A quantitative test for heat-induced cell necrosis in vascular cambium and secondary phloem of *Eucalyptus obliqua* stems

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Yasika Medhavi Subasinghe Achchige, Liubov Volkova, Andrew Drinnan and Christopher J. Weston have conceived and designed the experiment. Yasika Medhavi Subasinghe Achchige performed the experiment. Yasika Medhavi Subasinghe Achchige, Liubov Volkova and Christopher J. Weston analysed the data. Andrew Drinnan provided equipment for microscopic analysis. All authors contributed to writing of the manuscript.

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This article has been accepted For publication And undergone full peer review but has Not been through the copyediting, typesetting, pagination And proofreading process, which may lead to differences between this

Version And the Version of Record. Please cite this article as doi: 10.1093/jpe/rtaa081.

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Abstract

Aims

Exposure of *Eucalyptus* tree stems to the radiant heat of forest fires can kill cambial cells and their embedded regenerative meristems, thus preventing epicormic resprouting and recovery of the tree. Currently there is no tissue-level method to quantify the viability of cambial cells in *Eucalyptus* following heat exposure. The first aim of this study was to adapt and validate the tetrazolium reduction method of testing for cell viability in *Eucalyptus*. The second aim was to apply the method to establish a threshold level of cambium cell viability in *Eucalyptus obliqua* to enable the identification of a critical temperature.

Methods

The study used the tetrazolium reduction method to quantitatively determine phloem-cambium cell viability in *Eucalyptus*. Circular sections of bark with underlying phloem and cambium were cut from mature *Eucalyptus obliqua* and samples ranging in mass from 1 mg to 30 mg were exposed for 1 minute to temperature treatments ranging from 20°C to 85°C and kept for 20-22 hours at room temperature in 0.8% 2,3,5 triphenyl tetrazolium chloride (TTC) to test for cell viability. The 1,3,5 triphenyl tetrazolium formazan (TPF) formed was cold extracted with ethanol and quantified as absorbance at 485 nm.

Important findings

The TTC reduction method reliably quantified a decline in cell viability with rising temperature in tissue sections that included vascular cambium, and identified 60°C as the critical temperature for cambium-phloem cells of *Eucalyptus* species. Cell viability, calculated as [TPF Treatment°C] / [TPF 20°C], declined by 90% between 20°C and 85°C. The cell viability results confirmed that significant tissue necrosis occurred in *Eucalyptus* at temperatures between 50°C and 70°C, after one minute of *in-vitro* tissue heating. The decline in cell viability with increasing temperature shown by the TTC method was consistent with an independently derived count of live cells following temperature treatment and neutral red staining.

Key words: cell viability, critical temperature, *Eucalyptus*, neutral red, 2,3,5 triphenyl tetrazolium chloride.

Introduction

In plant cell systems 2,3,5 triphenyl tetrazolium chloride (TTC) is the most commonly used tetrazolium salt to detect the presence of respiring cells, with the number of viable cells directly related to the production of triphenyl tetrazolium formazan (TPF or pink formazan; e.g. Towill and Mazur 1975, Ruf and Brunner 2003). In plants, TTC reduction to TPF has been applied as either a qualitative test staining for live cells (e.g. Pellett and Heleba 1998, Bova and Dickinson 2005) or as a quantitative assay for cell viability (e.g. Steponkus and Lanphear 1967, Towill and Mazur 1975, Caldwell 1993). Among these TTC reduction studies of cell degradation only a few have considered tree tissues (e.g. Clemensson-Lindell 1993, Bova and Dickinson 2005). The latter authors used a tetrazolium staining technique, with the formation of pink formazan in live cells, to indicate the depth of necrosis in heat-treated *Acer* and *Quercus* stems subjected to fires - measured with callipers in thin cross sections of trunk removed from the tree after fire.

