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A mathematical and statistical analysis of the curves illustrating vulnerability of xylem to cavitation

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Summary When vulnerability curves are used to assess the susceptibility of plants to drought, the water potential corresponding to 50% loss of conductivity is frequently used as a measure of susceptibility. However, this value does not distinguish between different patterns of conductivity loss, such as a rapid loss over a narrow water potential range versus a gradual decline in conductivity. We have applied an exponential sigmoidal equation to vulnerability curves obtained from four closely related *Eucalyptus* clones differing in drought tolerance.The coefficients of the equation were evaluated and statistically compared among the clones. If the air-seeding hypothesis of cavitation is accepted, these coefficients have biological relevance. One of the coefficients describes the position of the curve on the water potential axis and is equivalent to the water potential corresponding to 50% loss of conductivity. This coefficient could reflect the size of the largest pit pore per xylem vessel. Another coefficient is related to the slope of the conductivity loss, and could reflect the range in maximum pit pore size per vessel.

Keywords: embolism, hydraulic conductivity, vulnerability curve.

Introduction

Sperry et al. (1988*a*) devised a method to relate the loss of hydraulic conductivity caused by cavitation to leaf water potential. Since this study, hydraulic vulnerability curves have been constructed for numerous species (reviewed in Tyree and Ewers 1996) and for some clonal material (Neufeld et al. 1992). The loss of hydraulic conductivity with increasing water stress is a curvilinear response and consequently the analysis and comparison of these curves are difficult.

In many studies, the assessment of vulnerability curves has simply involved visual inspection and comparisons at arbitrarily chosen water potentials (Sperry and Tyree 1988, Sperry and Sullivan 1992, Tyree et al. 1993); for example, the water potential corresponding to 50% loss of conductivity (PLC_{50}) is frequently used as a measure of susceptibility (cf. Sperry et al. 1988*b*, Tyree and Yang 1992, Cochard et al. 1994, Machado and Tyree 1994, Zotz et al. 1994). However, this value does not distinguish between different patterns of conductivity loss, such as a rapid loss over a narrow water potential range versus a gradual decline in conductivity. Neufeld et al. (1992) also found that PLC_{50} values were not of great use when comparing vulnerability curves of different clones within the same genus because they were likely to have similar values.

Several models have been used to analyze vulnerability curves. Cochard (1992) and Kolb and Davis (1994) have used various polynomials, Neufeld et al. (1992) used the Weibull equation (Rawlings and Cure 1985) to compare curves from a range of sugarcane clones, and Mencuccini and Comstock (1997) employed the Gompertz function to compare arid zone shrubs. These equations have the drawback that the coefficients of the equations have no biological significance. Furthermore, the complexity of the equations does not allow for easy statistical comparisons of the coefficients of the equations and consequently any analysis reverts to comparisons at arbitrary values.

For the coefficients of an equation fitted to vulnerability curves to have biological significance, they must in some way relate to the processes believed to occur during a cavitation event. The air-seeding hypothesis (Zimmermann 1983) suggests that air bubbles are drawn through the pores in pit membranes separating water- and air-filled xylem conduits. If this is the case, the tension required to generate a cavitation event is related to the size of the largest pore of a vessel. Cavitation at low tensions (high water potentials) implies large pit membrane pores, and cavitations occurring over a range of tensions imply a wide range in maximum pore size per vessel. In this study, an exponential sigmoidal equation was fitted to vulnerability curves of four closely related *Eucalyptus* clones. The biological significance and statistical comparisons of the coefficients of the equation are discussed.

The model

In the model, data for the vulnerability curve are fitted to an exponential sigmoidal equation (Equation 1):

$$PLC = 100/(1 + \exp(a(\Psi - b))), \quad (1)$$

where Ψ is the water potential, PLC is the corresponding percent loss of conductivity and a and b are constants.

Unlike previous equations used to describe vulnerability curves, a sensitivity analysis of the effects of the coefficients of Equation 1 indicate that they may be related to anatomical characteristics pertinent to vulnerability loss. The value of 100 is used to represent the maximum loss in conductivity. Coefficient *b* determines the position of the curve on the abscissa (Figure 1a) and gives the water potential corresponding to 50% loss of conductivity. According to the air-seeding mechanism of cavitation, greater vulnerability would be associated with a large maximum pit membrane pore, and coefficient *b* could thus be related to the mean of the largest pit pore per vessel. Coefficient *a* is primarily related to the slope of the curve, and thus the range of water potentials over which conductivity is lost (Figure 1b). A gentle gradient suggests that the conduits cavitate across a wide range of xylem tensions, and thus *a* could be related to the distribution of maximum pore size per vessel.

