

## Control of *Eutypa* dieback in grapevines using remedial surgery

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**Summary.** A series of long-term trials was established to monitor the effect of remedial surgery for the control of *Eutypa* dieback in commercial vineyards of South Australia. Removing infected cordons and trunks has been used as a strategy for managing *Eutypa* dieback in grapevines for many years and this study is the first to provide evidence of its effect in controlling the disease. Between 42 and 100% of vines produced watershoots after removal of infected wood, depending on time after surgery and height of the trunk remaining. Remedial surgery reduced the incidence of vines with foliar symptoms of *Eutypa* dieback in vineyards, and the severity of those symptoms, but the efficacy varied with the presumed extent of pre-existing infection and the origin of the watershoot. It is recommended that all stained wood and a further 10 cm of healthy tissue be removed to reduce the likelihood that symptoms of *Eutypa* dieback will recur.

**Key words:** *Eutypa lata*, reworking, trunk renewal, top-working, *Vitis vinifera*.

### Introduction

*Eutypa* dieback, one of the most important trunk diseases of grapevines (*Vitis* spp.) worldwide, is caused by the fungus *Eutypa lata* (Pers.) Tul. & C. Tul (= *E. armeniaca* Hansf. & M.V. Carter) (Carter, 1991). Vines infected with *E. lata* produce stunted shoots with chlorotic leaves, often cupped and with tattered margins. Necrosis of wood results in wedge-shaped areas of stained tissue on cross-sections made through the external cankers that form around sites of infection in the trunks or cordons (Moller and Kasimatis, 1981).

Ascospores infect grapevines through pruning wounds and germinate in xylem vessels, after which the fungus colonises the wood (Carter, 1960; Moller and Kasimatis, 1978). The mean rate of progression of wood staining due to *E. lata*

ranged between 12 and 18 mm year<sup>-1</sup> in a study of eight cultivars, with a maximum of 50 mm/year recorded in individual Cabernet Sauvignon and Shiraz grapevines (Sosnowski *et al.*, 2007b). Furthermore *E. lata* colonised the non-stained tissue up to 80 mm ahead of the front of necrotic tissue.

In the vineyard, foliar symptoms are generally expressed 3 to 8 years after inoculation (Carter, 1988; Tey-Rulh *et al.*, 1991). Although it has long been considered that foliar symptoms are caused by toxins produced by the fungus in the wood and transported to the foliage (Moller and Kasimatis, 1981; Tey-Rulh *et al.*, 1991), Mahoney *et al.* (2005) recently suggested that fungal metabolites may affect the wood at the point of production to inhibit nutrient transport, thereby inducing foliar symptoms. Subsequently, Sosnowski *et al.* (2007b) showed that the most severe foliar symptoms were not necessarily associated with the presence of isolates that caused the most extensive staining.

Eradicating *E. lata* from infected grapevines is challenging. The application of nutrient solutions to foliage, and injection of fungicides, nutrients and

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Table 1. Remedial surgery trials established on ‘Shiraz’ vines in two regions of South Australia between 1999 and 2003. Foliar and wood symptoms of *Eutypa* dieback were recorded for vines in some trials where possible, and incidence of watershoot production and recurrence of foliar symptoms were assessed for all trials each spring post-surgery until 2008.

Trial	Region <sup>a</sup>	Year planted	Cut	Year of surgery	Incidence of symptoms pre-surgery (%)		Vines that produced watershoots (%) 1–9 years after surgery									Vines with foliar symptoms (%) 1–9 years after surgery								
					Foliage	Trunk	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9
					1	EV	1971	low	1999	89	-	-	-	-	100	100	100	*	-	-	-	5.6	17	0
2	EV	1971	low	2000	35	71	65	62	75	76	77	81	81	81	81	0	0	0	1.4	5.8	5.8	3.6	5.1	5.1
3	CW	1967	high	2002	-	-	91	86	87	87	86	85	85	0	0	0	3.6	3.5	8.9	8.9				
4	CW	1970	high	2003	56	-	79	89	89	91	92	91	4	0.9	5.2	16	21	29						
5	EV	1971	low	2003	-	36	42	76	76	81	77	75	0	0	1.3	1.1	5.0	8.8						
6	EV	1971	high	2003	-	-	92	92	93	89	87	87	0	0	8.1	11	39	59						

<sup>a</sup> EV, Eden Valley; CW, Coonawarra.