As far as we are aware there is only one study of heat degradation of tree stem cells based on quantifying TPF production following heat treatments. Dickinson et al. (2004) applied a quantitative test of cell viability to estimate tissue necrosis arising from heat transfer through the bark into the cambium of the northern hemisphere gymnosperms *Pinus ponderosa* and *Pseudotsuga menziesii*, and the angiosperms *Acer rubrum* and *Quercus prinus*. The quantitative TTC test allowed Dickinson et al (2004) to model stem tissue damage and to predict tissue necrosis and tree mortality after fires.

Given the increasing incidence of fires in forests globally, driven by climate change and increasing anthropogenic ignitions (Ryan and Frandsen 1991, van der Werf et al. 2010, Gričar et al. 2020), it is surprising there are so few detailed studies linking fire characteristics with stem heating and the fate of phloem and cambial cells (e.g. Bova and Dickinson 2005). A better understanding of the specific impacts of fire on tree survival is also highly relevant to the application of prescribed fire that has become important in the management of forests for fuel reduction, regeneration and maintenance of ecological values (McCaw 2013). For example, a better knowledge of the interaction between fuel load, fire-line intensity and bark properties protecting cambium from critical temperatures would allow for more confident specification of planned burn conditions to maximize tree survival (or limit tree mortality). The characteristics of bark, such as thickness and moisture content, directly impact heat transfer to the vascular cambium and the development of lethal temperatures (Vines 1968; Costa et al. 1991; Bova and Dickinson 2004; Jones et al. 2006; Michaletz and Johnson 2007; Lawes et al. 2011; Wesolowski et al. 2014).

When phloem and cambial cells are raised to critical temperatures, often assumed to be 60°C (Alexandrov 1964; Wagner 1973), cell viability declines when proteins of the plasma membrane and cytoplasm denature, disrupting enzyme-mediated cell metabolism (Alexandrov 1964; Daniell et al. 1969; Levitt 1956). The death of a large proportion of cambial tissue can limit production of secondary phloem and xylem (Bauer et al. 2010; Bär et al. 2019, Bova and Dickinson 2005; Costa et al. 1991; Reifsnyder et al. 1967), and disrupt water and photosynthate movement (Michaletz and Johnson, 2007). Tree death may follow as root starvation and water stress develop (Kramer and Kozlowski 1960; Nobel 2005). For *Eucalyptus*, a large genus of trees that dominates Australian forest formations, the elevation of stem cambial tissues to critical temperature may also kill epicormic meristems from which many species regenerate photosynthetic capacity following fire that scorches or removes the tree canopy.

The genus *Eucalyptus* includes species of widely-different bark type growing in environments that burn at frequencies from every few years to multiple decades between fires. In *Eucalyptus* epicormic resprouting is a common regeneration strategy (Keeley et al. 2012; Burrows, 2013), and it is well developed in *E. obliqua*, the most widely distributed species in south eastern Australia (Brooker and Kleinig 1990, Boland et al. 2006). Although epicormic sprouting is found in numerous families of tree species globally, *Eucalyptus* is unique because meristems are buried deep in the cambium and protected by the full thickness of the bark, a feature contributing to their description as the world's most successful epicormic resprouters (Clarke et al. 2013). Therefore the development of tissue-level techniques to determine critical temperature and cell viability in *Eucalyptus* relates also to regeneration and survival capacity following fire.

According to our knowledge, no previous study has attempted to identify an explicit critical temperature for any woody species. Therefore this study aimed to first adapt and test a TTC method for quantifying phloem and cambial cell viability following heat treatments of tissue sections *in vitro*. A second aim was to apply the method to establishing a threshold level of cambium cell viability for *Eucalyptus obliqua* to enable the identification of a critical temperature. According to Dickinson et al (2004) tissues will be killed if cell viability drops by 63.2%. Therefore, the temperature at which the cell viability reaches 36.8% is considered the critical temperature.

It is anticipated the results of the study will provide rich opportunities for more detailed analysis of relationships between bark characteristics, heat transfer into stems, epicormic regeneration and cambium tissue viability in *Eucalyptus* more broadly.