Material and methods

Experimental material and study sites

Four clones, developed and selected by a commercial forestry company were used in this study. Two *Eucalyptus grandis* W. Hill *ex* Maiden. × *camaldulensis* Dehnh. hybrids, GC1 and GC2, a *Eucalyptus grandis* × *urophylla* S.T. Blake hybrid, GU and a pure *Eucalyptus grandis* clone, GG were chosen on the basis of their presumed drought susceptibilities. Material from trees of the four clones was collected from clonal competition trials at one xeric and two mesic sites in the coastal region of KwaZulu-Natal, South Africa, during winter 1995 (June–July) and summer 1996 (January).

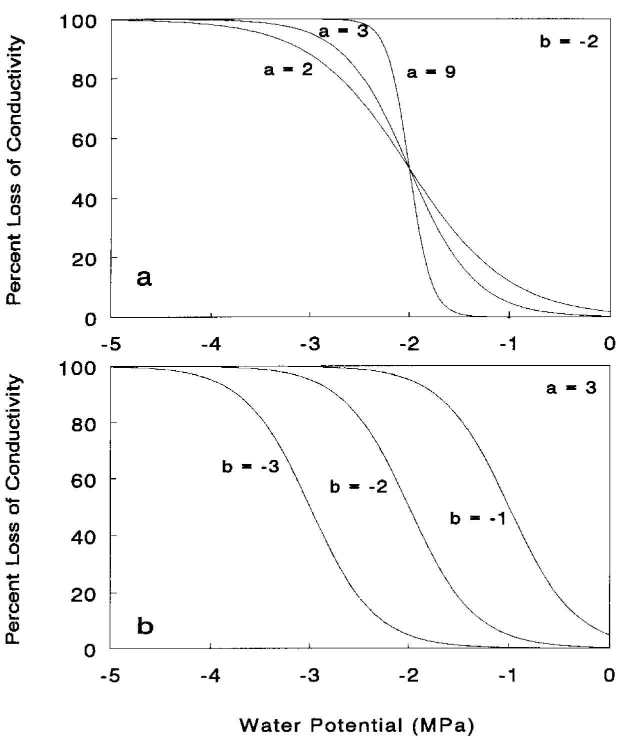

Figure 1. Models of the exponential sigmoidal equation used to describe the vulnerability curves, showing increasing values of coefficient *a* (a) and coefficient *b* (b).

An extendable aluminum pruning pole was used to cut branches approximately 5 m in length, with a proximal diameter of less than 4 cm, from slightly below the crown (usually 15–20 m high) of 7–8-year-old trees. Because all the original GG trees growing at the xeric site had died back, coppice shoots of a similar size to the excised branches of the other clones were used. Branches were collected at dawn and transported to a field laboratory in black plastic bags after the cut end had been sealed with parafilm to prevent excessive water loss.

Measurement of hydraulic conductivity and vulnerability curves

Hydraulic vulnerability curves were constructed for five to eight branches, each from a separate tree of the same clone, by plotting the loss of hydraulic conductivity against xylem water potential. Because the side shoots on a branch were shorter than the longest xylem vessel (approximately 1.3 m, measured according to the method of Zimmermann and Jeje (1981)), hydraulic conductivities (k_h) of whole branches were measured. Conductivity was calculated by measuring the water mass flow passing through a branch connected to a constant pressure reservoir, based on a modification of the method described by Sperry et al. (1988*a*). A 0.01 M HCl solution (prepared with degassed, distilled water) was used in the reservoirs. The excised branch was allowed to dehydrate for two to three days. During dehydration, five to seven measurements of water potential and the corresponding hydraulic conductivity were taken. After each dehydration period, water in the tissue was allowed to equilibrate for 1 to 1.5 h in a sealed plastic bag before measurements. Both ends of the branch were recut under water before measurement and the reduction in branch length was taken into account in the calculation of hydraulic conducuctivity. A Scholander pressure chamber was used to determine xylem water potentials of twigs from the side shoots.

