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Studies on the effect of water and temperature stress on grapevines inoculated with *Eutypa lata*

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Summary. Three independent studies were conducted in Australia and Spain to examine the effect of water and temperature stress on the development of Eutypa dieback in grapevines. In Adelaide, South Australia, 'Red Grenache' vines in pots were inoculated with *Eutypa lata* and subjected to various temperature and soil water regimes, then assessed for foliar symptoms, wood staining and colonization by the fungus. Vines subjected to a combination of heat or cold plus low or high soil moisture displayed more severe foliar symptoms, the most severe occurring on vines subjected to the hottest (30°C) and wettest (20–40% soil water content) conditions. Wood staining was not related to foliar symptom severity or to the temperature and moisture combination. Furthermore, there was no relationship between staining and mycelial growth, indicating that staining may not be an accurate reflection of the spread of *E. lata*. In Cabrils, Spain, 'Tempranillo' vines were subjected to water stress in pots and showed a significant reduction in shoot diameter growth, stomatal conductance and leaf water potential. Inoculation with *E. lata* also decreased the leaf water potential of stressed vines. Wood staining in inoculated vines was similar irrespective of watering treatment. Further investigations are needed to elucidate the relationship between stress and growth of *E. lata* in infected vines. Field experiments in two climatically different regions (Barossa Valley and Riverland) of South Australia suggested that water-stressed vines in a warm, dry environment may be more susceptible to infection of pruning wounds by *E. lata* than vines receiving standard watering.

Key words: *Vitis vinifera*, abiotic stress, drought, eutypiose, trunk disease.

Introduction

The fungus *Eutypa lata* (Pers.) Tul. & C. Tul. (= *E. armeniacae* Hansf. & M.V. Carter) causes Eutypa dieback, one of the major trunk diseases of grapevines (*Vitis* spp.) in many parts of the world (Carter, 1991). The ascospores of this fungus are dispersed by wind and rainsplash, enter grapevines through pruning wounds and germinate in the xylem vessels, after which the fungus colonizes the wood (Carter, 1960; Moller and Kasimatis, 1978). Wood symptoms include wedge-shaped areas of necrotic tissue on cross-sections of the external cankers that form around the sites of infection on the trunks or cordons (Moller and Kasimatis, 1981). Foliar symptoms, including stunted shoots and chlorotic leaves, often cupped and with tattered margins, are thought to be caused by toxic metabolites produced by the fungus in the wood and transported to the foliage (Moller and Kasimatis, 1981; Tey-Rulh *et al.*, 1991). However, Mahoney *et al.* (2005) suggested that fungal metabolites may interact with the wood at the point of

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production, disrupting the vascular structure and inhibiting nutrient transport, and thereby causing foliar symptoms. More recently, it was found that foliar symptoms were not directly related to extent of wood infection (Sosnowski *et al.*, 2007b). In the vineyard, foliar symptoms are expressed 3 to 8 years after inoculation (Carter, 1978; Tey-Rulh *et al.*, 1991); however, in greenhouse assays, after inoculation of cuttings or of young vines before bud break, symptoms have been reported to occur within 4 weeks (Péros and Berger, 1994), 6 weeks (Jung *et al.*, 2010) or 8 months (Sosnowski *et al.*, 2007b) .

Environmental factors are thought to be important in the expression of foliar symptoms of Eutypa dieback, and variation in the severity of symptoms has been recorded from year to year in France (Dumot *et al*., 2004), USA (Butterworth *et al*., 2005) and Australia (Sosnowski *et al*., 2007a). Sosnowski *et al*. (2007a) reported an increase in incidence of foliar symptoms to be associated with greater winter rainfall and suggested that increased water availability may facilitate the transport of toxins to the foliage in spring. They also reported that a lower disease incidence was associated with higher temperature in spring. It was proposed that as vines grow more vigorously in warmer conditions, the ability of fungal metabolites to reach the foliage at toxic concentrations is reduced, so that the increased plant biomass leads to a reduction in the expression of foliar symptoms.

Regulated deficit watering and partial rootzone drying involve controlling and managing water stress by watering to less than the full requirement of the vines, to control vigour and increase fruit quality while conserving water (McCarthy *et al*., 2002). Furthermore, climate change may lead to more extreme environmental conditions for grapegrowing in the future, especially by increasing water stress in the summer months (Schultz, 2000; Jones, 2005). Low soil water content or a water deficit (Hardie and Considine, 1976; Smart and Coombe, 1983; Lovisolo and Schubert, 1998) plus high temperature (Kriedemann and Smart, 1971) have been implicated as causes of stress on grapevines. Water stress exacerbates disease symptoms associated with *Phaeomoniella chlamydospora*, a pathogen involved in esca and Petri disease (Ferreira *et al*., 1999; Edwards *et al*., 2007a, 2007b), and *Xylella fastidiosa,* which causes Pierce's disease (Goodwin *et al*., 1988; Thorne *et al*., 2006).

The environment and vine health are therefore likely to influence the expression of foliar symptoms of Eutypa dieback, and the colonisation of the wood by this pathogen; however, to date there are no reports on this topic. Research on the effects of water and temperature stress on grapevines was initiated independently in Australia and Spain. In this paper, we report on three experiments conducted concurrently in the two countries and draw some conclusions from the combined results.

