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Vascular cambium necrosis in forest fires: using hyperbolic temperature regimes to estimate parameters of a tissue-response model

M. B. Dickinson[A,B], *J. Jolliff*[A] *and A. S. Bova*[A]

[A]USDA Forest Service, Northeastern Research Station, Forestry Sciences Laboratory, 359 Main Road, Delaware, Ohio 43015-8640, USA.
[B]Corresponding author. Email: mbdickinson@fs.fed.us

Abstract. Hyperbolic temperature exposures (in which the rate of temperature rise increases with time) and an analytical solution to a rate-process model were used to characterise the impairment of respiration in samples containing both phloem (live bark) and vascular-cambium tissue during exposures to temperatures such as those experienced by the vascular cambium in tree stems heated by forest fires. Tissue impairment was characterised for red maple (*Acer rubrum*), chestnut oak (*Quercus prinus*), Douglas fir (*Pseudotsuga menziesii*), and ponderosa pine (*Pinus ponderosa*) samples. The estimated temperature dependence of the model's rate parameter (described by the Arrhenius equation) was a function of the temperature regime to which tissues were exposed. Temperatures rising hyperbolically from near ambient (30°C) to 65°C produced rate parameters for the deciduous species that were similar at 60°C to those from the literature, estimated by using fixed temperature exposures. In contrast, samples from all species showed low rates of impairment, conifer samples more so than deciduous, after exposure to regimes in which temperatures rose hyperbolically between 50 and 60°C. A hypersensitive response could explain an early lag in tissue-impairment rates that apparently caused the differences among heating regimes. A simulation based on stem vascular-cambium temperature regimes measured during fires shows how temperature-dependent impairment rates can be used to predict tissue necrosis in fires. To our knowledge, hyperbolic temperature exposures have not been used to characterise plant tissue thermal tolerance and, given certain caveats, could provide more realistic data more efficiently than fixed-temperature exposures.

Introduction

Temperature-dependent rate processes are ubiquitous in biology, including photosynthesis, respiration and bioluminescence (Johnson *et al.* 1974). The rates of these processes first rise as temperatures increase through an ambient range and then, as temperature rise continues, peak and decline as the cellular and biochemical systems that carry out these functions are impaired at rates that overwhelm repair processes (Levitt 1980). Above-ambient temperatures, at which functional impairment and, ultimately, tissue necrosis occurs, are of most interest in fire ecology. One important question is what fire-induced temperature regimes can a tissue (e.g. root cortex, stem and branch vascular cambium, bud, foliage, seed) withstand? In the literature, tissue necrosis often is assumed to occur at a threshold temperature (e.g. 60°C). Although this has been a useful approximation it obscures underlying tissue response processes. Tissue response models have been used to describe more completely the effects of elevated temperatures on plants (Lorenz 1939; Caldwell 1993; Dickinson and Johnson 2004). Tissue response models, in turn, allow fire behaviour and heat transfer to be linked with their effects on organisms (Martin 1963; Martin *et al.* 1969; Mercer *et al.* 1994; Dickinson 2002; Dickinson and Johnson 2001, 2004).

Tissue response model parameter estimates are required in order to use linked models to compare tissues, individuals, and species in their response to fires (Dickinson and Johnson 2004). In this paper, we estimate the temperature dependence of a rate parameter that describes tissue impairment at elevated temperatures for stem-phloem and vascular-cambium tissues from two deciduous tree species, red maple (*Acer rubrum* L.) and chestnut oak (*Quercus prinus* L.), and two coniferous tree species, Douglas-fir (*Pseudotsuga menziesii* Mirbel) and ponderosa pine (*Pinus ponderosa* Douglas ex Lawson & C.Lawson). We quantify impairment with an indicator of tissue respiration activity. Rather than estimating the rate parameter at a series of fixed temperatures (Lorenz 1939; Caldwell 1993; Dickinson and Johnson 2004), we estimate the parameters of a temperature dependence (Arrhenius) equation directly by heating tissues over a non-isothermal temperature regime (the hyperbolic, in which the rate of temperature rise increases through time). The hyperbolic regime allows an analytical solution to the rate process model and, thus, a means of estimating

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Arrhenius parameters statistically (Zsakó 1970, 1975; Rhim *et al.* 1989). Estimating the parameters of the Arrhenius equation directly may reduce the labour associated with describing tissue thermal tolerance and, because of the use of non-isothermal temperature regimes, provides parameter estimates more relevant to fire-effects modelling than those estimated from constant-temperature exposures.