\*, Vineyard was removed in 2006 after foliar symptoms had been assessed.

-, data not collected.

biocontrol agents into the trunk have not controlled *Eutypa* dieback (Sosnowski *et al.*, 2006). Many growers ‘renew’ infected vines by removing diseased wood and reworking, which has been termed “remedial surgery” (Creaser and Wicks, 2004). This procedure involves the removal of wood from cordons and trunks until the cross-sectional wedge of stained wood is no longer visible. Then a further 10 cm of wood is removed to increase confidence that all infected wood has been removed (Sosnowski *et al.*, 2007b). A cane or watershoot (sucker) is then trained to replace the cordons.

Here we present observations, for up to 9 years, on the production of watershoots and recurrence of foliar symptoms of *Eutypa* dieback on vines subjected to remedial surgery. The aim was to determine the efficacy of remedial surgery as a strategy to control *Eutypa* dieback disease, taking advantage of a management practice already underway in commercial vineyards in South Australia.

## Materials and methods

Between 1999 and 2003, trials were established on *Vitis vinifera* cv. Shiraz, 28–35 years old, in six commercial vineyards located in two wine regions of South Australia (Eden Valley and Coonawarra) to determine the efficacy of remedial surgery for the control of *Eutypa* dieback. The number of vines in each trial varied between 36 and 202, and vines were subjected to remedial surgery using ei-

ther “low-cut” or “high-cut” methods, as outlined below (Table 1).

### Remedial surgery

The low-cut method involved removing the trunk 30–40 cm above the ground by cutting with a chainsaw or hydraulic loppers, then training a resultant watershoot to replace the trunk and cordons. The high-cut method involved removing the cordons from the top of the trunk (crown) and then selecting and training the lowest available shoot to form a new trunk and cordons. Shoots that developed were grouped into “low” (bottom third), “mid” (middle third) or “high” (top third) according to the point of emergence from the trunk. After new shoots were established onto the wire, excess wood above the point of emergence was removed, usually several years after surgery.

### Field trials

Trial 1 was established on 36 vines in the Eden Valley. During each spring from 1996 to 1998, vines were assessed for severity of foliar symptoms of *Eutypa* dieback. Severity of *Eutypa* dieback symptoms was expressed as the difference in length of stunted shoots on diseased vines and those on symptomless vines in the same block as a percentage of the length of shoots on symptomless vines (Sosnowski *et al.*, 2007b). In the winter of 1999, 18 randomly selected vines were cut low and the remaining 18 vines were left untreated.

No treatments were applied to wounds remaining on cut vines. This trial was established independently of other trials in this study and, in 2004, on learning of its existence, foliar symptoms were assessed, as described above, each spring until the vines were removed in 2007.

Trial 2 was established on 140 vines in Eden Valley. In spring 1999, vines were assessed for incidence of foliar symptoms of *Eutypa dieback*. During the following winter, vines were cut 30–40 cm above ground level and the presence of discoloured wood in trunk cross-sections was recorded. Wounds were treated with either Benlate (1 g L<sup>-1</sup> water; active ingredient (a.i.) benomyl, Du Pont, Sydney, Australia), Nustar (0.5 g L<sup>-1</sup> water; a.i. flusilazole, Du Pont), Trichoseal (10 g L<sup>-1</sup> water; Agrimm Technologies, Christchurch, New Zealand) or water (control). The treatments were set up in a completely randomized design with 35 replications.

Trials 3 and 4 were established in Coonawarra on 200 and 129 vines, respectively. Foliar symptoms were recorded only in Trial 4 and this was done during the spring preceding remedial surgery. Cordons were removed at the crown (high-cut) in 2002 and 2003 for Trials 3 and 4, respectively. Benlate (1 g L<sup>-1</sup> water) was applied to all wounds in both trials immediately after surgery. During the following spring, the lowest available watershoots were trained up to the wire to form new cordons.

Trials 5 and 6 were established during the winter of 2003 on two adjacent blocks of 157 and 202 vines, respectively, in a vineyard in Eden Valley. Vines were cut low for Trial 5 and cut high (above the crown) for Trial 6. Immediately after low-cuts were made in Trial 5, wounds were assessed for presence of wood discolouration typical of *Eutypa dieback*. All wounds were treated with Garrison (a commercial pruning wound paste containing 2.5 g L<sup>-1</sup> cyproconazole and 1 g L<sup>-1</sup> iodocarb, Chemcolour Industries, Christchurch, New Zealand). During the following spring, watershoots were trained up to the wire to form new cordons; the lowest available shoots were chosen in Trial 6.

