoculation of grapevines in field trials.

8.2.2 Studies of the period of susceptibility of grapevine buds to infection by powdery mildew

Glasshouse Trial 1A (Verdelho, 2003/2004)

The increase in powdery mildew severity on leaves of shoots during the disease epidemic that developed after inoculation in Season 1 (2003/04) is shown in Figure 7. The severity of disease on leaves decreased substantially after the two sprays of Stylet Oil® were applied to terminate the epidemic five weeks after inoculation.

At the time of inoculation, colours of Verdelho buds that were aged 1-10, 11-23, 24-33, 34-42 and 43 or more days were green, pink-green, pink, pink-brown and brown, respectively. Buds of different age exposed to powdery mildew infection in Season 1 (2003/04) that died over winter or produced flag shoots in spring of Season 2 (2004/05) on Verdelho grapevines in Trial 1A are shown in Table 10. From 0.9-1.8% of buds that were aged 1-18 days at the time of inoculation and when disease epidemics commenced produced flag shoots in the following spring. In this trial, buds aged more than 18-21 days were either not susceptible to infection or were not infected enough to produce flag shoots. Depending on bud age, 6.3-19.1% of buds died after exposure to powdery mildew infection.

Buds on shoots at different growth stages exposed to powdery mildew infection in Season 1 (2003/04) that died over winter or produced flag shoots in the spring of Season 2 (2004/05) are shown in Table 11. On shoots with 3, 6 and 9 leaves infected with powdery mildew, 0.4%, 0.9% and 0.6% of over-wintered buds produced flag shoots, respectively.

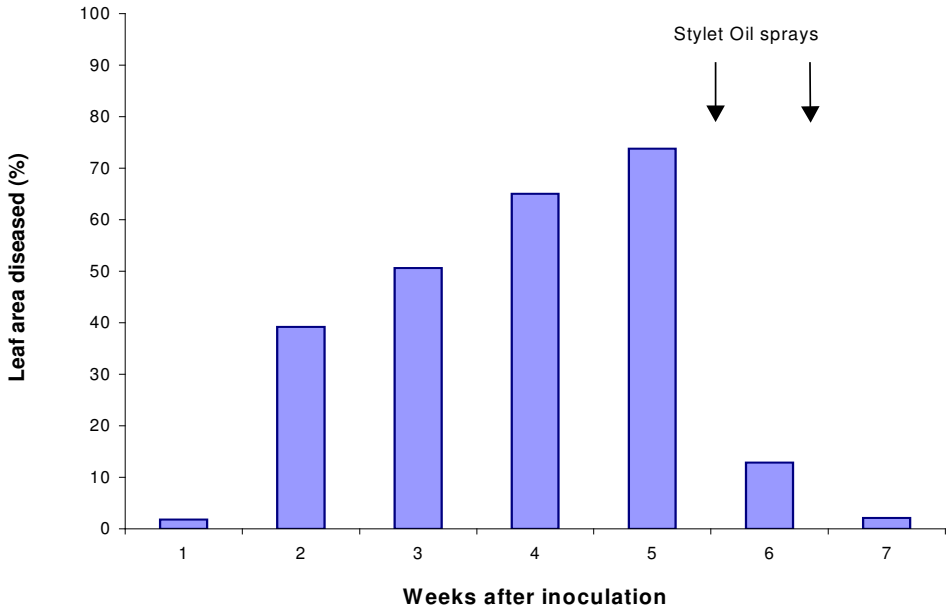


Figure 7. The severity of powdery mildew on leaves of shoots on Verdelho grapevines (percent leaf area diseased) in Glasshouse Trial 1A at Irymple Vic. during the disease epidemic that developed after inoculation in Season 1 (2003/04).

Table 10. Buds of different age exposed to powdery mildew infection in Season 1 (2003/04) that died over winter or produced flag shoots in spring of Season 2 (2004/05) on Verdelho grapevines in Glasshouse Trial 1A at Irymple Vic.

Bud age[1] (days)	Buds assessed (number)	Dead Buds[2] [number (%)]	Buds with flag shoots [number (%)]
1	183	35 (19.1)	2 (1.1)
4	310	49 (15.8)	3 (1.0)
7	396	39 (13.5)	4 (1.4)
10	338	39 (11.5)	3 (0.9)
13	370	30 (14.5)	3 (1.5)
16	222	11 (10.6)	1 (1.0)
18	111	7 (6.3)	2 (1.8)
21	118	10 (8.5)	0 (0.0)

[1] Age of buds when exposed to infection by *E. necator* during inoculation (primary infection) or during the epidemic that developed after inoculation (secondary infection).
[2] Buds that died after exposure to powdery mildew infection. Numbers of buds exclude numbers of basal buds that often did not burst and terminal buds that died when shoots died back during winter because of stress caused by disease or other factors.

Table 11. Buds on shoots at different growth stages exposed to powdery mildew infection in Season 1 (2003/04) that died over winter or produced flag shoots in spring of Season 2 (2004/05) on Verdelho grapevines in Glasshouse Trial 1A at Irymple Vic. in 2003/2004.

Shoot growth stage[1]	Buds assessed (number)	Buds dead[2] [number (%)]	Buds with flag shoots [number (%)]
3 leaves	256	60 (23.4)	1 (0.4)
6 leaves	553	113 (20.4)	5 (0.9)
9 leaves	709	104 (14.7)	4 (0.6)

[1] Growth stage of shoots when exposed to infection by *E. necator* during inoculation (primary infection) or during the epidemic that developed after inoculation (secondary infection).
[2] Buds that died after exposure to powdery mildew infection. Numbers exclude numbers of basal buds that often did not burst and terminal buds that died when shoots died back during winter because of stress caused by disease or other factors.

Glasshouse Trial 1B (Chardonnay, Loxton 2003/2004)

The increase in powdery mildew severity on leaves of Chardonnay shoots during the disease epidemic that developed after inoculation in Season 1 (2003/04) is shown in Figure 8. The severity of disease on leaves decreased substantially after the two sprays of Stylet Oil® were applied to terminate the epidemic five weeks after inoculation.

In this trial, substantial numbers of terminal buds on shoots died over winter because of the effects of the disease and other factors on vine health. Very low numbers of buds on shoots of all of the growth stages that were exposed to powdery mildew infection in Season 1 (2003/04) survived to produce shoots in the spring of Season 2 (2004/05). Consequently, no conclusions could be drawn about bud infection and flag shoot formation in Season 2 in relation to inoculated buds on shoots at different phenological stages in Season 1.

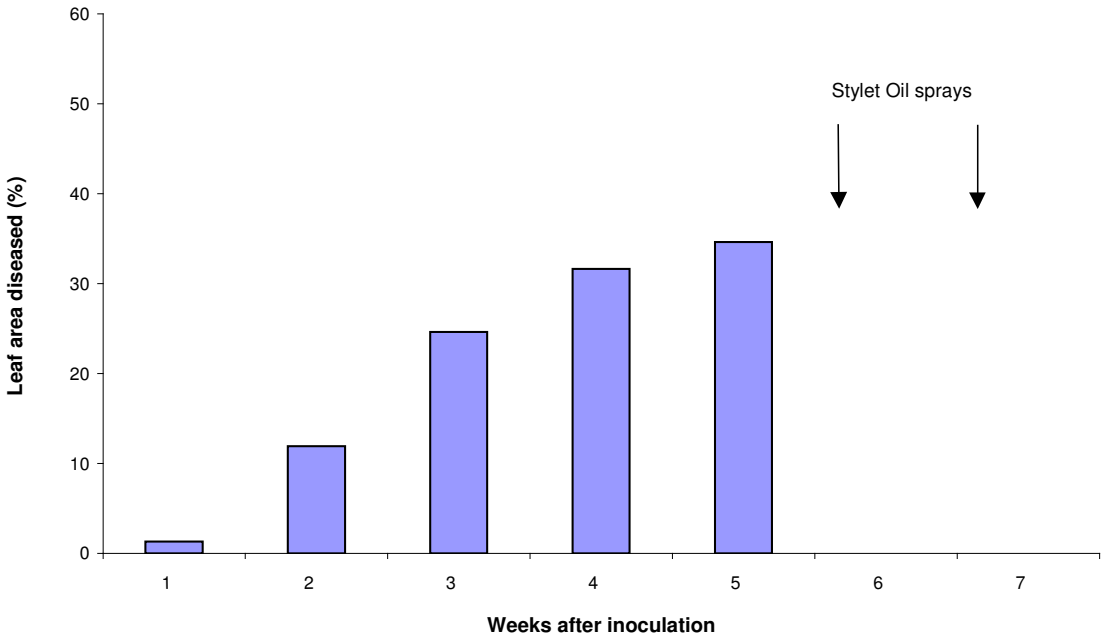


Figure 8. The severity of powdery mildew on leaves of shoots on Chardonnay grapevines (percent leaf area diseased) in Glasshouse Trial 1B at Loxton SA during the disease epidemic that developed after inoculation in Season 1 (2003/04).

Glasshouse Trial 1C (Sultana, Irymple 2004/2005)

Five weeks after inoculation in Season 1, 74% of the inoculated Sultana buds (260 out of 350 buds) were diseased externally. Sporulating powdery mildew and/or scarring by mycelia of *E. necator* was observed on the surface of these buds and/or on their sheaths. The incidence of external infection by *E. necator* on buds of different age and on adjacent tissues (stems, leaf petioles and lateral shoots) on shoots at different growth stages at five weeks after inoculation in Season 1 (2004/05) is shown in Figure 9. External infection of buds and/or the sheaths of buds was significantly associated with external infection of adjacent stems and/or leaf petioles (Phi coefficient = 0.29, $P < 0.001$). Nearly all buds (94%) with surface infection and/or sheath infection had adjacent stems and/or leaf petioles that were also infected.

The incidence of buds of different age on shoots at different growth stages exposed to infection by *E. necator* in Season 1 (2004/05) that died over winter or produced flag shoots in the spring of Season 2 (2005/06) is shown in Figure 10. The number of inoculated buds that died over winter declined with bud age at the time of inoculation ($P < 0.0001$, Cochran-Armitage test for trend). The percentage of buds on shoots with 0-1, 3, 6 and 9 leaves at the time of inoculation that died during winter was 28.5, 27.0, 13.6 and 9.8, respectively. The results indicate that younger Sultana buds exposed to infection in Season 1 are more like to die during the following winter than older buds.

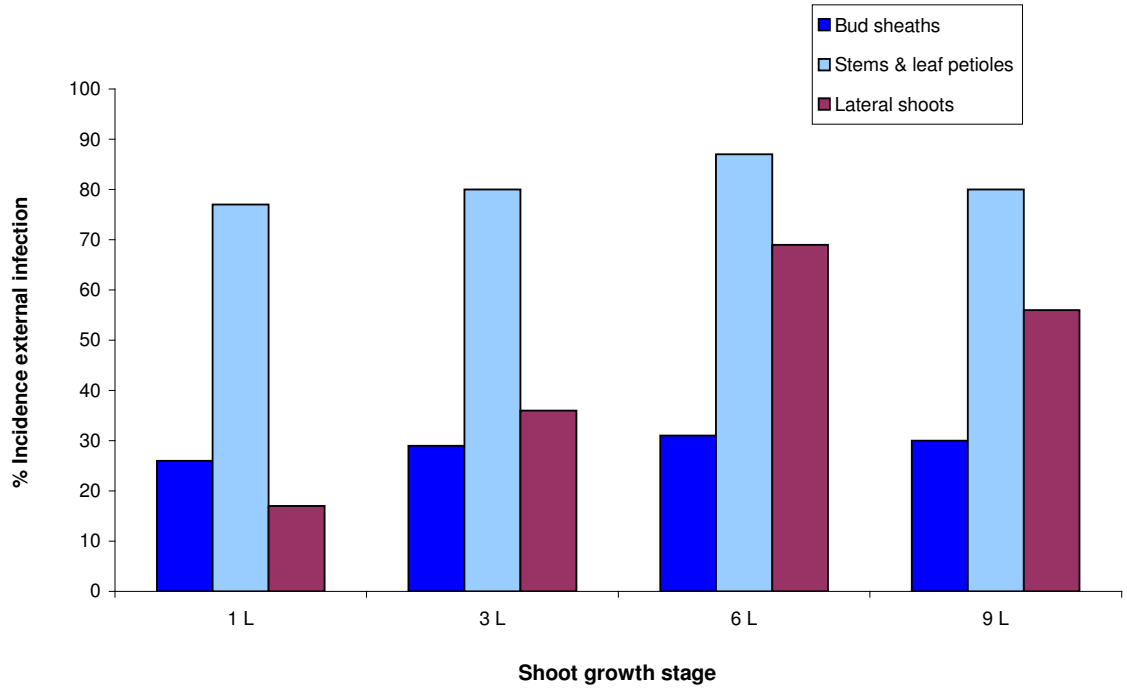


Figure 9. Incidence of external infection by *E. necator* (powdery mildew) on buds of different age and on adjacent tissues (stems, leaf petioles and lateral shoots) on shoots at different growth stages on Sultana grapevines in Glasshouse Trial 1C at Irymple Vic. at 5 weeks after inoculation in Season 1 (2004/05).

