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Optimising management of eutypa dieback



FINAL REPORT to

GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

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1 ABSTRACT

Management strategies for eutypa dieback have been optimised by identifying new pruning wound treatments that control eutypa dieback, generating efficacy data for fungicide label registration and demonstrating the use of commercial sprayers to apply treatments to pruning wounds effectively. Folicur (tebuconazole), Shirlan (fluazinam) and Cabrio (pyraclostrobin) were the most effective treatments evaluated and high-volume recycle and home-engineered sprayers provided the most effective means of applying the treatments. A greenhouse assay was also developed for rapid evaluation of pruning wound treatments under different disease pressures. Five new fungal species have been isolated from trunk cankers associated with the eutypa dieback complex, but none are more pathogenic than *Eutypa lata,* and their growth was restricted by the same fungicides that inhibit *E. lata*. Surveys of naturally infected grape vine collections, and inoculation experiments, suggest that some grapevine cultivars and rootstocks may resist or tolerate trunk disease pathogens. These outcomes provide new information that will assist with management of eutypa dieback and contribute to the long-term sustainability of the Australian wine industry.

2 EXECUTIVE SUMMARY

Eutypa dieback is a major disease of grapevines worldwide which causes considerable economic loss to the $8.3 billion Australian wine industry and is caused by the fungus *Eutypa lata*. The fungus infects vines through pruning wounds and colonises wood tissue causing dieback of cordons, stunting of green shoots, leaf distortion, poor fruit set, uneven berry ripening and, if not controlled, eventually kills vines. Eutypa dieback is now recognised as a significant problem in most cool climate growing regions of southern Australia where it threatens the sustainability of many vineyards.

Currently, the only method of controlling eutypa dieback, once established in vines, is by removal of all infected wood tissue from the vine using remedial surgery. A more cost effective method of control of the disease is to prevent entry of *E. lata* into the vines by protecting pruning wounds.

A number of pruning wound treatments, including fungicides and alternative products, have been evaluated for efficacy in controlling eutypa dieback. Fungicides from three fungicide activity groups, Folicur (tebuconazole), Shirlan (fluazinam) and Cabrio (pyraclostrobin), provided control of the disease at the label rates currently recommended for control of other grapevine diseases. Permits are being sought for their use as pruning wound protectants during vine dormancy. Use of these products would provide a greater range of options for growers to manage the disease.

The “alternative” products, garlic, lactoferrin and the biocontrol product Serenade, provided some control of eutypa dieback, but less than that of conventional fungicides. These may provide options for organic growers and those wishing to reduce use of synthetic fungicides.

A detached cane assay (DCA) was developed that allows assessment of pruning wound protectants using live single-node cuttings under controlled conditions. This assay can provide efficacy data on wound treatments in as little as 6 weeks from establishment, compared with up to 18 months for a field trial. The method also allows for evaluation of treatments at decreased disease pressure, more reflective of that occurring naturally. In order to assess the ability of treatments to control eutypa dieback, field trials must be conducted, but the DCA allows for a rapid screening of treatments to support field results and to generate additional data to assist with product registration.

Control of eutypa dieback was achieved with Folicur applied to pruning wounds using commercial sprayers. The best disease control was achieved with recycle sprayers and a home-engineered cordon sprayer, which maximised spray coverage on the wounds and provided control equivalent to treatments applied with a paintbrush. Other types of sprayers were able to provide some control, but adjustment of spray nozzles and water spray volumes of at least 600 L/ha were required to maximise coverage of wounds and achieve control. Spray application of effective fungicides provides an economically viable method of preventing eutypa dieback in vineyards where hand-application of pruning wound protectants is not cost effective.

Five species of fungi related to *E. lata* were isolated from trunk cankers on grapevines and other woody host plants in grapegrowing regions of Australia. All were pathogenic to grapevines in greenhouse studies but none were more virulent than *E. lata*. Fungicides that controlled *E. lata* were also inhibitory to the related fungi suggesting that effective pruning wound treatments will control all grapevine trunk diseases.

Field assessment of a collection of grapevine cultivars from around the world, located in the Barossa Valley, South Australia, showed significant variation in eutypa dieback and trunk disease symptoms among cultivars. CSIRO grapevine rootstocks were screened using the DCA, revealing significant variation in colonisation by *E. lata*. Further investigation is needed to screen and develop cultivars with resistance or tolerance to trunk disease, which if successful, may reduce the need for wound protectants or other control measures.

Extension activities, including workshops and industry publications, have delivered project outcomes to industry, leading to adoption of management strategies by growers. Australia continues to lead research on the management of grapevine trunk diseases, with limited research being carried out elsewhere around the world. Therefore, it is important that research continues, to ensure the long-term sustainability of the Australian wine industry.

3 BACKGROUND

Eutypa dieback, caused by the fungus *Eutypa lata,* is a major disease of grapevines in most cool climate wine regions in Australia and world-wide. This disease threatens the sustainability of vineyards, especially those 8 years or older, and is becoming an increasing problem in many regions of southern Australia (Sosnowski and Wicks 2012), causing considerable economic loss to the $8.3 billion Australian wine industry. It contributes to vineyard decline by reducing growth and yield (Munkvold *et al*. 1994, Creaser & Wicks 2001). In Australia, yield losses of up to 1,500 kg/ha have been reported for Shiraz vineyards (Wicks & Davies 1999) and, in California, economic losses of at least US$260 million per annum have been attributed to trunk disease (Siebert, 2001).

Spores of *E. lata* are released from fruiting bodies on dead, infected wood and are spread by rain-splash and wind. Infection occurs when spores land on exposed pruning wounds, germinate and eventually colonise the cordons and trunk, causing cankers and a characteristic wedge of dead tissue (Figure 1 a&b). The fungus produces toxic metabolites which are translocated to the foliage, resulting in stunted shoots, distortion and necrosis of leaves, reduced bunch size and uneven ripening (Figure 1 c,d&e; Moller & Kasimatis 1981, Tey-Rulh et al. 1991, Molyneux et al. 2002). If not controlled, the fungus eventually kills infected vines.

Spread of the disease within vines can be controlled by the removal of infected wood (Sosnowski *et al.* 2009; 2012). However, the most effective control strategy is to protect pruning wounds from infection (Sosnowski *et al.* 2008).

Previous research on eutypa dieback conducted by SARDI and the University of Adelaide, with funding by GWRDC and CRCV has produced the following outcomes:

- A DNA assay was developed for the identification of *E. lata* in pure culture and in wood tissue, giving more accurate and potentially cost-effective pathogen detection compared to morphological methods.
- Foliar symptoms of eutypa dieback were directly related to yield losses although symptom expression varied from year to year. Evidence suggested that environmental factors may contribute to this phenomenon.
- The development of a bioassay, in which foliar symptoms are induced within 8 months and isolates of *E. lata* varied in their ability to induce foliar symptoms.
- Growth rate of *E. lata* in wood of various grapevine cultivars ranged from 10 to18 mm per year in the field and the fungus was isolated up to 75 mm beyond staining in stems of potted vines.
- Paints, pastes, fungicides and natural products have been evaluated as wound protectants.
- Conventional spray machines were found to be an effective means of applying fungicide to pruning wounds.
- Remedial surgery was the most effective method for treating infected vines in the medium-term (up to 10 years), and is likely to contribute to long-term sustainability of vineyards.
- The susceptibility of vines to eutypa dieback was influenced by water and temperature stress, and deficit irrigation was shown to increase susceptibility of vines in warm, dry climates.
- Vineyard surveys showed eutypa dieback to be widespread in grapegowing regions of Australia. In new growing areas in particular, grape growers need to adopt control strategies to avoid production loss and increase the sustainability of grapevines.

Currently, only two pruning wound treatments are registered in Australia for the control of eutypa dieback; Greenseal, a paint containing tebuconazole fungicide, and Vinevax, a trichoderma-based biological control. There is a need to provide more options for growers to prevent infection of pruning wounds, particularly treatments that can be applied with sprayers.

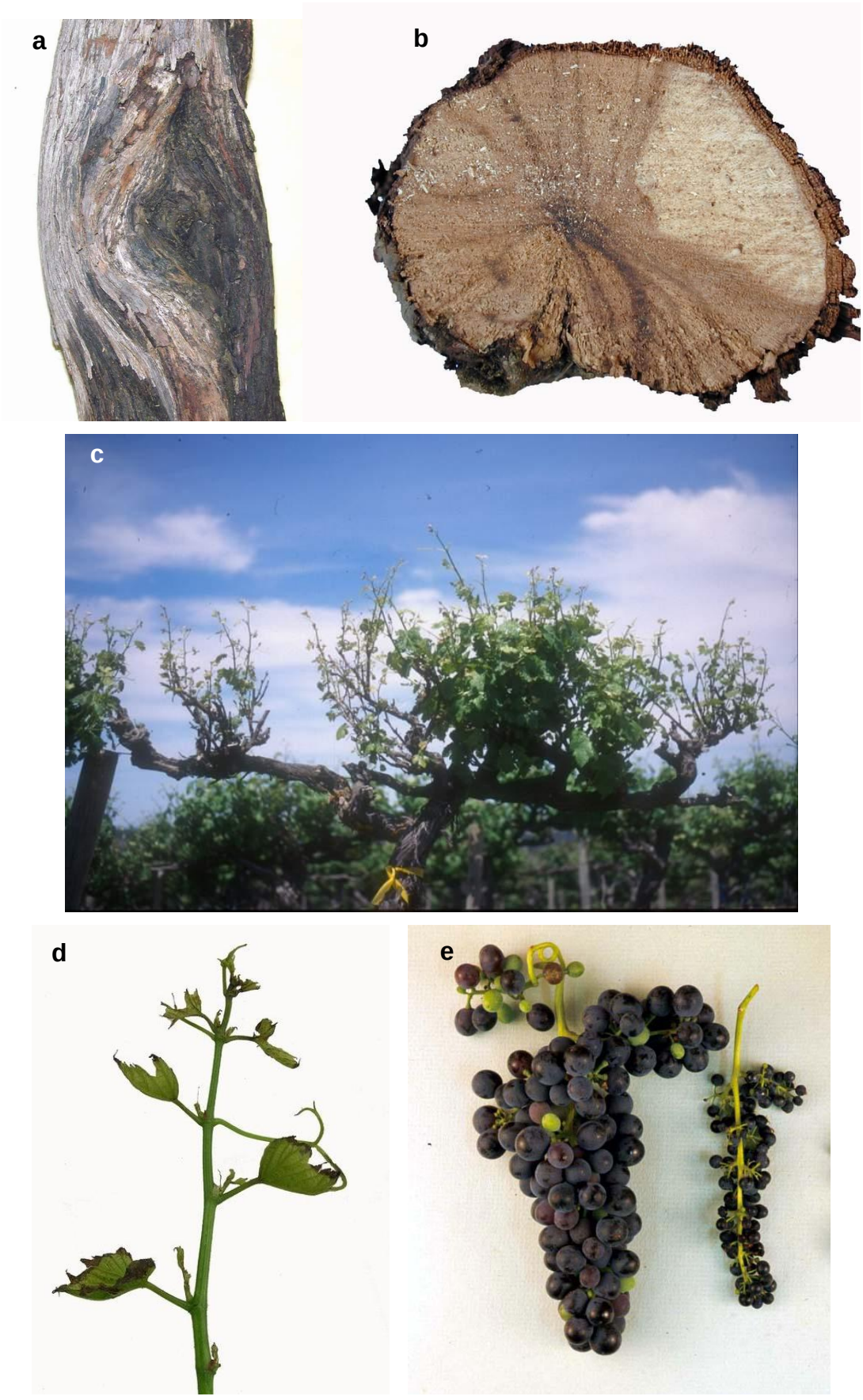


Figure 1. Symptoms of eutypa dieback in grapevines. Wood symptoms including (a) external canker and (b) wedge of internal staining. Characteristic foliar symptoms including (c) mature vine displaying stunted shoots, (d) single shoot with cupped leaves with necrotic margins and (e) uneven ripening and reduced bunch size.

A number of fungicides registered for use on grapevines to control other fungal diseases such as powdery mildew, downy mildew, botrytis and phomopsis have shown potential as wound protectants (Sosnowski *et al*. 2013). There is a need to further evaluate fungicides such as tebuconazole (Folicur) pyraclostrobin (Cabrio), pyrimethanil (Scala) and fluazinam (Shirlan) to determine optimum application rates for preventing infection by *E. lata* on pruning wounds.

Furthermore, in laboratory studies, a number of "alternative" products, including garlic and lactoferrin (derived from milk), inhibit the growth of *E. lata*, (Sosnowski *et al*. 2013). Garlic and lactoferrin have antimicrobial properties (Ankri and Mirelman, 1999, Lahoz *et al*, 2008) and, if effective, may be useful in organic viticulture or to reduce pesticide usage in conventionally managed vineyards. Further evaluation of these treatments on pruning wounds is required.

Initial laboratory screening uses fungicide-amended agar plates inoculated with spores and mycelium of *E. lata*. This identifies potentially effective fungicides within 2 weeks, providing candidates for evaluation on grapevine wounds. Field trials are undertaken to generate data in a vineyard environment, subject to natural climatic conditions and vineyard management influences, but these tests are very labour intensive and can take up to 18 months to complete. In order to generate data in a shorter timeframe, there is a need to develop a new system to evaluate products for use as wound protectants. A greenhouse bioassay (Sosnowski *et al.* 2007b) that uses detached grapevine canes growing in controlled conditions shows promise.

In previous research, pruning wound protectants have been applied by hand with paint brush or spray bottle. Hand application of pruning wound protectants is not economically viable in larger commercial vineyards, primarily due to labour costs. A spraying secateur was developed in the early 1980s to apply benomyl to apricot and grapevine wounds at the time of pruning (Carter and Perrin, 1985) but factors such as occupational health and safety regulations have prevented widespread adoption. Carter and Price (1977) applied benomyl to apricot pruning wounds using a turbomist sprayer, only slightly reducing the incidence of infection following inoculation with *E. lata.* Ramsdell (1995) subsequently reported reduced symptoms of eutypa dieback in vines treated with benomyl using an air-blast sprayer following pruning over a 5-year period. Using a recycle spray system with a different treatment mixture of flusilazole and carbendazim, Lecomte *et al* (2003) reported reduced infection of pruning wounds by *E. lata.* More recently, Herche and Gubler (2010) used a tractor-driven sprayer to apply myclobutanil to pruning wounds.There is a need to evaluate methods of applying fungicide to pruning wounds using conventional spray equipment. Demonstration of the efficacy of spray application would increase adoption of pruning wound protectants in Australia.

Diatrypaceous fungi occur worldwide and comprise a number of pathogens of woody crops, forest and ornamental tree species. Trouillas *et al* (2010) conducted surveys of Californian vineyards where eleven diatrypaceous fungal species related to *E. lata* were isolated from grapevine wood with eutypa dieback symptoms. Pathogenicity tests showed that many of these species caused eutypa dieback wood symptoms (Trouillas and Gubler 2010). Recent surveys in Australian vineyards identified six species of diatrypaceous fungi other than *E. lata* associated with eutypa dieback (Trouillas *et al.* 2011). It is important to evaluate the pathogenicity of these species relative to *E. lata* and to ensure that wound protectants being developed to control *E. lata* are also effective against these other fungi.

This project builds on the knowledge acquired in previous research with the following objectives:

- To develop effective chemical and non-chemical pruning wound treatments, determine optimum application rates, and evaluate additives that may improve the penetration of fungicides into woody tissue.
- To evaluate means of large scale application of pruning wound treatments.
- To determine pathogenicity of related fungal species associated with eutypa dieback and evaluate the efficacy of fungicides being developed for control of *E. lata.*

Outcomes will provide grapegrowers with practical options for pruning wound protection which will lead to rapid grower adoption, alleviate economic losses and enhance the sustainability of production in regions affected by eutypa dieback.

4 DEVELOPMENT OF A DETACHED CANE ASSAY

Introduction

Eutypa dieback can be controlled by treating pruning wounds with fungicides and other substances, however, the number of products available for this treatment is limited and more data are required to register products for this purpose.

Initial screening of products is carried out in laboratory trials using agar plates amended with fungicide. This identifies potential fungicides within 2 weeks, providing candidates for field evaluation on grapevine wounds. Field evaluations can take up to 18 months to generate data. There is a need for development of a more efficient technique to evaluate wound protectants on grapevines to reduce the time taken for evaluation and to increase the capacity for generating data for product registration.

In this project, a detached cane assay (DCA), initially developed for other purposes, was adapted to evaluate products for control of eutypa dieback using live plantlets under controlled conditions. Previously, J. Bennett at the Marlborough Wine Research Institute in New Zealand used single node cuttings to assess cane fruitfulness and Sosnowski *et al.* (2007a) modified it to screen table grapes for blackspot disease of grapes. Mundy and Robertson (2010) also recently adapted the technique as a model for studying grapevine trunk diseases.

Methods

Grapevine canes (cv Shiraz) were collected during dormancy and stored at 3-4ºC before being cut into 10 cm single-node sections with the top surface 1 cm above the node (Figure 2a). Canes were placed into holes in 2 cm thick polystyrene boards, ensuring that the bottom of the canes extended approximately 1 cm below the boards. The boards with canes were floated on tapwater in plastic tubs (Figure 2b) in a greenhouse maintained at approximately 25°C. The water was changed weekly for the first 4 weeks, thereafter, soluble fertiliser (Campbells Diamond Special T) was added weekly at a rate of 0.2 g/L.

Experiment 1 was carried out to determine the extent of colonisation of the canes by *E. lata* by re-isolating the fungus from the canes at different times after inoculation. *E. lata* ascospores were obtained from fruiting bodies, using methods similar to those described by Carter (1991). This involved soaking dead grapevine wood containing perithecia in water for 1 hour in a plastic container, suspended by attaching to the lid and leaving overnight. A suspension of spores was prepared and adjusted to 25,000 or 50,000 spores /mL using a haemocytometer. In this experiment, the top wound on each cane was moistened by spraying with distilled water before inoculating with a 20 µL water droplet (Figure 2b) containing a suspension of either 500 or 1000 *E. lata* spores. Spore concentrations were chosen based on those used in past field trials. Controls were not inoculated.

At 4, 8 and 12 weeks after inoculation, six canes from each treatment were removed from the board. Bark was removed and the canes sterilised in bleach for 10 minutes prior to rinsing twice in sterile water. Starting immediately below the inoculation point, cuts were made using sterile secateurs every 2 mm along the canes for a distance of 50 mm. Segments were placed sequentially on plates of potato dextrose agar (PDA) and incubated for a week with 12 hr day/night light cycle at 25°C. Each wood segment was then assessed for presence or absence of *E. lata* growth (Figure 2c), and the distance the fungus extended from the inoculation point recorded.