Materials and methods

Approach and experiments

The first step in developing the TTC test was to identify the minimum mass of phloem-cambial tissue required to confidently characterize the impact of temperature treatments on cell viability. Based on typical sample sizes adopted in previous studies of the TTC test in plant tissues (Dickinson et al. 2004), three mass groupings of tissue slices were tested (1 mg, 10 mg, and 30 mg) at 20°C, 60°C and 85°C prior to selecting the optimum sample mass to test over more temperature treatments. According to the results, the 10 mg mass group was selected as the optimum tissue mass for the TCC method.

Furthermore, in the expectation that *Eucalyptus* cambium-phloem tissue samples contain extractives, such as polyphenols, that may reduce TTC to TPF to give a false positive result for cell viability, we also included an independent measure of cell viability based on staining for live cells with Neutral Red followed by counting, as a cross-check of the magnitude of TTC method results. Subsequently both the TTC method and Neutral Red method were tested on tissue sections at temperatures from 20°C to 85°C in the expectation of revealing a decline in the cell viability with increasing temperature. A Cell Viability Index (CVI) was calculated as described below.

Sampling of live-bark and excision of tissue samples

Sections of bark with underlying phloem, cambium and xylem were obtained from ten, 28-year-old *Eucalyptus obliqua* L'Hér. var. *obliqua* trees in Creswick State forest, Victoria, Australia (Latitude:- 37.42493, Longitude: 143.90269). A circular window of 8.0 cm diameter and average depth of 2 cm containing the bark and the secondary xylem (Fig. 1a) was removed from the stem at 1.3 m height with a drill-mounted circular cutting tool. Samples were immediately wrapped into a paraffin film to reduce the rate of cell dehydration and transported to the laboratory within 15 minutes and cooled to 4°C.

After removing the outer bark (phellum) and the xylem, clean cuboids of cambium-phloem cells, about 1 cm transverse x 1 cm radial by 5.0 cm tangential, were cut with a sharp scalpel blade (Fig. 1b). To identify the optimum mass of sample required for the TTC test, slices of 0.1 cm thickness but varying transverse and radial lengths (comprised of phloem and cambial cells), ranging from about 1 mg to 30 mg were hand-cut from the cuboids and immediately transferred to 0.05M potassium phosphate buffer solution (pH 7.4). For the Neutral Red method uni-cellular to bi-cellular thickness transverse cross sections were carefully shaved off from the cuboid and placed in 0.05M potassium phosphate buffer solution (pH 7.2). Both slices and cross sections in the buffer solutions were held at room temperature (20°C), prior to testing for cell viability by the TTC method and the Neutral Red method (Fig. 1c, Table 1).

Temperature treatments

The slices and thin cross sections of cambium-phloem tissue samples were placed in plastic vials containing buffer solution and submerged for 1 minute in a continuously stirred water bath held at temperatures from 20°C (control) to 85°C (positive control); maintained with ice (20°C) or electric element heating (40°C, 50°C, 60°C, 70°C and 85°C). Three slices and five cross sections were tested for each treatment temperature. The temperatures of the solution in the plastic vial and of the water bath were checked during the treatment to confirm consistency. Following the application of temperature treatments, all tissue slices in buffer solution were cooled to 20°C prior to assay for cell viability as a function of the mass of formazan produced from TTC according to the method described below. As a check on the cell viability derived from the TTC test, cell viability was also determined by neutral red staining for the same temperature treatments as described further below.