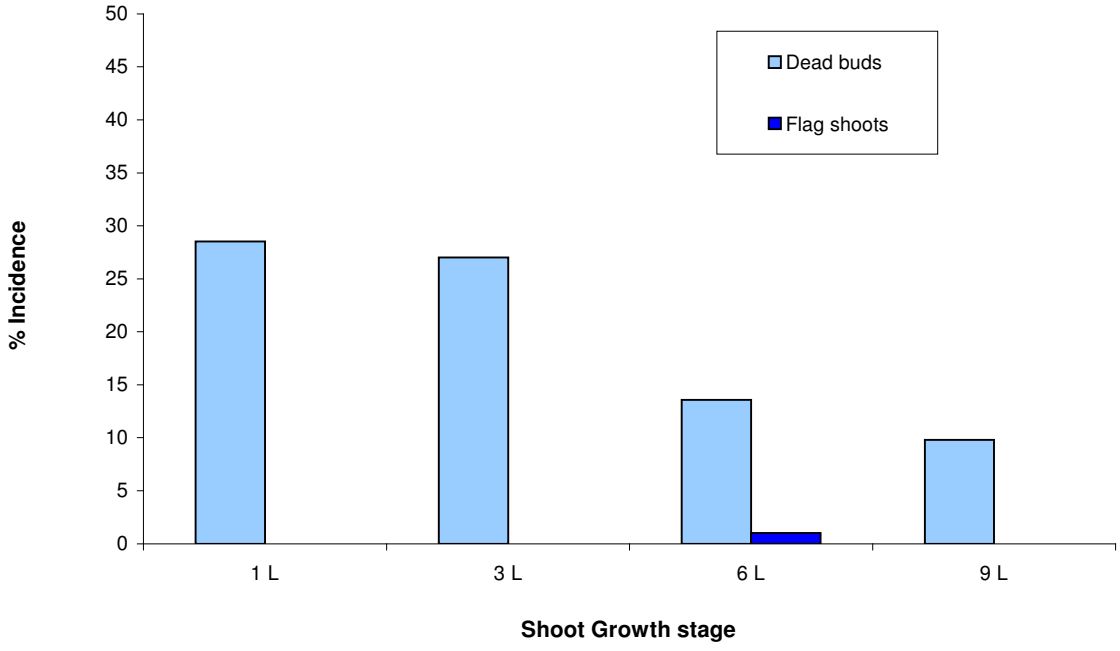


Figure 10. Incidence of buds of different age on shoots at different growth stages exposed to infection by *E. necator* in Season 1 (2004/05) that died over winter or produced flag shoots in the spring of Season 2 (2005/06) on Sultana grapevines in Glasshouse Trial 1C at Irymple Vic. in 2004-2005.

Glasshouse Trial 1D (Verdelho, Loxton 2004/2005)

After inoculation, powdery mildew severity on leaves of Verdelho shoots increased substantially until the first of three fortnightly sprays of Stylet Oil® was applied 5 weeks after inoculation to terminate the epidemic. Table 12 shows the severity of disease on leaves at each node (bud) position on shoots at the end of the epidemic that followed inoculation in Season 1 (2004/05). The incidence of flag shoots in Season 2 (2005/2006), produced from over-wintered buds that were exposed to infection during the epidemic in Season 1, is also shown. Table 13 shows the mean severity of disease on leaves adjacent to buds on shoots of different phenological stage at five weeks after inoculation, and the mean flag shoot incidence in Season 2 in relation to buds on shoots of different phenological stage exposed to infection in Season 1.

Although not significant, there was a trend towards higher disease severity on younger leaves (adjacent to buds aged 4-18 d) than on older leaves (adjacent to buds aged 22 or more d) (Table 12).

In this trial, flag shoots developed from over-wintered primary buds in Season 2 that were aged 2-24 d when exposed to infection in Season 1. The incidence of over-wintered primary buds that produced flag shoots ranged from 7.95% to 13.58% (Table 12). Again, although not significant, there was a trend towards higher production of flag shoots from over-wintered primary buds that were aged 4-20 d when exposed to infection in Season 1. This trend appeared to align with the trend towards higher disease severity on leaves adjacent to these buds in Season 1 (noted above). Increased death of terminal buds on shoots during winter moderated the numbers of flag shoots produced from buds that were younger (aged 2-6 d) when exposed to infection during the severe epidemic in Season 1.

After flag shoots produced from over-wintered primary buds were removed in Season 2, some secondary buds on the same nodes also produced flag shoots. Furthermore, after flag shoots produced from the secondary buds were removed, some tertiary buds on the same nodes produced further flag shoots. The incidence (%) of over-wintered primary, secondary and tertiary buds that produced flag shoots in Season 2 in relation to numbers of buds at each bud position and buds on shoots of each phenological stage exposed to infection in Season 1 is shown in Tables 12 and 13.

Glasshouse Trial 1E (Chardonnay, Loxton 2004/2005)

After inoculation, powdery mildew severity on leaves of Chardonnay shoots also increased substantially until the first spray of Stylet Oil® was applied 5 weeks after inoculation. This spray and two further sprays at two-week intervals terminated a severe epidemic. Table 14 shows the severity of disease on leaves at each node (bud) position on shoots at the end of the epidemic that followed inoculation in Season 1 (2004/05), and the incidence of flag shoots in Season 2 (2005/2006), produced from over-wintered buds that were exposed to infection during the epidemic in Season 1. Table 15 shows the mean severity of disease on leaves adjacent to buds on shoots of different phenological stage at five weeks after inoculation, and the mean flag shoot incidence in Season 2 in relation to buds on shoots of different phenological stage exposed to infection in Season 1.

Although not significant, there was a trend towards higher disease severity on younger leaves (adjacent to buds aged 4-12 d) than on older leaves (Table 14).

In this trial, flag shoots developed from over-wintered primary buds in Season 2 that were aged 2-20 d when exposed to infection in Season 1. The incidence of over-wintered primary buds that produced flag shoots ranged from 0 to 4.37% (Table 14). A conditional logistic regression analysis showed that there was a significant trend towards higher production of flag shoots from over-wintered primary buds that were younger (especially those aged 2-12 d) when exposed to infection in Season 1 ($P < 0.001$). This trend appeared to align with the trend towards higher disease severity on leaves adjacent to most of these buds in Season 1.

As in Trial 1D, after flag shoots produced from over-wintered primary buds were removed in Season 2, occasional secondary buds on the same nodes also produced flag shoots. However, no tertiary buds on the same nodes produced further flag shoots after shoots from the secondary buds were removed. The incidence (%) of over-wintered primary and secondary buds that produced flag shoots in Season 2 in relation to numbers of buds at each bud position and buds on shoots of each phenological stage exposed to infection in Season 1 is shown in Tables 14 and 15.

Table 12. The severity of powdery mildew on leaves adjacent to buds at five weeks after inoculation in Season 1 (2004/05) in relation to the percentage of primary, secondary or tertiary buds that over-wintered and produced flag shoots in Season 2 (2005/2006) on Verdelho grapevines in Glasshouse Trial 1D at Loxton SA.

Season 1				Season 2		
Epidemic commencement[1]			Epidemic end[2]			
Shoot growth stage[3] (number of leaves)	Bud or node number[4]	Bud age[5] (days)	Leaf area diseased[6] (%)	Primary buds with flag shoots[7] (%)	Secondary buds with flag shoots[7] (%)	Tertiary buds with flag shoots[7] (%)
3 Leaves	12	2	NR[8]	8.61	0.05	0
	11	4	79.2	10.10	0.83	0
	10	6	85.2	12.75	1.00	0
6 Leaves	9	8	NR	13.58	0.83	0
	8	10	88.3	13.25	1.00	0
	7	12	82.0	13.41	0.83	0
9 Leaves	6	14	NR	12.25	0.50	0.20
	5	16	73.8	11.26	0.50	0
	4	18	68.4	12.25	0.33	0
12 Leaves	3	20	NR	11.75	1.00	0
	2	22	40.2	9.44	0	0
	1	24	25.9	7.95	0	0

[1] Epidemic commencement = day of inoculation in Season 1.
[2] Epidemic termination occurred when Stylet Oil® sprays were applied from 5 weeks after inoculation.
[3] Buds on shoots at each growth stage exposed to infection also include those for the earlier stages presented in the Table. For a summary, see Table 13.
[4] Buds (nodes) were numbered from the base of each shoot.
[5] Estimated bud age based on a leaf generation time of one leaf every two days in glasshouse conditions.
[6] Mean area diseased on the primary leaf adjacent to each bud.
[7] Flag shoots were produced in Season 2 from over-wintered primary, secondary and tertiary buds within buds exposed to infection in the previous season. Secondary and tertiary shoots developed after primary and secondary shoots were removed, respectively. Values are percentages of 604 buds exposed to infection at each node in Season 1 that produced flag shoots in Season 2.
[8] NR = Not recorded.

Table 13. The mean severity of powdery mildew on leaves adjacent to buds on shoots of different phenological stages at five weeks after inoculation in Season 1 (2004/05) in relation to the mean percentage of primary, secondary or tertiary buds that over-wintered and produced flag shoots in Season 2 (2005/2006) on Verdelho grapevines in Glasshouse Trial 1D at Loxton SA.

Season 1				Season 2		
Epidemic commencement [1]			Epidemic end [2]			
Shoot growth stage[3] (number of leaves, L)	Bud or node number[4]	Bud age[5] (days)	Leaf area diseased[6] (%)	Primary buds with flag shoots[7] (%)	Secondary buds with flag shoots[7] (%)	Tertiary buds with flag shoots[7] (%)
0-1 L	12	0-2	NR[8]	8.61	0.05	0
3 L	10-12	2-6	82.2	10.47	0.63	0
6 L	7-12	2-12	83.7	11.95	0.76	0
9 L	4-12	2-18	79.5	11.94	0.65	0.02
12 L	1-12	2-24	67.9	11.38	0.57	0.02

[1] Epidemic commencement = day of inoculation in Season 1.
[2] Epidemic termination occurred when Stylet Oil® sprays were applied from 5 weeks after inoculation.
[3] Shoots with 0-1, 3, 6, 9 and 12 separated (unfolded) leaves.
[4] Buds (nodes) related to each growth stage on a shoot with 12 leaves with buds numbered from the base of the shoot.
[5] Estimated bud age based on a leaf generation time of one leaf every two days in glasshouse conditions.
[6] Mean area diseased on the primary leaf adjacent to the buds on each shoot.
[7] Flag shoots were produced in Season 2 from over-wintered primary, secondary and tertiary buds within buds exposed to infection in the previous season. Secondary and tertiary shoots developed after primary and secondary shoots were removed, respectively. Values are percentages of 604 buds exposed to infection at each node in Season 1 that produced flag shoots in Season 2.
[8] NR = Not recorded.

Table 14. The severity of powdery mildew on leaves adjacent to buds at five weeks after inoculation in Season 1 (2004/05) in relation to the percentage of primary, secondary or tertiary buds that over-wintered and produced flag shoots in Season 2 (2005/2006) on Chardonnay grapevines in Glasshouse Trial 1E at Loxton SA.

Season 1				Season 2		
Epidemic commencement[1]			Epidemic end[2]			
Shoot growth stage[3] (number of leaves)	Bud or node number[4]	Bud age[5] (days)	Leaf area diseased[6] (%)	Primary buds with flag shoots[7] (%)	Secondary buds with flag shoots[7] (%)	Tertiary buds with flag shoots[7] (%)
3 Leaves	12	2	NR[8]	3.23	0	0
	11	4	88.2	3.42	0	0
	10	6	87.1	4.37	0	0
6 Leaves	9	8	NR	3.61	0	0
	8	10	81.8	2.66	0	0
	7	12	75.9	2.47	0.19	0
9 Leaves	6	14	NR	1.90	0	0
	5	16	63.1	0.90	0	0
	4	18	50.2	1.00	0	0
12 Leaves	3	20	NR	0.20	0	0
	2	22	71.6	0	0	0
	1	24	69.4	0	0	0

[1] Epidemic commencement = day of inoculation in Season 1.
[2] Epidemic termination occurred when Stylet Oil® sprays were applied from 5 weeks after inoculation.
[3] Buds on shoots at each growth stage also include those for the earlier stages presented in the Table. For a summary, see Table 15.
[4] Buds (nodes) were numbered from the base of each shoot.
[5] Estimated bud age based on a leaf generation time of one leaf every two days in glasshouse conditions.
[6] Mean area diseased on the primary leaf adjacent to each bud.
[7] Flag shoots were produced in Season 2 from over-wintered primary and secondary buds within buds exposed to infection in the previous season. Secondary shoots developed after primary shoots were removed. Values are percentages of 526 buds exposed to infection at each node in Season 1 that produced flag shoots in Season 2.
[8] NR = Not recorded.

