Centrifuge technique consistently overestimates vulnerability to water stress-induced cavitation in grapevines as confirmed with high-resolution computed tomography

Vulnerability to cavitation is a key variable defining the limits to drought resistance in woody plants (Kursar et al., 2009). This trait is typically assessed by a vulnerability curve, which can be generated by a range of methods, including dehydration (Sperry et al., 1988), air injection (Cochard et al., 1992), and centrifugation (Alder et al., 1997). Results from two recent papers suggest that one of the most widely used methods, the centrifuge technique, overestimates vulnerability to cavitation in species with very long vessels (Choat et al., 2010; Cochard et al., 2010). Typically, the centrifuge technique produces characteristic ‘R shaped’ curves for long-vasseled species, compared with ‘S shaped’ curves produced by the dehydration method (Cochard et al., 2010). Both research groups proposed that open vessels contained in the centrifuged samples were responsible for this artifact. Grapevine (Vitis vinifera L.), a liana species known to have unusually long and wide vessels, appears to be particularly susceptible to artifacts with the centrifuge method (Choat et al., 2010), but the conclusions of this paper have been challenged by Jacobsen & Pratt (2012). They contend the dehydration technique actually underestimates vulnerability to cavitation in grapevine because the production of gels and/or tyloses causes a decline in maximum specific hydraulic conductivity ($K_{\text{max}}$) over time. On the basis of their results and previously published hydraulic data, they concluded that the centrifuge technique is the most appropriate technique for estimating vulnerability to embolism (see details in Jacobsen & Pratt, 2012).

Here we demonstrate that declining $K_{\text{max}}$ did not influence the results of Choat et al. (2010) and present new evidence from high-resolution computed tomography (HRCT) to support our original conclusions and refute those of Jacobsen & Pratt (2012). We also contend that the analysis of previous literature presented in Jacobsen & Pratt (2012) was oversimplified and obscured the specific comparison of cavitation resistance in current year shoots of grapevine. Overall, the findings presented in Jacobsen & Pratt (2012) for V. vinifera cv Glenora are in direct contrast to published and unpublished results generated by our research groups for other V. vinifera varieties.

Noninvasive techniques for imaging of cavitation in planta

The ultimate goal of vulnerability curve generation and analysis should be to accurately reflect patterns of cavitation that occur in live/intact plants. Therefore, nondestructive visualization of cavitation in planta has become the most reliable means for assessing xylem functionality (Scheenen et al., 2000; Holbrook et al., 2001). Ideally, these measurements can be combined with concurrent or subsequent measurements of water transport based on sap flow, transpiration, and hydraulic conductivity. Choat et al. (2010) used nuclear magnetic resonance (NMR) imaging of live grapevines (cv Chardonnay) to corroborate vulnerability curves generated via dehydration and air injection methods. We recently collected the most compelling evidence to date by utilizing HRCT (see Brodersen et al., 2010, 2011 for complete details of this method) to visualize the extent of embolism in V. vinifera cv Chardonnay stems in planta at a resolution of 4.5 μm (Fig. 1). Using HRCT, we compared the functional status of xylem in droughted grapevines with excised stems centrifuged to xylem tensions shown to induce significant cavitation (Fig. 1). Less than 10% of the vessels in intact stems of Chardonnay vines were cavitated at a mean stem water potential ($\Psi_{\text{stem}}$) = −1.22 MPa (Fig. 1). C. R. Brodersen et al. (unpublished) similarly found few vessels cavitated in Chardonnay vines (identical to those used here) under mild water stress, and calculated that stems dropped to 50% of their conductivity at $\Psi_{\text{stem}}$ = −2.2 MPa. By contrast, excised Chardonnay stems centrifuged to generate xylem tension of only −0.5 MPa exhibited significantly greater cavitation at this higher $\Psi_{\text{stem}}$ (i.e. what would be considered minimal/no stress under field conditions; Fig. 1a). Similarly high cavitation induced by the centrifuge technique for grapevine stems was reported by Choat et al. (2010) and Jacobsen & Pratt (2012). The visualizations captured here by HRCT (Fig. 1b) are consistent with every technique we used to generate vulnerability curves (i.e. benchtop dehydration, air injection on long stems, and NMR imaging) except for the centrifuge method and air injection on short segments for V. vinifera cv Chardonnay (Choat et al., 2010).

Jacobsen & Pratt (2012) raise questions about the interpretation of NMR observations in Choat et al. (2010), suggesting that undifferentiated vessels close to the cambium would appear as functional vessels. It is possible that some vessels close to the cambium may not have been conductive (i.e. as a result of intact protoplasts, as observed by Halis et al., 2012). However, complementary percentage loss of conductivity (PLC) measurements on...
segments were spun to induce a complete collapse of the vessels. Overall, agreement was not flushed before centrifugation. Means for three samples were: \( \Psi_{\text{stem}} = -0.5 \pm 0; \) percent vessels cavitated = 29.2 \( \pm 2.2; \) mean lumen area of cavitated vessels (\( \mu m^2 \)) = 2571 \( \pm 317 \). The mean lumen area of the cavitated vessels was significantly larger in centrifuged vessels from a theoretical PLC analysis would have little effect on the overall PLC exhibited in the stems that we studied (Choat et al., 2010). Furthermore, dye perfusion of the samples used for HRCT data presented here demonstrated that no vacuole-filled vessels were present in these stems. Overall, agreement between NMR and HRCT imaging, dehydration curves, and native PLC measurements in the field suggests that dehydration curves estimate vulnerability to embolism more accurately than the centrifuge technique in grapevine. Why such great differences exist between cultivars remains to be resolved. Findings reported by Jacobsen & Pratt (2012; i.e. vessel lengths and rapid vessel occlusion) may be specific to the unusual grapevine variety used in their study and potential differences in occlusion rates associated with its cold hardiness.