Triphenyl tetrazolium chloride test for cell viability (TTC method)

Following exposure to heat treatments the tissue slices were placed in vials containing 1.5 ml of 0.8% tetrazolium chloride (TTC) solution prepared in 0.05M potassium phosphate buffer (pH 7.4; (Chen et al. 1982)), vacuum infiltrated for six cycles of 15 seconds each and kept at room temperature (20°C) for 20-22 hours in the dark. Samples were then rinsed twice with deionized water, blot dried, macerated using a mortar and pestle, transferred to Falcon™ conical tubes containing 4 ml of 95% ethyl alcohol, vortexed for 30 seconds and kept in 95% ethyl alcohol for 12-16 hours to allow formazan extraction at room temperature (20°C). The supernatant was then centrifuged at 3000 rpm for 5 minutes and the absorbance at 485 nm (A_{485}) was measured using a UV-1800 spectrophotometer (SHIMADZU™, Japan). The formazan concentration was calculated from the standard curve (Fig. S1). The macerated tissue remaining in the Falcon™ tubes was then

oven dried to 70°C for 12 hours to allow the mass of formazan formed to be reported on a sample dry weight basis. The CVI was calculated by dividing the formazan concentration for each temperature treatment by the mean formazan concentration of the samples kept at 20°C.

$$\text{CVI (TTC method)} = \frac{\text{Formazan concentration of the treated slice}}{\text{Mean formazan concentration of the slices kept at 20°C}} \quad \text{Eqn 1}$$

Neutral red staining test for cell viability (Neutral Red method)

Following temperature treatments, the thin cross sections were transferred to freshly prepared neutral red (3-amino-7-dimethylamino-2-methylphenazine hydrochloride, Toluylen red) solution (20 ppm), made up in a 0.05M potassium phosphate buffer (pH 7.2) and vacuum infiltrated for 30 seconds for consistent staining (Dickinson and Johnson, 2004). Tissue sections were steeped in the stain for 1 hour and then washed with distilled water to remove background residual stain prior to microscopy. Cell staining was observed with slides of tissue sample mounted in a compound light microscope set at magnification of $\times 40$ (XSP-103a, Xiamen Phio Scientific Instruments Co. Ltd., China). Three field view counts of live (red stained) phloem parenchyma cells and cambial cells were selected from the entire cross section based on minimal cell damage and even staining. Cells stained as light red were counted as live and unstained cells were counted as dead cells, with the total cell count per field view then scaled to 100 (Fig. 2). Phloem fibres were not counted, nor were ray parenchyma cells which stained dark red, whether live or dead, most likely due to the presence of tannin (Chattaway 1953). The arrangement of cambial and phloem cells in cross sections influenced the total cell count, with both the size of the bundles and the number of bundles varying among each field view (Chattaway 1953). Moreover, due to the arrangement and the size of ray parenchyma in a transverse section (Chattaway 1953), they occupy more space in a field view but contain fewer cells than other phloem parenchyma. In contrast, owing to the multiseriate tangential arrangement of the non-ray parenchyma in a transverse section (Chattaway 1953), there are more cells per unit area compared to ray parenchyma. Therefore, considering the arrangement of cells in the phloem tissue, cross sectioning may damage most phloem parenchyma cells, thereby affecting the live cell count relative to the total cell count, and producing a lower live cell percentage than anticipated. Thus, the total cell count per field view was scaled to 100 and the live cell count was recalculated accordingly.

The CVI for each field view was calculated by dividing the number of live cells for each field view by the mean number of live cells in the samples exposed to 20°C treatment.

$$\text{CVI (Neutral Red method)} = \frac{\text{Live cell count of each field view}}{\text{Mean live cell count of the samples exposed to 20°C}} \quad \text{Eqn 2}$$

Following the findings of Dickinson et al (2004) this study assumed that a loss of 63.2% or more of cell viability would kill the cambium-phloem tissue sample. Therefore a CVI=0.368 (≈ 0.37) was taken as the threshold CVI value for this study and the temperature at CVI=0.37 is identified as the critical temperature.

Statistical Analysis

Due to heteroscedastic distribution of the residuals of the CVI of each method, all CVI values were log transformed prior to statistical analysis. The effect of temperature on cell viability was assessed using one-way analysis of variance (ANOVA) followed by a Tukey post-hoc test of GenStat 18.1 (VSN International Ltd, Hemel Hempstead, UK). Linear regression models were developed to identify the relationship between temperature and CVI. These models were used to determine the critical temperature according to each of the two methods.

