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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.

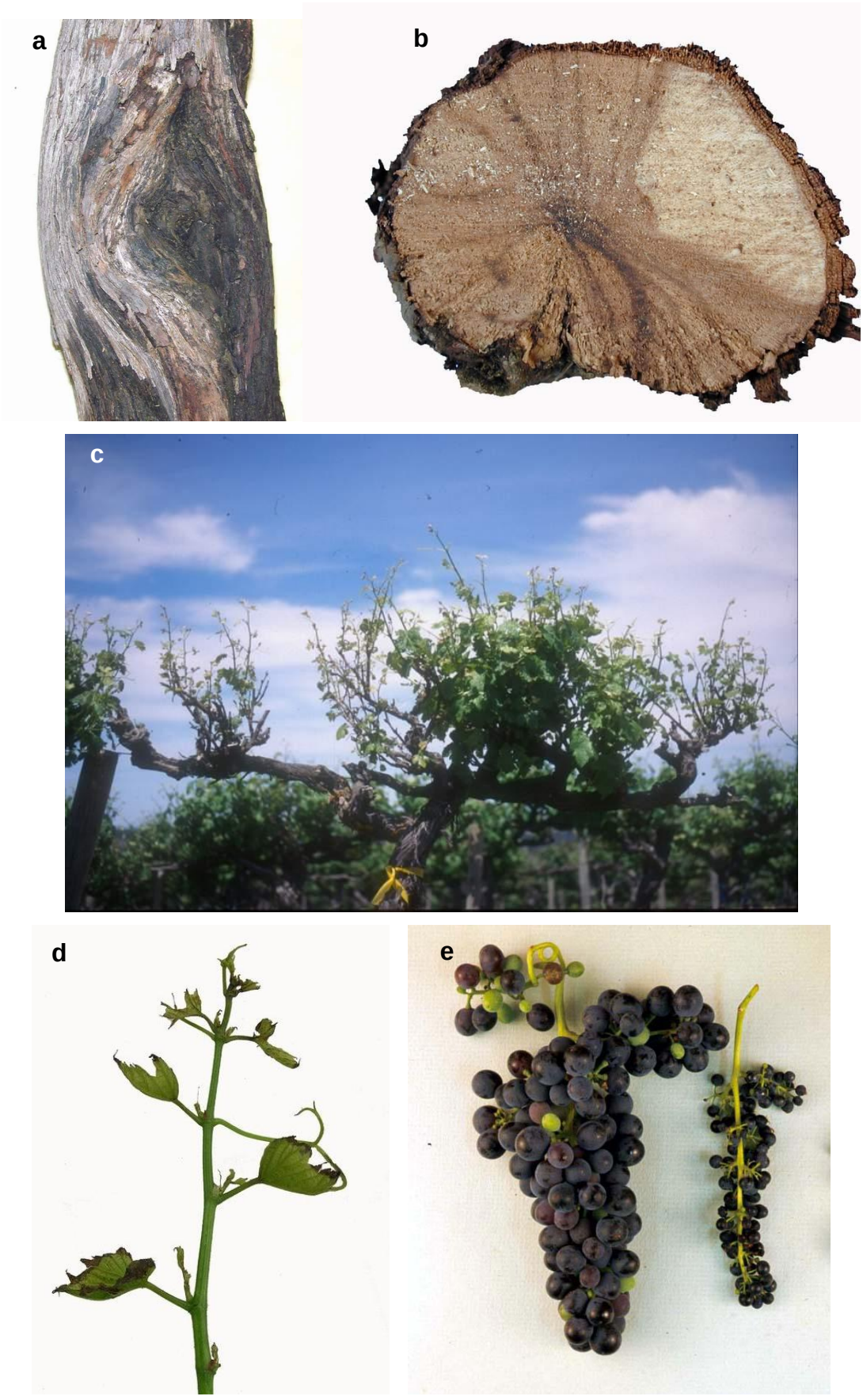

Figure 1. Symptoms of eutypa dieback in grapevines. Wood symptoms including (a) external canker and (b) wedge of internal staining. Characteristic foliar symptoms including (c) mature vine displaying stunted shoots, (d) single shoot with cupped leaves with necrotic margins and (e) uneven ripening and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

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.