Methods

Tissue impairment model

The one-hit model describes a process in which cells (or cellular components such as proteins or mitochondria) are unaffected by heating until being killed (denatured or impaired) in a one-step process by a heat-induced lesion. If the temperature remains constant, the probability that a lesion will occur at any given time after heating begins is constant. This process is defined by

$$\frac{dV}{dt} = -kV, \tag{1}$$

where V is an indicator of viability and k is the rate parameter (s^{-1}). In this equation, we assume that the rate of decline in viability $-kV$ is directly proportional to V itself; that is, the rate process is of first order (see Johnson *et al.* 1974). The one-hit rate-process model has been used to describe cell death in animal- (Dewey *et al.* 1977; Bauer and Henle 1979), bacterial- (Gould 1989) and plant-cell populations (Lorenz 1939; Dickinson and Johnson 2004) and decline in respiration rates in plant cells (Caldwell 1993) resulting from exposures to elevated temperatures.

Describing and understanding the variability in the temperature dependence of rate-process parameters, such as k, has been a central focus of rate-process research in biology (see Johnson *et al.* 1974). Typically, k is estimated at each of a series of fixed, elevated temperatures by solving Eqn 1 for constant k (i.e. a constant temperature) and then estimating the parameters of the temperature-dependence function with replicated experiments. The solution of Eqn 1 for constant k is

$$V(t) = V_0 \exp(-kt), \tag{2}$$

where V_0 is initial viability. The temperature dependence of k is commonly described by the Arrhenius equation for defined ranges in elevated temperatures as follows:

$$k(T) = Z \exp\left(-\frac{E}{RT}\right), \tag{3}$$

where T is temperature (K), Z is the frequency factor (s^{-1}), E is the activation energy ($J\,mol^{-1}$), and R is the universal gas constant ($8.31\,J\,mol^{-1}\,K^{-1}$). Values of the Arrhenius parameters Z and E often fall within a range characteristic of protein denaturation, suggesting that protein denaturation is central to the tissue-impairment process (Rosenberg *et al.* 1971).

A potentially less laborious alternative to estimating k at each of a series of fixed temperatures is to estimate the temperature-dependence function in a single (replicated) experiment. Equation 1 is first rearranged and Eqn 3 substituted for k as follows:

$$-\frac{dV}{V} = Z \exp\left(-\frac{E}{RT}\right) dt. \tag{4}$$

Then, dt is substituted by a suitable temperature regime, one that spans the temperature range of interest and allows analytical solution of Eqn 4. A hyperbolic temperature regime satisfies both of these requirements, i.e.

$$\frac{1}{T} = a - qt, \tag{5}$$

where a and q are the intercept (K^{-1}) and slope ($s^{-1}\,K^{-1}$), respectively, and t is time (s). The temperature-dependence equation is then solved for t, differentiated with respect to $1/T$, solved for dt, and finally, substituted into Eqn 4. The resulting equation must then be integrated over the appropriate limits as follows:

$$-\int_{V_0}^{V} \frac{dV}{V} = -\frac{Z}{q} \int_{\frac{1}{T_0}}^{\frac{1}{T}} \exp\left(-\frac{E}{RT}\right) d\frac{1}{T}. \tag{6}$$

Integration of the right side of Eqn 6 yields

$$-\int_{V_0}^{V} \frac{dV}{V} = -\frac{ZR}{Eq}\left[\exp\left(-\frac{E}{RT_0}\right) - \exp\left(-\frac{E}{RT}\right)\right]. \tag{7}$$

Integration of the left side of Eqn 7 requires definition of viability V.

We define viability as respiration activity indicated by a tetrazolium trichloride (TTC) assay (e.g. Parker 1953; Kayll 1963; Towill and Mazur 1975; Caldwell 1993). TTC is reduced to a pink formazan in live tissues with a peak absorbance at 485 nm. Absorbances (A_{485}) increase linearly with formazan concentrations (McNaught and Wilkinson 1997). Thus, viability is defined as $V = A_{485}$. Absorbances do not reach zero even in heat-killed tissues and it was necessary to further define viability as $V - V_{min}$, where V_{min} is the absorbance associated with heat-killed tissue. With a definition of viability, the left-hand side of Eqn 7 can be integrated as follows:

$$\begin{aligned} g(V) &= -\int_{V_0}^{V} \frac{dV}{V} \\ &= \ln(V_0 - V_{min}) - \ln(V - V_{min}), \end{aligned} \tag{8}$$

where $g(V)$ is the impairment function, V_0 is absorbance for unheated control samples and V is absorbance at some time after the heating trial began. Thus, the final equation is

$$g(V) = -\frac{ZR}{Eq}\left[\exp\left(-\frac{E}{RT_0}\right) - \exp\left(-\frac{E}{RT}\right)\right]. \tag{9}$$