Results

Optimum tissue mass for TTC method and comparison of TTC and Neutral Red methods

For each sample mass grouping (1 mg, 10 mg, 30 mg) the A_{485} , and hence TPF or formazan formation, increased from lowest in the 85°C treatments, through 60°C to highest in the 20°C treatments (Fig. 3). Within the 1 mg sample mass group, A_{485} did not vary significantly between temperature treatments, while 10 mg and 30 mg sample mass groups provided sufficient TPF formation to significantly separate temperature treatments (Table S1). The A_{485} for 85°C treated cells increased with sample mass, indicating an absorbance associated with heat-killed tissues (Fig. 3; $r^2=0.95$). This increasing production of TPF as the mass of dead cells increased from 1 mg to 30 mg may be due to TTC reduction by cambium-phloem tissue extractives such as polyphenols (Clemensson-Lindell 1993). This result shows that background reduction of TTC needs to be accounted in calculating cell viability. For further testing of the TTC method, a sample mass of around 10 mg was selected as this minimized sample preparation time, especially at the maceration step, whilst also providing sufficient mass to separate temperature treatments.

The change in the CVI with increasing temperature treatments

The cell viability index was negatively linearly correlated with temperature ($p<0.001$) according to results from both methods for measuring cell viability (Fig. 4). For the TTC method the CVI declined by 63.2% at 60.1°C ($\approx 60^\circ\text{C}$), while for the neutral red method the CVI declined by 63.2% at 82.5°C ($\approx 83^\circ\text{C}$) (Fig. 4).

In the TTC method, the CVI of samples kept at 20°C (1.000 ± 0.104) was ten times greater than samples treated at 85°C (0.114 ± 0.006) (Fig. 4); comparisons among all temperatures for significant differences in formazan concentration are shown in Table 2.

Similarly, in the Neutral Red method the highest mean CVI (0.994 ± 0.033) was observed in the control (20°C) tissue samples and the lowest (0.348 ± 0.023) was recorded in the positive control (85°C) tissue samples, while cell viability gradually decreased from 40°C to 70°C (Fig. 4, Table 3). In the Neutral Red method, the CVI decreased by about 40% from 20°C to 60°C and by 65% at 85°C. The mean CVI of each temperature treatment (excluding 50°C and 60°C) was significantly different from all the other temperature treatments ($p<0.001$).

When compared to the cell viability of the control samples of the TTC method, results depict a loss of nearly 50% of cell viability when reaching 50°C, a loss of 60%-80% of cell viability when reaching

60°C - 70°C, and a loss of nearly 90% of cell viability when reaching 70°C - 85°C. In addition, there is no significant difference between the mean CVI at 70°C and CVI at 85°C (Table 2).

Discussion

The TTC method optimized in this study consistently identified in a quantitative way the gradual decrease in cell viability with increasing temperature and yielded reproducible CVI's for *Eucalyptus* phloem-cambium tissues. The cell viability reached a minimum at around 70°C as treatment temperature increased from 20°C, similar to the results of studies by Chen et al (1982); Caldwell (1993); Dickinson and Johnson (2004) and Dickinson et al (2004).

The results of this study suggest 60°C (TTC method) as the critical temperature for cambium-phloem cells of *Eucalyptus* species. This finding broadly supports the idea of critical temperature, which is often assumed to be 60°C in previous studies by Alexandrov (1964), van Wagner (1973), Bauer et al, (2010), Dickinson and Johnson (2004) and Dickinson et al (2004). According to our knowledge no prior study has provided sufficient evidence to establish 60°C as the critical temperature for woody cambium-phloem cells. Therefore this study marks the first successful attempt to establish a threshold temperature for cambium-phloem cell viability following heat exposure.