Table 15. The mean severity of powdery mildew on leaves adjacent to buds on shoots of different phenological stages at five weeks after inoculation in Season 1 (2004/05) in relation to the percentage of primary, secondary or tertiary buds that over-wintered and produced flag shoots in Season 2 (2005/2006) on Chardonnay grapevines in Glasshouse Trial 1E at Loxton SA.

Season 1				Season 2		
Epidemic commencement[1]			Epidemic end[2]			
Shoot growth stage[3] (number of leaves, L)	Bud or node number[4]	Bud age[5] (days)	Leaf area diseased[6] (%)	Primary buds with flag shoots[7] (%)	Secondary buds with flag shoots[7] (%)	Tertiary buds with flag shoots[7] (%)
0-1 L	12	0-2	NR[8]	3.23	0	0
3 L	10-12	2-6	87.7	3.67	0	0
6 L	7-12	2-12	83.3	3.29	0.03	0
9 L	4-12	2-18	74.4	2.62	0.02	0
12 L	1-12	2-24	73.4	1.98	0.02	0

[1] Epidemic commencement = day of inoculation in Season 1.
[2] Epidemic termination occurred when Stylet Oil® sprays were applied from 5 weeks after inoculation.
[3] Shoots with 0-1, 3, 6, 9 and 12 separated (unfolded) leaves.
[4] Buds (nodes) related to each growth stage on a shoot with 12 leaves with buds numbered from the base of the shoot.
[5] Estimated bud age based on a leaf generation time of one leaf every two days in glasshouse conditions.
[6] Mean area diseased on the primary leaf adjacent to the buds on each shoot.
[7] Flag shoots were produced in Season 2 from over-wintered primary and secondary buds within buds exposed to infection in the previous season. Secondary shoots developed after primary shoots were removed. Values are percentages of 526 buds exposed to infection at each node in Season 1 that produced flag shoots in Season 2.
[8] NR = Not recorded.

Glasshouse Trial 1F (Chardonnay, Loxton 2005/2006)
After inoculation, powdery mildew severity on leaves on the Chardonnay shoots increased substantially until fortnightly Stylet Oil® sprays were applied from 5 weeks after inoculation to terminate the epidemic. The severity of disease on leaves at each node (bud) position on shoots at the end of the epidemic that followed inoculation in Season 1 (2005/06) reached levels similar to those recorded for Trial 1E. Again, there was a slight trend towards higher disease severity on younger leaves than on older leaves.

In Season 2 (spring 2006), however, no flag shoots were produced from over-wintered buds that had been exposed to infection during the epidemic in Season 1. Frequent exposure of inoculated buds on vines in the shade house to minimum temperatures as low as –7°C during a cold winter followed by exposure to maximum temperatures exceeding 30°C in early spring (just after bud burst) appeared to have prevented flag shoot development.

Histological studies of infected buds
Anamorphic stages of *E. necator*, especially hyphae and haustoria, were observed in thin sections of inoculated Verdelho buds from shoots at different phenological stages in Trial 1D. While levels of bud infection in Verdelho were lower than in Carignane, relationships between external and internal bud infection and flag shoot formation (Section 8.1 'R&D in the United States') were similar (data not presented).

8.2.3 Evaluation of treatment programs to reduce bud infection and expression of flag shoots

Glasshouse Trial 2A (Verdelho, Irymple 2004/2005)
After inoculation, a powdery mildew epidemic developed on the Verdelho vines in Trial 2A. The severity of powdery mildew on internodes of inoculated shoots at two weeks after inoculation and just before fungicide treatments were applied is shown in Figure 11. The mean disease severity on the internodes of untreated shoots (percent internode area diseased) two weeks after inoculation was 35%. Disease severity was substantially higher on Internodes 1-3 than on Internodes 4-5 ($P < 0.05$, Figure 11). Disease severity was highest on Internode 2.

The effects of the fungicide treatment programs applied in Season 1 (2004/05) on the severity of scarring caused by powdery mildew on internodes of inoculated shoots (percent internode area diseased) during vine dormancy and just before bud burst in Season 2 is shown in Table 16. All treatments reduced disease severity on dormant stem internodes. The mixture of spiroxamine and tebuconazole applied at 2 or 4 weeks after inoculation (T3 or T4) in Season 1 reduced the severity of scarring caused by powdery mildew on internodes of the dormant inoculated shoots more than the other fungicide treatments ($P < 0.05$).

Penconazole applied at 2 weeks after inoculation (T1) in Season 1, or a mixture of spiroxamine and tebuconazole applied at 4 weeks after inoculation (T4) in Season 1, reduced flag shoot incidence at 7 and 9 weeks after bud burst in Season 2 ($P < 0.05$, Figure 12) in relation to the untreated control (Treatment 6, T6). Penconazole applied just after bud break in Season 2 (T5) subsequently reduced flag shoot incidence at 9 weeks after bud break. The proportion of inoculated buds that died during winter (1.9-2.9%) was not affected by fungicide treatment.

Field Trial 2B (Chardonnay, Loxton 2004/2005)
Heat-wave conditions with daily maximum temperatures exceeding 35°C and a minimum day time relative humidity of 10-25% occurred in the week before and the week after shoots on Chardonnay vines in this field trial were inoculated with *E. necator*. While some infection of leaves occurred, the weather conditions after inoculation were unfavourable for further spread of disease.

The incidence of powdery mildew on leaves adjacent to buds on inoculated shoots six weeks after inoculation and two weeks after the first fungicide treatment was applied in Season 1 is shown in Table 17. Penconazole (Topas®) applied at four weeks after inoculation reduced the incidence of powdery mildew on leaves. No powdery mildew developed on the surface of inoculated buds in Season 1. In spring of the following season, no flag shoots developed on vines that were treated with penconazole and only one flag formed on untreated vines (Table 17). Consequently, the effects of the fungicide treatment program could not be determined statistically because of the low incidence of flag shoots.

Field Trial 2C (Sultana, Irymple 2004/2005)

A powdery mildew epidemic developed in Season 1 (2004/05) after distal sections (youngest 5-6 leaves, buds and stems) on selected shoots on vines in each plot were inoculated three times at weekly intervals, commencing when shoots had 5-6 leaves. In the 8 days following the first inoculation, conditions favoured disease development. However, disease development was retarded after the last two inoculations, apparently because of the occurrence of hot days with maximum temperatures of 30-40^{o}C and low humidity in the 10 d after each inoculation.

At 4-6 weeks after inoculation and just before treatment with penconazole in Treatment Program 1 (T1) was applied, disease severity on stem internodes (percent internode area diseased) was 4.2-4.9% (Table 18). When berries were at 9 mm diameter, disease severity on vine foliage and bunches was lower on vines with the pre-flowering penconazole treatment (T1) than on untreated vines (Treatment Programs 2 and 3, T2 and T3). At harvest, severity of powdery mildew on bunches (percent bunch area diseased) with T1 (35.6%) compared to T3 (89.7%, untreated) was reduced by 47.8%. The weight of bunches on treated vines (427.5 g, T1) was 99.8% higher than on untreated vines (214.5 g, T3).

During dormancy, the severity of scarring caused by the disease on the internodes of canes (internode area diseased) was low. Nevertheless, scarring (disease) severity was lower on treated vines (T1) than on vines without treatment at the time of assessment (T2 and T3) ($P < 0.001$, Table 18). The severity of scarring in relation to internode position on dormant canes is shown in Figure 13. Disease severity was lower on Internodes 1-5 on dormant canes of treated vines than on canes of untreated vines. Although not significant, there was also a trend towards lower disease severity on Internodes 6-16 on canes of treated vines. On canes of untreated vines (T3), disease severity on Internodes 1-5 was higher than on Internodes 10-16 ($P = 0.05$).

The incidence of partially and fully formed flag shoots produced on treated and untreated vines in the spring of Season 2 (2005/06) is shown in Table 19. Flag shoots appeared on vines 4-9 weeks after bud burst. When most flag shoots had up to 4 separated leaves and secondary spread of disease to adjacent foliage had commenced, flag shoot development was restricted by hot weather in the period from 5-9 weeks after bud burst. Most flag shoots that started to develop either died or partially died during days when temperatures were 31-35^{o}C and the relative humidity was as low as 14%. As a consequence, the incidence of flag shoots was unevenly distributed through trial plots and statistically, there were no significant effects of treatments. Nevertheless, although not significant, there was a trend towards lower flag shoot formation on vines treated with penconazole at pre-flowering in Season 1 (T1) than on vines treated with penconazole at one week after bud burst in Season 2 (T2). Generally, flag shoot formation on untreated vines was higher than on treated vines. On untreated vines, 0.31% of over-wintered buds produced flag shoots (Table 19). In this trial, only 28% of all flag shoots were produced on the hotter, more exposed (west) side of vine canopies. In relation to the position of buds on over-wintered canes, most flag shoots (51.7%) were produced from buds on Nodes 1-6 that were exposed to infection when weather conditions were most favourable for disease development.

Field Trial 2D (Chardonnay, Irymple 2005/2006)

After inoculation when shoots had 5-6 leaves, a disease epidemic slowly developed on the foliage of inoculated vines. Treatments with penconazole at 4 weeks after bud burst (T1), and especially at 6 weeks after bud burst (T2), reduced disease severity on the internodes of shoot stems and scarring on the internodes of over-wintering spurs.

In the first 9 weeks after bud burst in Season 2 (spring 2006), however, no flag shoots were produced from over-wintered buds that had been exposed to infection during the epidemic in Season 1 and the effects of the fungicide treatment programs on flag shoot formation could not be determined. Frequent exposure of inoculated buds on vines to minimum temperatures from 0^{o}C to –7^{o}C during a cold winter followed by exposure to maximum temperatures of 30-38^{o}C with low relative humidity appeared to have prevented flag shoot development. During April to September 2006, vines were exposed to 53 d when ground temperatures were 0^{o}C to –7^{o}C. In the first 9 weeks after bud burst in spring 2006, vine foliage was exposed to 12 d with maximum temperatures of 30-38^{o}C and 30 d with a daytime minimum relative humidity of less than 20%. On seven of the latter days, daytime minimum relative humidity was 2-10%.

Table 16. The effects of fungicide treatment programs applied in Season 1 (2004/05) on the severity of scarring caused by powdery mildew on internodes of inoculated shoots (percent internode area diseased) on Verdelho vines in Glasshouse Trial 2A during vine dormancy and just before bud burst in Season 2.

Treatment (T) number[1]	Internode area diseased (%)					Mean internode area diseased per shoot (%)
	Internode number					
	1	2	3	4	5	
1	15.3[2]	20.6	17.9	10.8	4.9	13.9bc[3]
2	15.2	22.5	22.3	16.9	8.0	17.0b
3	14.2	17.3	14.3	10.0	6.9	12.5c
4	10.4	14.5	17.6	12.4	6.2	12.2c
5	17.0	24.4	22.6	15.0	7.9	17.4b
6	19.8	27.1	28.8	20.1	12.2	21.6a

[1] The treatment programs were as follows:
T1 = DMI fungicide [penconazole (Topas®)] applied 2 weeks after inoculation in Season 1.
T2 = DMI fungicide [penconazole (Topas®)] applied 4 weeks after inoculation in Season 1.
T3 = Morpholine + DMI fungicides [spiroxamine (Prosper®) tank mixed with tebuconazole (Folicur®)] applied 2 weeks after inoculation in Season 1.
T4 = Morpholine + DMI fungicides [spiroxamine (Prosper®) tank mixed with tebuconazole (Folicur®)] applied 4 weeks after inoculation in Season 1.
T5 = DMI fungicide [penconazole (Topas®)] applied 1 week after bud burst in Season 2 (ie. after this disease assessment).
T6 = No fungicide treatment (Control).

[2] Values for mean disease severity (% internode area diseased) for Internodes 1-5 for each treatment with a lower case letter in common were not significantly different (P = 0.05, LSD = 5.57)

[3] Values for mean disease severity (% internode area diseased) per shoot for treatments with a lower case letter in common were not significantly different (P = 0.05, LSD = 4.02)

Table 17. Incidence of powdery mildew on leaves adjacent to inoculated buds on Chardonnay vines in Field Trial 2B six weeks after inoculation and two weeks after the first fungicide treatment in Season 1 (2004/05) in relation to numbers of flag shoots produced in Season 2 (2005/06) on vines with and without fungicide treatment.