Live-bark samples

Phloem-tissue samples were prepared from 35–50-cm-long trunk sections cut near ground level from trees 10–25 cm in diameter at breast height. Red maple and chestnut oak trunk sections were collected from the United States Department of Agriculture Forest Services' (USFS) Vinton Furnace Experimental Forest in south-eastern Ohio, USA (elevation 275 m; N 39 : 11.956, W 82 : 23.776), whereas Douglas fir and ponderosa pine sections were collected at the University of Montana's Lubrecht Experimental Forest near Missoula, Montana, USA (elevation 1500 m; N 46 : 53.503, W 113 : 27.012). Trunk sections were transported in insulated chests with ice packs to the USFS Forestry Sciences Laboratory in Delaware, Ohio, and were either used immediately or stored vertically in deionised water at 4°C for up to 3 days post-harvest. Phloem-tissue sections (1 cm long, 0.5 cm wide

and 1 mm thick), including vascular cambium, were excised from the trunk sections and placed in towels soaked in 0.05 M potassium phosphate buffer (pH 7.5) in preparation for heat treatments (see Caldwell 1993).

Heat treatments

Tissue sections were threaded on monofilament and immersed in a water bath at the initiation of each heat treatment. The water bath was filled with potassium phosphate buffer (0.01 M, pH 7.5). Five replicate tissue sections were removed simultaneously from the bath at fixed time intervals after initiation of the prescribed temperature regime. Tissue temperatures were monitored with a 0.25-mm-diameter type-K thermocouple inserted into a single tissue section. Control samples were retained in buffer at ambient temperature for the duration of the heating treatments. Heat-killed tissues were obtained by heating five tissue sections at 70–80°C for 5–10 min after a hyperbolic temperature exposure. Control, heated and heat-killed sections were placed in test tubes containing 1.5 mL of 0.8% (w/v) TTC solution made in 0.05 M potassium phosphate buffer (pH 7.5). All samples were vacuum infiltrated for six cycles of 15 s each. Samples were kept in the dark for at least 18 h (18–24 h) at room temperature. After incubation, the samples were rinsed twice in deionised water and blot dried. Then, samples were macerated in a mortar and pestle and the formazan extracted with 1.5 mL of 95% ethyl alcohol at room temperature (Ruf and Brunner 2003). The samples were left in ethanol from 4 to 24 h. The supernatant was transferred to microfuge tubes and spun before absorbance at 485 nm (A_{485}) was quantified.

The water bath was programmed to approximate a hyperbolic rise over the temperature range of interest (Eqn 5). The controller required specification of a series of time segments in which temperature rose linearly from an initial to a final temperature. In our tests, the final temperature at the end of a segment became the initial temperature of the subsequent segment. A 1-min time step was used because it produced a smooth temperature rise in samples. We used two temperature ranges. First, a relatively narrow range (50–60°C) was used to heat red maple, chestnut oak, ponderosa pine and Douglas fir tissue. The hyperbolic temperature rise occurred over 1800 s. Second, a wider range (30–65°C) was used to heat red maple and chestnut oak samples. The hyperbolic temperature rise occurred over four heating times ranging from 420 to 1080 s.

Arrhenius parameter estimation

Arrhenius parameters were estimated from A_{485} data and Eqn 9 by a non-linear least-squares routine (NLIN, SAS Institute Inc.: Cary, NC). Absorbance measurements made on each of the five tissue sections for each time-of-exposure within an experimental run were averaged before analysis. The difference between sample and prescribed (hyperbolic) temperature averaged over experimental runs was 0.1°C, ranging between 0.2 and −0.4°C (standard deviation = 0.07°C), and had no consistent relationship with temperature. Temperatures were corrected for average bias before analysis.

Results

The activation energy E and the frequency factor Z were highly correlated in all experimental runs ($r > 0.99$), a common feature of kinetics data (Reich 1981; Rosenberg *et al.* 1971). The consequence is that similar fits between Eqn 9 and data can be achieved by many pairs of E and Z within a rather large range of parameter values. As such, parameter estimates tended to vary considerably among experimental runs even when the $g(V)$ trends were similar. This unsatisfactory result extended to negative, and physically non-sensical, activation energies, except when E was constrained to be greater than zero (e.g. E replaced by exp[ln(E)] when specifying Eqn 9 in SAS). In order to obtain stable estimates of either parameter it proved necessary to fix one of the parameters. We fixed the frequency factor at its value estimated when Eqn 9 was fitted to the data from all experimental runs from the same species conducted over the same temperature range (i.e. 30–65 or 50–60°C). With Z fixed, we estimated the activation energy separately for each experimental run. Model fit to all replicate experimental runs was significant ($P < 0.01$). The mean activation energy and its standard deviation and 95% confidence interval were calculated from the replicate estimates of activation energies (Table 1).