After dehydration to the extent that all conductivity had been lost (no flow of water through the branch), the branch was flushed at a pressure of approximately 200 kPa for 20 min. The flushing solution had been passed through a 0.22-µm membrane filter to prevent particulate matter from blocking the conduits. After flushing, hydraulic conductivity was measured as described previously. The entire process was repeated until a maximum conductivity (k_{max}) was reached. The percentage loss in conductivity (PLC) was then calculated as:

$$PLC = 100(k_{max} - k_h)/k_{max}. \quad (2)$$

This technique for determining a vulnerability curve involved numerous measurements from the same branch, rather than individual measurements from numerous segments of a branch, as described by Sperry et al. (1988*a*). This modification was necessary because the long vessel lengths found in the

Eucalyptus clones made it impossible to use numerous segments from a branch. To validate the modification of the technique, preliminary experiments using both methods were made on a local shrub, *Tecomaria capensis* (Thunb.) Spach. The results of this investigation revealed no differences in the vulnerability curves obtained with either method and it was assumed that the technique used with the *Eucalyptus* clones produced results comparable to those obtained with the original method outlined by Sperry et al. (1988*a*).

Analysis of the vulnerability curves

Coefficients *a* and *b* were determined from a linearized form of Equation 1:

$$\ln(100/\mathrm{PLC} - 1) = a\Psi - ab. \quad (3)$$

A plot of $\ln(100/\mathrm{PLC} - 1)$ against Ψ yielded a straight line of slope *a* with an ordinate intercept $-ab$.

The vulnerability curve from each branch was treated as a single replicate that yielded a single value of each of *a* and *b*. The replicate values of *a* and *b* from the five to eight branches of each clone were compared among clones by analysis of variance and Scheffé's multiple range test.

Results and discussion

Hydraulic vulnerability curves were constructed for the four *Eucalyptus* clones during summer and winter, from both the mesic and xeric sites (Figure 2). The coefficients determined when Equation 1 was fitted to these curves are summarized in Table 1. There were no marked seasonal or site effects on vulnerability of xylem to cavitation (Figure 2, Table 1), and analysis of variance confirmed that there were no significant differences in the values of *a* and *b* between sites and seasons within each clone.

To study clonal effects on the values *a* and *b*, the data for each clone were pooled across season and site. The lines in Figure 2 were derived by fitting Equation 1 to the combined data sets. Anaysis of variance of the mean values of *a* and *b* for each clone revealed significant differences in the values of these coefficients among the clones (Table 2). Clone GG had the highest value of coefficient *b* (Table 2), implying that cavitation events in the xylem water column were initiated at higher water potentials in Clone GG than in the other clones. The GC clones had the lowest coefficient values, suggesting that these clones were more resistant to cavitation events than the other clones. The wide ranges of water potentials over which the GC clones lost conductivity (Figure 2) were reflected in the low values of coefficient *a* in these clones (Table 2). The high values of *b* in Clones GG and GU suggest these trees have wide maximum pit membrane pores per vessel, and the high values of *a* in these clones suggest a narrow range in