The decline in TPF production with increasing temperature demonstrates the utility of the TTC method as an indicator of cell viability in *Eucalyptus* cambium-phloem tissues, as there is good evidence from the literature that phloem parenchyma cell viability is seriously impaired at 60°C, with no cells surviving at 85°C (Dickinson et al. 2004).

Prolonged exposure to temperatures below 60°C can also cause cambium death leading to tree mortality (Dickinson and Johnson, 2004). Because temperature and exposure time each affect the degree of loss of cell viability (Chen et al. 1982), it is necessary to investigate interactions between them to gain the most detailed understanding of controlling factors (Hare 1961; Kayll 1963, Dickinson et al. 2004). Identification of critical exposure time will also allow for better comparisons of the cambium-phloem cell viability of different *Eucalyptus* species and to estimate their level of heat tolerance.

We selected a 10 mg tissue mass group for the TTC method because a lower rather than a higher tissue mass reduces sample preparation time, especially the maceration step, allowing the consistent application of temperature treatments, whilst also minimizing potential interference from polyphenols (Steponkus and Lanphear 1967; Clemensson-Lindell 1993). The TTC method is technically simple, facilitating many samples to be tested for CVI over 24 hours in robust experimental designs. It also allows for cambium-phloem samples to be taken from tree trunks subjected to heating in both laboratory conditions and from field environments, and tested for CVI so that the insulating properties of bark can be quantified.

We note that the Neutral Red method for assessing viability of the cambium cells of *Eucalyptus* species confirmed the same trend in results as the TTC method but yielded higher average CVI than the TTC method at each treatment temperature, except for 20°C (Fig. 4 and Fig. S2), suggesting that the critical temperature derived (83°C) from the Neutral Red method is an overestimation. This may result from human error in differentiating live and dead cells when the underlying cell layer stained in red, so that the dead top-most layers appeared to be alive. In this way, the Neutral Red method

requires consistent and accurate preparation of thin sections, preferably of unicellular or bicellular layers; the method is thus more technically demanding and more prone to human error than the TTC method.

In *E. obliqua* the epicormic meristem is deeply embedded in the cambium, a rare characteristic in woody species. In most commercially important families of tree species the meristem is located on the surface of the bark with no epicormic trace extending through the xylem to the pith. This includes *Pinus*, the subject of most of the detailed cell death studies (Stone and Stone 1954, Waring and Pitman, 1985, Ryan and Reinhardt 1988, Finney 1999, Fowler et al. 2004, Gucker 2007, Pausas and Keeley 2014). In *Eucalyptus*, bark plays a significant role in protecting the epicormic meristems from heat-related death during fire (McArthur, 1967, Gill and Ashton, 1968, Nicolle 2006, Clarke et al. 2013, Burrows et al. 2010, Wesolowski et al. 2014). Because bark slows the rate of heat transfer, it protects the vascular cambium from overheating that can cause cell death (Gill and Ashton 1968; Odhiambo et al. 2014). Insulation capacity increases with the thickness, density and moisture content of the bark (Wesolowski et al. 2014), so that tree diameter and height, fire history, and recent climate all become important in fire survival (Brando et al. 2012, Wesolowski et al. 2014).

Conclusion

In this study we developed a fast and reliable method to test cambium cell viability of *Eucalyptus* via a Cell Viability Index (CVI) following heat exposure, using the 2,3,5 - triphenyl tetrazolium chloride (TTC) method. We found that a 10 mg sample of tissue is the optimal mass for preparation of a sample while providing consistent application of temperature treatments. The study revealed that 60°C is the explicit critical temperature for cambial cells of *Eucalyptus*. The developed method and CVI can be applied in further studies to investigate heat and exposure time interactions of cambium viability in *Eucalyptus* species.

Funding

Authors received no funding for this study.

Acknowledgments

Authors acknowledge Dr Lisa Wittick for sharing the spectrophotometer, Rajitha Ratnayake for field and technical assistance during project implementation. Yasika Subasinghe Achchige acknowledges the Melbourne Research Scholarship from The University of Melbourne.