Red maple and chestnut oak live-bark tissues appeared to exhibit either no change or a slight increase in respiration activity when heated over a hyperbolic temperature regime between approximately 30 and 50°C, followed by sharp declines in respiration activity above 50°C (Fig. 1). Recall that the tissue impairment function $g(V)$ increases as TTC reduction, our indicator of respiration, declines (Eqn 8). Steady increases in impairment were observed when samples from the four species were heated over a hyperbolic temperature profile from 50 to 60°C (Fig. 2). Chestnut oak respiration activity reached its lower limit, indicated by a plateau in $g(V)$, as temperatures approached 60°C in the 50–60°C exposures (Fig. 2). Thus, we truncated the chestnut oak data at 58°C for Arrhenius parameter estimation (Table 1).

Table 1. Arrhenius parameters estimated from hyperbolic temperature exposures

Species	Temperature		Arrhenius parameter	
	n	Range (°C)	Z (s^{-1})	E (s.d., 95% CI) (J mol^{-1})
Red maple	8	30–65	1.0903×10^{24}	166 729 (730, 166 118 to 167 340)
Chestnut oak	7	30–65	7.1466×10^{34}	235 446 (712, 234 788 to 236 105)
Red maple	6	50–60	7.1052×10^{3}	41 702 (473, 41 206 to 42 198)
Chestnut oak	6	50–58	7.4752×10^{16}	123 102 (602, 122 470 to 123 734)
Douglas fir	6	50–60	1.4413×10^{12}	95 217 (870, 94 304 to 96 130)
Ponderosa pine	5	50–60	2.1970×10^{4}	46 402 (571, 45 692 to 47 110)

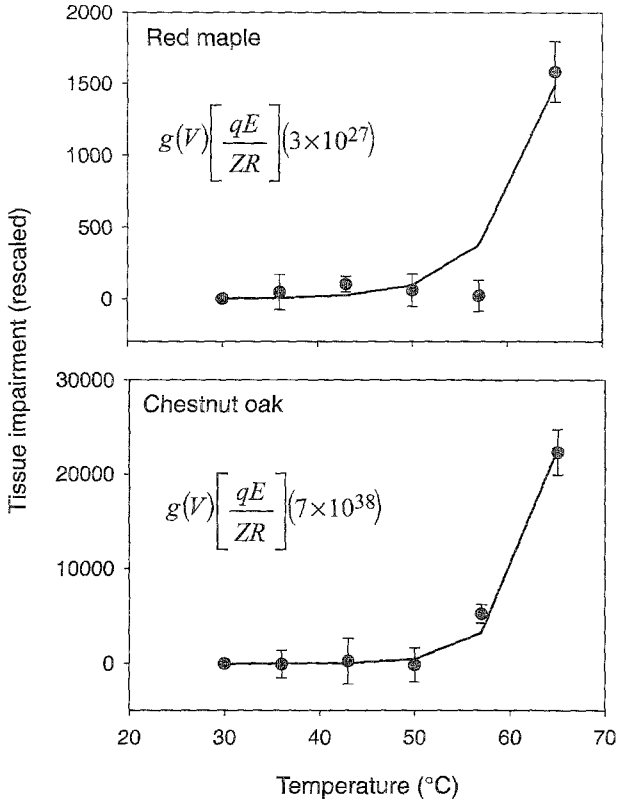

Fig. 1. Increase in red maple and chestnut oak tissue impairment over a 30–65°C hyperbolic temperature exposure. The impairment function $g(V)$ is rescaled because a range of heating rates q were used. The model-fitted values are those calculated from Arrhenius parameters estimated from all experimental runs (Table 1). For clarity, the means ± s.e. of observed data from only two heating rates are shown.

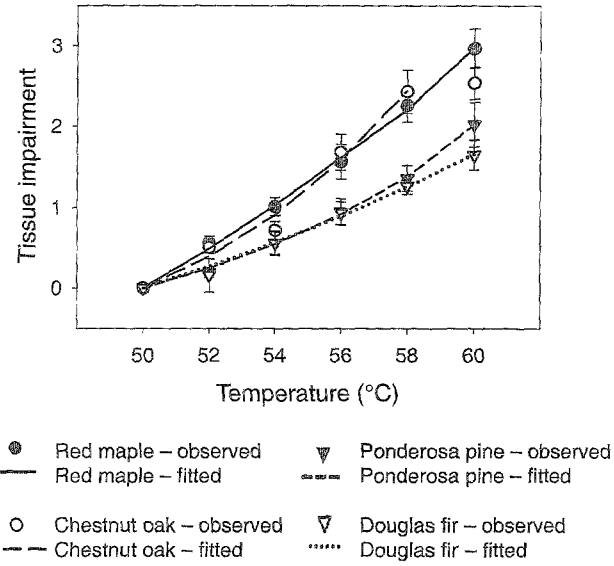

Fig. 2. Increase in red maple, chestnut oak, Douglas fir and ponderosa pine tissue impairment over a 50–60°C hyperbolic temperature exposure. The means of the impairment function $g(V)$ ± s.e. are given, along with lines denoting fitted values. No fitted value for chestnut oak is given at 60°C because of an apparent cessation in tissue respiration above 58°C. Arrhenius parameters estimated from these data are given in Table 1.