Materials and methods

Effect of combined water and temperature stresses on potted vines inoculated with *Eutypa lata* (Adelaide, South Australia)

An experiment was undertaken at the Plant Research Centre on the Waite Campus, Urrbrae, South Australia to evaluate the combined effect of soil water content and air temperature on the expression of foliar symptoms of Eutypa dieback and the growth of *E. lata* in the vine wood. One hundred and sixty two 1-year-old grapevines (*V. vinifera* cv. Red Grenache) were planted into pots filled with a potting mix on 8 February 2008. Vines were transferred to a shade house, pruned to two-bud spurs, watered by hand as required and fertilised with Thrive (Yates, Padstow, Australia) following the manufacturer's instructions.

Eutypa lata (isolate DAR79088, formerly B003) was cultured on PDA (Oxoid Ltd, Basingstoke, UK) amended with 0.025 g L^{-1} streptomycin sulphate (Sigma-Aldrich, St Louis, MI, USA) and incubated at 23°C under fluorescent lighting (Phillips TLD 36W/865 cool daylight; Philips Electronics N.V., Amsterdam, the Netherlands) for 12 h each day. On 21 February 2008, a mycelium plug (5 mm diameter) taken from the margin of 7-day-old cultures was inserted into a 5-mm diameter hole drilled into the stem of each of 81 vines, as described by Péros and Berger (1994) and Sosnowski *et al*. (2007b). Sterile PDA plugs were inserted into a further 81 vines, which served as controls. All inoculation sites were sealed with Parafilm® (Pechiney Plastic Packaging, Menasha, WI, USA).

Vines were maintained in the shade house until 15 June 2008, when a canopy made of clear polyethylene sheets was placed over the vines to pro-

tect them from rain. Soil moisture regimes were imposed by hand watering with the same volume of water at varying time intervals to maintain the soil at three levels of volumetric water content: high (20–40%), moderate (10–25%) or low (5–20%) during dormancy. The volumetric soil moisture content was measured periodically using time domain reflectrometry (TDR; Trase, Soilmoisture Equipment Corp., Santa Barbara, CA, USA).

At the onset of bud burst (17 September 2008) the pots were transferred to one of three controlled environment rooms (CER; Phoenix-E, Camarillo, CA, USA) set at 14, 22 or 30°C. Temperature was monitored using data loggers (Tinytag; Gemini data loggers, Chichester, UK) and confirmed that the air temperature varied between 28 and 31.5°C in the CER set at 30°C, between 20 and 25°C in the 22°C CER, and between 14 and 19°C in the 14°C CER. The experiment comprised a split-plot factorial design with 162 potted vines, nine vines (replicates) per treatment. The three temperatures formed the main plots and the three soil water regimes formed the sub-plots.

Watering continued as described above. On 4 December 2008, the leaf water potential of all vines was measured with a 3000 series Pressure Chamber (Soilmoisture Equipment Corp.) using industrial grade nitrogen (BOC Gases, Sydney, Australia) to pressurize the chamber.

Foliar symptoms were assessed on 7 November 2008, when the shoots of the control vines were between 50 and 70 cm long. The severity of the Eutypa dieback foliar symptoms was expressed as the difference in the length of stunted shoots on inoculated vines and symptomless control vines as a percentage of the length of shoots on control vines (Sosnowski *et al.,* 2007b).

Vines were removed from the pots on 22 April 2009 to assess the extent of stained wood and spread of mycelium of *E. lata*. Green shoots and roots were discarded and the bark was then removed using a sharp knife. The extent of staining above and below the inoculation point was measured. Stems were surface sterilised for 12 minutes in 2.5% sodium hypochlorite containing a drop of Tween 20 surfactant (Sigma-Aldrich, St Louis, MI, USA) per 500 mL of solution. Cross-sections (2 mm thick) of the stems were cut at 5-mm intervals to 25 mm above and 75 mm below the inoculation point using secateurs, which were sterilised between samples by immersing the blades in 99% ethanol followed by flaming. The wood sections were then placed on PDA amended with 0.025 g L^{-1} streptomycin sulphate and incubated at 23°C for 7 days under fluorescent lighting for 12 h each day and then assessed for *E. lata* based on colony morphology (Carter, 1991).

All data were subjected to ANOVA using Statistix for Windows v. 4.1 (Analytical Software, Tallahassee, FL, USA). Differences between foliar symptom severity (%), length of stained vascular tissues and maximum distance of colonisation by mycelium of *E. lata* were compared by the least significant difference (LSD) test for all treatments.