Treatment program[1] (Number)	Fungicide applied	Time of spray application			Leaves diseased 6 weeks after inoculation in Season 1 (%)	Flag shoots[1] in Season 2 (Number)
		Weeks after inoculation in Season 1		Weeks after bud burst in Season 2		
		2	4	1		
1	DMI[2]	-	+	+	14.8	0
2	No treatment	-	-	-	59.3	1

[1] Flag shoots produced in Season 2 on vines with or without treatment with 3600 buds inoculated in Season 1.

[2] DMI = Demethylation inhibiting fungicide, penconazole (Topas®).

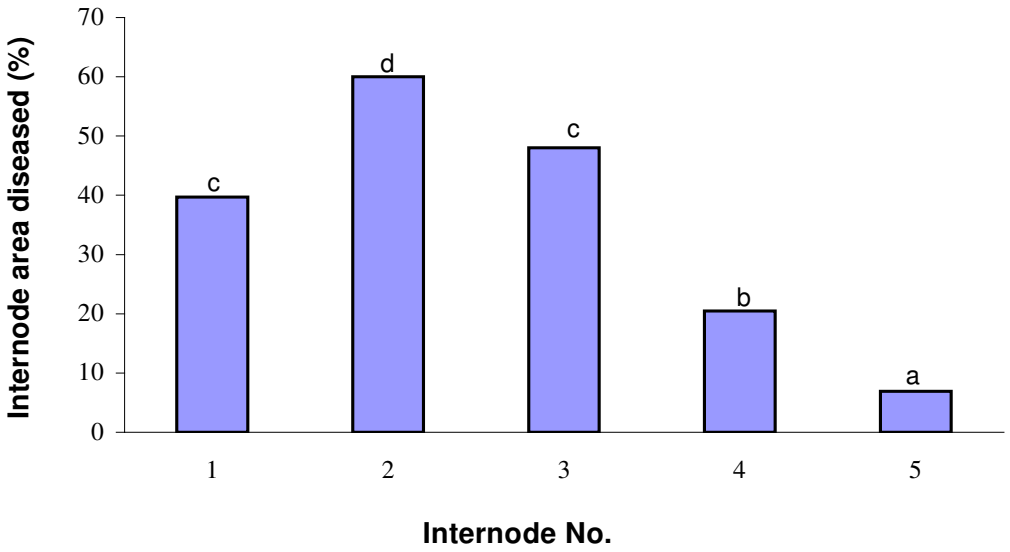


Figure 11. Mean severity of powdery mildew on stem internodes of untreated Verdelho shoots two weeks after inoculation in Glasshouse Trial 2A. Values for disease severity with a lower case letter in common were not significantly different ($P < 0.05$, LSD = 9.07).

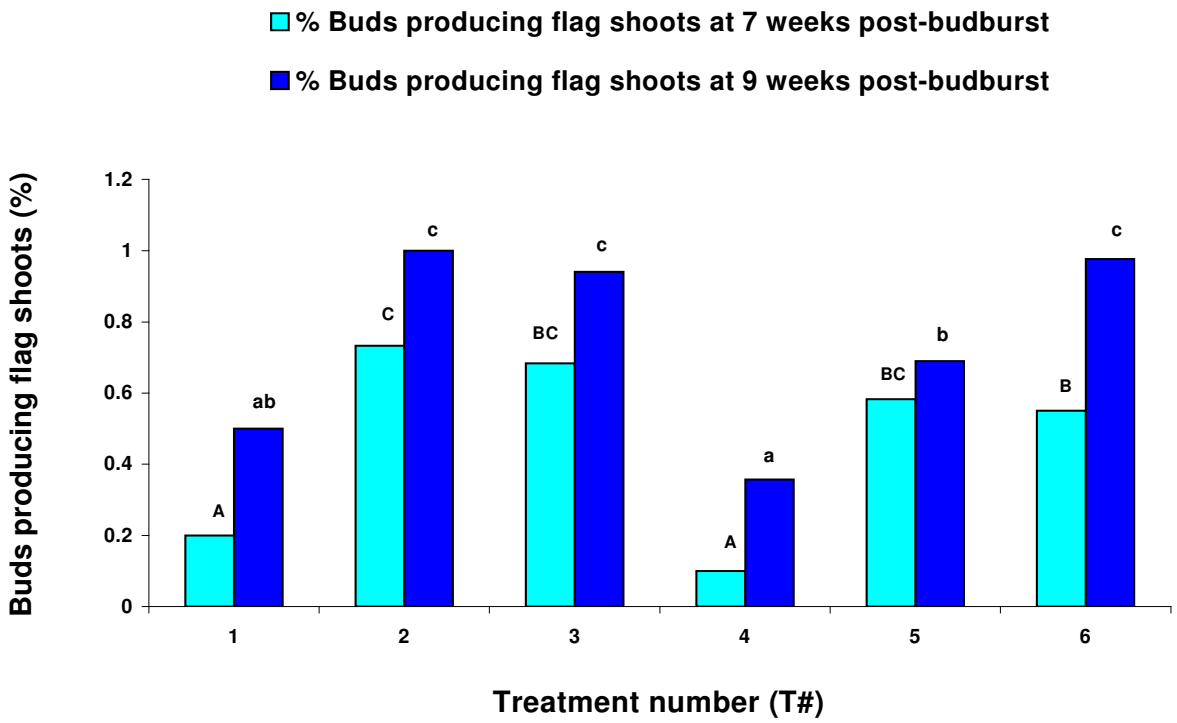


Figure 12. Effects of chemical treatments on the development of flag shoots on Verdelho grapevines at seven and nine weeks after budburst in Glasshouse Trial 2A. Values with a lower case letter in common were not significantly different ($P < 0.05$).

Table 18. The severity of powdery mildew on stem internodes of shoots 4-6 weeks after inoculation (just before fungicide treatments were applied) and during vine dormancy on Sultana vines in Field Trial 2C at Irymple Vic (2004/05).

Treatment program[1] (Number)	Fungicide applied	Time of spray application[1]			Internode area diseased at 4-6 weeks after inoculation in Season 1 (%)	Internode area diseased at vine dormancy in Season 2 (%)
		Weeks after inoculation in Season 1		Weeks after bud burst in Season 2		
		2	4-6	1		
1	DMI[2]	-	+	-	4.2a[3]	0.45 a[3]
2[4]	DMI[2]	-	-	+	NR	1.06 b
3	No treatment	-	-	-	4.9a	1.34 b

[1] See Table 6 for further information about the spray programs.
[2] DMI = demethylation inhibiting fungicide, penconazole (Topas®).
[3] Values for mean disease severity (% internode area diseased) per shoot for treatments with a lower case letter in common were not significantly different ($P < 0.05$).
[4] Treatment in Treatment Program 2 was applied after disease assessment.

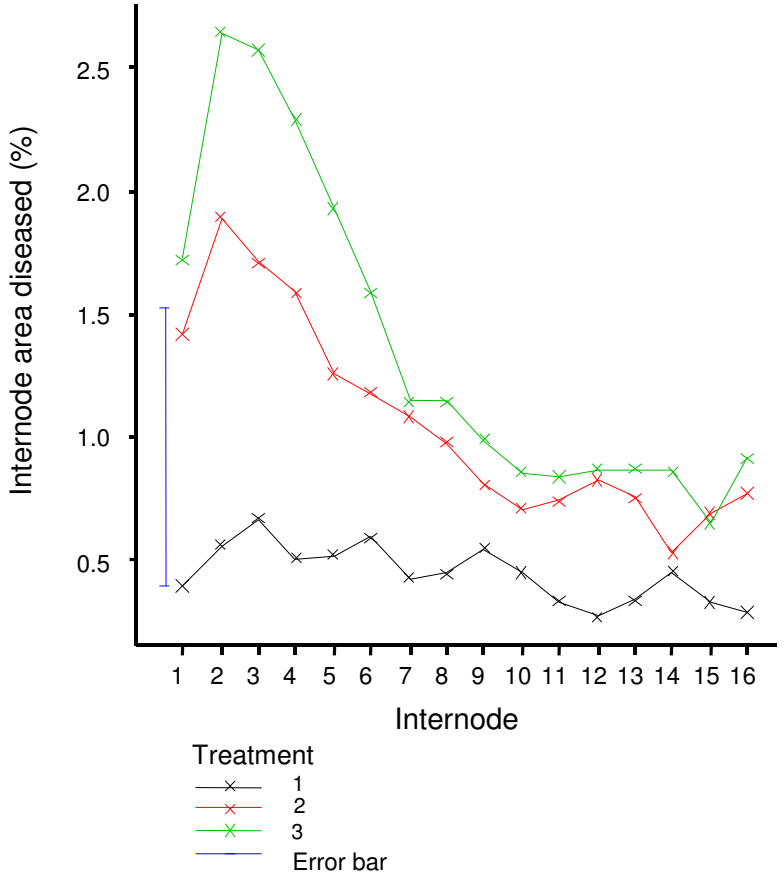


Figure 13. Severity of powdery mildew on stem internodes at different positions on inoculated treated and untreated shoots (numbered from the base) during dormancy on Sultana vines in Field Trial 2C at Irymple Vic. (2004/05).

Table 19. The effects of fungicide treatment programs on the incidence of flag shoots produced on Sultana vines in Field Trial 2C at Irymple Vic. (2004/05).

Treatment program[1] (Number)	Fungicide applied[2]	Time of spray application[1]			Flag shoots produced from over-wintered buds in Season 2 (Number)	Flag shoots produced from over-wintered buds in Season 2[3] (%)
		Weeks after inoculation in Season 1		Weeks after bud burst in Season 2		
		2	4-6	1		
1	DMI	-	+	-	13	0.14
2	DMI	-	-	+	18	0.19
3	No treatment[2]	-	-	-	29	0.31

[1] See Table 6 for further information about the spray programs.
[2] DMI = demethylation inhibiting fungicide, penconazole (Topas®).
[3] Percentage of 9360 over-wintered buds per treatment that produced flag shoots.

Figure 14. Verdelho grapevines inoculated with *E. necator* and treated with different fungicides to reduce bud infection and flag shoot formation in Trial 2A in the glasshouse at DPI Irymple Vic. (Photo by Terry Hunt, DPI).

9 Discussion

[This discussion is based on the results and observations from R&D conducted in the United States at UCD and in Australia that were summarised in the previous Section].

Bud inoculation techniques

Studies of different bud inoculation techniques were conducted at the start of the R&D program in Australia to ensure that the most appropriate inoculation methods were used in the bud infection trials. The studies showed that deionised water with Tween 20® at 25µL/100mL could be used to efficiently wash conidia from grapevine leaves with young sporulating colonies of *E. necator* and produce suspensions with high numbers of conidia (1.3×10^4 to 2.3×10^5). These spore suspensions were used successfully to inoculate young grapevine leaves or shoots, provided that the suspensions were stored at 5-6°C and used quickly (preferably within 30 min) before numbers of viable conidia declined substantially. Inoculations with high numbers of conidia were required to maximise infection of the external surface of buds for flag shoot production in the following season (see below). "Wet' inoculations using suspensions of conidia were easier than 'dry' inoculations, especially in the field. 'Dry' inoculations were less successful because the removal of conidia from sporulating leaves was difficult and the number of conidia placed on buds was harder to regulate.

In the trials reported here, either individual buds were inoculated with a measured volume of spore suspension (inoculum) using a pipette or fine brush, or entire shoots were sprayed with inoculum. While infection of bud surfaces was achieved with both techniques, the latter technique appeared to produce more extensive epidemics more efficiently. However, this technique required larger volumes of inoculum.

Bud susceptibility to infection

The disease severity, bud infection and flag shoot formation data produced from the studies reported here indicate that grapevine shoots have a stage-specific susceptibility and their surfaces (buds, stems and leaves) develop age-related (ontogenic) resistance. In most studies, younger buds, stems and leaves were infected more than older buds, stems and leaves. Studies of infection and disease development on shoots inoculated at different phenological stages (E-L 5 to E-L 17, shoots with 0-1 to 12 separated leaves, respectively) indicated that susceptibility of shoots to infection was highest when shoots had 3-6 leaves. The estimated age of buds on these shoots was 1-16 d, depending on growth rate of the shoots in the glasshouse.