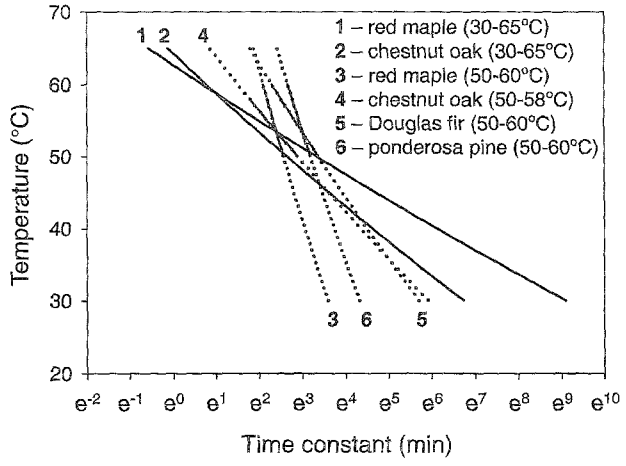

Fig. 3. Time constants of the rate process associated with the impairment of tissue respiration. The time constant is the predicted time-of-exposure to a constant temperature required to reduce respiration activity by 63.2% as indicated by the tetrazolium trichloride (TTC) assay. Calculations were based on Arrhenius parameters in Table 1 estimated from tissues exposed to hyperbolic temperature regimes. The temperature ranges over which Arrhenius parameters were estimated are noted and depicted as solid lines, whereas the dashed lines are extrapolations.

Four heating rates q were used during the 30–65°C hyperbolic temperature runs (see Methods). There was no evidence from analysis of variance ($P > 0.3$) that these different heating rates affected Arrhenius parameter estimates for either red maple or chestnut oak, the species exposed to this regime.

We used the Arrhenius parameters (Table 1) to predict the time of exposure at each of a range of fixed temperatures required to produce a 63.2% decline in viability (Fig. 3). A 63.2% decline defines the time constant of the rate process at a fixed temperature, that is, the time at which $kt = 1$ and $V_t / V_0 = 1 / e$ in Eqn 2. The Arrhenius parameters estimated from tissues exposed to hyperbolic regimes from 30 to 65°C resulted in substantially shorter time constants at temperatures above about 55°C than tissues exposed to hyperbolic regimes from 50 to 60°C.

Discussion

We compare rates of tissue damage from our study with those from studies based on constant-temperature exposures by calculating the expected time required to cause necrosis after exposures to constant temperatures (Fig. 3). For convenience, and because no information exists, we assume that the tissue will be killed if there is a 63.2% decline in tissue

viability (e.g. respiration activity). A widely used rule-of-thumb in fire ecology is that necrosis results if tissues reach 60°C (Brown and DeByle 1987; Steward *et al.* 1990; Gutsell and Johnson 1996). An approximate 60°C threshold is supported by data from constant-temperature exposures in which necrosis occurs after 1–3-min exposures (Lorenz 1939; Nelson 1952; Kayll 1963). The 60°C threshold has been justified for use in modelling necrosis of tissues heated non-isothermally in fires because of (1) the exponential dependence between temperature and the rates associated with tissue impairment (e.g. Eqn 3; Hare 1961) and (2) the characteristic, and relatively rapid, rise and fall of vascular-cambium temperatures resulting from heat transfer into stems during fires (Dickinson 2002; Dickinson and Johnson 2004). The time constants for red maple and chestnut oak tissue impairment estimated over the 30–65°C hyperbolic temperature exposures fall between 1 and 3 min, consistent with the literature. In contrast, the time constants at 60°C calculated from 50–60°C heating regimes were much longer than expected.

Differences in time constants (Fig. 3) and Arrhenius parameters (Table 1) between the 30–65 and 50–60°C hyperbolic exposures show that different heating regimes can result in different rate-parameter estimates. Part of the reason for the heating-regime effect may be that the temperature dependence of the rate parameter k changes as temperatures rise. Temperature-dependence relationships for animal and plant tissues often exhibit an abrupt change in slope (e.g. $\ln(k)$ *v.* temperature) over the relevant temperature range (e.g. 38–65°C) (Dewey *et al.* 1977; Levitt 1980; Caldwell 1993; Dickinson and Johnson 2004). Changes in slope would not be identified by the method we outline because we estimated Arrhenius parameters from the entire temperature range. In contrast, when constant-temperature exposures are used, the rate parameter k can be estimated from Eqn 2 for each of a range of treatment temperatures. Hyperbolic temperature regimes and the derivative of $g(V)$ could be used to identify regions of similar temperature dependence (e.g. Zsakó 1973), but a greater sampling frequency than ours would be required for sufficient accuracy.