Effect of water stress on potted vines inoculated with *Eutypa lata* (Cabrils, Spain)

An experiment was conducted at Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Cabrils, Spain to study the effect of water stress on grapevines infected with *E. lata*. In March 2008, 160 1-year-old grapevines (*V. vinifera* cv. Tempranillo grafted on Richter 110 rootstock) were planted into 3 L pots filled with a sand:peat mixture (6:1, v:v; Floratorf peat, Floragard, Oldenburg, Germany). Vines were maintained in an air-conditioned greenhouse (35°/20°C ± 3°C; 40/80% ±10% RH; natural light), and were trimmed to one shoot per vine. Vines were fertilised weekly with 10 mL of Hoagland's solution (Hoagland and Aaron, 1950). Vines were divided into four groups (40 plants per group) each of which received one of the following treatments : 1) water stress with inoculation, 2) water stress without inoculation, 3) sufficient watering with inoculation, 4) sufficient watering without inoculation. Watering of vines from groups 1 and 2 was restricted from 9 June to 13 October 2008 in order to induce water stress. Water restriction was imposed by periodically estimating water loss after weighing five additional vines having the same characteristics (cultivar and potting conditions), and then watering all vines in the stress groups with a volume equivalent to the mean water loss. On average, each water-stress cycle was 5–7 days, and the water supplied was 300–600 mL per vine. Vines with sufficient watering were supplied with 120 mL of water each day.

Plants were inoculated on 20 June 2008 with *E. lata* (strain CBS 121487) prepared by growing the fungus on potato dextrose agar (PDA; Difco

Laboratories, Detroit, MI, USA) at 25°C for 7 days under fluorescent light (Osram L 18W/840; Osram GmbH, Munich, Germany) for 12 h each day. A wound (15×5 mm) into the xylem was made with a sterilised scalpel on the stem of each vine, 10 cm below the graft union. A mycelial plug (5 mm diameter) obtained from the margin of a colony was placed in the wound with the mycelium facing the stem, and the wound was wrapped with Parafilm®. Control plants received sterile PDA plugs instead of mycelial plugs.

A linear variable differential transformer (LVDT; Solartron Metrology, Bognor Regis, UK) was attached to the shoot of eight plants in each treatment group on 26 June 2008 in order to monitor the growth of the shoot diameter. The LVDT signals were recorded with a data logger (Campbell Scientific Ltd., Logan, UT, USA; model CR10X, with AM416 multiplexer) and the daily recording at 06:00 hours was used in the graphical representation of the growth of the shoot diameter over time. The accumulated growth of the shoot diameter at the end of the experiment was used to compare the effect of the treatments. Recordings were carried out from 28 June 2008 to the end of the experiment, 13 October 2008.

Five vines randomly selected from the remaining 32 plants of each treatment group were used to measure the stomatal conductance and the leaf water potential every 7–14 days, starting on 17 July 2008 and ending on 10 October 2008. All measurements were taken between 11:00 and 13:00 hours. Stomatal conductance was measured with a Li-1600 steady-state porometer (Li-Cor, Li-Cor Inc., Lincoln, NE, USA) on one leaf in the upper third of the canopy of five randomly selected vines per group. The leaf water potential was measured for one leaf detached from each of the vines using a pressure pump (Soil Moisture Equipment, Santa Barbara, CA, USA) following Scholander *et al.* (1965).

At the end of the experiment, on 13 October 2008, the length of the vascular necrosis of each vine was recorded by removing the bark from the stem and measuring the extent of discolouration upwards and downwards from the edge of the inoculation wound. Wood pieces (two to four) were excised from the necrotic stem tissues about 3–5 mm from the inoculation site, surface sterilised (70% ethanol, 4 min), plated on PDA and incubated as described above to confirm the presence of the pathogen.

The experimental data were analysed using the SAS Enterprise Guide v4.1 statistical package (SAS Institute Inc., Cary, NC, USA), with 'water regime' and 'inoculation' as independent factors. Prior to analysis, the leaf water potential data were converted to positive values, and data for all dependent variables were log-transformed if necessary. All data were analysed with a two-way ANOVA design. Mean values were compared using the Tukey's test if appropriate.

Effect of deficit irrigation on field vines inoculated with *Eutypa lata* (Barossa Valley and Riverland, South Australia)

Two field trials were established in South Australia, at the Nuriootpa Research Centre in the Barossa Valley (34° 28' 34.98" S, 139° 0' 30.62" E) and at the Loxton Research Centre in the Riverland (34° 26' 17.59" S, 140° 35' 52.43" E), to evaluate the ability of *E. lata* to infect and establish in wounds of vines receiving different watering regimes. In the Barossa Valley, Ruby Cabernet vines (*V. vinifera*), planted on their own roots in 1993 were either watered fully (100% of the standard allocation each year based on vine requirements) or not watered at all (0%), beginning in September 2006. In the Riverland, Cabernet Sauvignon vines (*V. vinifera*) grafted on Ramsey rootstock and planted in 1992 received one of three watering levels; 100, 60 and 25% of the standard watering program, beginning in September 2006. All vines were spur-pruned with two (Barossa Valley) or four (Riverland) cordons.

Long-term weather data for the two locations were provided by the Australian Bureau of Meteorology. Mean annual rainfall in the Riverland and the Barossa Valley is 261 and 472 mm, respectively, and mean maximum temperature 23.8 and 21.5°C, respectively.

