A rapid technique for evaluating treatments for eutypa dieback control

By Matthew Ayres, Mark Sosnowski and Trevor Wicks
South Australian Research and Development Institute, GPO Box 397, Adelaide, SA 5001

INTRODUCTION

Eutypa dieback is a serious fungal disease affecting grapevines in most cool climate wine regions in Australia and internationally. If left unmanaged, the disease reduces yields and eventually kills vines. Typically known as a disease of older vines, recent extensive surveys indicate that eutypa dieback is becoming a threat to younger vines in emerging regions.

Spores of the fungus *Eutypa lata* are produced 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. Spread of the disease in infected vines can be controlled by the removal of infected wood material (Sosnowski *et al.* 2009). However, the most effective strategy for preventing the disease is to protect pruning wounds from infection (Sosnowski *et al.* 2008). Research has shown that treating pruning wounds with paints and some fungicides can prevent infection. However, the number of fungicides suitable for this treatment is limited and more data must be obtained in order for fungicides to be registered for this purpose.

Researchers at the South Australian Research and Development Institute (SARDI), in collaboration with The University of Adelaide, are evaluating methods to optimise management of eutypa dieback, with funding from the Grape and Wine Research and Development Corporation along with additional support from industry.

Initial screening of products is carried out in laboratory trials using agar plates amended with different rates of fungicides. This provides efficacy data on potential fungicides in two weeks, but may not reflect effectiveness on grapevine wounds. Field evaluation is imperative for registration but can take up to 15 months to generate data from one experiment. There is a need for a rapid technique to evaluate treatments on grapevine wounds in order to decrease the time taken to generate data for registration.

Product	Active ingredient	Label rate
Folicur	tebuconazole (430g/L)	0.3mL/L
Scala	pyrimethanil (400g/L)	2.0mL/L
Prosaro	tebuconazole + prothioconazole (210 + 210g/L)	0.3mL/L
Cabrio	pyraclostrobin (250g/L)	0.4mL/L
Shirlan	fluazinam (500g/L)	1.0mL/L

Table 1. Fungicides evaluated for pruning wound protection against infection by *E. lata*, including label rate for foliar application on grapevines.

Researchers at SARDI have developed a 'detached cane assay' to reduce the time needed to evaluate pruning wound treatments. It was adapted from a single-node cutting technique developed by Jeff Bennett, at the Marlborough Wine Research Institute, in New Zealand, for assessing cane fruitfulness, which was also used for a black spot bioassay screening of tablegrapes (Sosnowski *et al.* 2007). Mundy and Robertson (2010) also recently adapted the technique as a model for studying grapevine trunk diseases. Here, we report on the detached cane assay and results from preliminary experiments.

METHODS

Dormant grapevine canes (cv Shiraz) were taken from storage at 3-4°C and cut into 10cm single-node sections with the top surface 1cm above the node (Figure 1). Canes were placed into holes in 2cm thick polystyrene boards, ensuring that the bottom of the canes extended approximately 1cm below the boards. The boards with canes were floated on tap water in plastic tubs (Figure 2) on benches in a greenhouse and maintained at approximately 25°C. The water was changed weekly for the first four weeks. Thereafter, soluble fertiliser (Campbells Diamond Special T) was added weekly at a rate of 0.2g/L.

The initial experiment was carried out to determine the extent of colonisation by *E. lata* by re-isolating the fungus from the canes at different times. In this experiment, the top wound of each cane was moistened by spraying with distilled water and inoculated with a 20µl water droplet (Figure 2) containing a suspension of either 500 or 1000 *E. lata* spores (Carter 1991). Concentrations were chosen based on those used in past field trials. Controls were not inoculated.

At four, eight and 12 weeks after inoculation, six canes from each treatment were removed from the board. Bark was removed with a knife and the canes were sterilised in bleach for 10

Figure 1. Single-node cutting.

minutes prior to rinsing twice in sterile water. Starting just below the inoculation point, cuts were made using sterile secateurs every 2mm along the canes for a distance of 50mm. Segments were placed sequentially on plates of potato dextrose agar (PDA) and incubated for a week with 12-hour day/night light cycles at 25°C. Each segment was then assessed for presence or absence of *E. lata* growth (Figure 3, see page 52), and the distance the fungus extended from the inoculation point recorded.

In experiment 2, five commercial fungicide formulations were each applied by paintbrush to wounds on 20 canes at two, five or 10 times the label rate for foliar application on grapevines (Table 1). Fungicides and application rates were chosen based on results from previous research. The following day, wounds were inoculated with 500 spores as described previously. Untreated controls were either inoculated with the spore suspension or left un-inoculated.

After four weeks, canes were removed from the boards, and prepared for assessment as described previously. Longitudinal cuts were made at the treated ends of the canes using sterile secateurs and a transverse cut was made

Figure 2. Inoculating single-node cuttings in polystyrene boards floating on tap water in tubs.