In Experiment 2 (Seth Toalak 2011), six different *E. lata* spore suspensions (0, 10, 50, 100, 200 and 500 spores) were applied to wounds on canes which were then maintained as described above. After four weeks, canes were removed and prepared for assessment as described above. Starting immediately below the inoculation point, cuts were made using sterile secateurs every 2 mm along the canes for a distance of 30 mm. The resulting cane segments were then placed on PDA plates, incubated for 1 week and assessed for presence or absence of *E. lata* growth.

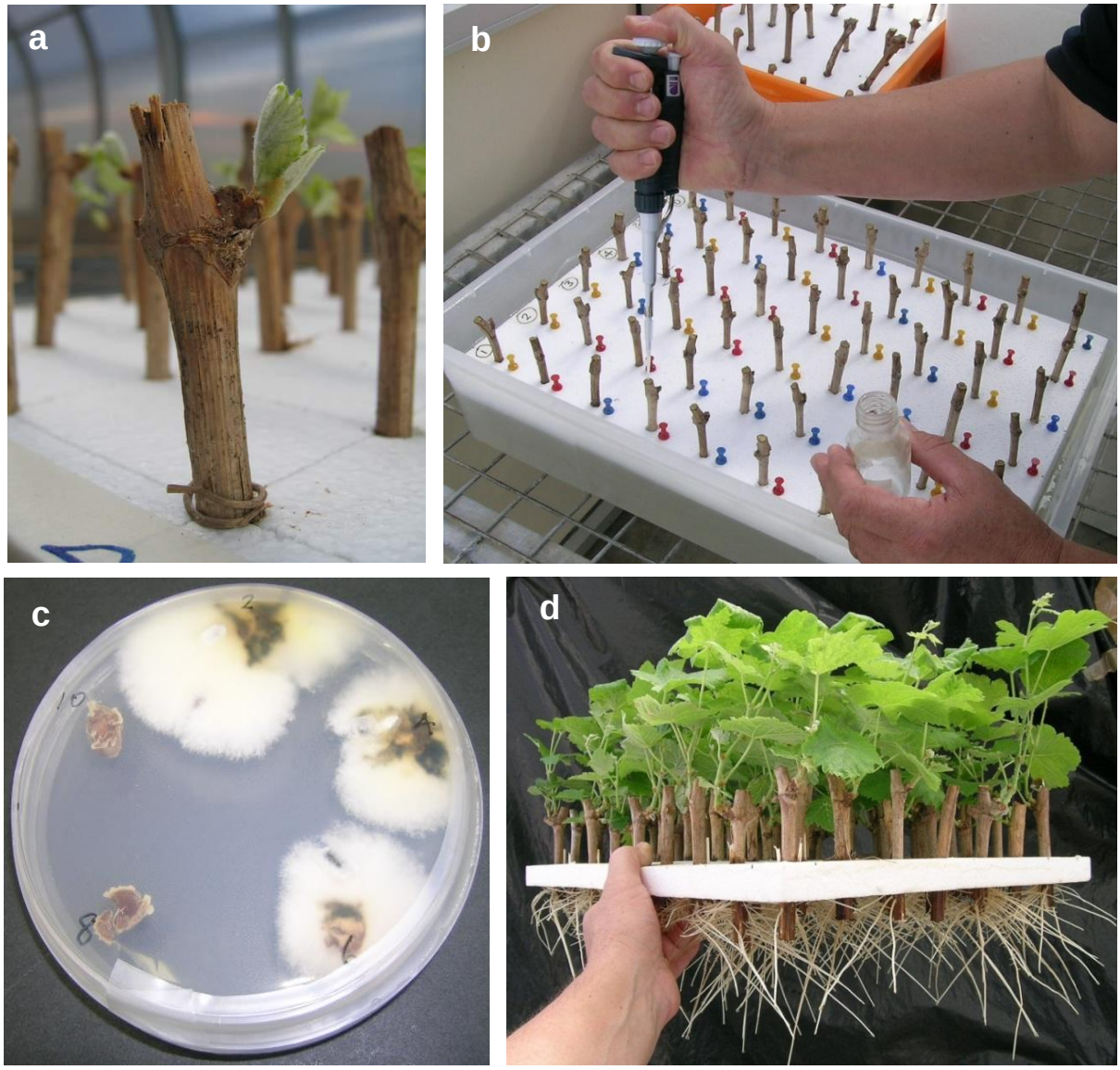


Figure 2. Detached cane assay; a) single node cutting, b) inoculating cuttings inserted into polystyrene boards floating on water (coloured pins indicate treatment), c) growth of *E. lata* from wood segments and d) shoot and root growth after 4 weeks.

Results

In both experiments, bud burst occurred within a week of establishment, and roots emerged after 2 weeks and continued to grow throughout the experiment (Figure 2d).

In Experiment 1, *E. lata* was recovered from all canes inoculated with both spore suspensions at each assessment time. *E. lata* was recovered from 2 to 20 mm and 2 to 18 mm below the inoculation point for the canes inoculated with 500 and 1000 spores, respectively. Figure 3 shows the combined mean distance colonised by *E. lata* at 4, 8 and 12 weeks after inoculation.

In the second experiment, *E. lata* was recovered from the canes inoculated with all suspensions but the level of recovery varied significantly. At 500 and 200 spores /wound, recovery was 80% and 75% respectively while recovery was 25% or less at 100 spores or less /wound (Figure 4).

Discussion

Eutypa lata readily colonised detached canes, growing up to 20 mm from the point of inoculation within 4 weeks. As a result, samples can be harvested four weeks after treatment and assessment of mycelia growth data available within a further two to four weeks. This technique reduces the time required to produce efficacy data compared with up to 18 months needed for field experiments.

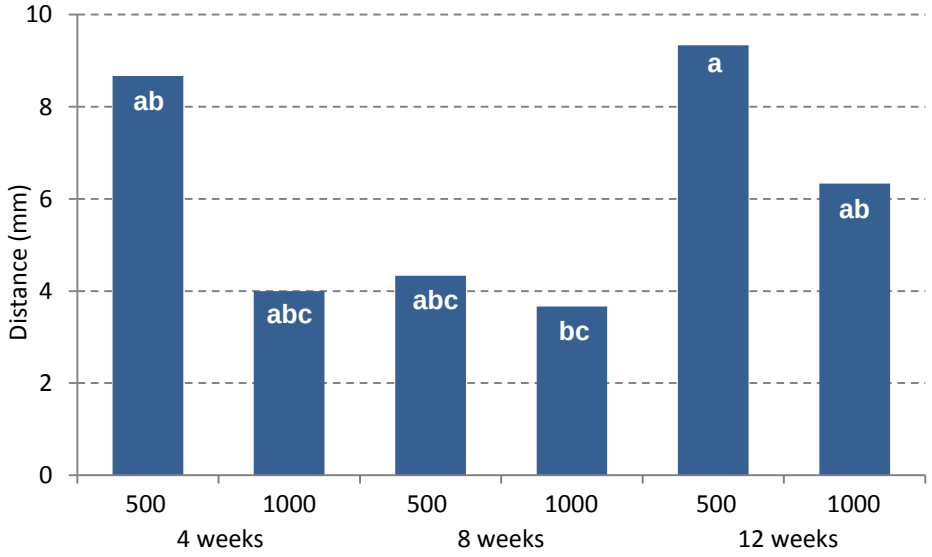


Figure 3. Experiment 1: Mean distance of *Eutypa lata* colonisation of canes at 4, 8 and 12 weeks after inoculation with 500 or 1000 spores. Bars with the same letter are not significantly different from each other.

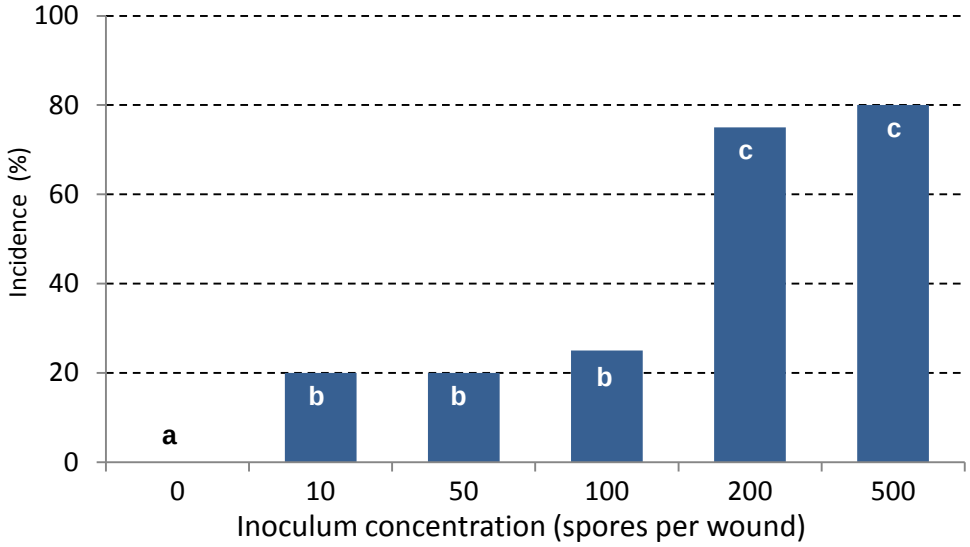


Figure 4. Experiment 2: Incidence of *E. lata* recovered from canes inoculated with different spore suspensions. Bars with the same letter are not significantly different from each other.

Furthermore, the DCA allows for the use of a range of inoculum levels and is therefore suitable for assessing efficacy of treatments under varying disease pressures. In the field, inoculum doses of 500 and 1000 spores are routinely used to ensure sufficient infection of the untreated controls for statistical evaluation. However, based on research by Carter and Moller (1971), as few as 10 spores could be expected to infect pruning wounds. The DCA technique enables evaluation of pruning wound treatments at inoculum doses more likely to reflect natural conditions.

Using a similar single-node plantlet technique, Mundy and Robertson (2010) showed that significantly longer lesions formed on detached canes than on attached canes, based on the growth of lesions attributed to *E. lata*. In that study, inoculation was made by applying agar plugs containing *E. lata* mycelium to wounds, whereas in the current study, a suspension of spores was used for inoculation which provides a closer simulation of field conditions.

The DCA method that uses fresh pruning wounds on live plantlets is a useful tool to screen potential products for use in field trials and provides data to support product registration. The technique will also provide a tool to screen alternative products for control of eutypa dieback and for epidemiological studies which underpin the development of disease management strategies.

5 EVALUATION OF WOUND PROTECTANTS

5.1 Background

Previous research (CRV 03/06S, SAR 06/01) showed that treating pruning wounds with the benzimidazole fungicides benomyl and carbendazim prevented infection by *E. lata.* However, these fungicides were withdrawn for use in grapevines in 2001 and 2010, respectively. With this, and the increased awareness of the threat posed by grapevine trunk diseases in Australia (Sosnowski and Wicks 2012), there is a need to find alternative products that are effective in preventing infection of pruning wounds. Only two products are currently registered in Australia for eutypa dieback; Vinevax (a *Trichoderma* biocontrol agent) and Greenseal (a paint containing tebuconazole fungicide). A number of other products have shown promise in controlling eutypa dieback in Australian and overseas trials but require further evaluation to optimise application rates.

Initial screening of products was carried out in laboratory trials using agar plates amended with different rates of fungicides. This provided efficacy data, but may not reflect effectiveness of fungicides on grapevine wounds. Fungicides demonstrating potential were then further evaluated in field trials.

Field trials were conducted during the project to evaluate a number of promising fungicides from various fungicide groups and some alternative products. In the greenhouse, detached cane assays (DCA) were conducted to generate additional efficacy data and to evaluate treatments at lower inoculum doses, more closely reflecting natural amounts of inoculum than the high rates used in the field trials.

The aim of these trials was to develop effective pruning wound treatments and evaluate additives that may improve the penetration of fungicides into woody tissue to provide management strategies for eutypa dieback. The trials also aimed to provide data to support registration or label extension of fungicides for the control of eutypa dieback.

5.2 Field trials

Introduction

Two field trials were conducted over two consecutive years to evaluate selected treatments (Table 1) for their ability to protect pruning wounds from infection by *E. lata*. Treatments were selected based on their efficacy against *E. lata* in previous research (SAR 06/01).

Methods

Trials were conducted in 2010 and 2011 on Cabernet Sauvignon vines planted in 1984 (Trial 1) and Sauvignon Blanc vines planted in 1985 and grafted to Shiraz in 2001 (Trial 2) at the Nuriootpa Research Centre, approximately 100 km north of Adelaide, South Australia.

In Trial 1, 1-year-old canes were pruned to two buds on 7 July 2010 and 28 June 2011 using secateurs (Figure 5a). Within 2 h of pruning, using a paintbrush, wounds were treated (Figure 5b) with Folicur (0.6, 1.5 and 3 ml/L water), Scala (4, 10 and 20 ml/L), Prosaro (0.6, 1.5 and 3 ml/L), Cabrio (0.8, 2 and 4 ml/L) or Shirlan (2, 5 and 10 ml/L), reflecting 2, 5 and 10 times the label rate registered for other grapevine diseases. Controls, positive (inoculated) and negative (non-inoculated) were treated with sterile distilled water (SDW). One day after treatment, wounds were moistened by spraying with SDW immediately prior to inoculation with approximately 500 spores of *E. lata* suspended in 20 µl SDW and applied using a pipette (Figure 5c). A second inoculation of 500 ascospores was made 6 and 3 days after treatment in 2010 and 2011, respectively. The trial was set up as a randomised block design with eight replications.

In Trial 2, 1-year-old canes were pruned to two buds on 7 July 2010 and 28 June 2011. Within 2 h of pruning, wounds were treated using a hand-held spray bottle with Folicur (0.3 and 0.6 ml/L with and without 15 ml/L Pentrabark), Cabrio (0.8 ml/L with and without 1.0 ml/L Du Wett), Pentrabark (15 ml/L) or Du Wett (1 ml/L) until runoff. Using a paint brush, other wounds were liberally treated with Serenade (2 ml/L), garlic (fresh juice, 5 ml/100 ml) or lactoferrin (5 g/100 ml). Controls, positive (inoculated) and negative (non-inoculated) were treated with SDW. Inoculation with *E. lata* spores was made as for Trial 1.

For both trials, canes were harvested from vines in late May 2011 and 2012 and returned to the laboratory for assessment (Figure 5d). Bark was removed from each cane using a sharp knife (Figure 5e). The exposed wood was surface sterilised in 2.5% sodium hypochlorite (NaOCl) for 10 minutes and then washed twice in SDW. Using sterilised secateurs, canes were cut into segments (3 × 2 × 2mm) taken from each side of the margin between live and dead wood tissue (Figure 5f). Five wood segments were plated in each plastic Petri dish filled with PDA amended with antibiotic (streptomycin sulfate 25 µg/L), with two plates used for each cane. Samples were incubated at 25°C under fluorescent light for 12 h each day for 7 days and then assessed for presence or absence of *E. lata* mycelial growth (Figure 5g). Efficacy was based on the mean percent recovery (MPR) of *E. lata* from the treated canes by isolation on PDA. Data are presented as mean percent disease control which was calculated as the reduction in MPR as a proportion of the inoculated control.

Table 1. Pruning wound treatments evaluated in field trials and detached cane assays.

Product	Active ingredient	Label rate	Supplier
Fungicides			
Folicur	Tebuconazole (430 g/L)	0.3mL/L	Bayer Crop Science
Cabrio	Pyraclostrobin (250g/L)	0.4mL/L	BASF Australia
Prosaro	Tebuconazole (210g/L) + prothioconazole (210g/L)	N/A	Bayer Crop Science
Scala	Pyrimethanil (400 g/kg)	2.0mL/L	Bayer Crop Science
Shirlan	Fluazinam (500 g/L)	1.0mL/L	Crop Care Australasia Pty Ltd
Surfactants			
Pentrabark	Alkylphenol ethoxylate, polysiloxane polyether copolymer	15mL/L	Quest Products corp.
Du Wett	Organosilicone / organic fluid blend	1.0mL/L	Nufarm Ltd
Alternatives			
Serenade	Bio-fungicide (*Bacillus subtilis*) (2mL/L)	N/A	BASF Australia
Garlic	Fresh garlic juice (5mL/100mL)	N/A	N/A
Lactoferrin	Lactoferrin (99.9%) (5g/100mL)	N/A	MG Nutritionals Australia

N/A not applicable

Results

Trial 1

In 2010/11, *E. lata* was recovered from 86% of inoculated control canes and 6% of naturally infected canes. When applied at 2-10 times the label rate for other grape diseases, Folicur reduced infection by 59-88%; Prosaro by 44-73%; Shirlan by 21-58%; Scala by 33-53% and Cabrio by 10-12% (Figure 6).

In 2011/12, *E. lata* was recovered from 53% of inoculated control canes and 4% of naturally infected canes. When applied at 2-10 times the label rate for other grape diseases, Folicur reduced infection by 83-87%; Prosaro by 92-97%; Shirlan by 8-31%; Scala by 31-71% and Cabrio by 72-75% (Figure 6).

Trial 2

In 2010/11, *E. lata* was recovered from 92% of inoculated control canes and 8% of naturally infected canes. Folicur applied at 0.6 ml/L with and without the addition of Pentrabark provided 48 and 46% control of *E. lata*, respectively. Folicur applied at 0.3 ml/L provided 35% control regardless of the addition of Pentrabark. Lactoferrin, garlic juice and Serenade provided 14, 8 and 7% control, respectively (Figure 7).

In 2011/12, *E. lata* was recovered from 55% of inoculated control canes and 13% of naturally infected canes. Folicur applied at 0.6 ml/L with the addition of Pentrabark provided 66% control of *E. lata*, while Lactoferrin provided 65% control. Folicur at 0.6 mL/L and 0.3 mL/L provided 60% and 57% control respectively, while garlic juice provided 52% control. Serenade provided 31% control (Figure 7).



Figure 5. Methods used to evaluate treatments in the field for the prevention of wound infection: (a) cutting canes to two-bud spurs on Cabernet Sauvignon vines at Nuriootpa; (b) applying treatment to wound with a paint brush; (c) inoculating treated wounds with ascospores of *Eutypa lata*; (d) spurs removed from vines during the following winter; (e) spurs with bark removed; (f) cutting chips from either side of the necrotic margin and (g) cultures of *E. lata* growing from wood chips.