Each spring following surgery, vines in Trials 2–6 were assessed for presence of watershoots and foliar symptoms of *Eutypa dieback* until 2008. For vines which were cut high (Trials 3, 4 and 6), the position from which the lowest available water-

shoot originated (low, mid or high) was also recorded.

### Statistical analysis

Foliar symptom severity data from Trial 1 were subjected to analysis of variance and compared by least significant difference (LSD) using Statistix for Windows v. 8 (Analytical Software, Tallahassee, FL, USA). Relationships between watershoot production and years after remedial surgery for Trials 2–6 were assessed by linear regression, and coefficients of determination ( $R^2$ ) were calculated using Microsoft Excel 2003. Statistical analysis of recurrence of foliar symptoms, based on data for incidence only, was not possible and comparison between trials was considered inappropriate due to variability in trial design, so the original data are presented and trends are explained.

## Results

Watershoot production and recurrence of foliar symptoms in new growth for up to 9 years following remedial surgery for six trials are shown in Table 1. Incidence of water shoot production ranged between 42 and 100% in all trials. Foliar symptoms typical of *Eutypa dieback* developed on new shoots within 4 years of remedial surgery. The greatest incidence of symptom recurrence was 59%, recorded 6 years after high-cut surgery in Trial 6.

The relationship between incidence of watershoot production and years after remedial surgery is shown in Figure 1. It reveals a trend that in the first year after surgery, low-cut vines had a lower incidence of watershoot production than high-cut vines, and incidence increased during the next 5 years until it was similar to that for high-cut vines.

Results from Trial 1, showing the effect of low-cut remedial surgery compared with taking no remedial action, are presented in Table 2. During the years 1996–1998, prior to remedial surgery, 89–100% of vines exhibited foliar symptoms of *Eutypa dieback*. From 2004 to 2006, inclusive, foliar symptoms were recorded on 72 to 100% of untreated vines, compared with 0 to 17% of vines subjected to remedial surgery. The mean severity of foliar symptoms, pre-surgery, on vines selected for treatment by remedial surgery (27, 68 and 62% in 1996, 1997 and 1998, respectively) was not significantly dif-

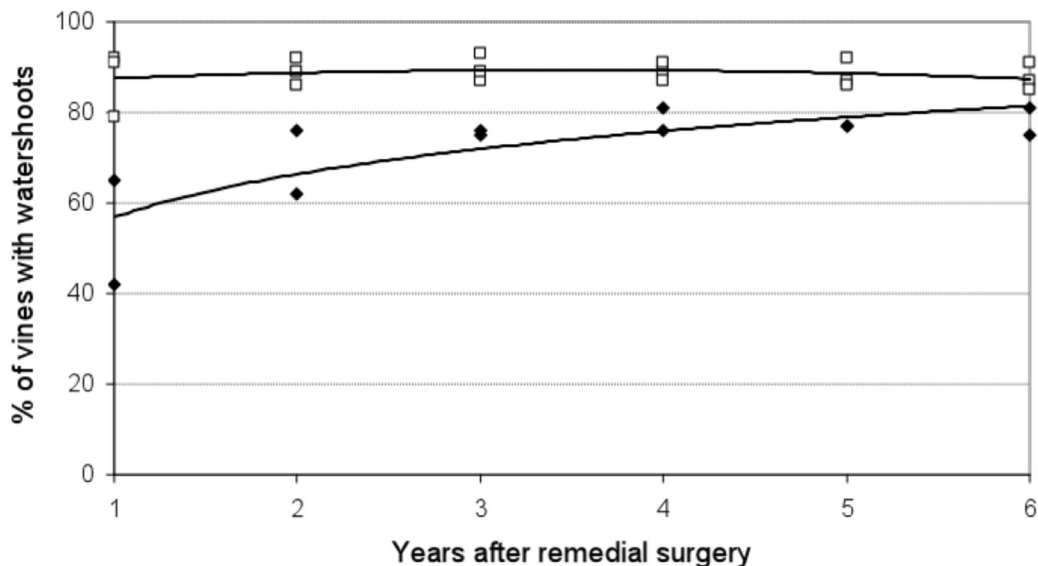


Figure 1. Relationship between incidence of watershoot production and years after remedial surgery for low-cut (◆ Trials 2 and 5,  $R^2=0.62$ ) and high-cut (□, Trials 3, 4 and 6,  $R^2 = 0.05$ ) 'Shiraz' vines in the Eden Valley and Coonawarra regions of South Australia.