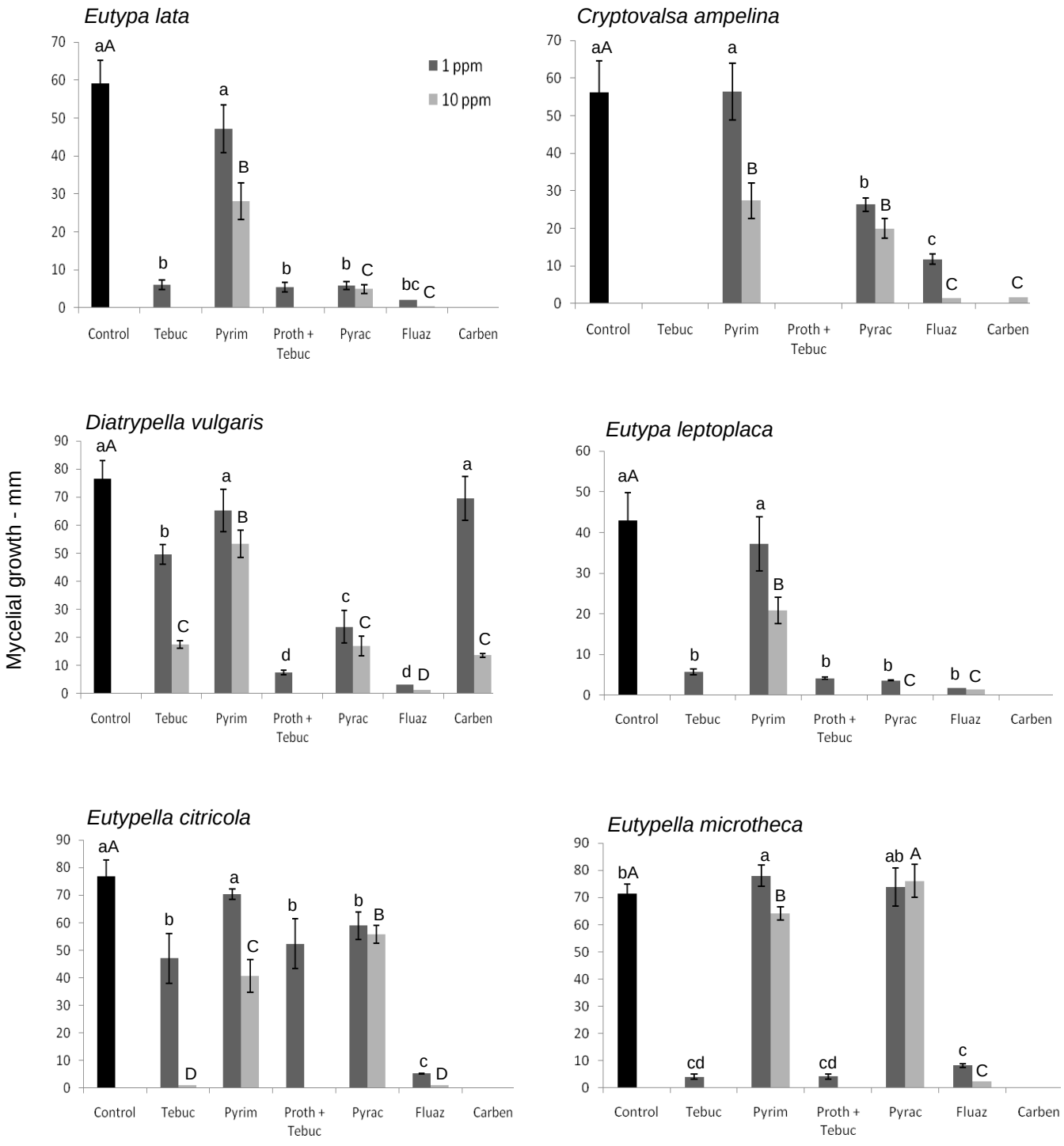

Figure 22. Effect of treatments *in vitro* on mycelial growth of Diatrypaceous species at 1 and 10 ppm active ingredient. Bars with a different letter are significantly different, indicated in lower case for 1 ppm and upper case for 10 ppm. Abbreviations: tebuc, tebuconazole; pyrim, pyrimethanil; proth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