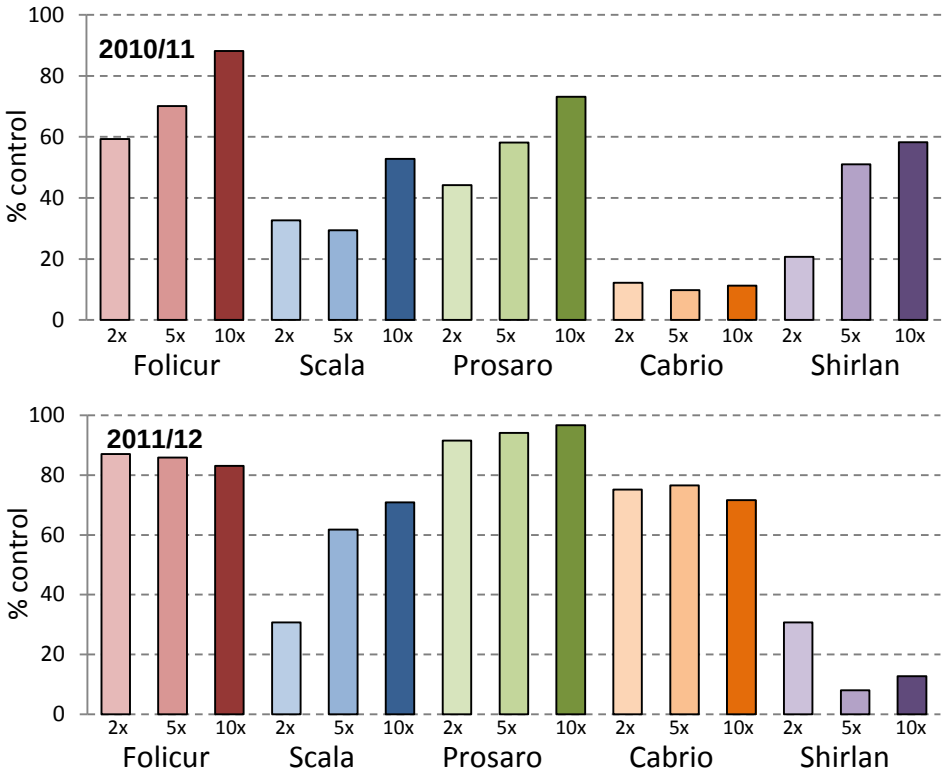


Figure 6. Mean percent control of infection of pruning wounds on Cabernet Sauvignon vines by *Eutypa lata* in Trial 1. Fungicides were applied at 2, 5 or 10 times the recommended label rate for other grapevine diseases (Table 1).

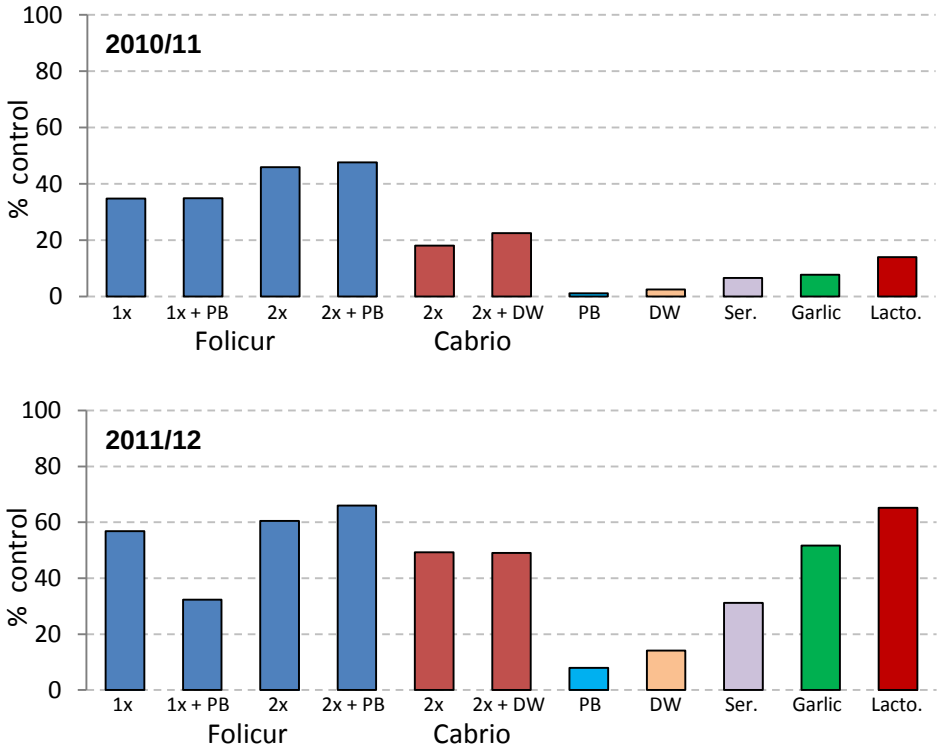


Figure 7. Mean percent control of infection of pruning wounds of Shiraz vines by *Eutypa lata* in Trial 2. Folicur was applied at one (1x; 0.3 ml/L) or two (2x; 0.6ml/L) times the recommended label rate for other grapevine diseases with or without Pentrabark (PB; 15 ml/L). Cabrio was applied at two times label rate with or without Du Wett (DW; 1 ml/L). Also evaluated were PB and DW alone, Serenade (Ser; 2 ml/L), garlic (50 ml/L) and lactoferrin (Lacto; 50 g/L).

5.3 Detached cane assays

Introduction

A detached cane assay (Section 4) was used to evaluate selected products as wound protectants against *E. lata*. The purpose of the DCA was to generate data to support field trial data for registration and to evaluate products at a range of inoculum doses. In field trials, high inoculum doses (1000 spores/wound) are applied in order to ensure sufficient infection by *E. lata* for statistical analysis. However, this high inoculum dose does not reflect natural infection of pruning wounds (Carter and Moller, 1971) and so may not give a true indication of the effectiveness of products in preventing infection in the field. In the following DCAs, products were evaluated at 1000, 500 and 200 spores/wound.

Methods

Three DCA experiments were conducted as described in Section 4. Four commercial fungicide formulations; Folicur, Scala, Cabrio and Shirlan, were each applied by paintbrush to wounds on 20 canes at 1, 2, 5 or 10 times the recommended label rate registered for other diseases on grapevines (Table 1). On the following day, wounds were inoculated with either 500 (DCA 1 & 2) or 200 spores (DCA 3). Three days later, a second inoculation of 500 spores was applied to wounds in DCA 1. Untreated controls were either inoculated with the spore suspension (positive control) or not inoculated (negative control).

After four weeks, canes were prepared for assessment as described in section 4. Longitudinal cuts were made at the treated ends of the canes using sterile secateurs and a transverse cut was made 3 mm below the wound surface, to include the interface between live and dead tissue. The resulting cane pieces were then placed on PDA plates, incubated for 1 week and assessed for presence or absence of *E. lata* mycelia. Efficacy was based on the mean percent recovery (MPR) of *E. lata* from the treated canes by isolation on PDA. Data are presented as mean percent disease control which was calculated as the reduction in MPR as a proportion of the inoculated control.

Results

In DCA 1, *E. lata* was recovered from 95% of controls inoculated with 2x500 spores per wound. When applied at 1-10 times the recommended label rate for other grapevine diseases, Folicur provided 47 - 100% control; Scala 5 – 58% control; Cabrio, 63 – 100% control and Shirlan, 53 – 100% control of *E. lata* (Figure 6a).

In DCA 2, *E. lata* was recovered from 95% of controls inoculated with 500 spores per wound. When applied at 1-10 times the recommended label rate for other grapevine diseases. Folicur provided 63 - 100% control; Scala, 42 - 84% control; Cabrio, 74 – 100% control and Shirlan 42 – 100% control of *E. lata* (Figure 6b).

In DCA 3, *E. lata* was recovered from 35% of controls inoculated with 200 spores per wound. Shirlan and Cabrio provided complete control of *E. lata* at all rates evaluated (Figure 6c). Folicur provided between 86 and 100% control of *E. lata* at all application rates. Scala was not effective at label rate, but provided between 43 and 100% control at 2-10 times the label rate.

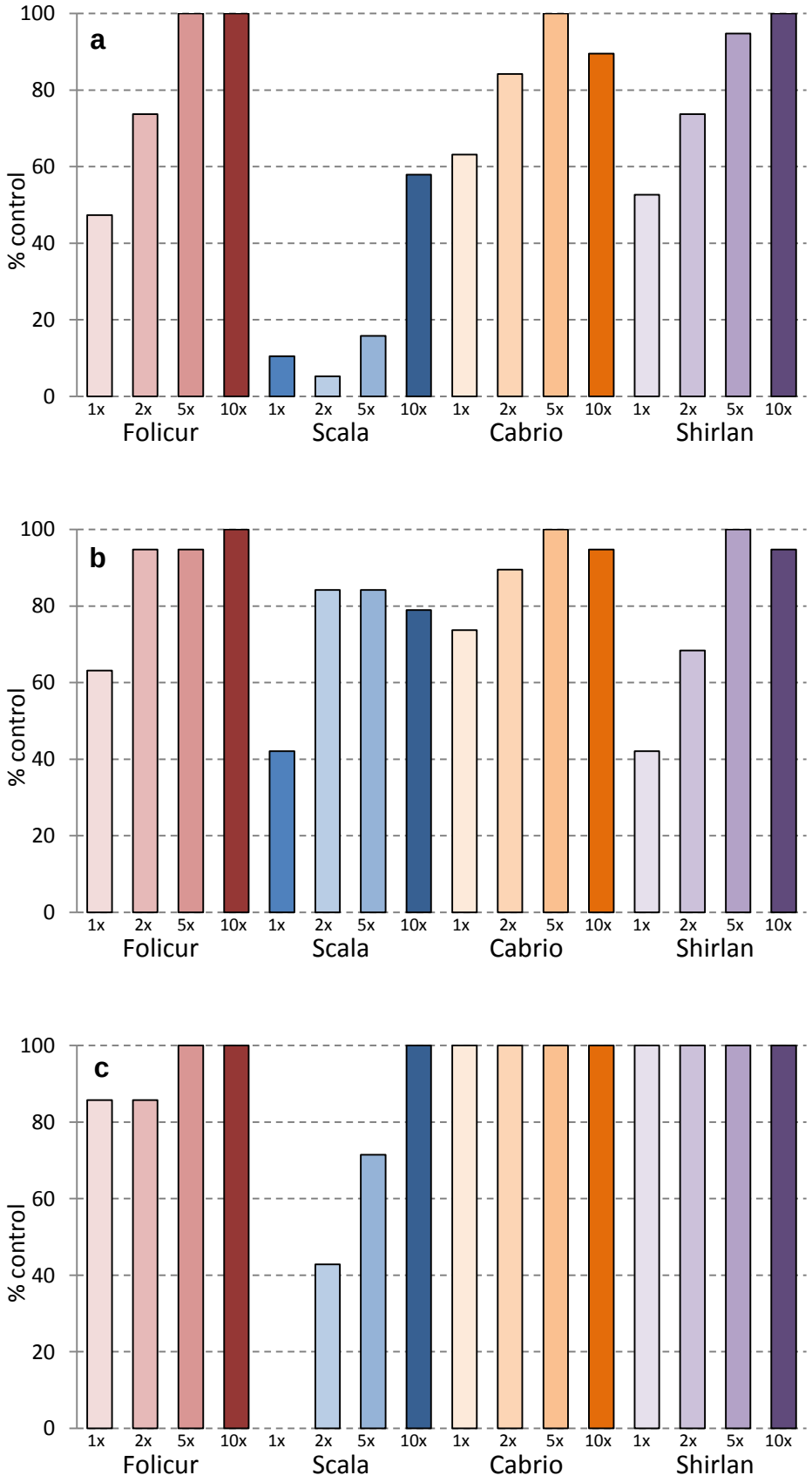


Figure 8. Mean percent control of eutypa dieback using a detached cane assay (DCA) following inoculations of a) 1000 (2x500), b) 500 and c) 200 spores of *E. lata* . Each chemical was applied at 1, 2, 5 or 10x the recommended label rate for foliar application to grapevines in Australia (Table 1).

5.4 Discussion

The field trials subjected treatments to extremely high disease pressure and resulted in *E. lata* recovery from 86-92% and 53-55% of inoculated controls in 2010/11 and 2011/12, respectively. Natural disease pressure, as reflected by recovery of *E. lata* from non-inoculated controls, never exceeded 6%. In order to ensure sufficient establishment of infection to compare efficacy of fungicides in the field trials, all wounds were inoculated with 1000 (2x500) ascospores, which is 100 times the number estimated to infect pruning wounds naturally (Carter and Moller 1971). DCAs were used in order to evaluate fungicides at reduced spore doses, to more closely reflect natural disease pressure (ie. 35% recovery of *E. lata* in controls inoculated with 200 spores /wound). This enabled more realistic evaluation of the treatments.

The demethylation inhibitors (DMI) fungicides Folicur and Prosaro were the most effective treatments in field trials. Tebuconazole is the main active ingredient of both treatments but Prosaro has the added addition of prothioconazole. Prosaro was initially included in the field trial as there was potential for its introduction to the Australian market for control of powdery mildew, but the suppliers have since decided not to proceed. In DCA1, where 2 x 500 spores /wound were used as an inoculum, similar control was achieved to that of the field trials which had the same inoculum dose. Folicur applied at label rate (0.3 ml/L) provided 47% control, whereas when inoculum dose was reduced to 200 spores /wound, control increased to 86%. Elsewhere, Folicur has been reported to control eutypa dieback (Halleen et al 2010) and botryosphaeria dieback in field trials (Amponsah *et al* 2012, Pitt *et al* 2012).

The Quinine outside Inhibitor (QoI) Cabrio (pyraclostrobin) controlled infection of pruning wounds by *E. lata* in the second field trial, where infection in controls was only 53%, compared with 86% in the first field trial, where little control was achieved. In DCA1, when applied at label rate (0.4 ml/L), Cabrio provided 63% control, whereas reducing the inoculum dose to 200 spores /wound, achieved complete disease control. Rolshausen *et al.* (2010) and Sosnowski *et al.* (2008) also reported on the efficacy of Cabrio to control eutypa and botryosphaeria dieback.

In contrast to Cabrio, the 2,6-dinitro-analine fungicide Shirlan (fluazinam) provided some control in the first field trial but little control in the second field trial. In DCA1, when applied at label rate (1 ml/L), Shirlan provided 53% control, whereas reducing the inoculum dose to 200 spores /wound achieved complete disease control. Bourbos and Barbopoulou (2005) and Pitt *et al*. (2012) also reported on the efficacy of Shirlan to control both eutypa and botryospharia dieback.

In field trials, the surfactants, Pentrabark and DuWett were added to Folicur and Cabrio, respectively, to evaluate their ability to improve efficacy of the fungicides by improving fungicide penetration into the grapevine canes. Results indicated that there was no benefit in adding these surfactants to the fungicides for protecting wounds from infection by *E. lata*. However, in these trials, treatments were applied until runoff. So, in the case of application with commercial sprayer where less than 100% coverage of wounds is achieved, it may be possible that surfactants could improve the efficacy of fungicides by increasing coverage.

The “alternative” products Serenade, garlic and lactoferrin were not effective in the first year of evaluation but in the second year, when evaluated at reduced disease pressure, each of the products provided significant control. Of these, greatest control was achieved by lactoferrin, but due to the lack of commercial products containing lactoferrin available on the market, it is no longer being pursued. Garlic extract, however, is available commercially and has been further evaluated and shown to be effective for eutypa dieback control at 1% concentration using the DCA (Babynec 2012). Field evaluation of garlic extract is continuing and may have potential as an alternative to synthetic chemicals for sustainable control of eutypa dieback.

These results show that three fungicide treatments, Folicur, Cabrio and Shirlan, applied at currently registered label rates for other grapevine diseases, are also effective as pruning wound protectants for the control of eutypa dieback. These data are currently being used to support an application to the Australian Pesticides and Veterinary Medicines Authority (APVMA) for registration for control of eutypa dieback.

A number of fungicides applied to pruning wounds on the day of pruning will provide effective control of eutypa dieback. Further research needs to be undertaken to determine the curative and preventative qualities of these fungicides to further improve recommendations for practical application of pruning wound treatments.

6 OPTIMISING SPRAY APPLICATION OF WOUND PROTECTANTS

Introduction

Protection of pruning wounds from infection by *E. lata* has been demonstrated by hand-application of treatments directly to the wounds. In large scale commercial viticulture operations, hand-application of products is not economically viable due to labour costs so there is a requirement for a more efficient means of delivering treatments to wounds.

In preliminary trials (SAR 06/01), it was shown that Bavistin (carbendazim) application using commercial sprayers provided efficacy similar to that of treatments hand-applied with a paint brush. Further evaluation of sprayers at a range of water spray rates was necessary to demonstrate optimal strategies for spray application.

In the current project, three field trials were established in conjunction with regional grower groups in McLaren Vale, Coonawarra and the Adelaide Hills in 2010 and repeated in 2011 to evaluate spray application of wound treatments for control of eutypa dieback. The aim was to evaluate commercial sprayers, typical of those being used in each of the grape-growing regions. Sprayers included air-blast, air shear, fan, recycle and purpose-built machines and were supplied and operated by local growers. Improved efficiency of application will lead to greater grower adoption and alleviate future economic losses from eutypa dieback to enhance the sustainability of production in regions affected by eutypa dieback.

In these spray trials, all treatments were subjected to very high disease pressure with spore doses of 1000 per wound, whereas natural field infection doses of *E. lata* are more likely to be in the order of 10 spores per wound (Carter and Moller, 1971). The aim of these trials were to demonstrate the ability of tractor-driven sprayers to apply pruning wound treatments, so to ensure efficacy could be demonstrated under these conditions, Folicur (tebuconazole) was applied at either 0.6, 1.5 or 3 ml/L for the spray treatments.

Methods

McLaren Vale

A trial was conducted on Shiraz vines, planted in 1996 near McLaren Vale, South Australia. One-year-old canes were pruned to two bud spurs on 15 July 2010 and 7 July 2011. In 2010, three sprayers were used; a Greentech, double-row, fan sprayer (Figure 9a), a Silvan Turbomiser, single-row, air-shear sprayer (Figure 9b) and a home-engineered, single-row, recycle sprayer (Figure 9c-d). Each sprayer applied Folicur at 3 ml/L in water spray volumes of 200, 400 and 600 L/ha.