Over-wintering buds with higher flag shoot production in the following season generally were those that had higher disease severity on adjacent leaves and stems at the end of epidemics. In the glasshouse trials conducted on Verdelho, Chardonnay and Sultana vines, disease severity on most leaves adjacent to inoculated buds at Nodes 1-9 (buds aged up to 21 d on shoots with up to 9 leaves at the time of inoculation) was high at the end of severe 5-week epidemics. In the following season, most flag shoots were produced from over-wintered buds that were aged 1-20 d at the time of inoculation in the previous season.

Bud infection pathway

The histological studies of buds indicated that infection is established in the interior of buds within 3 weeks of inoculation. Hyphae of *E. necator* appeared to access the interior of buds through a gap between the overlapping bud scales before changes in the surface (epidermis) of the bud scales (suberisation and lignification) restricted growth of the pathogen and made this pathway inaccessible. The studies of bud infection and flag shoot formation reported here indicate that resistance to bud infection develops at an early phenological stage, mostly from when shoots have at least 9 leaves. Investigations by other authors have also indicated that infection of grapevine buds occurs before flowering and before bud tissue is lignified (Gubler and Rademacher 2002, Rügner *et al.* 2002).

Survival of infected buds and flag shoot formation

After the pathogen has entered buds, it appears to grow slowly along the prophylls and leaf primordia until shoot emergence in the following spring. The studies of susceptibility of tissues in dormant Carignane buds indicate that the host and pathogen are physiologically active (with a latent period of 5-6 days) just before bud burst. Hence, increasing temperatures alone, instead of host biochemical stimuli (eg. increased sugar or starch concentration in bud tissues), may promote re-activation of the pathogen inside buds at the end of dormancy.

The data on external and internal bud infection, flag shoot production and bud survival indicate that not all bud infections produce flag shoots in the following season. The outcome of bud infections may be affected by various factors including the extent of colonisation of bud tissues, the fitness of individual buds and minimum winter temperatures. Competition with other microorganisms and/or hyperparasitism inside buds may also reduce growth of the pathogen.

In the studies reported here, extensive internal infection of buds and low bud fitness appeared to be the main causes of low flag shoot incidence. In spring, some buds did not burst after shoots that had emerged from other buds on the over-wintered canes of shoots inoculated in the previous season were removed. Many of these buds were at node positions similar to those of other buds that produced flag shoots. Furthermore, when shoots were severely diseased during epidemics, higher numbers of buds at the apical end of shoots died during winter than at the base of shoots, apparently because of the debilitating effects of disease. Some of these buds may have produced flag shoots if bud fitness was higher. Many of the apical buds that died during winter were the youngest and the least mature buds on shoots at the time of inoculation.

These observations indicate that flag shoot production in some years may be lower on vines that were severely diseased than on neighbouring vines with lower disease severity in the previous season. This was often observed during earlier studies of powdery mildew epidemics in unsprayed Sultana vineyards (R.W. Emmett, unpublished). When seasonal conditions were favourable, the foliage of vines with flag shoots was more severely diseased just after berry set than that of neighbouring vines. In the following season, most flag shoots were produced on vines in close proximity to vines that had flag shoots in the previous season.

Vine bud infection perpetuation?

Earlier studies of disease epidemics in unsprayed vineyards in the Sunraysia and Riverland districts (Emmett *et al.* 1995, Emmett, 2005) showed that inoculum of *E. necator* was often present in vineyards when buds were most susceptible to infection. Flag shoots were detected from two weeks after bud burst, ie. from when shoots had 5-6 leaves. In some seasons, sporulating leaf spots that developed after ascospore infection at or just after bud burst were also detected from around two weeks after bud burst. In most seasons, however, conidia produced on flag shoots were the most important source of inoculum (Emmett, 2005). As levels of this inoculum are likely to be higher in vines with flag shoots than in other vines when buds on young vine growth are most susceptible to infection, the risk of bud infection perpetuation is high.

Although infection may also occur in buds close to the apical end of shoots at later stages of shoot development, the importance of these bud infections will depend on the buds that are retained during pruning. On spur-pruned vines, infections of buds at the base of shoots (mostly on Nodes 1-3) are most important because these buds will be retained for the production of shoots in the following season. On cane-pruned vines, infections of buds on Nodes 1-16 on shoots that will be retained for the following season are important. On minimally pruned vines, infections of all buds are important because most shoots will be left on vines.

Prediction of flag shoot incidence

The positive correlation between infection of the exterior of Carignane buds in the first year and flag shoot formation in the second year indicated that incidence of flag shoots could be predicted by measuring the severity disease on the external surface of buds in the preceding year. The severity of disease on buds and/or bud sheaths, and adjacent internodes and leaves was also assessed in relation to flag shoot formation on Verdelho, Chardonnay and Sultana vines. Although not significant, there were trends towards higher flag shoot production from buds with higher severity of surface infection and adjacent leaf and/or internode infection in most trials. These trends or correlations were not as well defined for Verdelho, Chardonnay and Sultana because of the higher incidence of bud death and the apparent effects of temperature on flag shoot formation in some trials. Nevertheless, overall, these studies indicate that flag shoot incidence will be high when the severity of external infection on susceptible buds is high after exposure to high levels of inoculum over long periods during epidemics.

Effects of spring weather on bud infection

In the glasshouse trials conducted in this project, disease epidemics and bud infection on young vine shoots occurred in diffuse light when temperatures were 20-30^{o}C (mostly 22-28^{o}C) and relative humidity was 40-85%. Temperatures of 22-28^{o}C when relative humidity was 65-85% were most

favourable for growth of *E. necator* on the surface of leaves, stems and buds. In our studies, maintenance of the latter conditions was required from just before inoculation to at least 10 d after inoculation to quickly establish robust powdery mildew colonies on the surface of stems and buds. Relative humidity below 65% appeared to retard colony development and reduce colony survival. Rea and Gubler (2002) found that radial growth of powdery mildew colonies was highest at 25°C when the relative humidity was 65%. Carroll and Wilcox (2003) also reported that conidial germination and disease severity increased with increasing relative humidity up to around 85%.

The adverse effects of weather conditions in the early stages of disease epidemics were also evident during the field studies conducted in this project. In Field Trial 2C (Sultana, Irymple 2004/05), distal sections (youngest 5-6 leaves, buds and stems) on selected shoots on vines in each plot were inoculated three times at weekly intervals, commencing when shoots had 5-6 leaves. During the eight days after the first inoculation, conditions favoured disease development. However, the occurrence of days with maximum temperatures of 30-40°C and low relative humidity in the 10 d after each of the last two inoculations retarded disease development substantially. Hot dry weather (days with maximum temperatures exceeding 35°C and a minimum daytime relative humidity of 10-25%) in the week after inoculation also reduced the development of disease epidemics on vines in Field Trial 2B (Chardonnay, Loxton 2004/2005).

The above observations provide some insight into the environmental conditions influencing primary infection of susceptible leaves, stems and buds and the establishment of epidemics in vineyards each season. A combination of favourable conditions appears to be required for the establishment and survival of powdery mildew colonies on vines after primary infection. The conditions are required for periods of sufficient length (eg. 10 or more d) for colonies to establish and generate conidia for secondary infection and disease spread. These conditions appear to include daily maximum temperatures of 20-30°C and minimum daytime relative humidity of 65-85%. Reduced UV-B radiation may also be required (Austin *et al.* 2006).

In the absence of these conditions, host colonisation and epidemic initiation is likely to be delayed or, in highly adverse situations, may not occur. This is likely to occur especially in districts where days with high maximum temperatures (30-40°C) and low daytime relative humidity (0-40%, especially 0-20%) may occur between bud burst and flowering (eg. the Sunraysia and Riverland districts). Our studies indicate that increased frequency of these relatively hot dry days in the 10 d after ascosporic or conidial infection will substantially reduce the establishment and survival of new powdery mildew colonies on the surface of shoots, subsequent bud infection and incidence of flag shoots in the following season.

Effects of winter weather on the survival of infected buds

Low temperatures during winter may reduce the survival of infected buds or the survival of mycelia of *E. necator* in infected buds. The death of infected buds or *E. necator* in buds may account for the absence of flag shoot development on vines in Field Trial 2D (Chardonnay, Irymple 2005/06) in spring 2006. From April to September 2006, there were 53 d with minimum ground temperatures of 0°C to -7°C. During the same period, lignified shoots and buds on vines were exposed to minimum air temperatures of 0°C to -4°C on 19 d.

The occurrence of low ambient winter temperatures (lower than -13°C) may also account for the absence of flag shoots in some seasons and in some viticultural regions. Some examples of the latter are the Rheinhessen region in Germany (Hill 1990, Kast 2006), New York USA (Pearson and Gadoury 1987) and Eastern Washington USA (Grove 2004). In the absence of flag shoots, over-wintering cleistothecia are the only source of primary inoculum in vineyards.

Effects of spring weather on flag shoot development and disease epidemics

Spring temperatures appear to influence the formation and persistence of flag shoots. Temperatures and relative humidity also appear to affect sporulation of the pathogen on flag shoots and subsequently, the contribution of inoculum from flag shoots to disease epidemics. In preliminary growth chamber experiments with Carignane, more flag shoots developed on canes exposed to daytime temperatures of 22°C than those incubated at 30°C. This indicates that high spring temperatures in some seasons and regions may not be favourable for flag shoot formation.

In the first 9 weeks after bud burst in spring 2006, young grapevine shoots were exposed to 12 d with maximum temperatures of 30-38°C and 30 d with a daytime minimum relative humidity of less than 20%. These extreme environmental conditions appear to have prevented flag shoot development

in Field Trial 2D (Chardonnay, Irymple 2005/06). In Field Trials 2B (Chardonnay, Loxton 2004/05) and 2C (Sultana, Irymple 2004/05), the occurrence of days with maximum temperatures of 31-35^{o}C and low daytime humidity (as low as 14%) from 5-9 weeks after bud burst appeared to reduce flag shoot formation and survival. In Trial 2C, many flag shoots that had formed earlier, died or partially died during the hot dry weather and as a consequence, their contribution to conidium production and epidemic development was reduced substantially. Reduced flag shoot formation or survival because of hot dry spring weather may account for the delayed onset of epidemics in some seasons observed during other studies (Emmett 2005).

Varietal susceptibility to bud infection and flag shoot formation

In different experiments conducted under controlled conditions, numbers of flag shoots produced per 1000 primary buds on Nodes 1-6 of shoots with six leaves exposed to similar post inoculation (5-week) epidemics on Verdelho, Chardonnay and Sultana vines were 120, 33 and 2, respectively. These results indicate differences in bud susceptibility to infection and flag shoot production between vine varieties. In vineyards of varieties with a high potential for flag shoot production, crop loss is more likely to occur in the absence of adequate control measures because of the higher likelihood of early season powdery mildew epidemics.

An interesting discovery in this project was that low numbers of flag shoots also developed from secondary, and occasionally, tertiary buds within inoculated over-wintered compound buds after flag shoots produced from primary and secondary buds, respectively, were removed. Varieties with a high potential for flag shoot production, such as Verdelho, produced diseased shoots from primary, secondary and tertiary buds. This appeared to occur when the compound bud was infected extensively. This observation indicated that some infected buds remain latent and survive beyond bud burst in the season after infection occurred. During studies of flag shoot incidence on unsprayed Chardonnay and/or Sultana vines over 10 seasons, occasional flag shoots were produced from wood on vine cordons aged two or more years (R.W. Emmett, unpublished). These flag shoots probably developed from buds with latent infections.

The delayed formation of flag shoots may allow the pathogen to survive seasons when adverse spring conditions (eg. hot dry weather) prevent flag shoot formation. The occasional development of flag shoots from secondary or tertiary buds with latent infections may also ensure that there is a source of inoculum if primary shoots on vines are destroyed (eg. by frosts in early spring). The delayed appearance of flag shoots also has implications for disease management. After a disease outbreak, fungicide treatment programs will need to be applied for several seasons to prevent the spread of disease from flag shoots produced from buds infected during the disease epidemic.

Fungicide treatments to reduce bud infection and flag shoot formation

Fungicide treatment programs to prevent and/or reduce infection of buds and the formation of flag shoots in vineyards with a history of disease need to account for periods of high host susceptibility, expected periods of high exposure to inoculum and infection, and vine pruning practice. Halleen and Holz (2001) also noted that pruning methods and grape variety appeared to be two important factors that determined the occurrence of flag shoots in vineyards. In the glasshouse trials reported here, buds on shoots with 3-6 leaves were most susceptible to infection. At later stages of shoot development, buds close to the apical end of shoots were susceptible. In conditions that were highly favourable for infection, buds aged up to 20-24 d in most trials were infected sufficiently to produce flag shoots in the following season. In the vineyard, the latter is likely to be the worst case scenario.