One-year-old shoots were pruned to two spurs on 7 and 8 July 2008 in Barossa Valley and Riverland, respectively. In each trial, 50 shoots were inoculated for each watering level. Within an hour of pruning, the wounds were inoculated with a suspension of 500 ascospores mL^{-1} of *E. lata* as described by Sosnowski *et al.* (2008). On 12 July, wounds were re-inoculated with a suspension of 500 ascospores mL^{-1} of *E. lata* to improve the likelihood of successful infection. Uninoculated con-

trols were not included in these experiments since natural infection did not exceed 5% in previous experiments (data not shown), therefore the potential contribution of such natural infection was thought to be minimal.

On 12 June 2009, treated canes were excised from the vines and taken to the laboratory. Samples (3×2×2 mm) from the interface of stained and healthy wood were cultured on PDA amended with 0.025 g L^{-1} streptomycin sulphate as described above. The susceptibility of wounds to infection by *E. lata* was determined by the mean percent recovery of *E. lata* from the treated canes and all data were subjected to analysis of variance and compared by LSD (Statistix for Windows).

Results

Effect of combined water and temperature stresses on potted vines inoculated with *Eutypa lata* (Adelaide, South Australia)

ANOVA of the leaf water potential revealed an interaction effect between temperature and watering ($P<0.05$), but no effect of inoculation ($P>0.05$) on leaf water potential. The leaf water potential for vines growing in low soil moisture at 30°C (-0.87 MPa) was significantly less than that with all other treatments (Table 1). Vines in moist soil at 14°C exhibited the highest leaf water potential (-0.26 MPa). Foliar symptoms of Eutypa dieback, including shoot stunting, chlorosis and cupped leaves with necrotic margins, were seen on vines inoculated with *E. lata* 8 months after inoculation. No symptoms were seen on the non-inoculated controls. Vines grown at 30°C in moist soil expressed the most severe symptoms (mean symptom severity = 28%), significantly more severe ($P<0.05$) than the symptoms of vines grown at 14 and 30°C with moderate soil moisture, or at 22°C with low soil moisture (1–4% mean symptom severity) (Table 2). Furthermore, the four most extreme combinations of temperature and moisture (i.e. hot/wet, hot/dry, cold/wet and cold/dry) coincided with the most severe foliar symptoms (11–28%) and all the remaining combinations, involving moderate temperature and moderate moisture produced only slight symptoms (1–8%), although the difference was not significant.

The mean extent of staining associated with inoculation sites on stems ranged from 19 to 46 mm, and was not related to foliar symptom severity or to any temperature and moisture combination (Table 2). Staining was also observed on the control vines, associated with the point of insertion of blank PDA plugs, ranging in length from 11 to 42 mm (data not shown). Furthermore, staining was often associated with wounds made during propagation, which were above the point of inoculation.

The mean extent of colonisation by *E. lata* varied from 26 to 68 mm, although there were no significant differences between treatments (Table 2). The smallest extent of colonisation (26–46 mm) occurred in the four most extreme temperature/moisture combinations.

Effect of water stress on potted vines inoculated with *Eutypa lata* (Cabrils, Spain)

Monitoring of the shoot diameter showed that water-stressed vines grew less than non-stressed vines irrespective of the inoculation treatment (Figure 1). In general, vines grew from the begin-

Table 1. Leaf water potential (pressure in MPa) of all grapevines (*Vitis vinifera* cv. Red Grenache), inoculated and control treatments combined, subjected to nine combinations of soil water content and air temperature for 11 weeks in a shadehouse in South Australia on 4 December 2008 using a pressure chamber. Nine replications per treatment.

Soil water content	Leaf water potential[a]		
	14ºC	22ºC	30ºC
Low	-0.47 c	-0.65 b	-0.87 a
Moderate	-0.41 c	-0.46 c	-0.47 c
High	-0.26 d	-0.38 cd	-0.40 c

[a]Mean values within columns followed by the same letter are not significantly different (LSD; $P=0.05$).

Table 2. Severity of foliar symptoms, total distance of staining and mycelial colonisation of 1-year-old grapevines (*Vitis vinifera* cv. Red Grenache) above and below the point of inoculation with *Eutypa lata* and incubated in controlled environment rooms maintained at 14°C, 22°C or 30°C with high (20–40%), moderate (10–25%) or low (5–20%) soil water content for 7 months. Nine replications per treatment.

Air temperature (°C)	Soil water content	Foliar symptom severity [a] (%)	Staining distance [a] (mm)	Colonisation distance [a] (mm)
30	High	28 a	33 ab	26 a
30	Moderate	4 b	26 ab	46 a
30	Low	15 ab	19 b	39 a
22	High	8 ab	24 b	61 a
22	Moderate	6 ab	22 b	45 a
22	Low	1 b	46 a	50 a
14	High	11 ab	40 ab	39 a
14	Moderate	1 b	20 b	68 a
14	Low	11 ab	20 b	46 a

[a] See Table 1.

ning of the experiment until the first week of August 2008; thereafter shoot growth ceased and the diameter of the vines remained constant for the remainder of the experimental period. For three groups, *viz*. all stressed vines irrespective of the inoculation treatment and those non-stressed and inoculated vines, a decrease in shoot diameter preceded the cessation of growth, although this decrease was smaller in the stressed and inoculated vines (Figure 1). Foliar symptoms of Eutypa dieback were not observed during the experimental period.