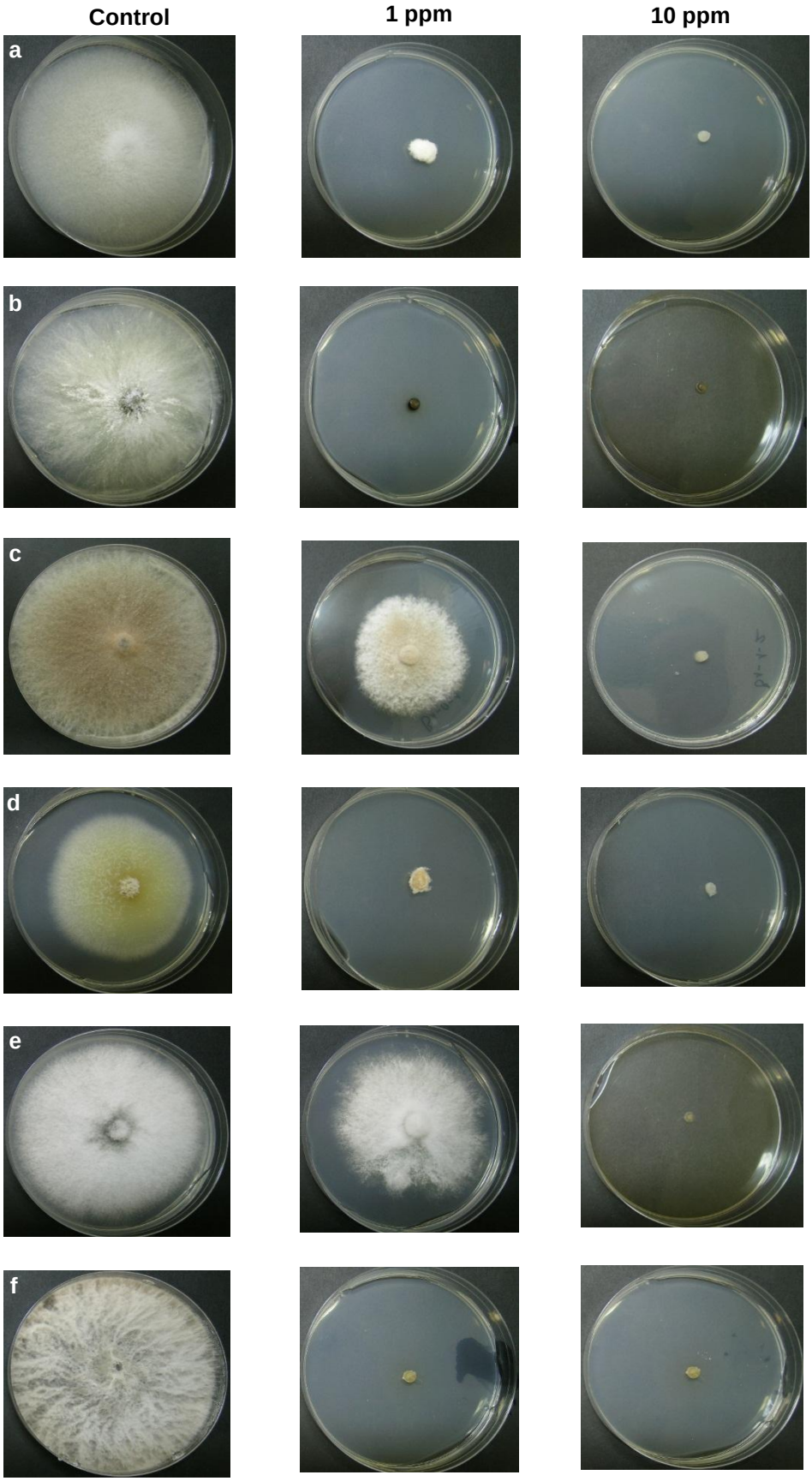

Figure 23. Mycelial growth of six Diatrypaceous fungi, a) *Eutypa lata*, b) *Cryptovalsa ampelina*, c) *Diatrypella vulgaris*, d) *Eutypa leptoplaca*, e) *Eutypella citricola* and f) *Eutypella microtheca* on unamended controls and on agar amended with tebuconazole (Folicur) at 1 and 10 ppm.

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.