Statement of Conflict of Interest

Authors declare no conflict of interest

Accepted Manuscript

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Accepted Manuscript

Tables

Table 1: Summary of sample volume and sample preparation for TTC method and Neutral Red method

Specifications	TTC method	Neutral Red method
Sample size (cm ³) (Approximately)	0.025	0.009
Length (cm)×Width (cm)×Thickness (cm)	(0.5×0.5×0.1)	(0.3×0.3×0.01)
Number of tissue samples per treatment	3	5
Total number of tissue samples	90	150
Temperatures × No of tissues × No of trees	(6 × 3 × 5)	(6 × 5 × 5)
pH value of the buffer solution	7.4	7.2
Heat exposure time for each treatment temperatures (minutes)	1	1

Table 2: Comparison of cell viability among different treatment temperatures in the TTC method

	20°C	40°C	50°C	60°C	70°C	85°C
20°C	-	-	-	-	-	-
40°C	NS	-	-	-	-	-
50°C	***	**	-	-	-	-
60°C	***	***	NS	-	-	-
70°C	***	***	***	***	-	-
85°C	***	***	***	***	NS	-

NS-non-significant; significance level: * <0.05, ** <0.01, *** <0.001

Table 3: The average ($\pm$ s.e., n=25) of cells counted in a field view according to status - live, dead, tannin filled and the average CVI for Neutral Red method

Treatment temperature	Average of live cells	Average of dead cells	Average of tannin filled cells	Average Cell Viability Index
20°C	50 $\pm$ 0.3	46 $\pm$ 0.2	4 $\pm$ 0.4	0.99 $\pm$ 0.03
40°C	40 $\pm$ 0.2	55 $\pm$ 0.4	5 $\pm$ 0.3	0.80 $\pm$ 0.05
50°C	33 $\pm$ 0.5	57 $\pm$ 0.4	10 $\pm$ 0.3	0.66 $\pm$ 0.05
60°C	30 $\pm$ 0.3	60 $\pm$ 0.5	10 $\pm$ 0.4	0.60 $\pm$ 0.03
70°C	21 $\pm$ 0.4	70 $\pm$ 0.2	8 $\pm$ 0.4	0.43 $\pm$ 0.02
85°C	17 $\pm$ 0.5	73 $\pm$ 0.4	10 $\pm$ 0.5	0.35 $\pm$ 0.02

Figure Legends

Figure 1: (a) A circular bark window, (b) Cuboid sample, (c) Cross sections (Top) and slices (Bottom) in the respective buffer solution

Figure 2: Microscopic image of a cross section of a stained secondary phloem tissue of *E. obliqua*. CM-cambium, DC- dead cell, FC-false live cell, LC-live cell, PF-phloem fibres, PS-phloem sieve tube

Figure 3: Formazan concentration produced at each treatment temperature vs dry weight of the tissue samples, where Size 1 is 1mg, Size 2 is 10 mg, Size 3 is 30 mg (n=18)

Figure 4: Effect of increasing temperature on cell viability. Upright closed triangles are mean live cell percentages (Neutral Red method) and closed circles are mean formazan concentrations (TTC method), for number of samples in each of the methods see Table 1. The horizontal dashed-line represents the 0.37 CVI. The interception points of the horizontal dashed-line and linear regression lines indicate the critical temperature derived from each method. Error bar is the standard error of the mean

Figures

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Title:

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Date:

2021-02-01

Citation:

Achchige, Y. M. S., Volkova, L., Drinnan, A. & Weston, C. J. (2021). A quantitative test for heat-induced cell necrosis in vascular cambium and secondary phloem of *Eucalyptus obliqua* stems. *JOURNAL OF PLANT ECOLOGY*, 14 (1), pp.160-169. <https://doi.org/10.1093/jpe/rtaa081>.

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