In view of the above, fungicide treatments to prevent bud infection should be applied at regular intervals to prevent infection of the surface of young buds on shoots as they develop. The interval between treatments will depend on shoot growth rate, which in turn, is related to temperature and other factors such as vine water availability and nutrition. In the Sunraysia and Riverland districts, most fungicide treatments to protect vine growth have to be applied every 14 d.

Buds that will be retained on vines for the following season are the targets of each treatment program. In relation to bud susceptibility alone, the period of treatment may be different for vines with different pruning systems. For example, a treatment program on spur pruned vines would need to commence just after bud burst and continue until all buds at the base of shoots at Nodes 1-5 were aged at least 24 d. In the Sunraysia and Riverland districts, this period of bud protection would be from one to five or six weeks after bud burst in most seasons (Table 21).

With cane-pruned vines, treatments would need to commence when shoots at the base of one-year old canes or in the crown of vines start to grow. The treatments then would need to continue until all buds on the shoots at Nodes 1-16 were aged at least 24 d. The shoots targeted would become the one-year old canes that would replace the two-year old canes in the next season. In most seasons in the Sunraysia and Riverland districts, the period of bud protection required would be from 1-2 weeks after bud burst to 11-12 weeks after bud burst (around 3-4 weeks after flowering commenced) (Table 21).

With minimally pruned vines, treatment programs would need to commence just after bud burst and continue until buds on all shoots were aged at least 24 d. In vineyards where shoot extension on vines is retarded after berry set while bunches develop, spray programs to prevent bud infection would either be similar to those for cane pruned vines or slightly more extensive (Table 21). After early season epidemics, unprotected minimally pruned vines are likely to have a higher incidence of flag shoots than cane or spur pruned vines because of the higher numbers of buds exposed to infection on the minimally pruned vines. This has been observed in other studies (Emmett *et al.* 2005b).

In addition to periods of bud susceptibility, potential periods of exposure to inoculum and infection should also be considered when planning fungicide treatment programs. In seasons when rains of 2.5 mm or more at temperatures of 10^{o}C or more occur just after bud burst, buds on young shoots could be exposed to infection from ascospores (Emmett 2005). Young shoots could also be exposed to infection from conidia produced on flag shoots from when shoots have 3-6 leaves (two weeks after bud burst) onwards, especially in the period from when shoots have 8-10 leaves until flowering (4-9 weeks after bud burst) (Emmett 2005). In the period from when shoots have 3-6 leaves to flowering (2-9 weeks after bud burst in the Sunraysia and Riverland districts), conidia from powdery mildew colonies produced after earlier ascosporic or conidial infections may also contribute to bud infection. Hence in most seasons, the risk of bud infection in relation to inoculum availability is also likely to be high from bud burst to just after flowering (Table 21). Periods of infection risk, however, are likely to be different from season to season, depending on the time when ascospores are released from cleistothecia and/or the appearance of flag shoots. Disease models could be developed and used to predict these periods more precisely each season (see 'Recommendations for future R&D').

Some examples of spray programs that could be applied to prevent bud infection and subsequent flag shoot formation are shown in Table 21. The treatments of highest importance in the programs for spur-pruned, cane-pruned or minimally pruned vines have the highest shading. Selection of the types of fungicide treatments is discussed below.

Selection of fungicide treatments

Currently, various fungicides with different chemistry are used to protect vine foliage and bunches from infection by *E. necator* and these should also prevent bud infection. They include sulphur, DMI, strobilurin, morpholine and quinoline fungicides.

Some of these fungicides also have some systemic activity and the potential to eradicate bud infections after they have occurred. Studies in this project (Section 8.2.3) confirmed that correctly timed post-infection treatments with some fungicides can reduce bud infection and flag shoot formation. Furthermore, fungicides or fungicide mixtures with different chemistry appeared to have different effects and could be used in spray programs at different times after bud infection. When buds on shoots with 5-6 leaves were exposed to infection, treatments with penconazole or a mixture of spiroxamine and tebuconazole were most effective when applied at two or four weeks after inoculation, respectively (see Section 8.2.3).

For efficient control of bud infection and prevention of flag shoot formation in the following season, the sequence of treatments with selected types of fungicides will depend on the time of bud exposure to infection. For example, as noted earlier, buds can be exposed to infection from ascospores during and just after rain from the week after bud burst (when shoots have up to three leaves) onwards. In this situation, an application of penconazole when shoots have 3-5 leaves and/or a mixture of spiroxamine and tebuconazole two weeks later (when shoots have 9-10 leaves), should substantially reduce bud infection and flag shoot formation in the following season on spur pruned vines (Table 21, Fungicide Program 1).

In another typical situation, buds can be exposed to infection from conidia produced on flag shoots from when shoots have 3-5 leaves onwards. In this scenario, an application of a mixture of spiroxamine and tebuconazole when shoots have 9-10 leaves should substantially reduce bud infection and subsequent flag shoot formation on spur-pruned vines (Table 21, Fungicide Program 2).

Differences in the efficacy of penconazole in comparison to the mixture of spiroxamine and tebuconazole at different times after inoculation may be related to the period of retention of each fungicide in bud tissues. The mixture of spiroxamine and tebuconazole was expected to have a longer retention time in vine tissue than penconazole (Sue Cross, pers. com.).

Studies in this project also showed that an application of penconazole just after bud burst (when shoots have up to two leaves) in the season following bud inoculation, will reduce the incidence of flag shoots at nine weeks after bud burst (Section 8.2.3, Figure 12). The efficacy of this treatment appeared to depend on its time of application in relation to shoot development. When applied at the one-leaf stage, the treatment prevented fungal growth and sporulation on shoots arising from infected buds, and as a result, the incidence of flag shoots was reduced. However, when the treatment was applied later in relation to shoot development, partially formed flag shoots were produced. On these shoots, sporulating growth of the pathogen was confined to lower leaves and stems, usually associated with Nodes 1-2. While these observations indicate that a single penconazole treatment just after bud burst could prevent growth of *E. necator* on shoots produced from infected buds, complete prevention of flag shoot development with this treatment is unlikely to be achieved because of the variability of bud burst and subsequent shoot growth.

During commercial grape production, the timing and selection of appropriate fungicide treatments should address the need to prevent disease development on grape bunches as well as the prevention of bud infection (see Section 10.2 'Recommendations for industry'). In most districts, fungicides with systemic activity are applied at 4-6 weeks after bud burst, at pre-flowering and/or at post flowering for optimum bunch disease control. However, applications of some of these fungicides will also be required earlier in the season to prevent bud infection, especially in vineyards with a high potential for disease (Table 21). Fungicide treatments for each purpose need to be selected carefully to ensure that no more than three treatments of each group of fungicides are applied each season in line with requirements for the management of fungicide resistance (Emmett *et al.* 2005a).

Further glasshouse and field studies are needed to determine the most cost-effective fungicide treatment programs for the control of bud infection and flag shoot formation (see 'Recommendations for future R&D'). This further research may show that other fungicides could also be used early in the season to reduce bud infection and flag shoot formation. A wider selection of effective treatments would facilitate the development of more cost-effective spray programs and reduce the risk of fungicide resistance because of fungicide over-use.

Cost of fungicide treatment programs

The estimated costs of some different fungicide treatment programs that could be used for the control of bud infection and bunch disease are shown in Table 21. These costs were estimated using the data shown in Table 20.

Table 20. Cost of items (fungicides, spray application and vineyard monitoring) used to estimate the total cost of fungicide programs for the control of bud infection and bunch disease in Table 21.

Item (Table 21)	Example	Cost/ha[1] ($)
S	Sulphur fungicide (eg. Thiovit Jet®, 600g/100L)	12
DMI	DMI fungicide (eg. Topas®, 12.5 mL/100L)	14
M	Morpholine fungicide (eg. Prosper®, 60mL/100L)	20
MD	Morpholine fungicide (eg. Prosper®, 40mL/100L) + DMI fungicide (eg. Folicur®, 11.6 mL/100L)	27
Strobn	Strobilurin fungicide (eg. Flint®, 15g/100L)	33
m	Vineyard monitoring ($22/hour/ha related to 10 ha)	2
.	Application of each fungicide spray (labour, machinery costs) (HortResearch Spray Calculator)	53

[1] Fungicides applied at 1000L/ha

Table 21. Susceptibility of grapevine buds to infection in relation to shoot growth stage, potential exposure to inoculum of *E. necator*, vine pruning system and potential fungicide treatments to reduce flag shoot formation in the following season.

Growth stage[1] (Shoots/bunch/berries)	**WB**	**0-5 leaves**		**6-10 leaves**		**11-15 leaves**			**PF**	**F**	**Bset 2-3 mm**		**Pea size 6-7 mm**		**PBC**	**BC**	**Veraison**		**Cost**[2] **$**
E-L stage	3	4-12		13-16		17-18			19-26		27-28	29	31		32-33		34-35		
Weeks after bud burst[3]	**-1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	
Leaves/shoot	0	2-3	5	7-8	10	11-12	13	14-15	16	17	19	20	22						
Buds /shoot - susceptible		3	5	8	8	7	6	5	5	4	5	4	5						
Buds/shoot – resistant[4]					2	5	7	10	11	13	14	16	17						
Bud susceptibility[5]																			
Spur-pruned																			
Cane-pruned																			
Minimally pruned																			
Inoculum[6]																			
Ascospores from cleistothecia																			
Conidia from flag shoots																			
Disease potential = High																			
Fungicide Program 1[7]																			
Spur-pruned	S		DMI		MD		S		DMI		Strobn		S		S				560
Cane-pruned	S		DMI		MD		M		DMI		Strobn		S		S				568
Minimally pruned	S		DMI		MD		M		DMI		Strobn		S		S				
Disease potential = Low																			
Fungicide Program 2[7]																			
Spur-pruned	S		S		MD		S		DMI		DMI		S		S				
Cane-pruned	S		S		MD		S		DMI		DMI		S		S				539
Minimally pruned	S		S		MD		S		DMI		DMI		S		S				
Disease potential = Very low																			
Fungicide Program 3[7]																			
Spur-pruned	S		S		m		m		DMI		DMI		S		m				
Cane-pruned	S		S		m		m		DMI		DMI		S		m				265
Minimally pruned	S		S		m		m		DMI		DMI		S		m				

[1] Growth stages: WB = Advanced woolly bud; PF = Pre-flowering; F = Flowering; Bset = Berry set; PBC = Pre-bunch close; BC = bunch close. [2] Cost = Total chemical and spray application cost/ha. [3] Weeks after bud burst in the Sunraysia and Riverland districts of south eastern Australia. [4] Buds aged more than 24 d. [5] Higher levels of bud susceptibility on vines with different pruning systems in relation to growth stage are indicated by higher intensities of shading. [6] Higher levels of ascosporic or conidial inoculum in relation to growth stage are indicated by higher intensities of shading. [7] Fungicide treatments of higher importance in relation to prevention or eradication of bud infection are indicated by higher intensities of shading. Types of fungicides: S = Sulphur. DMI = Demethylation inhibiting fungicide. MD = Morpholine + DMI fungicide mixture. M = Morpholine fungicide. Strobn = Strobilurin fungicide. m = monitor and spray if required.

In vineyards with a high disease potential (ie. vineyards with susceptible varieties with a recent history of disease), the cost of spray programs would be higher (eg. $560/ha for Fungicide Program 1, Table 21) because of the more frequent use of more expensive systemic fungicides. In comparison, a standard sulphur-DMI fungicide program for bunch disease control (Emmett 2003) would cost $526/ha. The latter program would comprise five sulphur sprays (at advanced woolly bud, mostly for mite control, and at 2, 4, 12 and 14 weeks after bud burst) and three DMI sprays (at 6 weeks after bud burst, and at pre- and post-flowering). To ensure the complete prevention of flag shoot development, treatment programs would need to be applied for at least three consecutive years after an epidemic because of the risk of delayed flag shoot appearance (see 'Varietal susceptibility to bud infection and flag shoot formation above). In the longer term, however, the cost of spray programs for the control of powdery mildew in the absence of primary infection from flag shoots, and ultimately, from cleistothecia (eg. $265/ha for Fungicide Program 3, Table 20) would be up to 50% lower than the standard sulphur-DMI program.

10 Project outcomes and recommendations

10.1 Project outcomes

The main objective of this project was to improve the management of powdery mildew in vineyards by reducing primary infection from over-wintering infected buds. Sub-objectives of the project were to:

(1) Determine bud susceptibility to infection by the powdery mildew pathogen in selected grapevine varieties grown in Australian vineyards (ie. Verdelho, Chardonnay and Sultana);
(2) Develop treatment programs to reduce bud infection, the expression of disease and/or primary infection in vineyards;
(3) Communicate results of the R&D to project stakeholders and the Australian viticultural industry.