ANOVA detected that the factor 'water regime' was significant ($P<0.05$) for all dependent variables analysed except for the length of necrosis, whereas 'inoculation' was only significant ($P<0.05$) for necrosis and leaf water potential. No interactions between these factors were detected.

At the end of the experiment, the water deficit was associated with a reduction in the shoot diameter, the stomatal conductance and the leaf water potential of the vines (Figure 1, Table 3), with a mean percent decrease of 48, 75 and 25%, respectively, compared with the corresponding figures for non-stressed vines (combining data of inoculated and non-inoculated vines). The mean leaf water potential of non-stressed vines during the experiment was -1.01 MPa, significantly greater ($P<0.05$) than that of the stressed vines, which was -1.26 MPa. In addition, *E. lata* significantly decreased the leaf water potential of stressed vines, from -1.17 MPa (non-inoculated) to -1.35 MPa (inoculated), while inoculation of non-stressed vines reduced the leaf water potential only slightly (Table 3).

The stomatal conductance and the leaf water potential of the vines during the experimental period are shown in Figure 2. The stomatal conductance of stressed vines was lower than that of non-stressed vines at all assessment dates. In addition, the values and trends for both inoculated and non-inoculated vines within each watering regime were very similar throughout the experiment, although the stomatal conductance of inoculated vines was often slightly greater than that of non-inoculated vines. The leaf water potential showed a similar trend, with stressed vines mostly having a lower leaf water potential (Figure 2).

All inoculation wounds in all treatment groups healed successfully within the experimental period, but necroses extending upwards and downwards from the wounds were observed in nearly all vines. The length of the necroses,

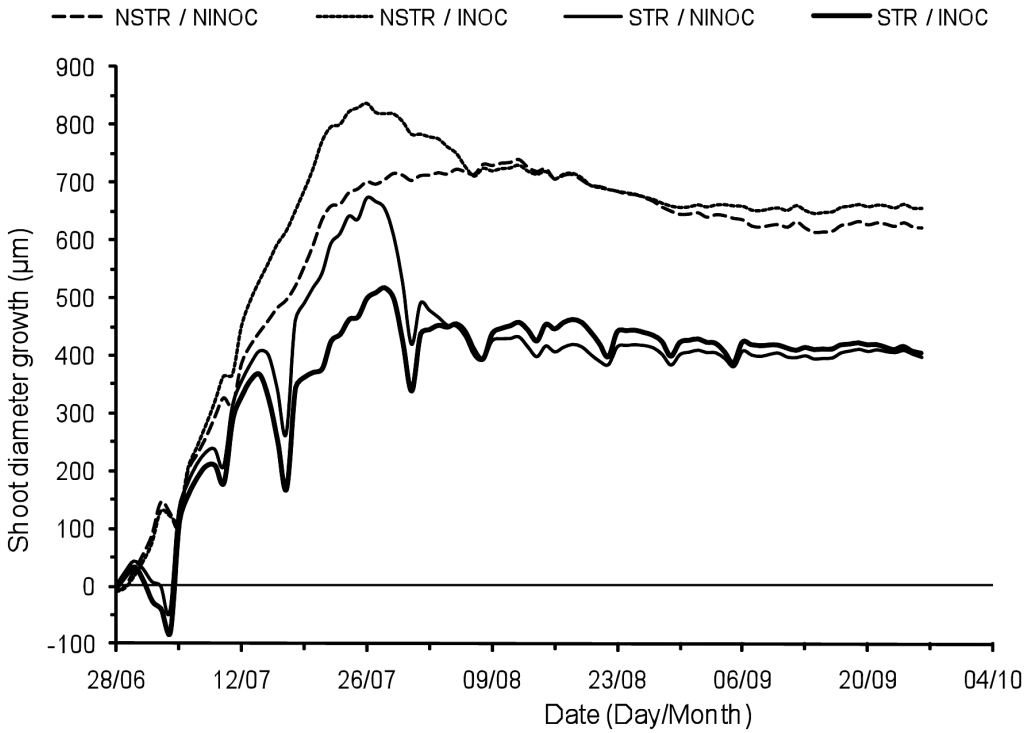

Figure 1. Shoot diameter growth (taken daily at 06:00 hours) of one-year old grapevines (*Vitis vinifera* cv. Tempranillo) inoculated with *Eutypa lata* or left uninoculated and subjected to one of two watering regimes. INOC, inoculated with *E. lata*; NINOC, non-inoculated, STR, water-stressed, NSTR, non-stressed.

as identified by the staining of vascular tissues, ranged from 5 to 37 mm in inoculated vines, and from 0 to 3 mm in control vines. The mean extent of necrosis in stressed and inoculated vines was 20 mm and in non-stressed and inoculated plants was 15 mm, but this difference was not significant (Table 3).

Effect of deficit irrigation on field vines inoculated with *Eutypa lata* (Barossa Valley and Riverland, South Australia)

In the Riverland, *E. lata* was recovered most frequently from vines that received reduced watering (Figure 3A). The frequency of recovery increased significantly ($P<0.05$) from 74% for fully

Table 3. Mean values of stomatal conductance and leaf water potential taken every 7–14 days, and shoot diameter growth and extent of necrosis taken at the end of the experiment, in one-year old grapevines (*Vitis vinifera* cv. Tempranillo) inoculated with *Eutypa lata* or left uninoculated and subjected to one of two watering regimes for 4 months.