Differences between heating regimes might also be related to the result that respiration rates remained unimpaired, and perhaps stimulated, early during 30–65°C hyperbolic exposures (Fig. 1). A lag in the development of rapid rates of tissue impairment is commonly observed in kinetics data (Lorenz 1939; Moats *et al.* 1971; Caldwell 1993; Jung 1986; Dickinson and Johnson 2004). One hypothesis to explain the lag effect in populations of mammalian cells is that non-lethal, heat-induced lesions (e.g. protein denaturation events) accumulate in cells early in the heating process. The accumulation of lesions would increase the likelihood that cells would die and, thus, would result in an acceleration of cell-mortality rates with the progression of heating (Jung 1986).

An alternative to Jung's (1986) lag hypothesis, and one specific to plants, is that respiration activity early during the 30–65°C hyperbolic exposures is mediated by a hypersensitive response. Hypersensitive responses are induced by mechanical wounding and insect and pathogen attack and involve the local and heightened production of secondary compounds around a wound, along with programmed cell death, that, in combination, result in compartmentalisation of damaged tissues (Shigo 1984; Karban and Baldwin 1997; Smith and Sutherland 2001). The apparent stimulation of respiration activity, indicated by negative or declining values of the $g(V)$ as red maple and chestnut oak tissues were exposed to temperatures between 30 and 50°C (Fig. 1), are consistent with a hypersensitive response. A similar stimulation of respiration activity can be seen in Caldwell's (1993) cucumber-leaf data.

The one-hit model (Eqn 1) on which the fitted lines in Fig. 1 are based does not contain a mechanism that would reproduce the effects of either the accumulation of non-lethal lesions (Jung 1986) or a stimulation of respiration activity. As a standard procedure, the lag is censored from constant-temperature data (Johnson *et al.* 1974; Caldwell 1993). However, it is unclear how relevant the rate parameters estimated from censored data are to the relatively short exposures to elevated temperatures that occur in stem vascular cambium heated by fires. *In lieu* of a more complete model than the one-hit (e.g. Jung 1986), a reasonable (and approximate) approach to estimating thermal-tolerance parameters for fire-effects modelling might involve exposing tissues to hyperbolic regimes that begin at ambient temperatures and rise at a rate characteristic of conditions that cause necrosis of that tissue in forest fires. The characteristic rate will differ considerably among tissues. For instance, root tissues, the stem vascular cambium and crown tissues (branch vascular cambium, bud meristems, foliage, and fruits) will experience different temperature regimes because they are both differentially insulated and experience different surface heat-flux regimes during fires.

Future experiments with hyperbolic heating regimes may consider the following issues. First, because the rates of tissue damage at ambient temperatures are effectively zero, it may be possible to assume that $T_0 = 0$ in Eqn 9 for hyperbolic temperature regimes that begin near the ambient (Zsakó 1970). The result is a linear equation after log-transformation that could be used to avoid certain problems associated with non-linear parameter estimation. We were not able to employ the $T_0 = 0$ assumption because of too many negative values for $g(V)$ (Fig. 1). Cell-mortality data (Lorenz 1939; Dickinson and Johnson 2004), rather than our tissue-respiration data, may be more suited to this approach. Second, a dampened response was observed after 58°C for chestnut oak in the 50–60°C hyperbolic exposures (Fig. 2), presumably because respiration activity had largely ceased. Higher heating rates would obviate this problem. Finally,