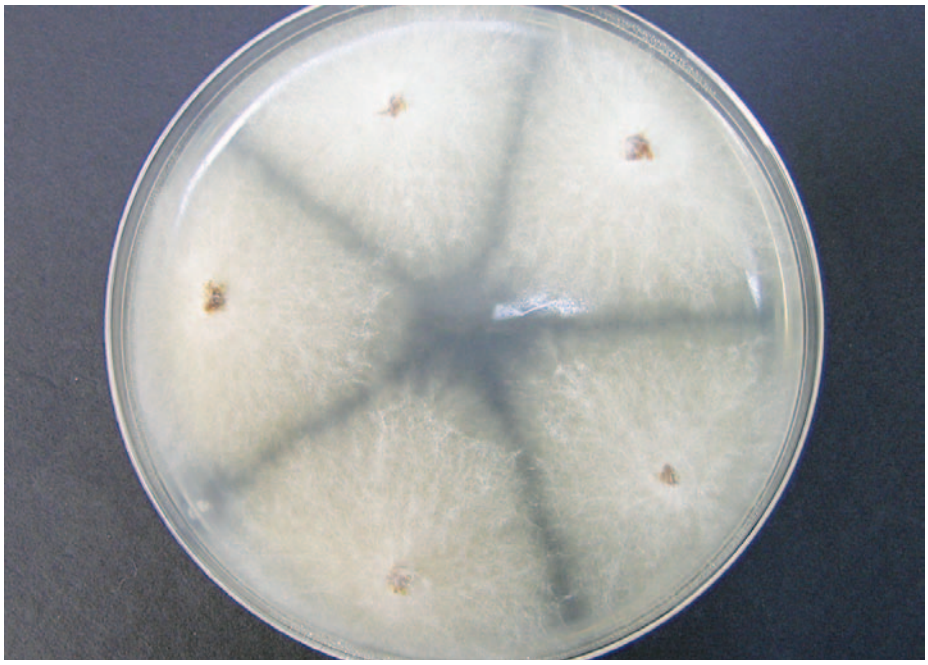

Figure 3. *E. lata* cultures growing from cane pieces on PDA plates.

3mm 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 one week and assessed for the presence or absence of *E. lata* cultures (Figure 3). Mean percent disease control was calculated as the reduction in incidence of recovery as a proportion of the inoculated control.

RESULTS

In both experiments, budburst occurred within one week of trial establishment, and roots began emerging after two weeks and continued to grow throughout the experiment (Figure 4).

In experiment 1, *E. lata* was recovered from all inoculated canes at both spore concentrations at all three assessment times. The distance that *E. lata* was recovered from the wound was variable, ranging from 2-20mm and 2-18mm for the canes inoculated with 500 and 1000 spores, respectively. There were no significant differences for the distance colonised between spore concentrations or assessment times.

In experiment 2, disease control differed among fungicide treatments and application rates (Figure 5). Cabrio provided complete control at the lowest concentration. Folicur and Shirlan provided between 67-100% control and Prosaro between 39-94% control. Scala was the least effective fungicide, providing between 4-63% control.

CONCLUSIONS

These results show that *E. lata* readily colonises detached canes, growing up to 20mm from the point of inoculation within four weeks. Samples can be harvested four weeks after treatment, with assessment data available within a further two to four weeks. This technique considerably reduces the time required to produce efficacy data, compared with 15 months needed to obtain field trial data. Furthermore, an inoculation concentration of 500 spores per wound was adequate for infection and will, therefore, be used for subsequent experiments.

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

Application of fungicides was effective in reducing or preventing infection of pruning wounds by *E. lata*. Cabrio, Folicur and Shirlan showed the greatest potential at the lowest rates

tested on detached canes. A field trial is currently under way using the same treatments and data will be compared.

The detached cane assay has shown to be a fast, high-throughput technique to evaluate pruning wound treatments for the control of eutypa dieback. Data from this and other experiments may lead to label extension for the aforementioned products to include control of eutypa dieback. The technique will also provide a tool to screen alternative products, and for epidemiological studies, which underpin the development of disease management strategies.

REFERENCES

Carter, M.V. (1991) The status of *Eutypa lata* as a pathogen. Monograph – Phytopathological Paper No.32 (International Mycological Institute: Surrey, UK).

Mundy, D.C. and Robertson, S.M. (2010) Evaluation of single-node plantlets as a model system for grapevine trunk diseases. New Zealand Plant Protection. 63:167-173.

Sosnowski, M.R.; Creaser, M.L.; Wicks, T.J.; Lardner, R. and Scott, E.S. (2008) Protecting grapevine wounds from infection by Eutypa lata. Australian Journal of Grape and Wine Research. 14:134-142.

Sosnowski, M.; Emmett, B.; Clarke, K. and Wicks, T. (2007) Susceptibility of table grapes to black spot (anthracnose) disease. The Australian and New Zealand Grapegrower and Winemaker. 521a:8-11.

Sosnowski, M.; Loschiavo, A.; Wicks, T. and Scott, E. (2009) Managing eutypa dieback in grapevines. The Australian and New Zealand Grapegrower and Winemaker Annual Technical Issue. 13-16.

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Figure 4. Shoot and root growth after six weeks.

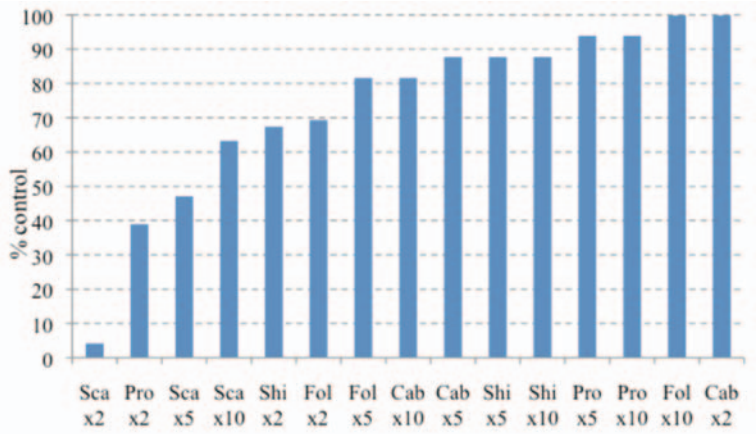

Figure 5. Mean percent control of *E. lata* infection by five fungicides (Sca-Scala, Pro-Prosaro, Shi-Shirlan, Fol-Folicur and Cab-Cabrio) at three concentrations (two, five and 10× label rate).