Water regime	Pathogen inoculation [a]	Stomatal conductance[b] (mmol H_2O m^{-2} s^{-1})	Leaf water potential[b] (MPa)	Shoot[b] diameter (µm)	Staining distance[b] (mm)
No stress	Non-inoculated	283 a	-0.95 a	617 a	1 b
No stress	Inoculated	311 a	-1.05 ab	647 a	15 a
Stress	Non-inoculated	75 b	-1.17 b	393 b	1 b
Stress	Inoculated	76 b	-1.35 c	395 b	20 a

[a] Replications per treatment: five plants for stomatal conductance and leaf water potential measurements, and eight plants for shoot growth and staining distance measurements.
[b] See Table 1 according to Tukey Test (P= 0.05).

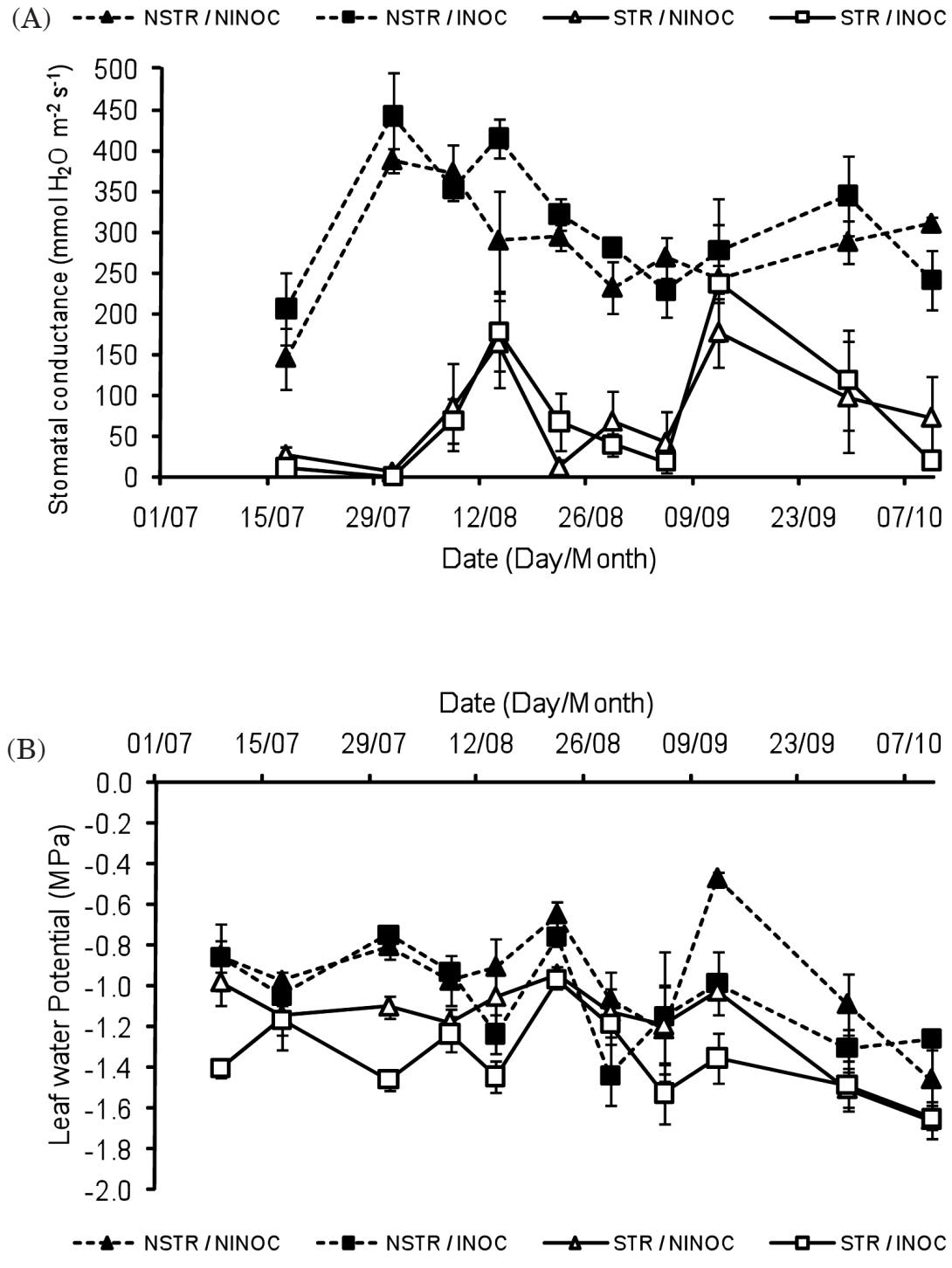

Figure 2. Stomatal conductance (A) and leaf water potential (B) data for one-year old grapevines (*Vitis vinifera* cv. Tempranillo) inoculated with *Eutypa lata* or left uninoculated and subjected to one of two watering regimes. INOC, inoculated with *E. lata*; NINOC, non-inoculated, STR, water-stressed, NSTR, non-stressed.

irrigated vines to 95% for vines that received only a quarter of the standard watering program. In the Barossa Valley, there was no effect of irrigation, with *E. lata* isolated from around 90% of inoculated shoots from both fully and unwatered vines (Figure 3B).