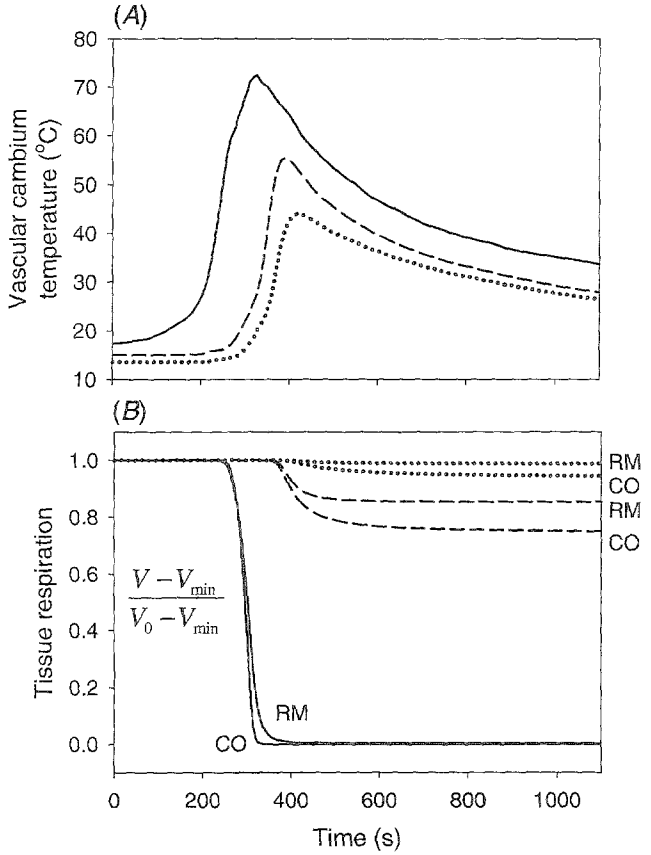

Fig. 4. Simulated decline in red maple (RM) and chestnut oak (CO) vascular-cambium respiration during exposures to elevated temperatures during fires. (*A*). Temperature regimes measured during experimental surface fires with thin, type-K thermocouple probes imbedded in the vascular cambium of live trees. The regimes are shifted along the time axis for ease of viewing. Differences in peak temperatures were caused by a combination of bark thickness and fire intensity. (*B*). The decline in tissue-respiration activity expressed in relation to unheated tissues. The finite-difference form of Eqn 1 was used to simulate tissue response on a 1-s time step (Dickinson and Johnson 2004). The temperature dependencies of the rate parameter k were determined from the Arrhenius equation by using parameters estimated from the 30–65°C hyperbolic temperature exposures (Table 1). The line style is maintained between *A* and *B* so that tissue response can be paired with its corresponding temperature regime.

results from the 50–60°C hyperbolic exposures suggest that conifer species may be more tolerant to elevated temperatures than the deciduous species, at least over this temperature range (Fig. 2).

Finally, we simulate the time-course of tissue impairment for red maple and chestnut oak (Fig. 4*B*) by using vascular cambium-temperature regimes measured during surface fires (Fig. 4*A*). The simulation is based on the assumption that stem vascular-cambium necrosis during fires is initiated by a rate process that is approximately described by the one-hit model (Eqn 1). Significant reductions in tissue viability would be expected only if vascular-cambium temperatures rose above 55°C in fires, whereas we would expect tissues to be killed quickly and outright if their temperatures rose above 70°C. Minimal differences in thermal tolerance are apparent between chestnut oak and red maple. Assuming that necrosis proceeds inexorably by a wounding response after a threshold level of tissue damage (e.g. 63.2%) has been reached, Fig. 4 demonstrates that the one-hit model can be used to obtain stem tissue-necrosis predictions that can, in turn, be tested against data on tree and stem mortality after fires. For practical purposes, given the high rates of tissue impairment at temperatures above 55°C and relatively rapid stem heating and cooling as flames pass a tree, the precise threshold may not be very important (Fig. 4; Hare 1961).

Conclusion

The traditional approach to estimating the temperature dependence of the one-hit model rate parameter (Eqn 1) has been to quantify tissue response to a range of fixed temperatures. Using constant temperatures can be labour intensive and does not match the temperature regimes experienced by tissues in fires. In this paper, for the first time to our knowledge for plant tissues, we describe a hyperbolic exposure (e.g. ambient to 65°C) that allows estimation of rate-parameter temperature dependence (i.e. parameters of the Arrhenius equation) from Eqn 9 and replications of a single non-isothermal experiment. Our results suggest that the one-hit model fails to capture important mechanisms, e.g. the hypersensitive response, and thus can only approximately describe tissue necrosis at elevated temperatures. In contrast to the assumptions of our necrosis model, exposures to different temperature ranges resulted in different relationships between time constants and temperatures for the species for which a comparison was made (Fig. 3). However, the 60°C time constants calculated from hyperbolic exposures that began near the ambient temperature were close to what we would expect from the constant-temperature literature. By matching heating regimes (temperature range and its rate of rise) to those experienced by tissue in fires, the hyperbolic regimes could provide an alternative to constant-temperature exposures for describing tissue impairment during heating.

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References

Bauer KD, Henle KJ (1979) Arrhenius analysis of heat survival curves from normal and thermotolerant CHO cells. *Radiation Research* **78**, 251–263.

Brown JK, DeByle NV (1987) Fire damage, mortality, and suckering in aspen. *Canadian Journal of Forest Research* **17**, 1100–1109.

Caldwell CR (1993) Estimation and analysis of cucumber (*Cucumis sativus* L.) leaf cellular heat sensitivity. *Plant Physiology* **101**, 939–945.

Dewey WC, Hopwood LE, Sapareto SA, Gerweck LE (1977) Cellular responses to combinations of hyperthermia and radiation. *Radiology* **123**, 463–474.

Dickinson MB (2002) Heat transfer and vascular cambium necrosis in the boles of trees during surface fires. In 'Forest fire research and wildland fire safety'. (Ed. X Viegas) Printed abstract, full-text on accompanying CD-ROM, 10 p. (Millpress: Rotterdam)

Dickinson MB, Johnson EA (2001) Fire effects on trees. In 'Forest fires: behavior and ecological effects'. (Eds EA Johnson, K Miyanishi) pp. 477–525. (Academic Press: New York)

Dickinson MB, Johnson EA (2004) Temperature-dependent rate models of vascular cambium cell mortality. *Canadian Journal of Forest Research* **34**, 546–559. doi: 10.1139/X03-223

Gould GW (1989) 'Mechanisms of action of food preservation procedures'. (Elsevier Applied Sciences: London)

Gutsell SL, Johnson EA (1996) How fire scars are formed: coupling a disturbance process to its ecological effect. *Canadian Journal of Forest Research* **26**, 166–174.