In 2011, two sprayers were used; a Croplands Quantum Mist double-row fan sprayer (Figure 9e-f) and the recycle sprayer described above. The Croplands sprayer was used to deliver Folicur at 1.5 and 3 ml/L with water spray volumes of 200, 400 and 600 L/ha. The recycle sprayer was used to deliver Folicur at 0.6, 1.5 and 3 mL/L with a water spray volume of 200 L/ha. In both years, a treatment of Folicur (3 ml/L) applied with a paint brush was included for comparison along with untreated positive (inoculated) and negative (non-inoculated) controls.

All pruning wounds were covered with plastic bags secured with rubber bands, except for the wounds that were about to be sprayed (Figure 12). Following a given spray treatment, the treated canes were covered with plastic bags and the next set of canes exposed for the next spray treatment. This was repeated until all pruning wounds had received the appropriate spray treatment.

All wounds (except for negative controls) were inoculated twice with 500 *E .lata* ascospores on 16 and 20 July 2010 and 7 and 8 July 2011. Each treatment was applied to 10 wounds and the trial was designed as a randomised block with 10 replications. Treated canes were removed in May 2011 and June 2012 and stored in a 4°C cool room prior to assessment in the laboratory.

Coonawarra

A trial was conducted on Cabernet Sauvignon vines, planted in 1994 near the township of Penola, South Australia. One-year-old canes were pruned to two-bud spurs on 22 July 2010 and 12 July 2011. In 2010, three sprayers were used; a Hardi, ducted, double-row, air-assisted sprayer (Figure 10a-b), a Greentech, double-row, fan sprayer (Figure 10c) and a Silvan Turbomiser, single row, air-shear sprayer (Figure 10d). Each sprayer delivered Folicur at 3 ml/L with spray volumes of 200, 400 and 600 L/ha. In 2011, three sprayers were used; the Greentech fan and Hardi air-assisted sprayers described above and an FMR Spray-Pro R Series, two-row, recycle sprayer (Figure 10e-f). The Greentech and Hardi sprayers delivered Folicur at 3 ml/L with a water spray volume of 200, 400 and 600 L/ha. The recycle sprayer delivered Folicur at 0.6, 1.5 and 3 ml/L with a water spray volume of 960 L/ha. In both years, a treatment of Folicur (3 ml/L) applied with a paint brush was included for comparison along with untreated positive (inoculated) and negative (non-inoculated) controls.

All pruning wounds were covered with plastic bags and uncovered as appropriate as described above. All wounds (except for negative controls) were inoculated twice with 500 *E. lata* ascospores on 22 and 23 July 2010 and 12 and 13 July 2011. Each treatment was applied to 10 wounds and the trial was designed as a randomised block with 10 replications. Treated canes were removed in July 2011 and June 2012 and stored in a 4°C cool room prior to assessment in the laboratory.

Adelaide Hills

A trial was conducted on Tempranillo vines planted in 1998 near the township of Charleston, South Australia. One-year-old canes were pruned to two-bud spurs on 27 July 2010 and 26 July 2011. In 2010, two sprayers were used; a Croplands Quantum Mist, single-row, fan sprayer (Figure 11a-b) and a home-engineered cordon sprayer (Figure 11b-c). The Croplands sprayer delivered Folicur at 1.5 and 3 ml/L with water spray volumes of 200, 400 and 600 L/ha. The cordon sprayer delivered Folicur at 3 ml/L with water spray volumes of 250, 450 and 600 L/ha. In 2011, three sprayers were used; The Croplands and cordon sprayers described above and a home-engineered, recycle sprayer (Figure 11e-f). The Croplands and cordon sprayers delivered Folicur at 3 ml/L with spray volumes of 200, 400 and 600 L/ha. The recycle sprayer delivered Folicur at 0.6, 1.5 and 3 ml/L with water spray volume of 400 L/ha. In both years, a treatment of Folicur (3 ml/L) applied with a paint brush was included for comparison along with untreated positive (inoculated) and negative (non-inoculated) controls.

All pruning wounds were covered with plastic bags and uncovered as appropriate as described above. All wounds (except for negative controls) were inoculated twice with 500 *E .lata* ascospores on 7 and 8 July in 2010 and 2011. Each treatment was applied to 10 wounds and the trial was designed as a randomised block with 10 replications. Treated canes were removed in July 2011 and June 2012 and and stored in a 4°C cool room prior to assessment in the laboratory.



Figure 9. Sprayers used at McLaren Vale trial in 2010 and 2011; a) Greentech, double-row, fan sprayer, b) Silvan Turbomiser, single-row, air-shear sprayer, c-d) home-engineered, single-row, recycle sprayer and e-f) Croplands, Quantum Mist, double-row, fan sprayer.



Figure 10. Sprayers used at Coonawarra trial in 2010 and 2011; a-b) Hardi, ducted, double-row, air-assisted sprayer, c) Greentech, double-row, fan sprayer, d) Silvan Turbomiser, single row, air-shear sprayer and e-f) FMR Spray-Pro R Series, two-row, recycle sprayer.



Figure 11. Sprayers used at Adelaide Hills trial in 2010 and 2011; a-b) Croplands Quantum Mist, single-row, fan sprayer, c-d) home-engineered cordon sprayer and e-f) home-engineered, recycle sprayer.



Figure 12. Plastic bags being applied to pruning wounds (a) and covered pruning wound (b).

Spray coverage

Spray coverage was assessed using water-sensitive papers (WSP) which were placed in four fixed positions on posts (front, back, left and right) and six random positions throughout the pruning wound zone of vines (Figure 13). For each spray treatment, a fresh set of 10 papers was placed into position and then collected once the sprayer had passed and the paper dried. WSP respond to contact with water by changing from yellow to blue. The percentage of each WSP that turned blue for each paper was quantified using Image J, a Java-based image processing program developed at the National Institutes of Health, Bethesda, Maryland, USA. The relative spray coverage of the wounds, as a mean of the 10 WSPs, was calculated for each of the spray treatments.

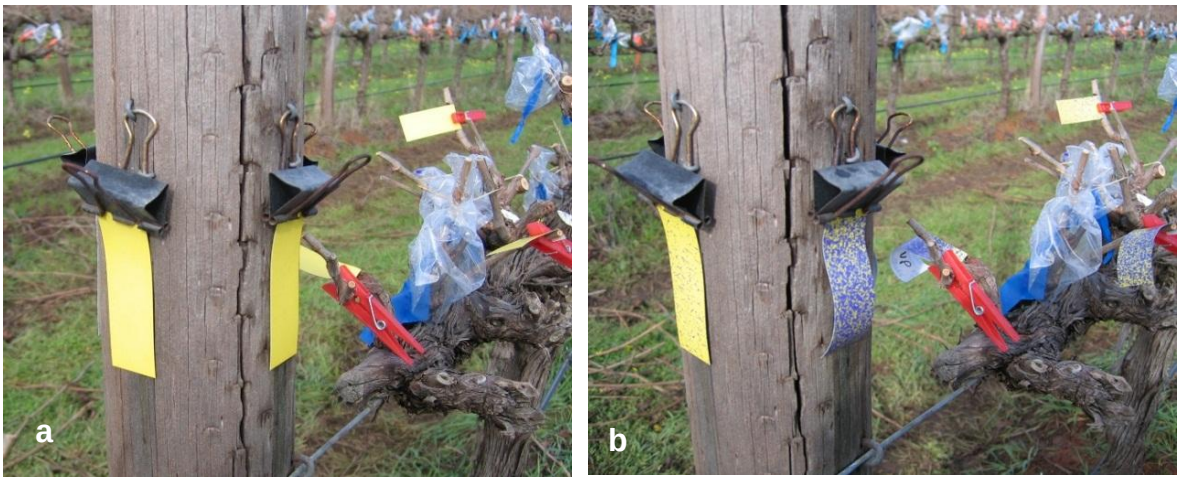


Figure 13. Water-sensitive paper cards placed at four fixed positions on a post and six random positions within the pruning wound zone of vines before (a) and after (b) a pass is made by a sprayer.

Efficacy Assessment

Canes were processed in the laboratory as described in Section 5.2. Efficacy was based on the mean percent recovery (MPR) of *E. lata* from the treated canes by isolation on PDA. Data are presented as mean percent disease control which was calculated as the reduction in MPR as a proportion of the inoculated control. Mean percent disease control was correlated with mean percent coverage for each spray treatment using Microsoft Excel.

Results

McLaren Vale

In 2010, with water spray volumes of 200, 400 and 600 L/ha, the Greentech sprayer provided 6, 22 and 25% mean coverage, respectively, the Silvan sprayer provided 71, 80 and 85% mean coverage, respectively (Figure 14a), and the Recycle sprayer provided 51, 69 and 83% mean coverage, respectively (Figure 15a).

E. lata was recovered from 69% of the inoculated control wounds and 2% of the naturally infected wounds. Applying Folicur in 200, 400 and 600 L/ha, the Greentech sprayer provided 1, 15 and 20% mean disease control, respectively, the Silvan sprayer provided 40, 48 and 49% mean disease control, respectively and the recycle sprayer provided 44, 80 and 93% mean disease control, respectively (Figure 15b). Folicur provided 94% mean disease control when applied with a paint brush at 3 ml/L.

In 2011, with water spray volumes of 200, 400 and 600 L/ha, the Croplands sprayer provided a mean coverage of 74-75%, 86-96% and 94-95% mean coverage, respectively (Figure 14b), for the two passes of Folicur at 1.5 and 3 ml/L (Figure 15c). The recycle sprayer was used to apply Folicur at 0.6, 1.5 and 3 ml/L using one water spray volume of 200 L/ha, providing 58-59% mean coverage.

E. lata was recovered from 86% of the inoculated control wounds and 5% of the naturally infected wounds. The Croplands sprayer applied spray volumes of 200, 400 and 600 L/ha providing 9, 65 and 61% mean disease control, respectively, when Folicur was applied at 1.5 ml/L and provided 29, 40 and 76% mean disease control, respectively, when Folicur was applied at 3 ml/L (Figure 15d). The recycle sprayer, when used at a water spray volume of 200 L/ha to apply Folicur at 0.6, 1.5 and 3 ml/L, provided 44, 61 and 67% mean disease control, respectively. When applied with a paint brush at 3 ml/L, Folicur provided 94% mean disease control.

Coonawarra

In 2010, with water spray volumes of 200, 400 and 600 L/ha, the Hardi sprayer provided 72, 79 and 92% mean coverage, respectively, the Greentech sprayer provided 31, 64 and 62% mean coverage (Figure 16a), respectively, and the Silvan sprayer provided 68, 84 and 88% mean coverage (Figure 16b), respectively (Figure 17a).

E. lata was recovered from 84% of the inoculated control wounds and 3% of the naturally infected wounds. With Folicur applied in spray volumes of 200, 400 and 600 L/ha, the Hardi sprayer provided 24, 39 and 58% mean disease control, respectively, the Greentech sprayer provided 22, 34 and 16% mean disease control, respectively, and the Silvan sprayer provided 35, 45 and 47% mean disease control, respectively (Figure 17b). When applied with a paint brush at 3 ml/L, Folicur provided 89% mean disease control.

In 2011, with water spray volumes of 200, 400 and 600 L/ha, the Greentech sprayer provided 43, 63 and 75% mean coverage, respectively, and the Hardi sprayer provided 82, 91 and 87% mean coverage (Figure 16c), respectively (Figure 17c). The FMR recycle sprayer used to apply Folicur at 0.6, 1.5 and 3 ml/L at one water spray volume of 960 L/ha, providing 88-90% mean coverage.

E. lata was recovered from 51% of the inoculated control wounds and 12% of the naturally infected wounds. With water spray volumes of 200, 400 and 600 L/ha, the Greentech sprayer provided 50, 58 and 30% mean disease control, respectively, and the Hardi sprayer provided 47, 29 and 62% mean disease control (Figure 17d). The FMR sprayer, when used at a water spray volume of 960 L/ha to apply Folicur at 0.6, 1.5 and 3 ml/L, provided 54, 55 and 80% mean disease control, respectively. When applied with a paint brush at 3 ml/L, Folicur provided 75% mean disease control.

Adelaide Hills

In 2010, with water spray volumes of 200, 400 and 600 L/ha, the Croplands sprayer provided a mean coverage of 23-32%, 58% and 75-81% mean coverage, respectively, for the two passes of Folicur at 1.5 and 3 ml/L, and the home-engineered cordon sprayer provided 53, 71 and 83% mean coverage (Figure 14c), respectively (Figure 18a).

E. lata was recovered from 96% of the inoculated control wounds and 15% of the naturally infected wounds. With water spray volumes of 200, 400 and 600 L/ha, the Croplands sprayer provided 15, 16 and 22% mean disease control, respectively, when used to apply Folicur at 1.5 ml/L, and provided 19, 30 and 39% mean disease control, respectively, when used to apply Folicur at 3 ml/L (Figure 18b). The cordon sprayer provided 33, 56 and 65% mean disease control, when used to apply Folicur at 3 ml/L using water spray volumes of 200, 400 and 600 L/ha, respectively. When applied with a paint brush at 3 ml/L, Folicur provided 63% mean disease control.

In 2011, with water spray volumes of 200, 400 and 600 L/ha, the Croplands sprayer provided 66, 86 and 90% mean coverage, respectively. The recycle sprayer applied Folicur at 0.6, 1.5 and 3 ml/L using one water spray volume of 400 L/ha, providing 88, 92 and 88% mean coverage, respectively (Figure 18c). The home-engineered cordon sprayer provided 83, 91 and 90% mean coverage, respectively.

E. lata was recovered from 60% of the inoculated control wounds and 13% of the naturally infected wounds. With water spray volumes of 200, 400 and 600 L/ha, the Croplands sprayer provided 16, 57 and 43% mean disease control, respectively, and the cordon sprayer provided 71, 60 and 83% mean disease control, respectively (Figure 18d). The recycle sprayer, using a water spray volume of 400 L/ha to apply Folicur at 0.6, 1.5 and 3 ml/L, provided 56, 56 and 77% mean disease control, respectively. When applied with a paint brush at 3 ml/L, Folicur provided 76% mean disease control.

Data for all trials were plotted on a regression graph (Figure 19). It revealed a correlation ($R^2 = 0.5$) between disease control and spray coverage, revealing that the greater the coverage achieved by sprayers, the better the disease control that was achieved.

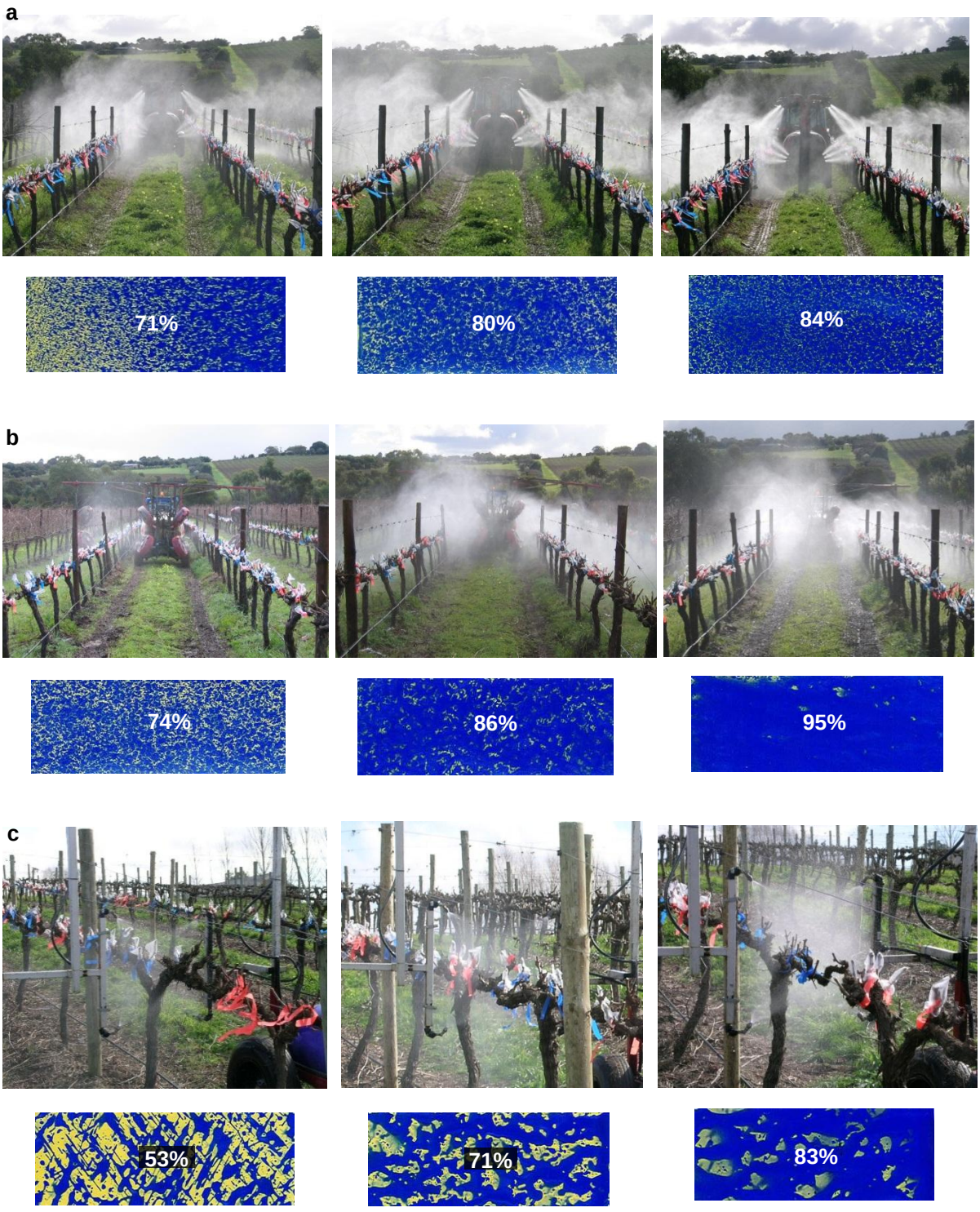


Figure 14. Spray mist produced from sprayers operating at 200, 400 and 600 L/ha (left to right). a) Silvan Turbomiser, single-row, air-shear sprayer in McLaren Vale, 2010, b) Croplands, Quantum Mist, double-row, fan sprayer in McLaren Vale, 2011 and c) home-engineered, cordon sprayer in Adelaide Hills, 2010. Below each image is a representative water sensitive paper with mean spray coverage as calculated by Image J.