Discussion

The results suggest that abiotic stress factors such as temperature and/or water availability may play a role as either predisposing or inciting factors (*sensu* Manion, 1991) causing symptoms of Eutypa dieback in grapevine. Vines subjected to a combination of heat or cold plus low or high soil moisture displayed more severe foliar symptoms; however, neither of these factors alone produced severe symptoms. This suggested that grapevine is more likely to express symptoms when its health is compromised by severe environmental stress (Kriedemann and Smart, 1971; Hardie and Considine, 1976; Smart and Coombe, 1983; Lovisolo and Schubert, 1998). In contrast, mycelial colonisation tended to be lower in vines subjected to a combination of extreme conditions, perhaps because optimal growth requirements for *E. lata* are 22–25°C and at least 90% relative humidity (Munkvold and Marois, 1995). In an earlier study by Sosnowski *et al*. (2007b), the maximum growth rate of *E. lata* in potted 'Red Grenache' vines was 115 mm per year. However, the experimental design in the present study did not permit the detection of *E. lata* in wood more than 75 mm above or 25 mm below the point of inoculation, and it is likely that at least in some instances where the pathogen was recovered from these points, colonisation was underestimated. This limitation may have contributed to the failure to identify significant differences between treatments. In future studies, *E. lata* should be isolated from the entire length of the stem. Moreover, the experiment was terminated one year after inoculation and a longer interval may have allowed further development of foliar symptoms and mycelial growth and hence

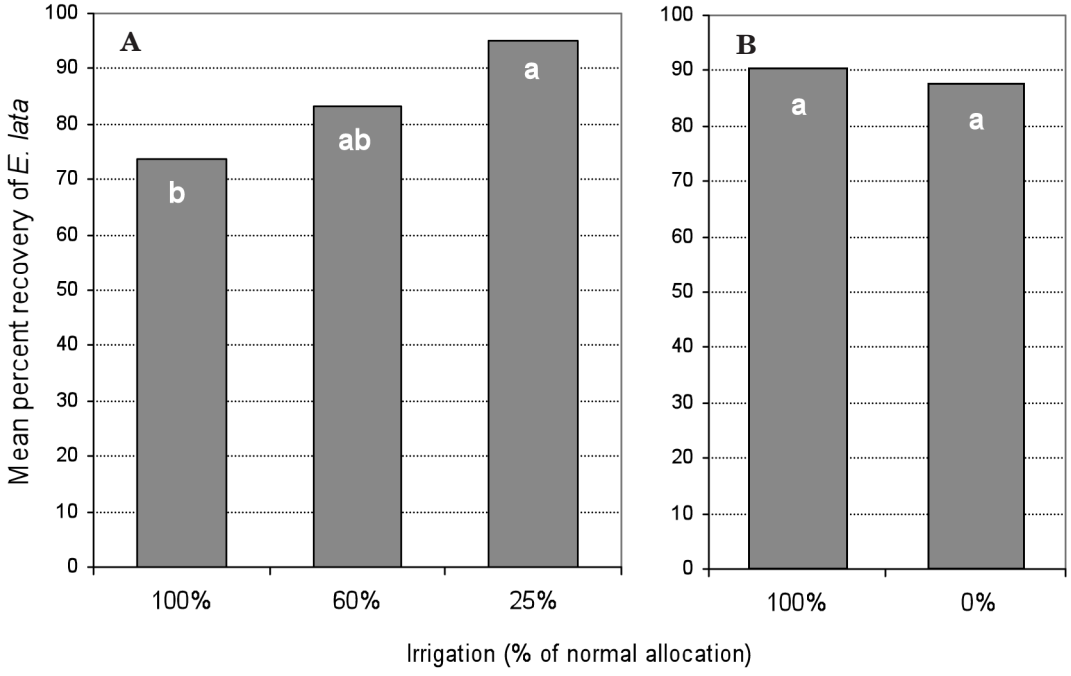

Figure 3. Effect of irrigation treatment (based on percentage of normal allocation in each year from 2006) on susceptibility of pruning wounds inoculated with *E. lata* in July 2008 on *Vitis vinifera* cultivars (A) 'Cabernet Sauvignon' in the Riverland and (B) 'Ruby Cabernet' in the Barossa Valley of South Australia. Mean percent recovery was based on assessments of treated canes collected in June 2009. Mean percentages with the same letter (a or b) are not significantly different (LSD; P=0.05).

may distinguish more clearly between treatments (Sosnowski *et al.,* 2007b).