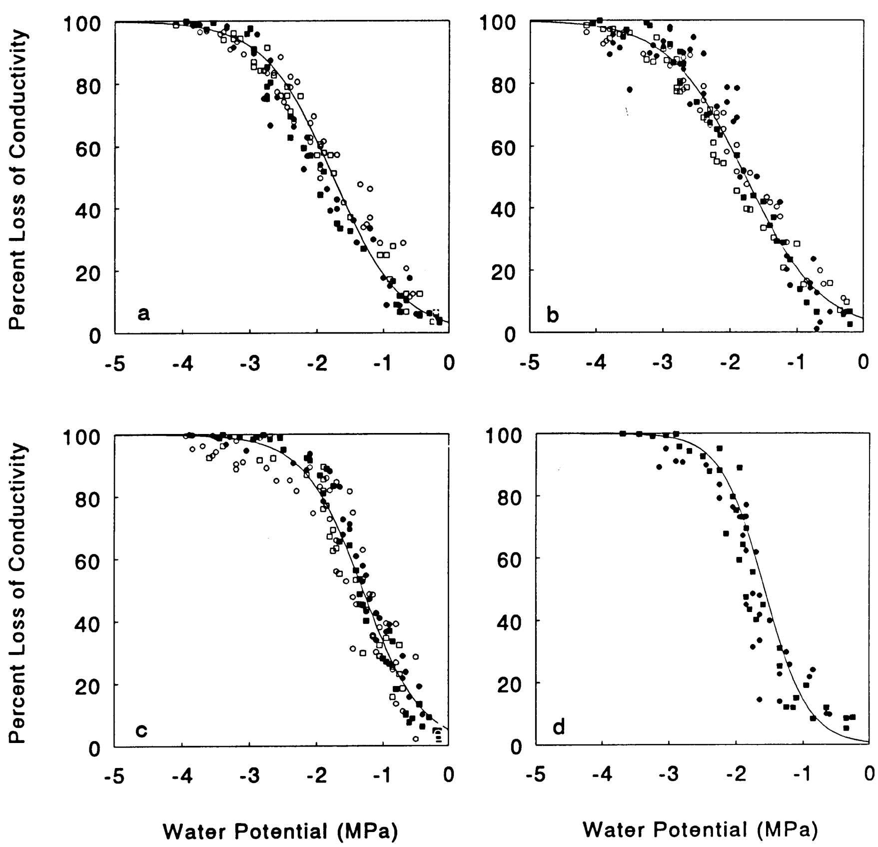

Figure 2. Vulnerability of xylem to cavitation illustrated as the percentage loss of conductivity with decreasing water potential, for branches from 7–8-year-old trees of the four *Eucalyptus* clones GC1 (a), GC2 (b), GG (c) and GU (d), measured during summer (■, □) and winter (●, ○) at both mesic (closed symbols) and xeric (open symbols) sites. There were five to eight replicate branches for each treatment (site and season). All data points from each replicate are shown. The lines describe the fitted equation for the pooled data sets.

Table 1. Mean values (five to eight replicates) of coefficients *a* and *b* from Equation 1 describing the vulnerability curves for branches from each of the four *Eucalyptus* clones during summer and winter measured at both the mesic and xeric sites. Within each clone, no significant differences between site or season were found ($P < 0.05$, $n = 5$–8). The R^2 values are from the linear regression of the transformed data (Equation 3).

Clone	Site	Season	*a*	*b*	R^2
GC1	Xeric	Summer	1.881	−1.729	0.969
		Winter	1.581	−1.630	0.955
	Mesic	Summer	2.228	−1.787	0.943
		Winter	2.050	−1.837	0.948
GC2	Xeric	Summer	1.66	−1.853	0.967
		Winter	1.450	−1.676	0.955
	Mesic	Summer	2.154	−1.742	0.936
		Winter	1.759	−1.795	0.806
GG	Xeric	Summer	2.132	−1.378	0.848
		Winter	1.706	−1.373	0.812
	Mesic	Summer	2.927	−1.277	0.954
		Winter	2.230	−1.202	0.965
GU	Mesic	Summer	3.537	−1.299	0.914
		Winter	2.072	−1.707	0.844

Table 2. Comparisons across clones of the coefficients *a* and *b* from the exponential sigmoidal equation used to describe the vulnerability curves of the branches from the 7–8-year-old trees of the four *Eucalyptus* clones. Values are means with 95% confidence limits derived from analysis of variance and Scheffé's multiple range test using the pooled data from all treatments for each clone. For each coefficient, means followed by the same letter are not significantly different.

Clone	*a*	*b*
GC1	1.923 a	−1.754 a
GC2	1.780 a	−1.762 a
GG	2.397 b	−1.331 b
GU	3.027 c	−1.578 b

maximum pore sizes across vessels. The data are in agreement with the observed relative drought susceptibilities of the four clones. Among the clones, the GC clones are considered to be the most drought tolerant and Clone GG is the most drought sensitive (B. Verbizier, Mondi Forests, South Africa, personal communication). The vulnerabilities of the clones studied here were higher than those obtained for two provenances of *Eucalyptus camaldulensis* where PLC_{50} ranged from −3.8 to −4.2 MPa (Franks et al. 1995). The clones used in the present study have *E. grandis* parentage and have been highly selected for properties other than hydraulic characteristics.

We have proposed a simple model for the analysis of curves describing the vulnerability of xylem to cavitation. The two major advantages of this model are that (1) the coefficients of the equation have biological significance, and (2) the coefficients can be statistically compared among material of different genetic constitution or subjected to different treatments. The use of the model also permits more detailed analysis than is possible by simple visual comparisons. For example, although plots of the vulnerability curves of Clones GG and GU appeared to be similar (Figure 2), the analysis demonstrated significant differences among the the clones. That is, Clone GG has a higher value of *b* than the other clones, suggesting onset of cavitation at higher water potentials. The higher value of *a* for Clone GU than for Clone GG suggests that conductivity loss in Clone GU occurs over a narrower range of water potentials than in Clone GG. The use of descriptive models, together with knowledge of maximum conductivity, permit prediction and comparison of absolute values of conductivity at any particular water potential. These data can be important when assessing the significance of maximum conductivity and conductivity loss. Another advantage of the proposed model is that coefficient *b* is equivalent to the water potential corresponding to PLC_{50}, thus enabling comparisons with earlier studies where data were presented as PLC_{50}.

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