The project was a part of a larger international project involving collaborative research on the biology and management of powdery mildew perennation in buds of grapevines conducted in Australia and the United States (see Section 3.2).

The main objective of the R&D project conducted in the United States was to examine bud colonisation and the perennation of the powdery mildew pathogen (*E. necator*) in the highly susceptible grapevine variety Carignane. Sub-objectives of the project were to:

(1) Determine the period of bud susceptibility to infection by *E. necator*;
(2) Examine the correlation between bud infection and grapevine phenological stage;
(3) Examine the susceptibility of dormant bud tissue to infection to increase understanding of the process of re-activation of *E. necator* perennating in dormant buds.

Key outcomes of R&D in this project and related R&D conducted in the United States include the following.

(1) Preliminary treatment programs to reduce bud infection, flag shoot formation and/or primary infection from infected buds in vineyards were developed (see 10.2 Preliminary recommendations for industry)

For the first time in Australia, spray programs designed to prevent bud infection and flag shoot formation were developed. These programs were based on information produced from studies in this project (Sections 8.2 and 9) and from related R&D conducted in the United States (Sections 8.1 and 9). The timing and type of fungicide treatments used in each program were aligned with vineyard disease potential, vine pruning system, periods of bud susceptibility and expected exposure to powdery mildew inoculum and infection.

Thorough application of these programs in at least three consecutive seasons should reduce primary infection from conidia produced on flag shoots, the risk of perpetuating bud infection, vine disease severity, cleistothecium formation later in the season and the potential for disease in vineyards. With time this will:

- Provide more reliable and improved long-term control of powdery mildew. Reliable disease control will be easier to achieve in vineyards with low disease potential.
- Reduce the use of fungicides in vineyards. Lowering the disease potential of vineyards will allow the use of strategic spray programs that require lower numbers of sprays for disease control

(Emmett 2005). When these programs are used, the annual cost of powdery mildew control in the absence of primary infection from flag shoots could be reduced by up to 50% (from $526/ha to $265/ha, see Section 9: Cost of fungicide treatment programs).

- Reduce the risk of chemical contamination in the environment and in grapes and wine. Chemical use and the risk of chemical contamination will be reduced when fewer sprays are used for powdery mildew control each season.
- Improve the quality of grapes and wine. More reliable disease control in vineyards with lower disease potential will make it easier for grape growers to reliably meet the low disease tolerances on grapes that are required to ensure that wines are not affected by powdery mildew.

(2) Bud susceptibility to infection by *E. necator* in selected grapevine varieties was determined.

Period of susceptibility of grapevine buds to infection

- The studies reported here confirmed that grapevine shoots have a stage-specific susceptibility to infection by *E. necator* and their surfaces (buds, stems and leaves) develop age-related (ontogenic) resistance.
- The risk of bud infection declined as buds aged and as their outer scales hardened.
- Young, green buds on shoots with up to 6 separated leaves were most susceptible to infection. The estimated age of buds on these shoots was 1-16 d, depending on growth rate of the shoots.
- Resistance to bud infection developed at an early phenological stage, from when shoots had at least 9 leaves. Nevertheless, after exposure to severe powdery mildew epidemics, buds aged 1-24 d on shoots of very susceptible varieties such as Verdelho were infected sufficiently to produce flag shoots in the following season.
- There was a significant trend towards higher production of flag shoots from over-wintered buds that were younger when exposed to infection in the previous season in some trials.

Varietal susceptibility to bud infection and flag shoot formation

- Susceptibility to bud infection and flag shoot formation was different between grapevine varieties. Verdelho was the most susceptible variety of the varieties studied. After inoculation with *E. necator*, more flag shoots were produced on Verdelho than on Chardonnay, and these varieties produced more flag shoots than Sultana.
- Verdelho and Chardonnay vines also produced low numbers of flag shoots from the secondary buds after flag shoots from primary buds at the same nodes had been removed. After the secondary flag shoots were removed, occasional flag shoots were produced from tertiary buds on Verdelho vines.
- In unprotected vineyards of varieties with a high potential for flag shoot production, crop loss is more likely to occur because of the higher likelihood of initiation of powdery mildew epidemics early in the season.

(3) Knowledge of the biology and management of powdery mildew perennation in buds of grapevines and flag shoot formation was increased substantially.

Colonisation of the surface and interior of buds by E. necator

- Surfaces of buds on vine shoots were colonised by *E. necator* during disease epidemics that continued for up to 5 weeks after inoculation. Disease development was slower on stems (and buds) than on leaves.
- Infection was established in the interior of buds within 3 weeks of inoculation. Hyphae of *E. necator* appeared to access the interior of young buds through a gap between the overlapping bud scales.
- As buds aged, physiological changes to the outer bud scales appeared to prevent entry of the pathogen and infection of the inner bud tissues.
- In infected buds, all green parts of the bud interior were colonised by hyphae with haustoria, except for the meristems. Conidiophores with attached conidia and germinated conidia with primary appressoria and haustoria were also found in colonised buds.
- Disease severity on the surface of buds (bud external infection) was positively correlated with flag shoot production in the following season.

Survival of infected buds and flag shoot formation

- Only a small proportion of infected buds produced flag shoots. Extensive internal infection of buds and low bud fitness appeared to be the main causes of low flag shoot incidence.
- When shoots were severely diseased during epidemics, higher numbers of buds at the apical end of shoots than at the base of shoots died during winter, apparently because of the debilitating effects of disease on shoot growth. Many of the apical buds that died were the youngest and the least mature buds at the time of inoculation.
- After severe epidemics on susceptible varieties, some infected buds may remain latent and survive for more than one season to produce flag shoots. Occasional flag shoots may arise from infected buds that did not burst in previous seasons, or from infected secondary or tertiary buds in compound buds at nodes where flag shoots produced from primary buds were killed or removed earlier.

Effects of temperature and relative humidity on bud infection, the survival of infected buds, flag shoot development and disease epidemics

- Temperatures of 22-28^{o}C and relative humidity of 65-85% from just before inoculation to at least 10 d after inoculation were most favourable for disease development on vine shoots and for bud infection. Reduced UV-B radiation may also be required.
- Hot dry weather (days with maximum temperatures exceeding 35^{o}C and a minimum daytime relative humidity of 10-25%) in the 10 d following inoculation appeared to substantially reduce disease establishment on the surface of shoots and buds and subsequent bud infection.
- Cold weather (frequent days with minimum temperatures of 0^{o}C to -4^{o}C) during winter may also reduce the survival of infected buds and/or the survival of *E. necator* within infected buds, and incidence of flag shoots in the following season.
- In spring, hot dry weather (days with maximum temperatures of 30-40^{o}C and daytime humidity as low as 10%) after bud burst, especially in the first 9 weeks after bud burst, appeared to reduce flag shoot formation and survival. These conditions also appeared to reduce sporulation on flag shoots and the contribution of inoculum from flag shoots to disease epidemics.

(4) Outcomes of the project and information about the management of powdery mildew in vineyards were communicated to project stakeholders and industry

Communication of R&D

- Information about grapevine bud infection and the management of powdery mildew *(E. necator)* in vineyards was communicated to scientific and industry audiences through more than 30 presentations and more than 20 publications.
- Presentations were made to grape growers and industry representatives at industry forums and seminars, scientific conferences, workshops, meetings and field days in Victoria, South Australia and New South Wales. Oral and/or poster presentations were also given at international scientific workshops in the USA and Italy.
- Publications relating to work on the project included articles in industry and scientific journals, papers in proceedings of scientific and industry conferences and workshops, and communications delivered through grape industry information services.
- Information on the project was incorporated into more than 10 progress and annual reports for key project stakeholders.

10.2 Preliminary recommendations for industry

The preliminary recommendations outlined below are based on current knowledge of the biology and management of bud infection and flag shoot formation produced from this project and from related R&D in the United States. Further studies are required, especially in relation to the efficacy of fungicide treatments, before robust recommendations can be presented (see Section 10.3).

Rationale for fungicide treatment programs to prevent bud infection

- Young buds aged up to 24 d that will be retained on vines in the following season are the targets of fungicide spray programs to prevent bud infection.

- Buds with an estimated age of 1-16 d on shoots with 3-6 leaves were most susceptible to infection. At later stages of shoot development, buds of this age close to the apical end of shoots were most susceptible. On very susceptible varieties (eg. Verdelho), buds aged 1-24 d, especially 1-20 d, were infected sufficiently to produce flag shoots after exposure to severe epidemics. In the vineyard, the latter is likely to be the worst case scenario.
- When designing spray programs, the pruning system of vines should be studied carefully to determine the buds that will be retained on vines after pruning for growth in the following season.
- The development period of shoots with the buds that will be left on pruned vines should also be considered. While most shoots will commence growth at vine bud burst, some may be slower to break dormancy and may grow later. Fungicide treatments will be required to protect buds on both sets of shoots.

- The buds that will be retained in the following season need to be treated with fungicides over periods when infection is likely to occur, ie. during periods of exposure to spores of powdery mildew (ascospores and conidia).
  - Buds could be exposed to infection from ascospores released from cleistothecia and/or conidia from flag shoots from bud burst onwards.
  - The time of ascosporic infection will depend on the time of rainfall and/or over-vine irrigation. The time of conidial infection will depend on the time of flag shoot appearance. These times can be different from season to season, and in the absence of models to predict them, growers should assume that infection could occur from bud burst onwards.
  - The likelihood of bud infection and subsequent flag shoot formation is increased with increasing severity of powdery mildew on the surface of buds and stems. The objective of fungicide treatments, therefore, is to prevent fungal growth on the surface of susceptible buds.
- Fungicide sprays should be applied at regular intervals to prevent external and internal infection of young buds. The spray intervals will depend on shoot growth rate.
  - In most regions and seasons, adequate protection of new vine growth is achieved when sprays are applied every 10-14 d.
- Some grapevine varieties are more prone to bud infection and flag shoot formation than others. Varieties that do not produce flag shoots will not require fungicide treatments to prevent bud infection.
  - In this project, flag shoot formation was highest on Verdelho and Chardonnay. In assessments of varietal susceptibility to powdery mildew in the field (Wicks *et al.* 1993, Emmett *et al* 2005a), these varieties had 'very high' disease susceptibility.
  - While the relationship between susceptibility to bud infection and/or flag shoot formation and field varietal susceptibility is unclear, varieties with low to very low field disease susceptibility (eg. Shiraz) are unlikely to produce flag shoots. Spraying vines of these varieties to prevent bud infection is unlikely to be worthwhile economically.
- The number and type of fungicide treatments required to prevent bud infection will depend on the disease potential of vineyards.
  - Vineyards with high disease potential are those with a high risk of disease. For example, the risk of disease is high in vineyards with susceptible varieties that have had powdery mildew in the last 1-2 years and that have conditions favourable for disease development (eg. vines with vigorous large dense canopies in sheltered areas that frequently have high relative humidity).
- Vineyards with a high potential for disease will require treatments with selected systemic fungicides that have post- and pre-infection activity in vine buds early in the season. The number of treatments required will depend on the number of buds on each shoot that will need to be protected. This, in turn, will depend on the type of vine pruning system.
  - Spur pruned vineyards will require treatments when shoots have 5 and 10 leaves (Table 21).
  - Cane pruned and minimally pruned vines will require treatments when shoots have 5, 10 and 13 leaves, at pre-flowering (when shoots have around 16 leaves) and at berry set (Table 21).
- The time when each type of fungicide should be applied in relation to the time of bud exposure to infection (ie. the time of inoculation) will depend on the fungicide's maximum period of post-infection activity.

- In trials in this project, the period of post-infection activity for penconazole (Topas®) was 2 weeks (ie. this treatment reduced bud infection when applied 2 weeks after inoculation). The period of post-infection activity for a tank mixture of spiroxamine (Prosper®) and tebuconazole (Folicur®) was 4 weeks (see Section 8.2.3).