The water and temperature stress experiment conducted in Australia showed no relationship between wood staining and mycelial growth of *E. lata*, indicating that staining may not be an accurate reflection of the spread of *E. lata* in the woody tissue of grapevine. In fact, a considerable amount of staining not associated with *E. lata* occurred in the control vines. Factors such as wound response, induced by the vine defence system, may play a role in the presence and extent of staining (Sosnowski *et al.*, 2007b). Likewise, micro-organisms other than *E. lata,* such as the Botryosphaeriaceae (Úrbez-Torres and Gubler, 2009) and other species of the Diatrypaceae (Trouillas and Gubler, 2007), may cause staining in grapevine wood. Since staining was sometimes associated with wounds made during the propagation process, and above the point of inoculation, other microorganisms may have entered the vines through these wounds. However, in the water stress experiment conducted in Spain, staining in control vines had a length of only around 1 mm, and staining by other factors (e.g., infection by other agents) was infrequent, so that the extent of staining could be attributed to *E. lata*. Although the extent of discolouration attributed to *E. lata* in the water stress experiment in Spain was greater in water-stressed vines than in well-watered vines, the extent of colonisation by the pathogen was not assessed. Variation in the findings from both controlled environment experiments made it difficult to draw sound conclusions; however, the results suggest there is some interaction between Eutypa dieback and the stress caused by manipulating the water supply and the temperature. This would be consistent with Ferreira *et al.* (1999), who found that grapevines inoculated with the trunk-inhabiting fungus *P. chlamydospora* experienced significantly more severe dieback when they were also water-stressed than when they were not so stressed.

Vines are in water deficit at water potential below -1.0 MPa (Hellman, 2010). In the water stress experiment, the leaf water potential of vines with a watering schedule designed to impose water stress exceeded the water deficit (-1.2 to -1.3 MPa), indicating that they were stressed, whereas that for vines watered to sufficiency had a water potential exceeding -1.0 MPa throughout the experimental period. In comparison, the leaf water potential of plants in the temperature and water stress experiment did not fall below -0.9 MPa, but there appeared to be a gradient of leaf water potential from the warm and dry treatments to the cool and wet treatments. In general, the leaf water potential was lower for the vines growing in the warmer and drier conditions.

In this study, water- but not temperature-stressed vines inoculated with *E. lata* had a lower leaf water potential than non-inoculated vines, but this was not the case for vines receiving both water and temperature stress. In the latter experiment, however, the leaf water potential was measured only once, whereas in the former experiment it was measured at 7-14 day intervals. In comparison, Edwards *et al*. (2007a and 2007b) found that the leaf water potential was lower in potted vines inoculated with *P. chlamydospora* than in non-inoculated vines. Similarly, Goodwin *et al.* (1988) reported that the water potential of leaves of grapevines with Pierce's disease, caused by the bacterium *Xylella fastidiosa*, was lower than that of healthy vines. In another study, infected vines exposed to water deficit expressed more extensive symptoms of Pierce's disease than did well watered vines (Thorne *et al.*, 2006). In all these reports, the lower water potential was attributed to the fungal or bacterial pathogen occluding the xylem vessels, and the current study suggests that *E. lata* also causes such occlusions. This needs to be confirmed in further research. Stomatal conductance was not affected by *E. lata*, however, it was stated to be greater in vines inoculated with *P. chlamydospora* and *X. fastidiosa* than in non-inoculated vines (Edwards *et al*., 2007a and 2007b; Goodwin *et al.*, 1988). This difference may be due to the leaf necrosis associated with both Pierce's disease and esca/Petri disease. In the current study no foliar symptoms of Eutypa dieback were observed in the water stress experiment where stomatal conductance was measured.

Since foliar symptoms of Eutypa dieback take up to 8 months to develop when young vines are inoculated (Sosnowski *et al*., 2007b), it was not surprising that foliar symptoms were not observed during the water stress experiment, since the vines were were grown in these regimes for only 4 months. Furthermore, a preliminary experiment on the effects of water and temperature stress in

Australia, which also lasted for only 4 months, did not produce any foliar symptoms (data not shown). In the subsequent experiment, the vines were inoculated before dormancy and stress treatments were -applied for 9 months before symptom expression was assessed.

In a warm, dry region, deficit watering may decrease the plant defence response and hence increase the susceptibility of pruning wounds to infection by *E. lata*. In the Riverland of South Australia, reducing the watering from 100% of the standard program to 25% significantly increased the susceptibility of pruning wounds to *E. lata*. However, in the Barossa Valley, there was no difference between irrigated and non-irrigated vines. The mean annual rainfall in the Barossa Valley is almost twice that of the Riverland and the mean maximum temperature is 2°C lower. This suggests that vines at the former site were less stressed by environmental factors, and this may in part explain why the percentage of vines infected with *E. lata* was similar irrespective of the watering regime. Ruby Cabernet is a cross between Cabernet Sauvignon and Carignan (Jackson, 2000), and its susceptibility to Eutypa dieback is not known, so this could also be a factor.

This is the first report that the abiotic stresses caused by temperature and soil water influence the infection of grapevine by *E. lata*, and the expression of Eutypa dieback symptoms. Since increasingly extreme environmental conditions have been predicted as a result of global climate change (Schultz, 2000; Jones, 2005), it is suggested that Eutypa dieback may be exacerbated in the future. Further research on the effects of heat, drought or deficit watering and other abiotic stresses on Eutypa dieback and other trunk diseases may assist in developing disease management strategies for a changing environment.

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