Hare RC (1961) Heat effects on living plants. USDA Forest Service, Southern Forest Experimental Station, Occasional Paper 183, New Orleans, LA.

Johnson FH, Eyring H, Stover BJ (1974) 'The theory of rate processes in biology and medicine.' (John Wiley and Sons: New York)

Jung H (1986) A generalized concept for cell killing by heat. *Radiation Research* **106**, 56–72.

Karban R, Baldwin IT (1997) 'Induced responses to herbivory.' (University of Chicago Press: Chicago, IL)

Kayll AJ (1963) Heat tolerance of Scots pine seedling cambium using tetrazolium chloride to test viability. Canada Department of Forestry, Forest Resources Branch, Publication 1006, Ottawa, Ontario.

Levitt J (1980) 'Responses of plants to environmental stresses. Vol. 1. Chilling, freezing, and high temperature stresses.' (2nd edn) (Academic Press: New York)

Lorenz R (1939) High temperature tolerance of forest trees. University of Minnesota Agricultural Experiment Station, Technical Bulletin 141, St Paul, MN.

Martin RE (1963) A basic approach to fire injury of tree stems. In 'Proceedings of the second annual tall timbers fire ecology donference'. pp. 151–162. (Tall Timbers Research Station: Tallahassee, FL)

Martin RE, Cushwa CT, Miller RL (1969) Fire as a physical factor in wildland management. In 'Proceedings of the ninth annual tall timbers fires ecology conference'. pp. 271–288. (Tall Timbers Research Station: Tallahassee, FL)

McNaught AD, Wilkinson A (1997) 'IUPAC compendium of chemical terminology'. (2nd edn) (Blackwell Press: New York)

Mercer GN, Gill AM, Weber RO (1994) A time-dependent model of fire impact on seed survival in woody fruits. *Australian Journal of Botany* **42**, 71–81.

Moats WA, Dabbah R, Edwards VM (1971) Interpretation of nonlogarithmic survivor curves of heated bacteria. *Journal of Food Science* **36**, 523–526.

Nelson RM (1952) Observations of heat tolerance of southern pine needles. USDA Forest Service, Southeastern Forest Experiment Station, Station Paper 14, Asheville, North Carolina.

Parker J (1953) Some applications and limitations of tetrazolium chloride. *Science* **118**, 77–79.

Reich JG (1981) On parameter redundancy in curve fitting of kinetic data. In 'Kinetic data analysis: design and analysis of enzyme and pharmacokinetic experiments'. (Ed. L Endrenyi) pp. 39–50. (Plenum Press: New York)

Rhim JW, Nunes RV, Jones VA, Swartzel KR (1989) Determination of kinetic parameters using linearly increasing temperature. *Journal of Food Science* **54**, 446–450.

Rosenberg B, Kemeny G, Switzer RC, Hamilton TC (1971) Quantitative evidence for protein denaturation as the cause of thermal death. *Nature* **232**, 471–473.

Ruf M, Brunner I (2003) Vitality of tree fine roots: reevaluation of the tetrazolium test. *Tree Physiology* **23**, 257–263.

Shigo AL (1984) Compartmentalization: a conceptual framework for understanding how trees grow and defend themselves. *Annual Review of Phytopathology* **22**, 189–214. doi: 10.1146/ANNUREV.PY.22.090184.001201

Smith KT, Sutherland EK (2001) Terminology and biology of fire scars in selected central hardwoods. *Tree-Ring Research* **57**, 131–139.

Steward FR, Peter S, Richon JB (1990) A method for predicting the depth of lethal heat penetration into mineral soils exposed to fires of various intensities. *Canadian Journal of Forest Research* **20**, 919–926.

Towill LE, Mazur P (1975) Studies on the reduction of 2,3,5-triphenyltetrazolium chloride as a viability assay for plant tissue cultures. *Canadian Journal of Botany* **53**, 1097–1102.

Zsakó J (1970) Hyperbolic temperature variation program in kinetic investigation. *Journal of Thermal Analysis* **2**, 459–460.

Zsakó J (1973) Kinetic analysis of thermogravimetric data, VI, some problems of deriving kinetic parameters from TG curves. *Journal of Thermal Analysis* **5**, 239–251.

Zsakó J (1975) Empirical formula for the exponential integral in non-isothermal kinetics. *Journal of Thermal Analysis* **8**, 593–596.

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