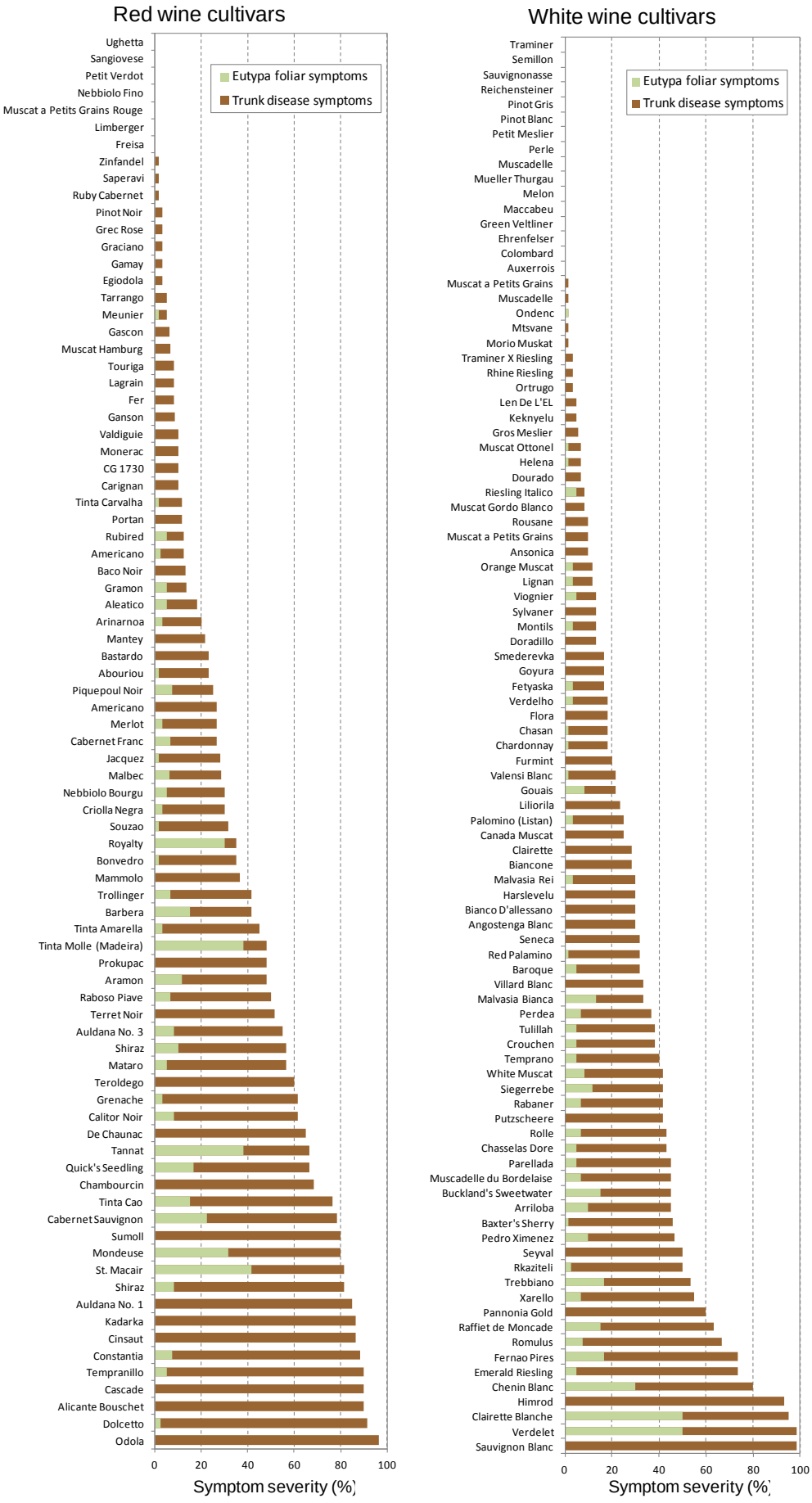

Figure 26. Severity of eutypa dieback foliar symptoms (green bars) overlayed on overall severity of trunk disease symptoms (brown bars) which include foliar and dieback symptoms on red and white wine grape cultivars planted at the Nuriootpa Research Centre between 1977 and 1982.

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.