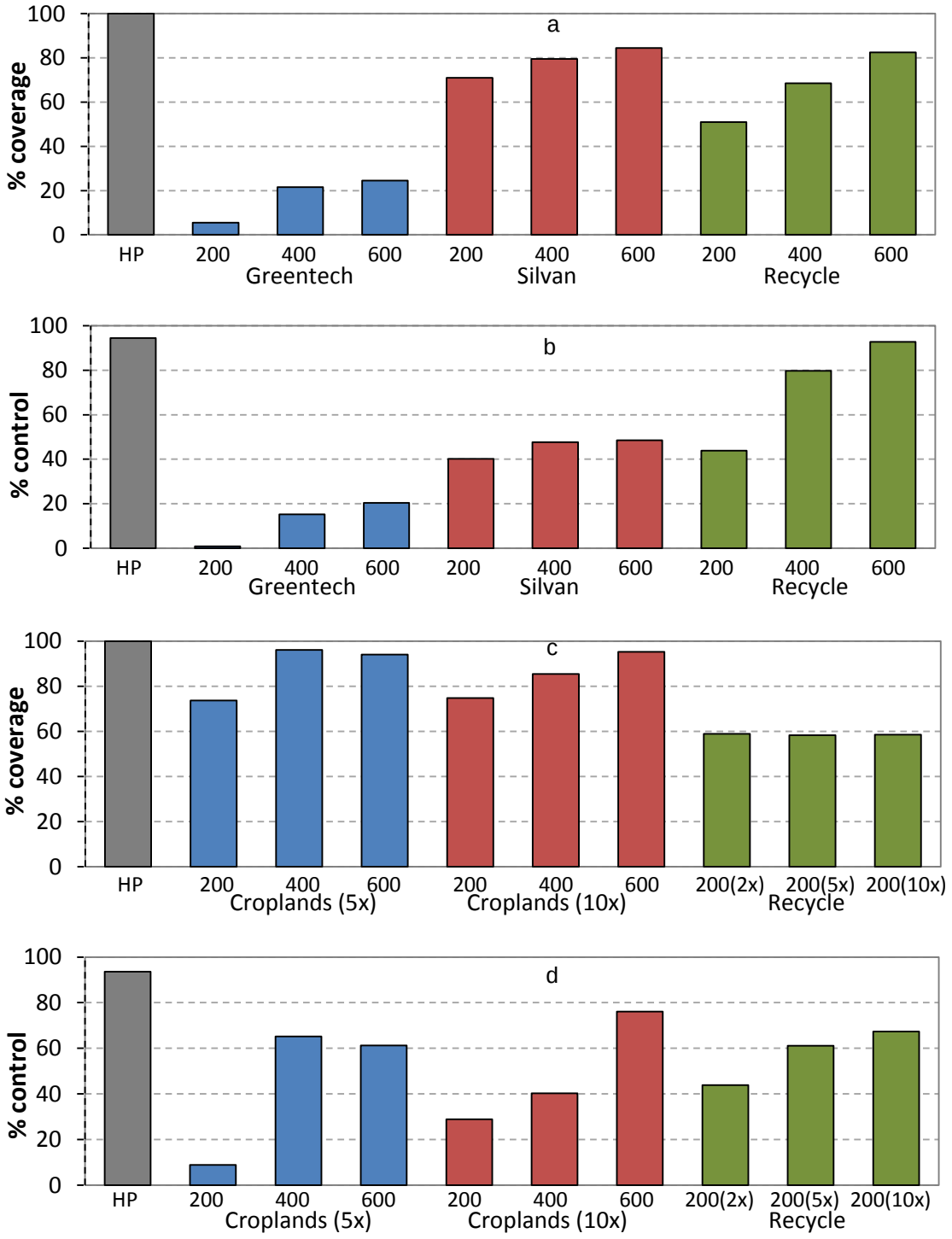


Figure 15. Spray coverage and disease control in spray trials conducted in July 2010 (a&b) and 2011 (b&c) in McLaren Vale on Shiraz grapevines using 4 sprayers; Greentech fan (Greentech), Silvan Turbomiser air-shear (Silvan) Croplands Quantum Mist fan (Croplands) and a home-engineered recycle sprayer (Recycle). Each sprayer was used to apply Folicur at 3 ml/L in 2010 and at 0.6, 1.5 and 3 ml/L in 2011 at water spray volumes of 200, 400 and 600 L/ha. Folicur was also hand-painted (HP) at 3 ml/L for comparison. Recovery of *E. lata* from the positive (inoculated) control was 69% (2010) and 86% (2011).

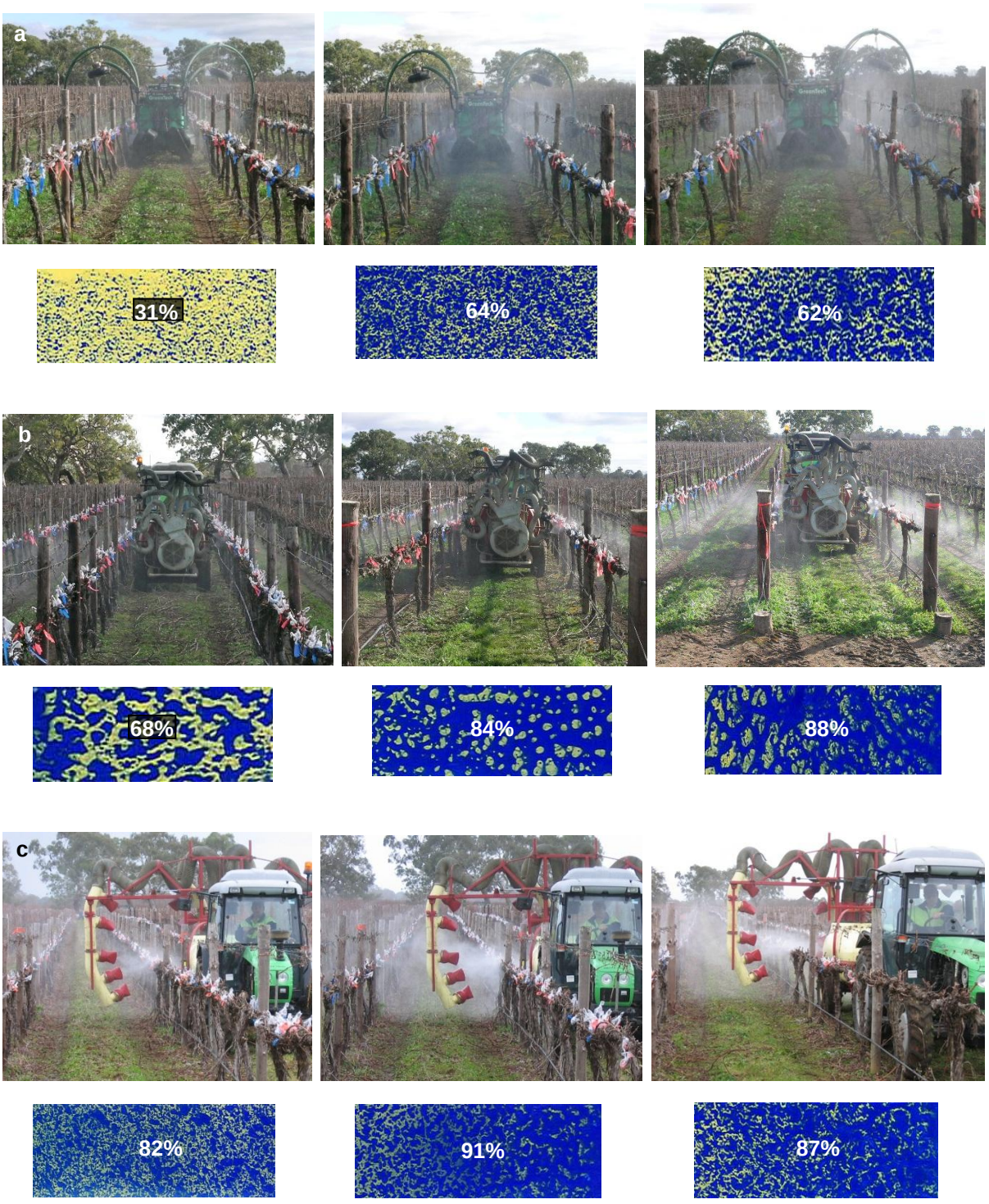


Figure 16. Spray mist produced from sprayers operating at 200, 400 and 600 L/ha (left to right) in Coonawarra. a) Greentech, double-row, fan sprayer in 2010, b) Silvan Turbomiser, single row, air-shear sprayer in 2010 and c) Hardi, ducted, double-row, air-assisted sprayer in 2011. Below each image is a representative water sensitive paper with mean spray coverage as calculated by Image J.

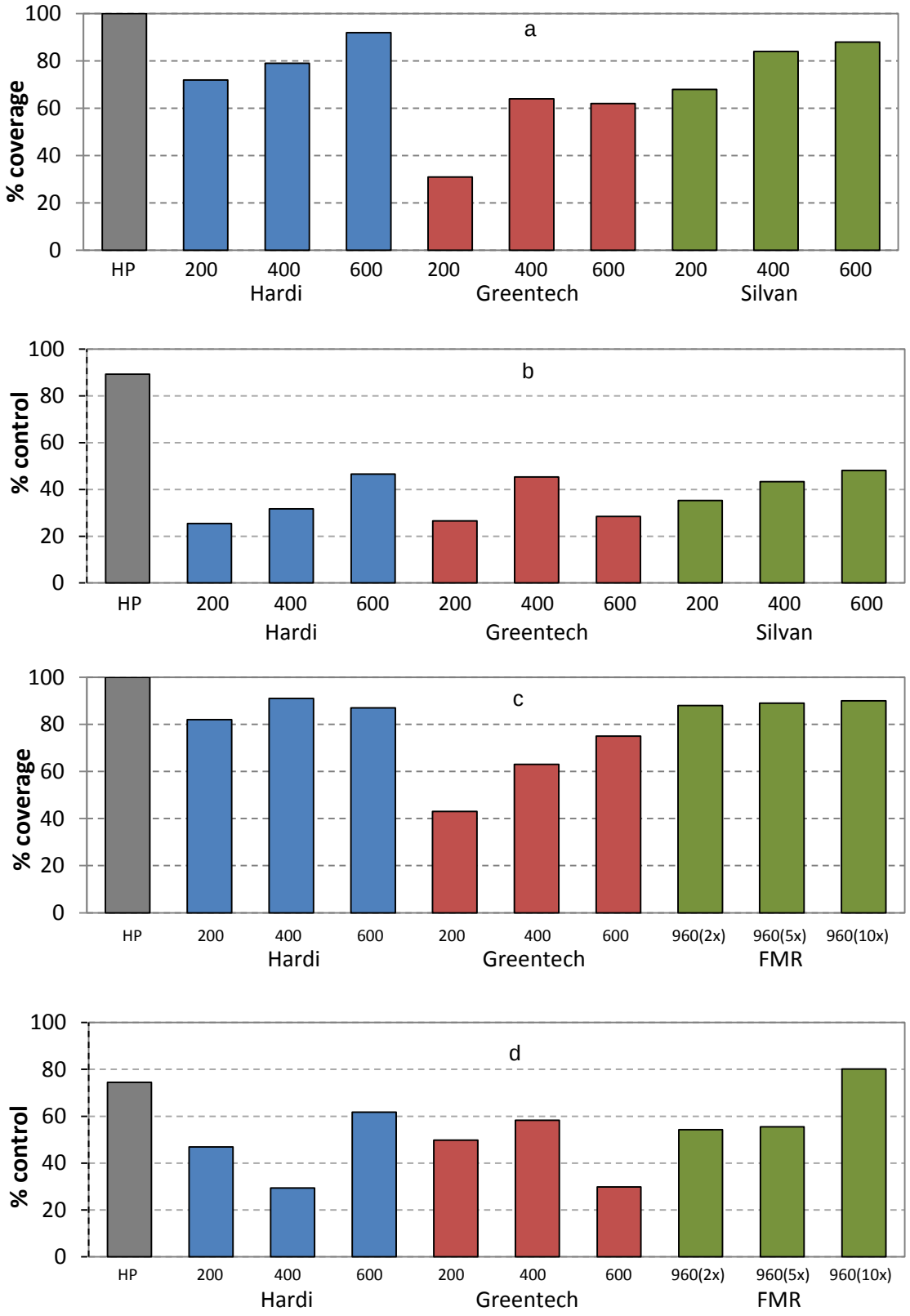


Figure 17. Spray coverage and disease control in spray trials conducted in July 2010 (a&b) and 2011 (b&c) in Coonawarra on Cabernet Sauvignon grapevines using 4 sprayers; Hardi ducted, air-assisted (Hardi), a Greentech fan (Greentech), a Silvan ducted, air-shear (Silvan) and an FMR Spray-Pro recycle sprayer (FMR). Each sprayer was used to apply Folicur at 3 ml/L at water spray volumes of 200, 400 and 600 L/ha, except the FMR, which was used to apply Folicur at 0.6, 1.5 and 3 ml/L using a water spray volume of 960 L/ha. Folicur was also hand-painted (HP) at 3 ml/L for comparison. Recovery of *E. lata* from the positive (inoculated) control was 84% (2010) and 51% (2011).

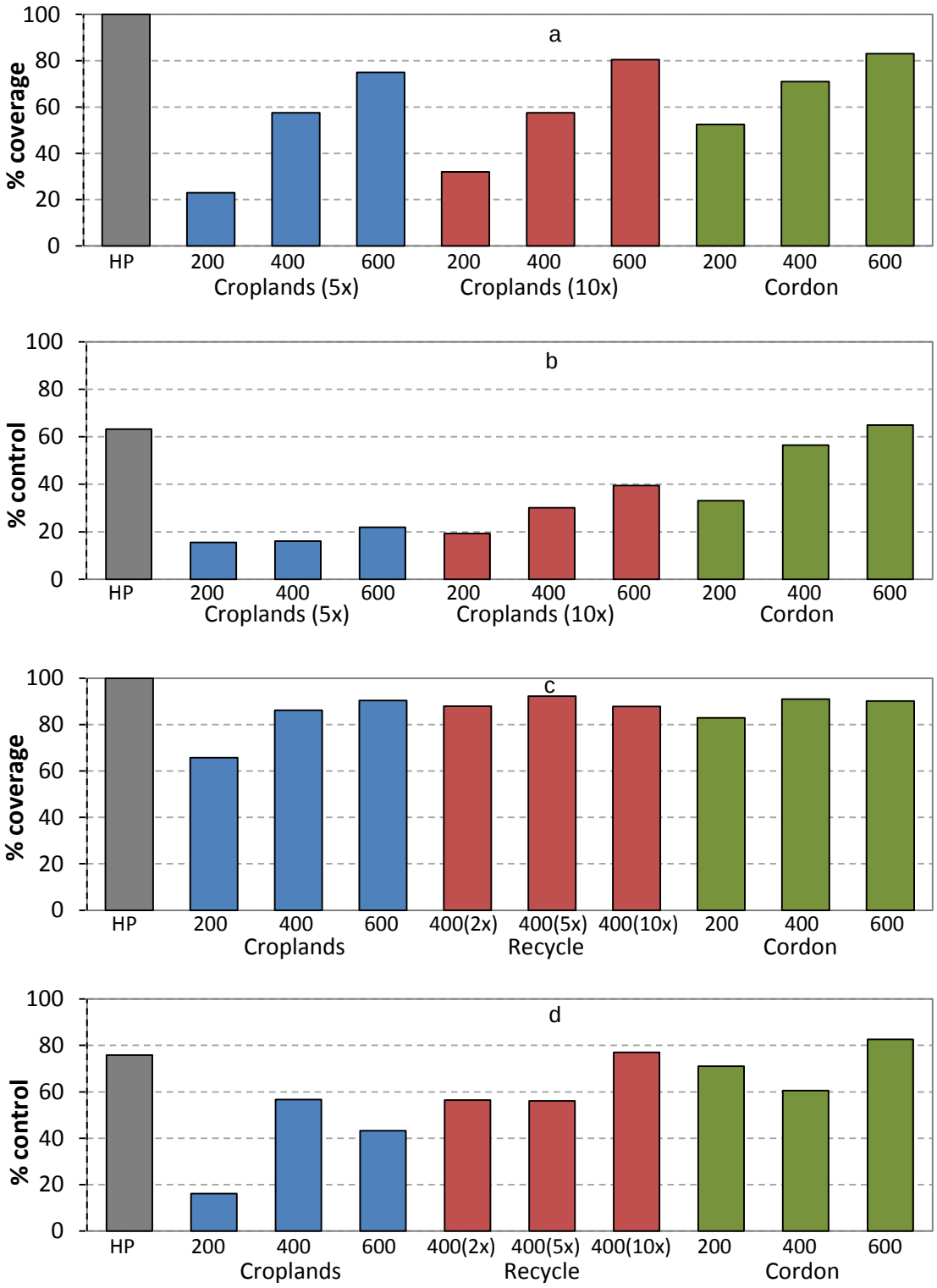


Figure 18. Spray coverage and disease control in spray trials conducted in July 2010 (a&b) and 2011 (b&c) in the Adelaide Hills on Tempranillo grapevines using 3 sprayers; Croplands Quantum Mist fan (Croplands), a home-engineered cordon sprayer (Cordon) and a home-engineered recycle sprayer (Recycle). The Croplands sprayer was used to apply Folicur at 1.5 and 3 ml/L, and the cordon sprayer was used to apply Folicur at 3 ml/L, each using water spray volumes of 200, 400 and 600 L/ha. The recycle sprayer was used to apply Folicur at 0.6, 1.5 and 3 ml/L using a water spray volume of 400 L/ha. Folicur was also hand-painted (HP) at 3 ml/L for comparison. Recovery of *E. lata* from the positive (inoculated) control was 96% (2010) and 60% (2011).