ferent ( $P > 0.05$ ) from that on vines designated untreated controls (30, 73 and 53%). However, when assessed between 2004 and 2006, foliar symptoms were significantly less severe ( $P < 0.05$ ) on treated (2, 1 and 0% in 2004, 2005 and 2006, respectively) than untreated (17, 26 and 18%) vines.

Nine years after remedial surgery, seven (5%) of the treated vines with watershoots had developed foliar symptoms in Trial 2 (Table 1). There was no effect of pruning wound treatment on the recurrence of foliar symptoms. The large proportion of zeros in the data precluded statistical analysis,

however, there was no obvious difference among the seven symptomatic vines with respect to wound treatment applied, as two had been treated with either Benlate or Trichoseal, one with Nustar and two were untreated controls.

The incidence of foliar symptoms on 'Shiraz' vines pre-surgery in Trials 2 and 4 was 35 and 56%, respectively. Six years after surgery, 6% of low-cut vines in Trial 2 (Eden Valley) developed symptoms as did 29% of high-cut vines in Trial 4 (Coonawarra). Trials established in adjacent blocks of vines planted in 1971 allowed compari-

Table 2. Incidence (%) and severity (%) over time of vines with foliar symptoms of *Eutypa dieback* pre and post-remedial surgery which was conducted by low-cuts in 1999 on 18 'Shiraz' vines in the Eden Valley, South Australia, compared with 18 vines left untreated (Trial 1). Mean values within rows followed by the same letter are not significantly different (LSD;  $P=0.05$ )

Year	Untreated		Remedial surgery	
	Incidence	Severity	Incidence	Severity
1996 (pre-treatment)	100	27 <sup>a</sup>	100	30 <sup>a</sup>
1997	100	68 <sup>a</sup>	100	73 <sup>a</sup>
1998	89	62 <sup>a</sup>	94	53 <sup>a</sup>
2004 (post-treatment)	72	17 <sup>a</sup>	6	2 <sup>b</sup>
2005	100	26 <sup>a</sup>	17	1 <sup>b</sup>
2006	72	18 <sup>a</sup>	0	0 <sup>b</sup>

Table 3. Watershoot production on 'Shiraz' vines cut high during remedial surgery in Trials 3 and 4 (Coonawarra, South Australia) and Trial 6 (Eden Valley, South Australia), showing the number of vines from which the lowest available shoots arose from either low (bottom third), mid (middle third) or high (top third) positions on the trunk and the percentage of vines in each category.

Origin of watershoot	Trial 3		Trial 4		Trial 6	
	No. of vines	% of total	No. of vines	% of total	No. of vines	% of total
Low	45	25	78	69	121	72
Mid	13	7	21	19	42	24
High	126	68	13	12	6	4
Total	184	-	112	-	169	-

son of the efficacy of the low-cut (Trial 5) and high-cut methods (Trial 6). Six years after surgery, 9% of vines cut low developed foliar symptoms, compared with 59% of vines cut high (Table 1).

The position from which watershoots arose on the trunk following high-cut remedial surgery on vines in Trials 3 and 4 (Coonawarra) and Trial 6 (Eden Valley) is shown in Table 3. Six years after surgery, watershoots occurred on 184 of 200 vines in Trial 3 and shoots arose from high on the trunk on the majority (68%) of the vines. Watershoots were produced on 112 of 129 vines in Trial 4, with most (69%) occurring low on the trunk. In Trial 6,

169 of 202 vines produced watershoots, the majority (72%) of which arose from low on the trunk. The incidence of foliar symptoms which developed on shoots arising from different positions on the trunk is shown in Figure 2. Foliar symptoms were recorded on 7, 21 and 18% (Trials 3, 4 and 6, respectively) of shoots arising from low on the trunk, on 21, 38 and 85% of shoots arising from the mid trunk and on 83, 39 and 72% of shoots arising high on the trunk. Figure 3 shows a vine in Trial 6 with a symptomatic shoot arising from high on the trunk and an asymptomatic shoot arising from low on the trunk.