Spray programs to prevent bud infection

- In spur pruned vineyards with a high potential for powdery mildew, a DMI fungicide spray (eg. penconazole) should be applied when shoots have 5 leaves. This should be followed by a further spray of either a DMI fungicide (eg. penconazole) or a tank mixture of a morpholine and a DMI fungicide (eg. spiroxamine and tebuconazole) 2 weeks later, when shoots have 10 leaves (Table 21, Fungicide Program 1). In the Sunraysia and Riverland districts, these sprays should be applied at 2 and 4 weeks after bud burst.
  - In this spray program, the DMI sprays should prevent infection of treated buds for at least 2 weeks after spray application, ie. until the next spray is applied. The post-infection or 'kick-back' activity of DMI fungicides such as penconazole should also control infections of buds that were initiated up to 2 weeks earlier. The 'kick-back' activity of the first spray should control any infections that occur from bud burst to 2 weeks after bud burst.
  - The spray of the morpholine and DMI fungicide tank mix when shoots have 10 leaves (at around 4 weeks after bud burst), should also prevent infection of treated buds for at least 2 weeks after spray application, ie. until shoots have 13 leaves and the next spray is applied at around 6 weeks after bud burst. The 'kick-back' activity of the fungicide mixture (eg. spiroxamine and tebuconazole) should also control infections of buds that were initiated up to four weeks earlier.
- In cane pruned or minimally pruned vineyards with a high potential for powdery mildew, sprays should be applied when shoots have 5 and 10 leaves as recommended for spur pruned vineyards. This should be followed by further sprays of a morpholine fungicide (eg. spiroxamine, Prosper®) when shoots have 13 leaves, a DMI fungicide (eg. penconazole) when shoots have 16 leaves (at pre-flowering) and a strobilurin fungicide (eg. trifloxystrobin, Flint®), or another different type of fungicide with some systemic activity, at berry set (Table 21, Fungicide Program 1). In the Sunraysia and Riverland districts, these sprays should be applied at 2, 4, 6, 8 and 10 weeks after bud burst.
  - In this spray program, all of the recommended fungicide sprays should prevent infection of treated buds for at least 2 weeks after spray application. The post-infection or 'kick-back' activity of the DMI fungicide (eg. penconazole) should also control infections of buds that were initiated up to 2 weeks earlier. The 'kick-back' activity of the first spray should control infections that occur from bud burst to 2 weeks after bud burst.
  - The spray of the morpholine and DMI fungicide mixture (eg. spiroxamine and tebuconazole), when shoots have 10 leaves at around 4 weeks after bud burst, should also prevent infection of treated buds for at least 2 weeks after spray application, ie. until shoots have 13 leaves and the next spray is applied at around 6 weeks after bud burst. The 'kick-back' activity of the morpholine and DMI mixture should also control infections of buds that were initiated up to 4 weeks earlier.

Adjustment of spray programs in relation to the risk of bud infection

- In vineyards with a low disease potential, sulphur fungicide sprays could be used instead of the DMI fungicide spray when shoots have 5 leaves and the morpholine fungicide spray when shoots have 13 leaves (Table 21, Fungicide Program 2). The less expensive sulphur sprays should prevent infection of treated buds for at least 2 weeks after spray application. However, they are unlikely to control infections that occurred before spray application.
- Sufficient control of bud infection may still be achieved by early season applications of a sulphur fungicide applied thoroughly every 10-14 d, commencing when shoots have 2-3 leaves (at one week after bud burst). Ultimately, however, this spray program may be more expensive than the programs suggested above for cane pruned or minimally pruned vines because more spray applications may be required to protect buds between bud burst and berry set.
- After a substantial disease outbreak in a vineyard with a highly susceptible variety, early season fungicide spray programs may need to be applied for at least 3 seasons to prevent bud infection from conidia produced on flag shoots arising from buds infected during the disease epidemic.

    - Occasional flag shoots may be produced from buds that did not burst in the season after the epidemic, or from secondary or tertiary buds at nodes where flag shoots produced from primary buds were killed or removed earlier.
- The risk of bud infection in vineyards should be reduced progressively by the thorough application of appropriate spray programs in three or more consecutive seasons. Each season, the incidence of flag shoots and primary inoculum in each vineyard should be reduced because bud infection in the previous season has been reduced by the fungicide treatments. Disease severity and the potential for cleistothecium formation should also decline progressively. With time, the disease potential of each vineyard should decline until it is very low, provided that substantial inoculum does not come into the vineyard from adjacent sources (eg. adjacent diseased vineyards).
- With timely monitoring to ensure that disease control is maintained, the number of spray applications required for the control of powdery mildew could be reduced substantially in vineyards with very low disease potential. Control programs could be reduced to 2 sulphur sprays, the first when shoots have 5 leaves and the second when berries are pea size, and 2 consecutive DMI sprays applied at pre-flowering and then at berry set (Fungicide Program 3, Table 21).

Sulphur sprays at advanced woolly bud

- Bernard *et al.* (2003) recommended that a high volume spray of sulphur and synertrol oil should be applied to vine trunks and cordons at advanced woolly bud when temperatures were at least 15^{o}C for rust mite control. This spray should also reduce the risk of ascospore infection early in the season and contribute to the control of bud infection.
    - These sprays should reduce numbers of mature over-wintering cleistothecia by promoting the release of ascospores before bud burst (Emmett 2003).

Integration of spray programs to prevent bud infection and bunch disease

- Selection of fungicide treatments for the control of bud infection and those required for the prevention of disease development on grape bunches should be integrated to ensure that no more than three treatments of each fungicide group are applied each season.
    - In most spray programs for bunch disease control, fungicides with systemic activity (eg. DMI, morpholine or strobilurin fungicides) are applied at pre-flowering and/or at post flowering. When some of these fungicides are also applied earlier in the season to prevent bud infection, treatments need to be selected carefully to ensure that no more than 3 sprays of each fungicide group are applied each season. This strategy is in line with requirements for the management of fungicide resistance.

10.3 Recommendations for future R&D

Chemical control of bud infection and flag shoot formation

Glasshouse and field trials to evaluate the efficacy of fungicides for the control of bud infection and flag shoot formation were conducted for the first time in this project. These evaluations were confined to selected fungicide treatments (ie. penconazole or a tank mixture of tebuconazole and spiroxamine) applied at different times after inoculation. These initial investigations indicated that some treatments had sufficient pre- and post-infection efficacy to substantially reduce bud infection and flag shoot formation. The 'preliminary recommendations for industry' outlined above are based on these outcomes.

Further glasshouse and field studies are required to determine the effects of other fungicide treatments on bud infection and flag shoot formation. This research will expand the options for chemical control so that more cost-effective and robust recommendations can be developed.

Effects of temperature, humidity and moisture on bud infection, survival of the pathogen in infected buds and on flag shoot formation

The outcomes of some of the glasshouse and field trials in this project provided some insight into the effects of environmental conditions on grapevine bud infection and flag shoot formation. The studies reported here (Sections 8 and 9) and elsewhere (Hill 1990, Kast 2006) indicate that there are conditions (temperature, humidity and/or moisture) that either favour or prevent bud infection, the survival of *E. necator* in infected buds during winter and/or the formation of flag shoots in spring.

Further studies are needed to define these conditions and increase understanding of the effects of weather and environmental conditions on bud infection, flag shoot formation and the initiation of disease epidemics from flag shoot inoculum in vineyards. This knowledge could be used to estimate seasonal disease potential and the need for fungicide treatments in vineyards.

Studies of the effects of temperature and moisture on bud infection and flag shoot formation were included in the original research plan for this project (Section 5). Unfortunately, these studies were not undertaken because of project funding constraints.

Risk-based management of powdery mildew in vineyards

Spray programs to prevent bud infection in vineyards would be more effective and efficient if periods of bud exposure to powdery mildew inoculum (ascospores and/or conidia) could be predicted each season. If the effects of temperature, humidity and wetness duration on bud infection and flag shoot formation are clarified as outlined above, a simulation sub-model of primary infection from flag shoots could be developed. This sub-model could then be integrated into a more comprehensive generic disease model that could be used to reliably predict seasonal powdery mildew activity in vineyards and indicate the need for selected fungicide treatments. As a consequence, the efficiency of disease management would be increased and the number of sprays required for disease control would be reduced substantially (Emmett 2005). The cost of powdery mildew control programs would be reduced because sprays with selected fungicides would be applied only when they were needed.

Another major advantage of this risk-based approach is that spray programs for the control of powdery mildew could be readily adapted to regional climatic conditions, and/or seasonal environmental conditions in individual vineyards. The disease-forecast model could also be used to assess the potential long-term effects of climate change on disease development in vineyards.

Effects of strains of E. necator *on bud infection and flag shoot formation*

Some authors have reported the occurrence of two genetically different biotypes of *E. necator* related to the pathogen's mode of over-wintering in vineyards (Emmett *et al.* 2005a). One biotype ('flag shoot' biotype) may over-winter as conidia and mycelia in buds, while the other ('ascospore' biotype) may over-winter as cleistothecia (Miazzi *et al.* 2003, Hajjeh *et al.* 2005). In some population studies, isolates related to groups representing each biotype were clustered according to time in the season (Miazzi *et al.* 2003). However, other studies in Italy indicated that a mixture of sexual and clonal reproduction occurred in flag shoot sub-populations (Cortesi *et al.* 2004). In Spain, Nunez *et al.* (2006) also identified two sub-populations of *E. necator* with 63% genetic similarity but these sub-populations could not be separated according to origin of primary inoculum. In France, Amrani and Corio-Costet (2006) found that differences in the relative proportions of each sub-population varied with the presence of flag shoots and with the region where isolates of the pathogen were collected.

In the bud infection studies conducted in Australia and the United States reported here, mixtures of isolates of *E. necator* (including some isolates collected from flag shoots) were used for inoculations. While some isolates appeared to colonise young vine foliage more rapidly than others in the glasshouse, mixtures of isolates used in the experiments had no apparent effect on bud infection or flag shoot formation.

Nevertheless, some further research on the influence of selected isolates *of E. necator* on bud infection and flag shoot formation would determine whether disease management programs should be modified because of the occurrence of particular biotypes of the pathogen in vineyards. Some biotypes may persist in infected buds more than others. The importance of this in relation to disease management would depend on the incidence of these biotypes in vineyards, whether they could be readily identified and whether their management is warranted economically.

11 Appendices

11.1 Communication of R&D

Information about the progress and outcomes of R&D on grapevine bud infection and the improved management of powdery mildew in vineyards was communicated to scientific and industry audiences through a series of publications and presentations (see below).

Publications

Publications relating to work on the project include those listed below. Information on the project was incorporated into reports for project stakeholders and industry, into articles in industry journals and into papers in proceedings of scientific and industry conferences and workshops.

Technical publications (including conference papers and abstracts) and industry journal articles (non-refereed)

Brady, P. and Emmett, R.W. (2004). Cost rivals returns in disease control. *National GrapeGrowers* December 2004. p. 29.

Clancy, T. and Emmett, R.W. (2006). Shoots flag a potential control pathway. In: 'GWRDC R&D at Work', May 2006. p. 14-15.

Cronin, P. and Emmett, R.W. (2005). Powdery mildew and Aspergillus in researchers' sights. *National GrapeGrowers*, August 2005. p. 49.

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Emmett, R.W. (2003). DPI Victoria Horticulture Program Project Report July-December 2002: Improved management of grapevine powdery mildew (DNR 02/06) (30 January 2003).

Emmett, R.W. (2004). DPI Victoria Horticulture Program Project Report July-December 2003: Improved management of grapevine powdery mildew (DNR 02/06) (7 January 2004).

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Presentations

Presentations with the latest information on the biology and management of powdery mildew were made at industry and scientific conferences, seminars and workshops during the duration of this project: to grape growers at Grapecheque Meetings in November 2002, August, September and October 2004; at industry field days held at Irymple Vic. in October 2004 and at Loxton SA and Waikerie SA in September and November 2003 and 2004; at industry forums and workshops, and through grape industry information services in 2005 and/or 2006 at Irymple Vic. (January, October and November), Waikerie SA (August), Loxton SA (September), Berri SA (September) and Bendigo Vic. (September). Presentations include those listed below.

Clarke, K. (2005). Grapevine powdery mildew: biology and management. Presented at: Dried Grape Industry Workshop, Irymple Vic. (11 October 2005) (18*).

Emmett, R.W. (2003). Developments in research on grape diseases. Presented at: Australasian Plant Pathology Society and La Trobe University Mycology Symposium, La Trobe University, Bundoora Vic. (19 December 2003) (40*).

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Emmett, R.W., Clarke, K., Hunt, T., Magarey, P.A., and Learhinan, T. (2006). Grapevine bud infection by powdery mildew (*Erysiphe necator*): Varietal susceptibility and the evaluation of fungicide

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11.2 Intellectual property

No 'commercial in confidence' intellectual property was produced from R&D in this project. However, information in this report is confidential to project stakeholders until it is published.

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11.5 Project staff

Table 22. Staff on GWRDC Project DNR 02/06 'Improved management of grapevine powdery mildew' in 2002-2006.

Name	Position
Department of Primary Industries, Primary Industries Research Victoria, Irymple Vic.	
Dr Bob Emmett	Senior Plant Pathologist (Project Leader)
Kathy Clarke	Research Officer
Terry Hunt	Technical Officer
South Australian Research and Development Institute, Loxton SA	
Peter Magarey	Senior Plant Pathologist
Tash Learhinan	Technical Assistant

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