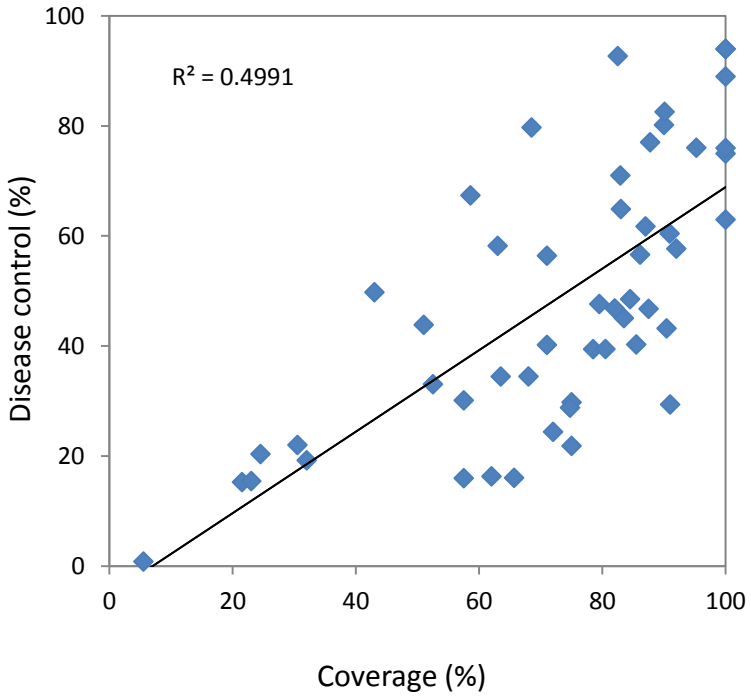


Figure 19. Correlation of spray coverage and disease control. Combined data from all trials in 2010 and 2011.

Discussion

The most effective treatments were achieved with recycle sprayers at each location and the cordon sprayer in Adelaide Hills, with spray water volumes of 600 L/ha or above. In each case disease control was equivalent to that of application by hand with a paint brush. The home-engineered cordon sprayer was fitted with four herbicide nozzles which produced large droplets, reducing drift and ensuring wounds were drenched. At the lowest chemical rate tested (0.6 ml/L), the FMR recycle sprayer with water spray volume of 960 L/ha, achieved 54% disease control. The recycle sprayers are suitable for application of high water spray volumes, not only providing good spray coverage and wound protection but can also reduce spray drift and loss of chemical to non-target areas and therefore allow for more efficient coverage of pruning wounds.

Although the fan sprayers varied greatly in their ability to apply pruning wound treatments for control of *E. lata*, up to 76% disease control was achieved with a Croplands sprayer at 600 L/ha. In this case the double-row set up provided two fans closely positioned either side of the cordon compared with the single-sided or greater distance of fans from cordons in other sprayers. Fan sprayers are designed for penetrating canopies during summer spray programs, making them less suitable for applying fungicides to dormant vines than other systems. Nevertheless, adjusting the position of fans may improve their effectiveness for applying pruning wound protection.

The Hardi air-assisted sprayer provided up to 62% disease control in Coonawarra, when using a water spray volume of 600 L/ha. This sprayer was adjusted by turning off unnecessary nozzles and adjusting active nozzle angles for optimal coverage.

The Sylvan air-shear sprayers provided up to 49% disease control at 600 L/ha in McLaren Vale and Coonawarra. The high pressure and small droplets produced by these sprayers led to more spray drift and less effective coverage of wounds.

In general, for spray treatments repeated over both years, disease control was greater in the second year of trials, with recovery of *E. lata* from inoculated controls greater in the first. For example, in the Adelaide Hills, the home-engineered cordon sprayer provided 33-65% disease control in 2010 when *E. lata* was recovered from 96% of inoculated controls compared with 60-83% disease control in 2011, when there was 60% infection in inoculated controls. In Coonawarra, the Hardi ducted, air-assisted

sprayer provided 25-47% disease control in 2010 when *E. lata* was recovered from 84% of inoculated controls compared with 29-62% disease control in 2011, when there was 51% infection in inoculated controls. Given that natural infection in all trials ranged from 2-15%, disease control is expected to be much greater for all treatments under normal conditions.

The correlation between spray coverage and disease control suggests that most sprayers may be able to provide good control of eutypa dieback, provided the sprayer is adjusted to maximise the spray coverage of pruning wounds. This can be achieved by re-directing the fans and nozzles, turning off unnecessary nozzles, increasing droplet size by reducing pressure or changing nozzles (Landers 2010).

8 INVOLVEMENT OF DIATRYPACEOUS SPECIES IN EUTYPA DIEBACK

8.1 Pathogenicity of diatrypaceous fungi

Introduction

Eutypa lata was originally described from apricot in South Australia and thereafter as a pathogen of grapevines, causing dead-arm disease, now known as eutypa dieback. The pathogen is a member of the Diatrypaceae family, and is spread by airborne spores, which infects fresh pruning wounds, destroys vascular tissue leading to formation of wedge shaped cankers in cross-section of cordons and trunks, and eventually kills the vine. Foliar symptoms that include stunting of shoots and necrosis and cupping of leaves are caused by phytotoxins produced by the fungus (Moller and Kasimatis 1978).

While *E. lata* was considered to be the main cause of eutypa dieback, both in the United States and Australia, recent surveys have shown that a number of other species in the Diatrypaceae family infect grapevines (Mostert *et al*. 2004; Trouillas and Gubler 2004; Trouillas *et al*. 2010). In Australia, Trouillas *et al*. (2011) reported six species within the Diatrypaceae both from grapevines and other woody hosts, with a further five species found only on other hosts such as poplar, ash and acacia. These included species of *Cryptovalsa, Cryptosphaeria, Diatrype, Diatrypella, Eutypa* and *Eutypella*.

There is a need to determine the pathogenicity to grapevines of the diatrypaceous species collected from grapevines and other hosts around vineyards throughout Australia, and determine how the virulence of these species compares with that of *E. lata*. This information will be used to ensure management strategies recommended for eutypa dieback are effective for all causal fungal species. This study was a collaborative effort between SARDI, University of California Davis and the National Wine and Grape Industry Centre.

Methods

Seventy fungal strains comprising nine species within the Diatrypaceae, collected from grapevines and other hosts including ornamental trees throughout Australia, were used in this study. They included *Cryptovalsa ampelina, Cryptovalsa rabenhorstii, Diatrypella vulgaris, Eutypa lata, Eutypa leptoplaca, Eutypella citricola, Eutypella microtheca* and unidentified species of *Diatrype* and *Cryptosphaeria*.

In Trial 1, conducted at the Waite Campus, South Australia, one-year old Cabernet Sauvignon rooted cuttings were inoculated with cultures of each fungal species. Using a sterile drill bit, a wound (one per cutting) ~5 mm in depth was created in the middle of the main stem of healthy grapevine rootlings. Agar plugs (5 mm in diameter) were removed from the margins of actively growing cultures using a sterile cork borer and one disc inserted into each wound (Figure 20a), which was then sealed with petroleum jelly and wrapped with Parafilm (Figure 20b). Sterile agar plugs were used as controls.

Nine months after inoculation, rootlings were removed from the soil, bark was removed from stems and extent of staining or discoloration from the point of inoculation was measured. To determine the extent to which diatrypaceous fungi colonised wood, rootlings were then surface sterilised in 2.5% sodium hypochlorite for 10 minutes and rinsed in sterile distilled water. Wood pieces (~2 mm discs) were taken at 10 mm intervals up to 100 mm above and below the inoculation point and transferred to agar. Following 7-10 days of incubation at 23ºC under fluorescent light, cultures were identified using colony morphology, and the distances at which fungi were reisolated, above and below the point of inoculation, recorded.

In Trial 2, conducted at National Wine and Grape Industry Centre in Wagga Wagga, one-year old cuttings of Cabernet Sauvignon were similarly inoculated with a range of diatrypaceous fungi. Eighteen months after inoculation, rootlings were removed from the soil and the total lesion length and colonisation distance in wood were assessed for each fungal strain as described above.

In all trials, rootlings were arranged in a completely randomised design. Analysis of variance (ANOVA) was used to compare differences in mean lesion lengths and colonisation distances among species, with means separated using Tukey's test or Least Significant Difference (P=0.05).

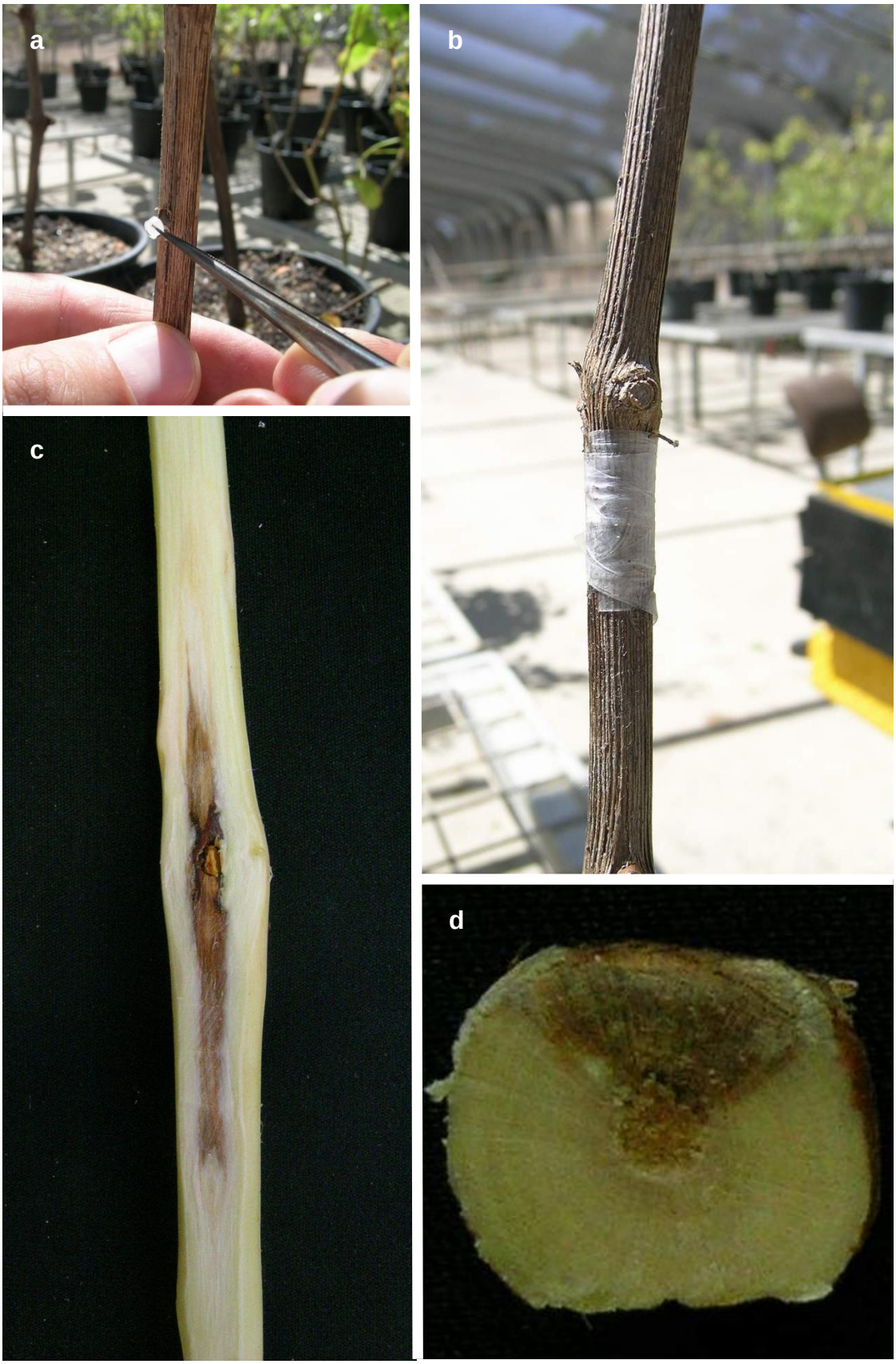


Figure 20. a) Inoculation of Cabernet Sauvignon grapevines with an agar plug of fungal mycelium, b) wound wrapped with parafilm, c) lesion development on main stem, and d) lesion development in cross section.

Results

Inoculations of 1-year-old Cabernet Sauvignon grapevines with diatrypaceous fungi were seldom accompanied by foliar symptoms. Only three vines inoculated with *E. lata* displayed foliar symptoms characteristic of eutypa dieback. However, around the inoculation point, stained or discoloured wood (Figure 20 c&d), developed after 9 months. In trial 1, the lengths of lesions caused by the various fungi differed significantly between species. Mean lesion lengths ranged from 16 mm for *E. leptoplaca* to 24 mm *for E. lata* (Figure 21a). In trial 2, additional strains and species were tested, but results were similar, with significant differences again among strains and species. Mean lesion lengths ranged from 10 mm for *Cryptosphaeria* sp. to 36 mm for *E. lata* (Figure 21b). In both trials, *E. lata* produced the longest lesions, while those produced by *Cryptosphaeria* sp. were the smallest.

Diatrypaceous fungi also colonised grapevines, with strains being reisolated and identified from lesions. The extent of colonisation varied between species. Mean colonisation distances ranged from 16 mm for *Cryptosphaeria* sp. to 53 mm for *E. lata* and *E. microtheca* (Figure 21a & b). In both trials *E. lata* colonised wood to the greatest extent, although *E. microtheca* behaved similarly in trial 1. *Eutypella citricola, C. ampelina* and *E. leptoplaca* also developed extensive lesions. *Cryptosphaeria* sp. was least able to colonise wood. In many cases diatrypaceous fungi were reisolated from asymptomatic wood in advance of lesions. Colonisation of wood by *E. lata* and *E. microtheca* exceeded lesion length by 29 mm, while *E. leptoplaca, E. citricola*, and *C. ampelina* advanced to 15 mm beyond discoloured wood (Figure 21a & b). However, some individual strains colonised wood to a much greater extent, with a number being reisolated more than 40 mm, and one strain 76 mm, in advance of disease symptoms.

Discussion

In this study, none of the diatrypaceous species isolated from grape vines and other hosts in and around Australian vineyards, with the exception of *E. lata*, produced foliar symptoms characteristic of eutypa dieback.

In the two pathogenicity trials, all nine species of fungi tested produced necrotic lesions on 1-year-old potted Cabernet Sauvignon grapevines. However, no other species were more pathogenic than *E. lata.* All species tested were reisolated from grapevines 9 or 18-months after inoculation and 85% of strains were reisolated from grapevine wood up to 76 mm in advance of lesions.

This has important ramifications for the management of these species. Remedial surgery, which involves pruning out all infected material, has been the method of choice for managing eutypa dieback and other grapevine trunk diseases such as botryosphaeria dieback (Sosnowski *et al*. 2011; Úrbez-Torres 2011). However, failure to remove all infected material has been shown to result in recurrence of symptoms, such that for eutypa dieback, the recommended approach to managing the disease now advocates the removal of all diseased material as well as an additional 10 cm of healthy tissue beyond the margin of visible lesions (Sosnowski *et al*. 2007b). The results of the current study suggest that this recommendation will also be sufficient for the management of the other diatrypaceous species that may infect pruning wounds.

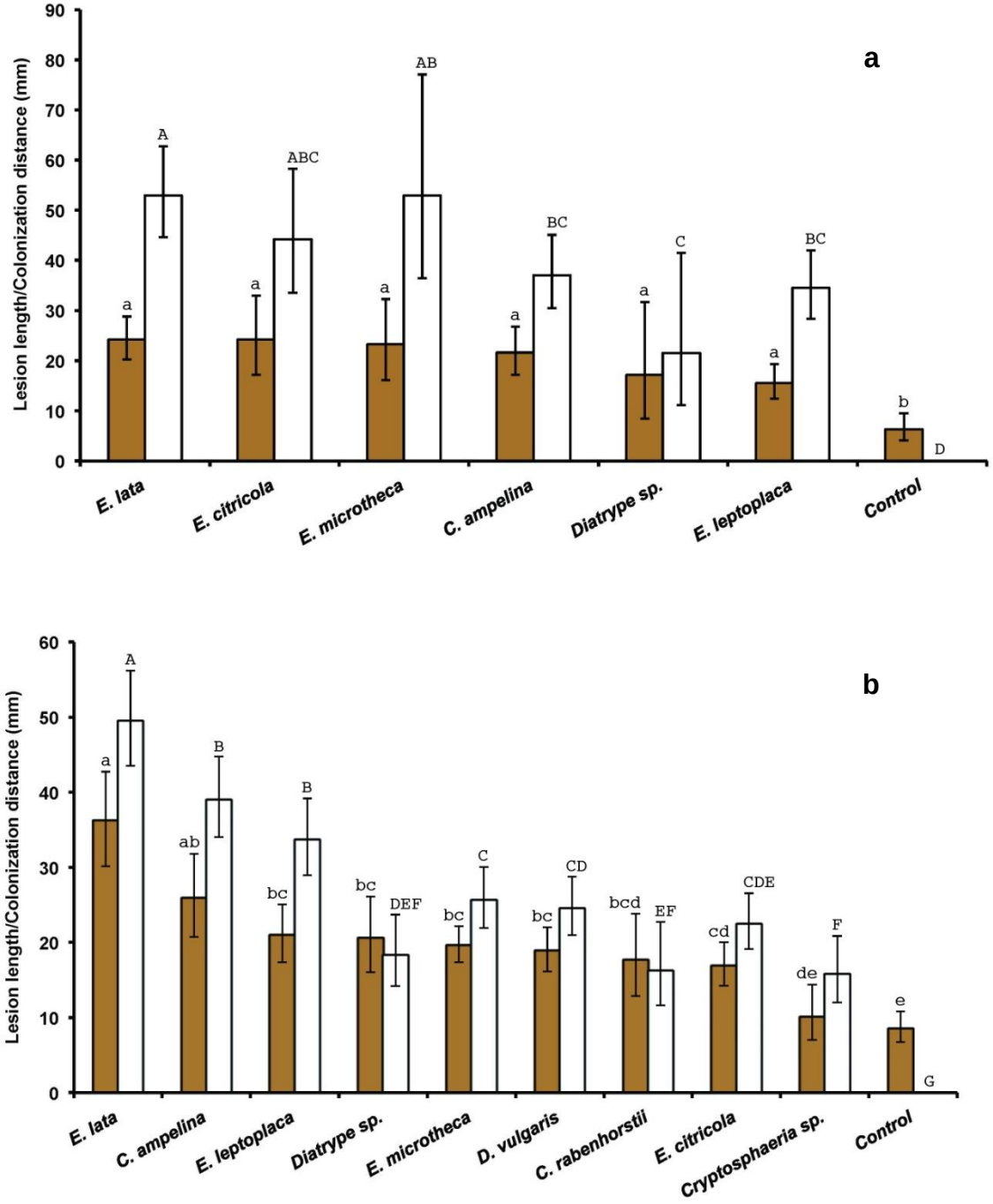


Figure 21. Mean lesion lengths (brown bars) and colonisation distances (white bar) of diatrypaceous fungi on Cabernet Sauvignon grapevines, a) nine (trial 1) and b) 18-months (trial 2) after inoculation. Means followed by the same lowercase or uppercase letters are not significantly different. Bars represent the 95% confidence interval of the mean.