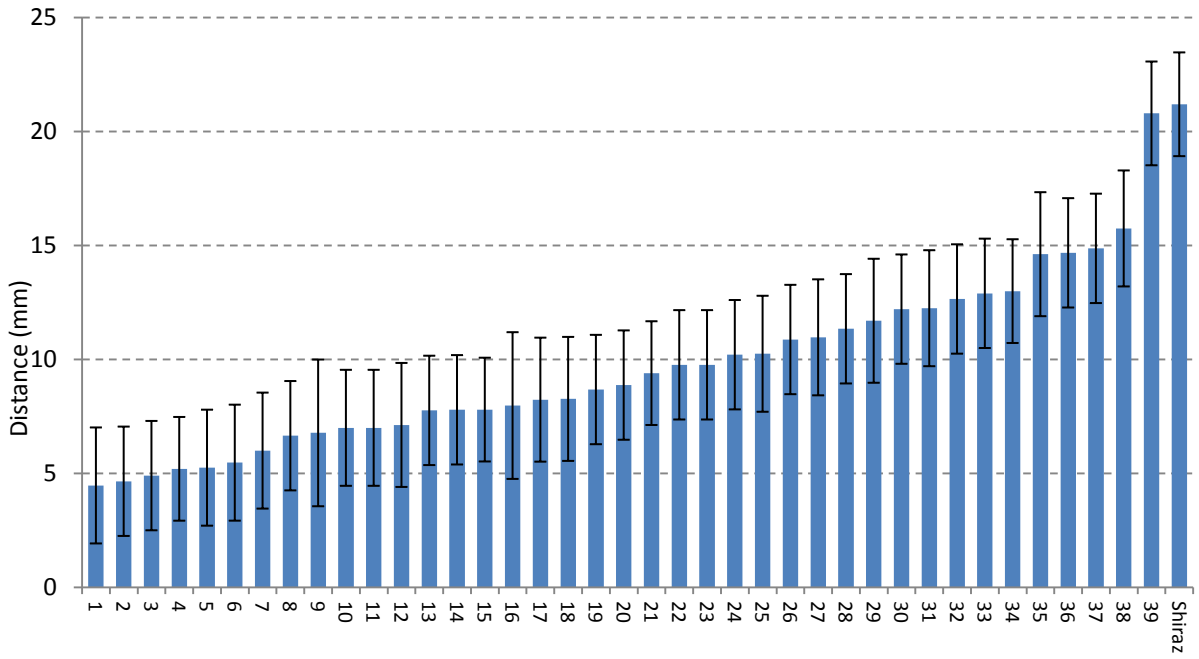

Figure 27. Mean distance of colonisation of canes from 39 grapevine rootstock genotypes plus Shiraz, by *E. lata* in a detached cane assay. Bars represent standard error of the mean.

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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12.2 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.

Ayres M (2012) Eutypa dieback project update. Eutypa and Vine Decline Meeting. Coonawarra Grapegrowers Association Office, Penola 5 June 2012.

Sosnowski M and Ayres M (2012) Eutypa dieback of grapevines – identification and management & research update. GrapeBarossaWorkshop, Managing the 'dying arm' in your vineyard. Peter Lehman Winery, Tanunda 31 May 2012.

Ayres M, Sosnowski M, Wicks T and Scott E (2012) Managing Eutypa Dieback- practical solutions for grape growers. SARDI Waite Seminar Series. 15 March 2012.

Sosnowski M (2102) Managing eutypa dieback of grapevines. Kangaroo Island Grapegrowers Association Workshop and Vineyard Tour. Kingscote, Kangaroo Island 14 Feb 2012.

Sosnowski M and Ayres M (2011) Eutypa dieback of grapevines – identification and management & research update. GrapeBarossa Workshop, Managing the 'dying arm' in your vineyard. Peter Lehman Winery, Tanunda 17 Nov 2011.

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.

Sosnowski MR (2011) Radio Interview on SARDI eutypa dieback project, Rural Report - ABC South Western Vic & South Eastern SA, 15 June 2011.

Sosnowski MR (2011) Eutypa dieback of grapevines – Identification & Management. Victorian Wine Industry Association Workshops, Heathcote, Myrtleford, Mooroolbark & Arrarat, Victoria, 16-19 May 2011.

Ayres M. Update of eutypa dieback research (2011). Adelaide Hills Wine Region Seminar, Lenswood Research Centre, SA, 15 Feb 2011.

Sosnowski M and Ayres M (2010) Barossa Viticultural Technical Group Field Visit and Workshop, Barossa Valley, SA, 24 Nov 2010.

Sosnowski MR (2010) Eutypa dieback of grapevines – Identification & Management. Hawke's Bay Winegrowers Winter Workshop Seminar, Mission Estate Winery, Hawke's Bay, New Zealand, 25 Jun 2010.

13 ACKNOWLEDGEMENTS

Staff

Project leader/supervisor: Mark Sosnowski

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SARDI technical assistance: David Sosnowski, Ian Bogisch, Cathy Todd, Lee Bartlett, Daniel Johnson, Alex Tittoto, Treva Hebberman.

Collaborators

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