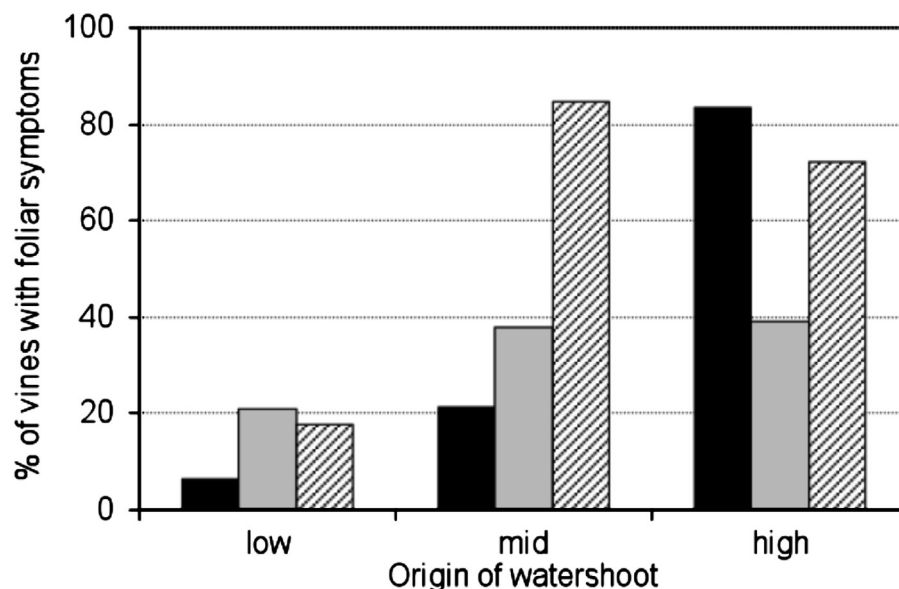


Figure 2. Incidence of foliar symptom recurrence on shoots of 'Shiraz' vines which originated from low (bottom third of trunk), mid (middle third) and high (top third) positions on the trunk, 6 years after remedial surgery using the high-cut method. Trial 3 (black) and Trial 4 (grey) were located in Coonawarra and Trial 6 (diagonal pattern) in Eden Valley, South Australia.



Figure 3. 'Shiraz' vine in Trial 6 with shoots exhibiting *Eutypa* dieback symptoms on the left cordon that emerged from high on the trunk, presumably from infected wood, and healthy shoots on the right cordon that emerged from the bottom of the trunk, presumably from uninfected wood.

## Discussion

Remedial surgery was generally an effective means of managing *Eutypa* dieback-affected grapevines in the commercial vineyards used in these trials. Reworking (trunk renewal, top-working) grapevines has been recommended for the management of *Eutypa* dieback since the 1980s (Carter, 1988). This is the first report of the effectiveness of remedial surgery in the medium to long term (4–9 years). Creaser and Wicks (2004) reported on the short-term effects of remedial surgery in a trial (Trial 2 in the present study) in which 63–76% of vines produced watershoots in the 3 years following surgery, and foliar symptoms were not detected on any of the new shoots. Con-

tinued assessment of these trials has revealed that symptoms appeared on a small number of vines in the fourth year after surgery, illustrating the importance of observing such trials in the longer term.

When rehabilitating diseased vines, the production of new watershoots is important for vineyard re-establishment. These results suggest that the greater the length of trunk retained, the greater the likelihood that watershoots will be produced in the first season following remedial surgery. However, the subsequent initiation of watershoots on low-cut vines over the next few years resulted in a similar incidence of shoots on both low and high-cut vines after 6 years. It is generally recom-

mended that vines failing to produce watershoots should be replaced either by layering shoots from a neighbouring vine (Nicholas *et al.*, 2001; Ahrens, 2010) or by planting a new vine.