8.2 Evaluation of fungicides for diatrypaceous species

Introduction

Recent studies have demonstrated that several fungi in the Diatrypaceae family, other than *Eutypa lata*, can be isolated from cankers associated with grapevine dieback (Pitt et al. 2010; Trouillas et al. 2010, 2011, see section 8.1).

To date, most eutypa dieback management strategies have been developed by evaluating their potential to control *E. lata* (Section 5). Therefore, there is a need to determine if fungicides that are effective at controlling *E. lata* are also effective for the control of associated diatrypaceous fungal species. The aim of this study was to determine *in vitro* the efficacy of a range of fungicides with different modes of action on the mycelial growth of members of the Diatrypaceae. This study was a collaborative effort between SARDI, the Polytechnic University of Valencia and the University of California Davis.

Methods

Isolates of six diatrypaceous fungi were obtained from cankers in infected grapevine spurs, cordons or trunks and from fruiting bodies on dead wood of grapevines in various wine regions of Australia (Sosnowski et al. 2007b, Trouillas et al. 2011). Commercial formulations of six fungicides, representing five chemical groups, were evaluated for inhibition of the mycelial growth of fungi. The fungicides were: Scala (pyrimethanil 400 g/L), Spin Flo (carbendazim 500 g/L), Shirlan (fluazinam 500 g/L), Prosaro (prothioconazole + tebuconazole 210 g/L+ 210 g/L), Folicur (tebuconazole 430 g/L) and Cabrio (pyraclostrobin 250 g/L).

Each fungicide was diluted in SDW, and aliquots added to PDA to give active ingredient concentrations of 1 and 10 ppm, based on methods of Sosnowski et al. (2008). Control PDA plates were prepared without the addition of fungicide or SDW. Mycelial plugs (5 mm in diameter), obtained from the margins of actively growing fungal cultures, were transferred to fungicide amended and control plates. The plates were incubated at 25°C under fluorescent light for 12 h each day for 9 days. Colony diameter was assessed by calculating the mean diameter from two perpendicular measurements and then subtracting 5 mm from each value to account for the original plug. There were four replicates of each fungicide concentration, and the experiment was repeated.

Results

As mycelial growth in the two experiments was similar between isolates of each species, the data were combined. The effect of treatments *in vitro* on mycelial growth of diatrypaceous fungi is shown in Figure 22 &Figure 23.

Mean colony diameter of *E. lata* mycelium on control plates was 59 mm after 9 days incubation. At 10 ppm, all fungicides prevented mycelial growth of *E. lata,* except pyrimethanil, pyraclostrobin and fluazinam which reduced colony diameter to less than 30 mm. At 1 ppm, carbendazim completely prevented mycelial growth, and all other fungicides, except for pyrimethanil, significantly reduced growth.

For *C. ampelina*, mean colony diameter of mycelium on control plates was 56 mm. Tebuconazole and prothioconazole + tebuconazole completely prevented mycelial growth at both concentrations. Fluazinam and carbendazim reduced colony diameter to less than 12 mm, while pyraclostrobin was similarly effective at both concentrations, reducing colony diameter to <30 mm.

Colony diameter of *D. vulgaris* mycelium on control plates was 77 mm. Prothioconazole + tebuconazole and fluazinam reduced colony diameter to less than 10 mm at both concentrations. Pyraclostrobin reduced colony diameter to less than 25 mm at both concentrations but tebuconazole and carbendazim only reduced growth significantly at 10 ppm.

With *E. leptoplaca*, mycelium growth on control plates was 43 mm. All fungicides except for pyrimethanil reduced colony diameter to less than 6 mm at both concentrations.

For *E. citricola*, colony diameter on control plates was 77 mm. tebuconazole, prothioconazole + tebuconazole, fluazinam and carbendazim reduced colony growth at 10 ppm to less than 1 mm. At 1 ppm, fluazinam and carbendazim reduced growth to less than 5 mm and all other fungicides reduced growth to a lesser extent.

Colony diameter of *E. microtheca* on control plates was 72 mm. Tebuconazole, prothioconazole + tebuconazole, fluazinam and carbendazim reduced colony diameter to less than 10 mm at both concentrations, whereas pyrimethanil and pyraclostrobin were ineffective.

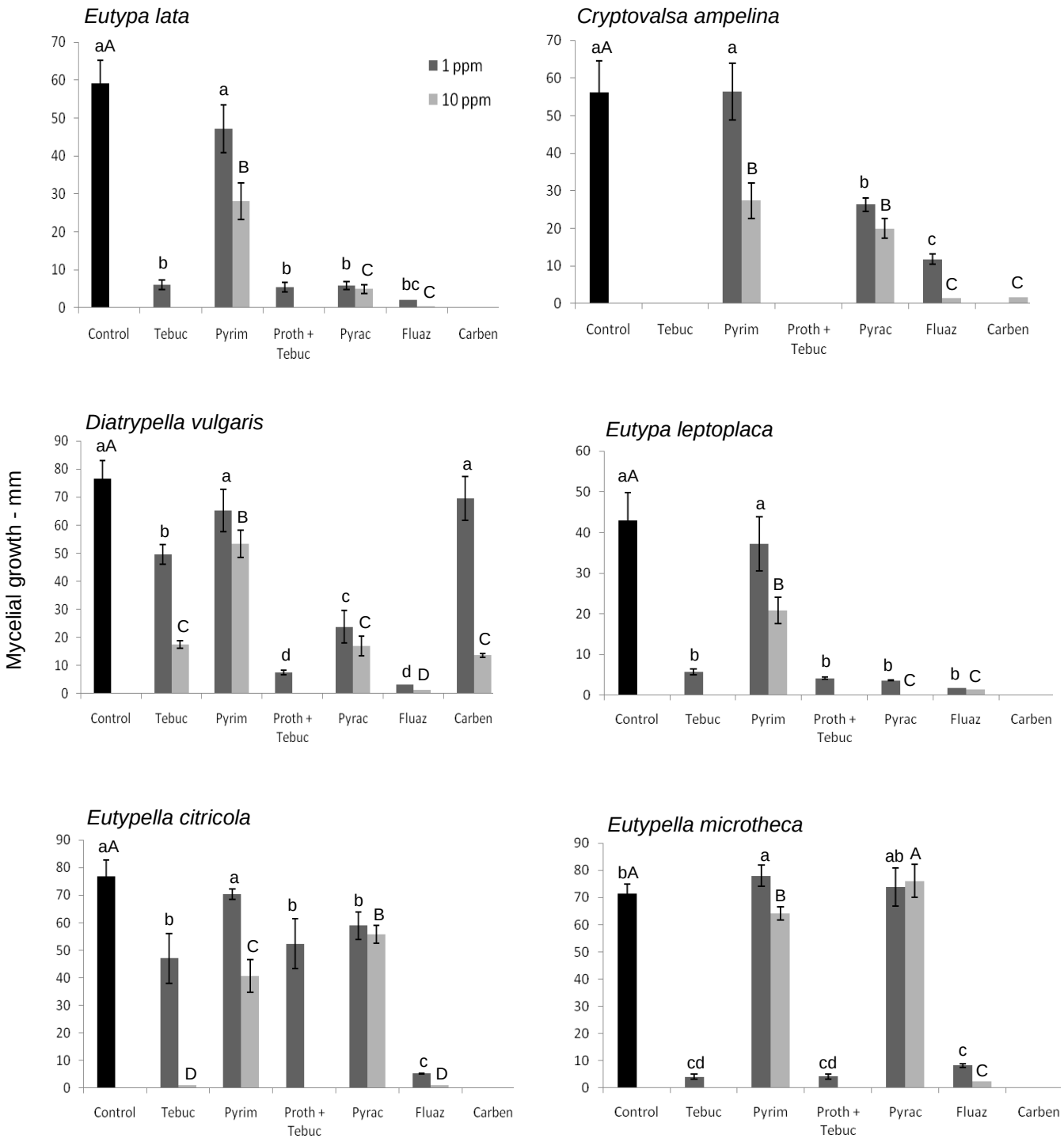


Figure 22. Effect of treatments *in vitro* on mycelial growth of Diatrypaceous species at 1 and 10 ppm active ingredient. Bars with a different letter are significantly different, indicated in lower case for 1 ppm and upper case for 10 ppm. Abbreviations: tebuc, tebuconazole; pyrim, pyrimethanil; proth + tebuc, prothioconazole + tebuconazole; pyrac, pyraclostrobin; fluaz, fluazinam; carben, carbendazim.

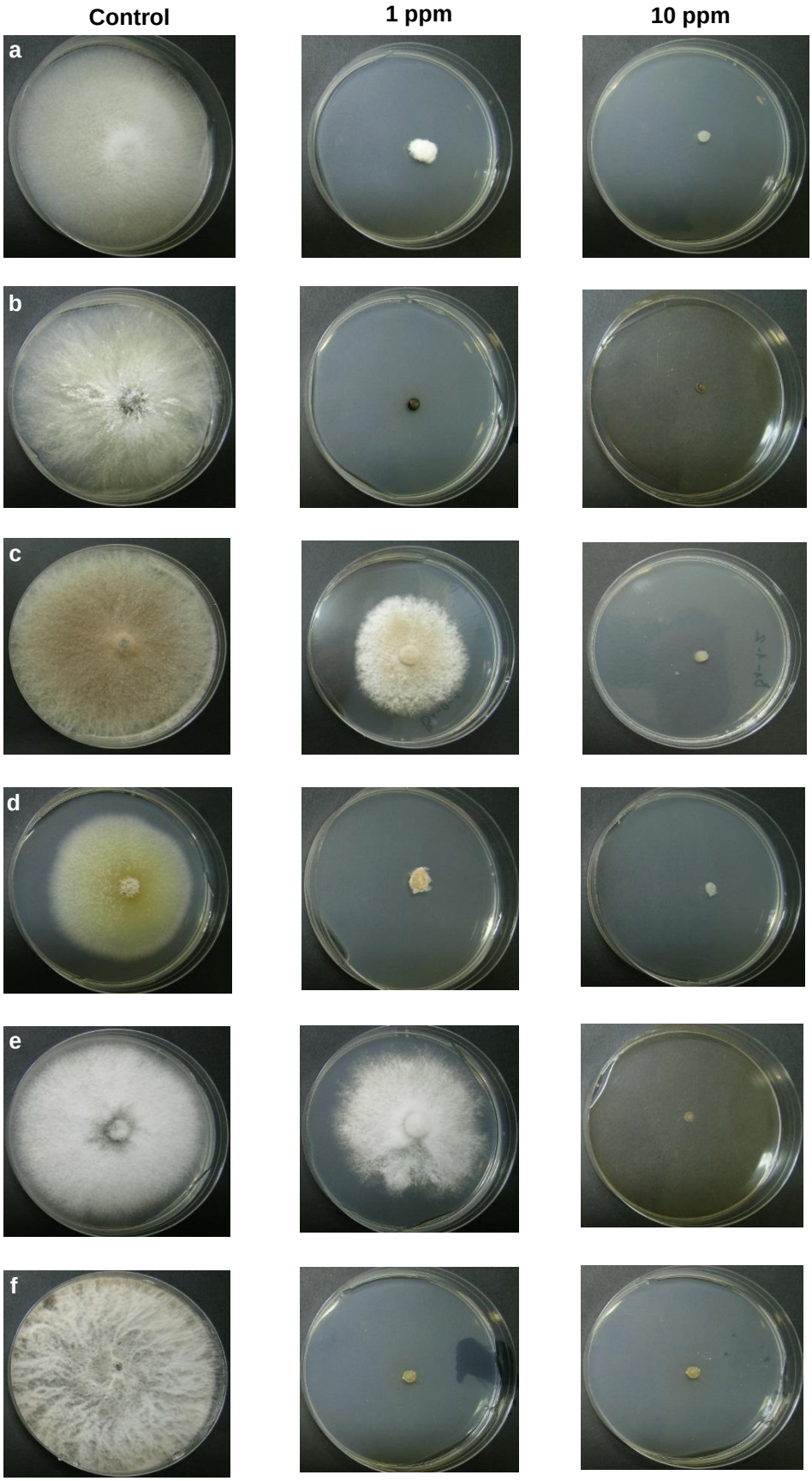


Figure 23. Mycelial growth of six Diatrypaceous fungi, a) *Eutypa lata*, b) *Cryptovalsa ampelina*, c) *Diatrypella vulgaris*, d) *Eutypa leptoplaca*, e) *Eutypella citricola* and f) *Eutypella microtheca* on unamended controls and on agar amended with tebuconazole (Folicur) at 1 and 10 ppm.

Discussion

This study represents the first approach for *in vitro* fungicide evaluation against mycelial growth of diatrypaceous fungi, other than *E. lata*. Of the products tested, four are currently registered in Australia for control of other grapevine pathogens, but carbendazim (Spin Flo) was prohibited for use on grapevines in 2010 due to occupational health and safety concerns and tebuconazole + prothioconazole (Prosaro) is not currently registered for use on grapes. Manufacturers are more likely to proceed with a label extension of a product, which is less expensive and can occur more quickly, than registration of a new chemical for use on grapevines.

Fluazinam (Shirlan) was the most effective fungicide, significantly reducing mycelial growth of all fungi. The demethylation inhibitor fungicides (DMI), tebuconazole (Folicur) and tebuconazole + prothioconazole also reduced mycelial growth, especially at the higher concentration. These results agree with other *in vitro* studies that reported high efficacy of DMI fungicides such as flusilazole, imazalil, penconazole, tebuconazole and tetraconazole on ascospore germination and mycelial growth of *E. lata* (Munkvold and Marois 1993; Halleen et al. 2001; Loschiavo et al. 2007; Sosnowski et al. 2008).

Mycelial growth of all fungal species was almost completely inhibited by carbendazim, except for *D. vulgaris* at the minimum chemical concentration in this study. In similar *in vitro* experiments, Loschiavo et al. (2007) demonstrated that carbendazim significantly reduced mycelial growth of *E. lata*.

Pyraclostrobin (Cabrio) reduced colony diameter of most of the fungal species by 50% or more, except for species of the genus *Eutypella*. This fungicide completely inhibited ascospore germination and reduced mycelial growth of *E. lata* by 50%, although other strobilurins such as azoxystrobin and trifloxystrobin exhibited poor efficacy *in vitro* (Sosnowski et al. 2008). Similar results were obtained by Halleen et al. (2010), who reported the ineffectiveness of azoxystrobin, kresoxim-methyl and trifloxyatrobin in reducing mycelial growth of *E. lata in vitro*.

Pyrimethanil (Scala) was ineffective at reducing mycelial growth. although some of the species tested showed a reduction of colony diameter by 50% when exposed to the highest concentration. These findings are in agreement with those obtained by Sosnowski et al. (2008) and Halleen et al. (2010), who reported this fungicide as ineffective in reducing mycelial growth of *E. lata*.

A number of the fungicides tested in this experiment proved effective against *E. lata* and other Diatrypaceous fungi. These products also demonstrated efficacy in field trials during the course of this project (section 5) for control of pruning wound infection by *E. lata,* suggesting that management strategies recommended for control of eutypa dieback may also prove effective in controlling these related pathogens.

9 RESISTANCE TO GRAPEVINE TRUNK DISEASE

Introduction

There have been limited reports of resistance or tolerance of *Vitis vinifera* cultivars to trunk disease. Carter (1991) cited a report on the resistance or susceptibility to eutypa dieback of cultivars grown in France (Dubos 1987) based on foliar symptoms in the vineyard. Of 32 cultivars assessed, five were categorised as resistant (cvs Aligote, Grolleau, Merlot, Semillon and Sylvaner) and all others listed as moderately to highly susceptible. Based on three surveys conducted in South Australia over the past 40 years (Wicks 1975, Highet and Wicks 1998, Loschiavo *et al* 2007), the cvs Grenache, Cabernet Sauvignon and Shiraz were recorded with the highest incidence of eutypa dieback foliar symptoms and cvs Merlot, Riesling, Pinot Noir, Sauvignon Blanc, Chardonnay and Semillon with the least. The growth of *E. lata* in grapevine wood also varies and cvs Merlot, Gamay, Grenache and Semillon were recorded to have half of the rate of dieback of cvs Cabernet Sauvignon and Shiraz (Sosnowski *et al.* 2006). For botryosphaeria dieback, there is no literature on resistance or cultivar susceptibility. However, observations during studies in North America suggest that many of the commonly grown *V. vinifera* cultivars such as; Chardonnay, Thompson Seedless, Riesling, Cabernet Sauvignon, and the interspecific hybrid cultivars; Chardonnel, Chambourcin, Catawba, Traminette and Niagara are highly susceptible to botryosphaeria dieback (J. Úrbez-Torres, personal communication).

A preliminary assessment of resistance or tolerance of vines in the SARDI germplasm collection in the Barossa Valley and of rootstock genotypes developed by Dr Brady Smith (CSIRO) was undertaken.

Methods

Symptom assessment in the field

The SARDI germplasm collection, located at the Nuriootpa Research Centre, South Australia, consists of 83 red and 95 white wine grape cultivars (*V. vinifera*) sourced from around the world. Vines were planted between 1977 and 1982 with a panel (three or four vines) per cultivar. All vines have been cordon trained and spur pruned, with no specific strategies to control trunk diseases.

On 7 November 2012, vines were visually assessed for (i) severity of eutypa dieback foliar symptoms and (ii) overall severity of trunk disease symptoms, which in addition to foliar symptoms, included presence of dead spurs, cordon die-back and trunk cankers. Ratings were given on a percentage scale (0% - non-symptomatic, 100% - dead). Ratings were averaged for each cultivar.

Detached cane assay assessment

A detached cane assay, as described in Section 4, was conducted in March 2012 using 39 CSIRO grapevine rootstock genotypes from *V. vinifera x cineria* and *berlandier* lines. Each genotype was represented by ten replicates, randomly arranged, five of which were inoculated with 200 *E. lata* spores per wound and the other five with 500 spores per wound. Shiraz canes used in previous DCA trials were used as a reference. The trial was harvested four weeks after establishment and analysed as described in section 4.

Results

Symptom assessment in the field

The severity of foliar and dieback symptoms varied substantially amongst the cultivars (Figure 24-25). In general, the severity of foliar symptoms was similar for red and white wine cultivars (5 and 4%, respectively). The severity of overall trunk disease symptoms was greater in red (35%) than white (26%) wine cultivars.