These trials indicate that remedial surgery can reduce the severity of foliar symptoms in vines affected by *Eutypa dieback*. In Trial 1, severity of foliar symptoms in vines 4 to 6 years after treatment by remedial surgery was 0–2%, significantly less than that of untreated vines (17–26%). The variation in foliar symptoms between seasons observed in this study is consistent with observations by Sosnowski *et al.* (2007a), who suggested that climatic influences may be responsible, at least in part. In Trials 2 and 4, where foliar symptoms were recorded pre-surgery, the incidence of recurrence was reduced substantially, more so in low-cut (from 35% down to 6%) than high-cut (from 56% down to 29%) vines after 6 years. Furthermore, in adjacent blocks of the same vineyard (Trials 5 and 6), where internal infection was assumed to be similar, 9% of vines cut low and 59% of vines cut high exhibited foliar symptoms. Foliar symptoms reappeared earlier and more frequently in Trial 4 than in Trial 3, both of which involved high cut vines in the Coonawarra region. However, the two vineyards were managed independently and, although it was not possible to assess symptoms before surgery, it is considered likely that disease pre-surgery was more severe on vines in Trial 4 than in Trial 3.

There was no apparent relationship between incidence of staining in the trunk and subsequent appearance of foliar symptoms. This was evident from Trials 2 and 5 where a similar incidence of foliar symptoms resulted from low-cut surgery of vines with initial staining in 71 and 36% of trunks, respectively. It is important to note here that *E. lata* can penetrate apparently healthy wood up to 80 mm beyond any visibly stained wood (Sosnowski *et al.* 2007b) and this may have contributed to recurrence of foliar symptoms from apparently healthy trunks. It is also possible that stumps with no visible symptoms on the cut surface, and even 10 cm of healthy wood below the last visible stained wood, may have had compartments of infected wood further down the trunk, which may have originated via wounds made when watershoots were removed during routine vineyard maintenance in spring (Lecomte and Bailey, 2011). Furthermore, foliar symptoms do not always occur on vines in

which wood is infected by *E. lata* (Sosnowski *et al.*, 2007b), which may confound diagnosis.

Particularly evident from this study was the effect on subsequent symptom expression of the position on the trunk from which watershoots arose. In Trials 3, 4 and 6, the lower the point of emergence of the watershoot, the less likely symptoms were to recur in a vine. It is likely that wood infected with *E. lata* extended for varying distances down into the trunk, evident by the varying incidence of wedge-shaped staining remaining in the cross-section of stumps at different heights following surgery. If shoots arose from above colonised or stained wood, symptoms would be more likely to recur. In this study, the lowest available shoot on high-cut vines originated from the middle and upper sections of the trunk in 28–75% of vines. Therefore, cutting vines high on the trunk is less likely to control *Eutypa dieback* than cutting lower down if infection has progressed into the trunk.

Wound protection using Benlate or Garrison in Trials 3–6 to minimise the likelihood of new infection (Sosnowski *et al.*, 2008) and the relatively low recurrence of symptoms in the other trials suggest that foliar symptoms in new growth may be attributed to pre-existing infection not removed by surgery, such that the residual fungus continued to grow in the trunk, producing toxins or impairing vascular transport, which led to symptom development. Destructive assessment of vines to examine symptoms in the wood may have provided more accurate results, but would have precluded assessment of foliar symptoms over time.

Trials were established in commercial vineyards where reworking of vines was in progress and, with the exception of Trial 1, it was neither practical nor desirable for vineyard managers to leave untreated vines to serve as controls, which confounded statistical design and analysis. Reworking in these commercial vineyards (Trials 2–6) was undertaken due to loss of productivity because of *Eutypa dieback*, so it could be assumed that if vines had remained untreated, disease incidence and severity would have continued to cause concern, as was the case for the untreated control vines in Trial 1. Nevertheless, the research did address the need to provide information to assist growers with appropriate strategies of remedial surgery for *Eutypa dieback* management.

Anecdotal evidence suggests that yield and fruit quality are restored within several years of surgery in which the established root system is retained, although further research is required to substantiate this. Trials are being maintained so that effects of remedial surgery over a longer period can be assessed in the future. There is a need for replicated experiments in which untreated, but otherwise similar, vines in the same vineyard are included as controls. Also, further research to relate the presence or absence of staining, or indeed the amount of staining if it is not possible to remove all of it, to subsequent development of foliar symptoms would be useful.

In conclusion, remedial surgery can be used to treat vines with *Eutypa* dieback, but wood infected by *E. lata* should be removed as far as possible to increase the probability that shoots emerge from trunks from which the pathogen has been removed. These findings support the recommendation that growers remove all visibly stained wood from vines and then make a cut a further 10 cm below to increase the likelihood that mycelium of the pathogen advancing in unstained wood is removed (Sosnowski *et al.*, 2007b).

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