Foliar symptoms of eutypa dieback developed on 39 of the 83 red wine cultivars with severity ranging from 2 to 42%, and on 44 of the 95 white wine cultivars with 2 to 50% severity (Figure 26). In terms of overall trunk disease symptoms, 76 red and 79 white cultivars were recorded with severity from 2 to 100%. There was a closer correlation between foliar and overall symptoms for white (R^2 = 0.41) than red (R^2 = 0.14) cultivars. No symptoms were observed on seven red and 16 white wine cultivars, whilst a further 16 red and 16 white cultivars were recorded with less than 10% mean severity of trunk disease symptoms. The mean severity of trunk disease symptoms was greater than 80% for 11 red and four white wine cultivars.



Figure 24. Red wine cultivars; (a) Sangiovese, (b) Merlot, (c) Cabernet Sauvignon and (d) Odola showing varying severity of trunk disease symptoms.



Figure 25. White wine cultivars; (a) Semillon, (b) Chardonnay, (c) Chenin Blanc and (d) Sauvignon Blanc showing varying severity of trunk disease symptoms.

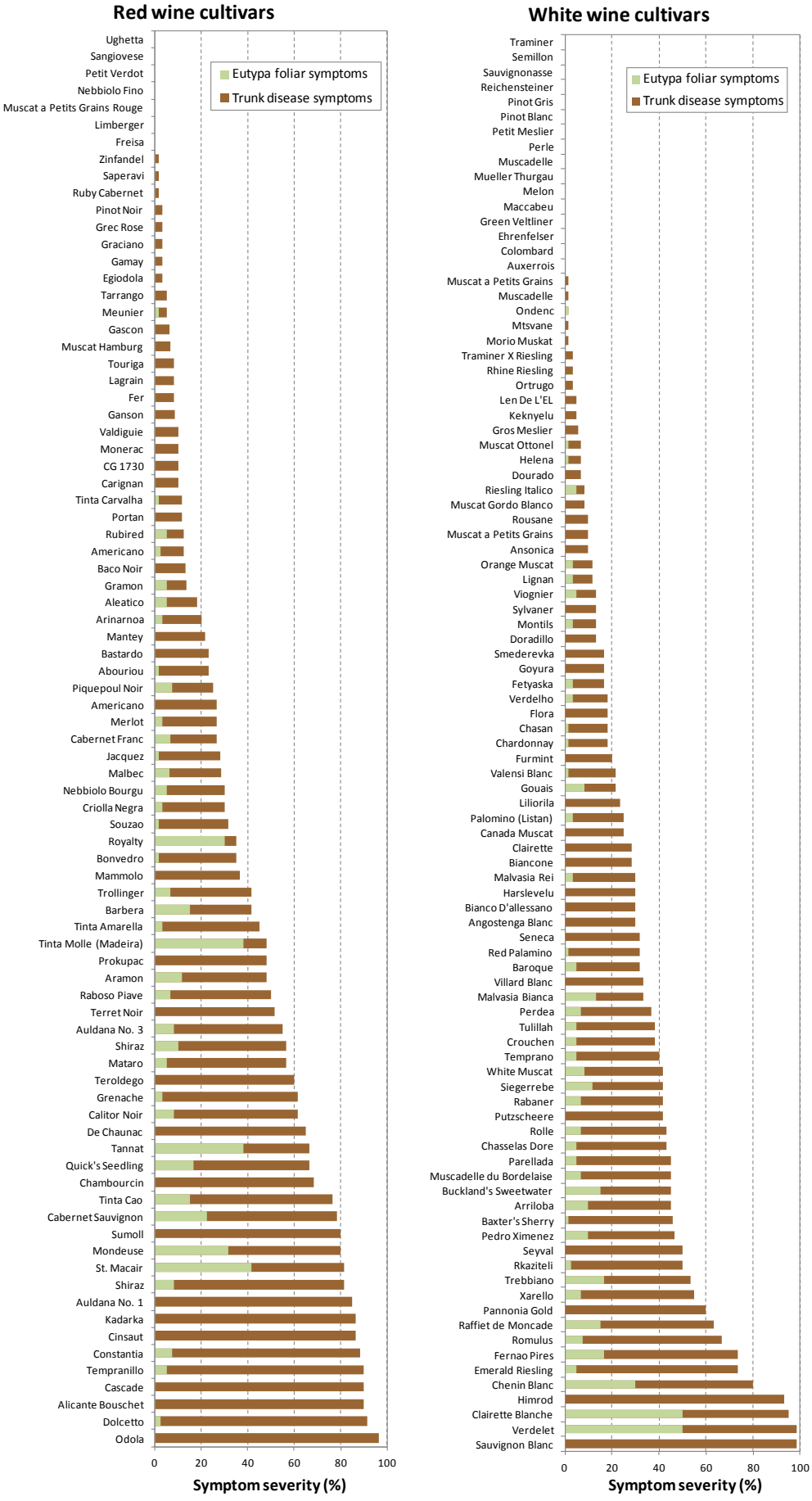


Figure 26. Severity of eutypa dieback foliar symptoms (green bars) overlayed on overall severity of trunk disease symptoms (brown bars) which include foliar and dieback symptoms on red and white wine grape cultivars planted at the Nuriootpa Research Centre between 1977 and 1982.

Detached cane assay assessment

The extent of colonisation of the detached canes varied substantially among genotypes (Figure 27). Shiraz showed the greatest length colonised at 21.2 mm from the point of inoculation, compared with less than 10 mm for 23 of the genotypes and less than 5 mm for three of the genotypes tested.

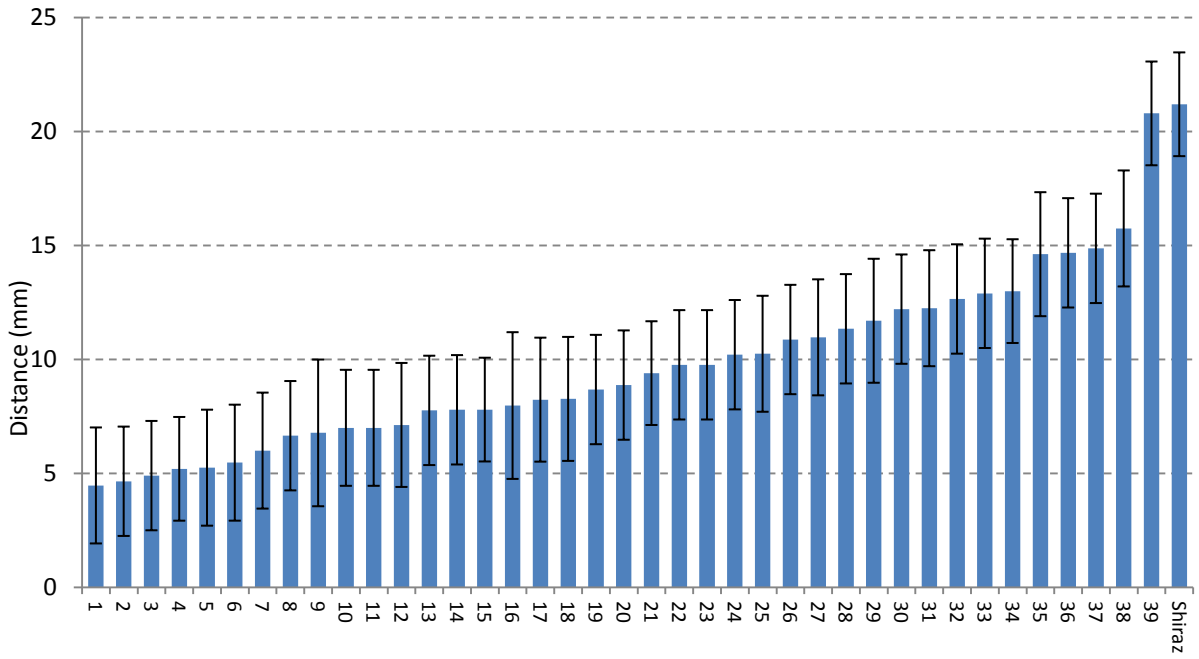


Figure 27. Mean distance of colonisation of canes from 39 grapevine rootstock genotypes plus Shiraz, by *E. lata* in a detached cane assay. Bars represent standard error of the mean.

Discussion

This preliminary study indicated that the severity of trunk disease varied considerably amongst the cultivars in the 31 to 36 year old vines located in the SARDI germplasm collection. Results were generally consistent with previous reports from eutypa dieback surveys in Australia, France and the USA. Twenty-three cultivars developed little to no trunk diseases, suggesting resistance or tolerance to trunk pathogens. Assessments will be repeated in spring 2013 to confirm these results. All vines were subjected to natural infection and, based on previous studies at the Nuriootpa Research Centre, up to 12% and 33% incidence of natural pruning wound infection has been reported for eutypa and botryosphaeria dieback pathogens, respectively. However, isolations of fungi from vines will be required to confirm the cause of symptoms and whether species responsible for botryosphaeria dieback are also involved.

The rootstock genotypes examined in the DCA varied in their ability to resist colonisation by *E. lata*. Colonisation of detached canes from certain rootstocks was 75% less extensive than in Shiraz, which is known to be susceptible to mycelial growth (Sosnowski et al. 2003), suggesting they may be tolerant of or resistant to eutypa dieback.

Further evaluation of promising material from this study could lead to development of cultivars and rootstock genotypes with resistance or tolerance to trunk disease in the future. This would, in turn, reduce inputs and routine management necessary to control trunk disease, contributing towards a more sustainable and profitable wine industry.

10 OUTCOMES AND RECOMMENDATIONS

Output 1

Effective pruning wound treatments

Activities:

- Establish field trials in Barossa Valley over two seasons
- Harvest and assess field trials

Ten treatments were evaluated in the field and greenhouse for their efficacy in protecting grapevine pruning wounds from infection by *Eutypa lata*. Of these, three fungicides, Folicur (tebuconazole), Cabrio (pyraclostrobin) and Shirlan (fluazinam), which are currently registered for application on grapevines for other diseases, controlled eutypa dieback. Permits are currently being sought for their use as pruning wound protectants against *E. lata*. As these chemicals represent different fungicide activity groups, this will provide a range of options for growers to manage fungicide resistance.

In addition, the "natural" products, garlic, lactoferrin and Serenade (derived from *Bacillus subtilis*), provided some control of eutypa dieback, but less than that of the abovementioned fungicides. These may provide alternatives for organic vineyards or to reduce chemical inputs for more sustainable integrated pest management.

Surfactants provided no additional benefit when added to products that were applied to pruning wounds by spray bottle to the point of runoff. However, when products are applied using spray machinery, where complete coverage of pruning wounds is not achieved, the surfactants may improve efficacy. Further research is required to determine if surfactants can assist efficacy when mixed in fungicide sprayed onto pruning wounds with commercial sprayers.

A detached cane assay (DCA) was developed during this project to provide a rapid means of evaluating pruning wound treatments. In most cases, it provided results similar to that of field trials when similar inoculum doses were applied. However, it enabled evaluation at doses as low as 200 spores per wound, reflecting disease pressure closer to that occurring naturally, which is not possible in field trials. Although field data is still important for pruning wound evaluation, DCA data complements field trials.

In this research, all treatments were applied to pruning wounds on the day of pruning, but it is now important to determine how long after pruning a product can be applied to pruning wounds and still prevent infection, and how long a product remains effective once it has been applied. Further research is required to determine the curative and preventative properties of fungicides to identify the critical time for application of wound protectants. Furthermore, with added information on the susceptibility of wounds at different times throughout the pruning season, practical recommendations on appropriate pruning practices could be provided specifically for Australian grapegrowers. Botryosphaeria dieback is also a serious threat to the sustainability of grapevines. Future research should focus on evaluating fungicides for both eutypa and botryosphaeria dieback.

Output 2

Optimise spray application methods

Activities:

- Establish field trials in three regions over two seasons
- Harvest and assess field trials

Trials were conducted to develop strategies for optimising application of pruning wound treatments using commercial sprayers. Sprayers, including air-blast, air-shear, fan-assisted, recycle and home-engineered cordon targeting types, were used to apply Folicur (tebuconazole) at various water spray volumes to control infection of grapevine pruning wounds by *E. lata*. The best disease control was achieved by applying fungicide with recycle sprayers and the home-engineered cordon sprayer, which were able to provide equivalent control to that of treatments applied with a paintbrush. Each of the other types of sprayers were able to provide some control, but water spray volumes of at least 600 L/ha were required.

These trials have proven the concept of spray application using Folicur (tebuconazole). Spraying other fungicides shown to have efficacy against *E. lata* when applied with a paintbrush will therefore also provide control of eutypa dieback.

As many sprayers are designed to apply products to the foliage of actively growing vines later in the growing season, they varied in their ability to target the pruning wounds effectively. It is possible to make adjustments to the spray nozzles or fan positions to target the pruning wound zone and improve the coverage of the pruning wounds but some sprayers may still produce unreasonable off-target spray drift. Home-engineered cordon sprayers can be designed to focus the spray solely on the pruning wound zone.

With recycle sprayers, any spray that misses the cordon is captured for re-use thereby minimising loss to the environment. They can therefore be used at a very high output rate which contributes to their ability to achieve good coverage of pruning wounds. As the level of disease control is directly correlated to the spray coverage of pruning wounds, the ability to achieve good wound coverage with minimal off-target spray drift makes recycle sprayers ideal for large scale application of pruning wound protectants to dormant grape vines.

Most sprayers can achieve control of eutypa dieback if they are adjusted to focus the spray onto the pruning wounds. It is recommended that vineyard managers assess and monitor spray application via the use of water-sensitive papers placed throughout the vines at the time of spraying, in order to ensure optimal coverage of pruning wounds and therefore control of eutypa dieback.

Output 3

Involvement of diatrypaceous species in eutypa dieback

Activities:

- Pathogenicity trials
- *In vitro* evaluation of fungicides

There is an increasing awareness of the role of diatrypaceous fungi other than *Eutypa lata* in grapevine trunk disease throughout the world. Eight of these related fungal species, isolated from grapevines and other plants in grape-growing regions throughout Australia, were assessed in greenhouse trials in South Australia and in collaboration with colleagues at the National Wine and Grape Industry Centre in Wagga Wagga, New South Wales, for their ability to cause disease in grapevines. All the species tested were found to be pathogenic to grapevines although none were more virulent than *E. lata*, based on the severity of lesions caused by the fungi. Foliar symptoms of eutypa dieback were not observed with any species other than *E. lata*, indicating the difficulty of early diagnosis of disease caused by the other species.

Currently, the only method of controlling eutypa dieback is by removal of all infected wood tissue in the vine using remedial surgery. These results indicate that the remedial surgery methods developed to control eutypa dieback will also be effective in eradicating these related fungi from infected vines.

Fungicides that control eutypa dieback were initially found to reduce growth of *E. lata* in laboratory experiments. In this project, laboratory experiments were conducted to evaluate the efficacy of fungicides to reduce growth of the five related diatrypaceous species which have been associated with eutypa dieback on grapevines in Australia. The fungicides that were most effective in controlling *E. lata*, Folicur (tebuconazole), Cabrio (pyraclostrobin) and Shirlan (fluazinam) were also effective against the related diatrypaceous species. This suggests that management strategies recommended for the control of eutypa dieback, will cover disease caused by these related pathogens.

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12 COMMUNICATION

12.1 Publications

12.1.1 Scientific publications

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12.1.2 Theses

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12.1.3 Conference papers

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12.1.4 Industry journals and articles

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12.2 Presentations

Sosnowski M (2013) Eutypa dieback of grapevines. Elders Viticulture Growers Information Evening, Tanunda Football Club, Barossa Valley, SA 5 June 2013. (85 attendees)

Sosnowski M (2013) Eutypa dieback of grapevines , Victorian Viticultural Association workshop. Healesville Memorial Hall, Yarra Valley, Victoria 23 April 2013. (69 attendees)

Sosnowski M and Ayres M (2012) Eutypa dieback of grapevines – identification and management & research update. Kangaroo Island Grape Growers and Wine Makers Association workshop. Dudley Wines Cellar Door, Kangaroo Island 16 Nov 2012. (12 attendees)

Sosnowski M and McCarthy M (2012) SARDI Viticulture – Science Capability. Presentation to members of Queensland Wine Industry Association. Wine Innovation Central Building, AWRI Boardroom, 6 November 2012.

Sosnowski M and Ayres M (2012) Eutypa dieback of grapevines – identification and management & research update. McLaren Vale Grape, Wine & Tourism Association workshop. Rosemount Estate, McLaren Vale 30 Oct 2012. (50 attendees)

Sosnowski M and Ayres M (2012) Eutypa dieback of grapevines – identification and management & research update. Langhorne Creek Grape & Wine Inc. workshop. Langhorne Creek Bowling Club, Langorne Creek, 19 Oct 2012. (23 attendees)

Sosnowski M and Ayres M (2012) Eutypa dieback of grapevines – identification and management & research update. Clare Region Winegrape Association workshop. Annies Lane Vineyard, Watervale, 16 Oct 2012. (40 attendees)

Sosnowski M and Ayres M (2012) Eutypa dieback of grapevines – identification and management & research update. Adelaide Hills Wine Region Workshop. Longview Vineyard, Macclesfield, 10 Sep 2012. (46 attendees)

Sosnowski M (2012) Eutypa dieback of grapevines – identification and management. Elders Viticultural Focus Group meeting. Aquarius Apartments, Mildura 29 Aug 2012. (20 attendees)

Sosnowski MR (2012) Grapevine trunk diseases in Australia. Presentation to researchers at the Institute for Food Research and Technology in Cabrils (Barcelona), Spain. 27 June 2012.

Sosnowski M and Ayres M (2012) Eutypa dieback of grapevines – identification and management & research update. Australian Vintage Annual Viticulture Meeting. Clare Country Club, Clare 13 June 2012.

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Sosnowski MR (2011) Limestone Coast field trial demonstration workshop. Menzies South Vineyard, Coonawarra, 13 July 2011.

Sosnowski MR (2011) Radio Interview on managing eutypa dieback in apricots and grapes – Talkback Gardening with Jon Lamb and Ashley Walsh - ABC Adelaide, 2 July 2011.

Sosnowski MR (2011) Grapevine trunk disease research. Presentation to Mudgee Future Leaders Troup. CSIRO Board Room, WIC West, Urrbrae, 30 June 2011.

Sosnowski MR (2011) Field trial demonstration and project update. Presentation to GWRDC Board, Nuriootpa Research Centre, 28 June 2011.

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