**Declines in \( K_s \) max with benchtop dehydration**

The declines in \( K_s \) max observed by Jacobsen & Pratt (2012) during benchtop dehydration should not be universally applied to all grapevine species or varieties, or to other long-voesed liana species or organs, although they appear to support their conclusions for measurements of \( V. \) vinifera cv Glenora. For data shown in Choat et al. (2010) vulnerability curves generated by dehydration for \( V. \) vinifera cv Chardonnay were not significantly influenced by declining \( K_s \) max (Fig. 2a); \( K_s \) max values did not decline until \( \Psi_{\text{stem}} < -3.0 \) MPa, and in fact, some of the highest \( K_s \) max values were measured at \( \Psi_{\text{stem}} \) between \(-2.0\) and \(-2.5\) MPa (Fig. 2a). The \( K_s \) max of severely stressed samples (\( \Psi_{\text{stem}} < -3.3\) MPa) was reduced, but recalculation of the PLC for these points makes little difference to the shape of the curve or the \( \Psi_{\text{stem}} \) when PLC = 50 (\( \Psi_{50} \)), and this was well beyond the window when cavitation starts to occur on the vulnerability curves. Alsina et al. (2007) found remarkably similar results for Chardonnay stems subjected to the benchtop dehydration technique: \( K_s \) max increased slightly with lower \( \Psi_{\text{stem}} \) (Fig. 2b). Most importantly, the dehydration curves are still significantly different from the centrifuge curve. Native PLC values (Fig. 2 in Choat et al., 2010) for Chardonnay vines subjected to drought stress in the field were also similar to those predicted by dehydration curves of Choat et al. (2010) and of Alsina et al. (2007), where \( \Psi_{50} \) was \(-2.09\) MPa. Therefore, the key conclusion of Jacobsen & Pratt (2012), that centrifuge-based vulnerability curves are able to accurately estimate grapevine vulnerability to cavitation, is not supported for \( V. \) vinifera cv Chardonnay.

Reductions in \( K_s \) max associated with the dehydration technique reported by Jacobsen & Pratt (2012) could be explained by differences in tylose or gel formation rates between grapevine varieties (Fritschi et al., 2008). Grapevines are known to form tyloses/gels and occlude vessels very rapidly and in close proximity to pruning wounds. For example, tyloses developed in grapevine stems as quickly as 1 d after pruning and were found in \( c. 85% \) of vessels after only 6 d (Sun et al., 2006, 2008), with the bulk of the occlusion occurring within 25 mm of the pruning wound. Choat et al. (2010) avoided issues with tyloses/gel formation by dehydrating very long stems (2 m long) on a benchtop and then excising the central portion of this sample for \( K_s \) and PLC measurements. This sampling technique avoided stem tissues located closely to the pruning wounds and therefore most likely to contain new tylose formation. If one allows a pruned segment to sit for 48 h after pruning (Jacobsen & Pratt, 2012), it invariably will exhibit reduced \( K_s \) max and care must be taken to...
utilize appropriate tissue sampling to avoid this response close to the pruning wounds. Sun et al. (2007) provided convincing evidence for the link between ethylene production and tylose formation in pruned grapevines. Soaking samples in water for 48 h likely enhanced ethylene and vessel occlusion in the recent experiments by Jacobsen & Pratt (2012). Curiously, their study also found extensive gel formation for cut samples, while Sun et al. (2010) and with potted vines in our current data-set, but not in Jacobsen & Pratt (2012).

**Previous reports of cavitation resistance in V. vinifera**

Jacobsen & Pratt (2012) present data (table 1 in their paper) suggesting that Schultz (2003) and Zufferey et al. (2011) found $\Psi_{50}$ ranging from $-0.32$ to $-0.51$; however, these data represent whole-shoot conductance across a longer portion of the soil to plant to atmosphere continuum, including leaves, petioles, and roots. It is essential to recognize that vulnerability to cavitation can vary dramatically between organs of the same plant (McElrone et al., 2004). Two research groups have found that grapevine petioles are more susceptible than stems to water stress-induced cavitation (V. Zufferey et al. and S. D. Tyerman et al., unpublished). This pattern is common across numerous V. vinifera wine grape cvs, including: Chasselas, Gamay, Pinot Noir, and Chardonnay. The data of Lovisolo et al. (2008) also indicate that petioles and roots of grapevines are more vulnerable than stems. Inclusion of these data in the summary by Jacobsen & Pratt (2012) are misleading, particularly if roots and leaves are more vulnerable to embolism than stems.

**Implications for irrigation practices**

We believe it is vitally important to accurately reflect the current state of knowledge about grapevine vulnerability to cavitation induced by water deficit. From an ecological perspective, grapevines would be considered highly vulnerable to water stress-induced cavitation regardless of whether $\Psi_{50}$ is $-0.5$ MPa, as proposed by Jacobsen & Pratt (2012), or $-1.7$ MPa as proposed by Choat et al. (2010), because $\Psi_{50}$ varies from $-0.18$ to $-14.1$ MPa across all plant species measured (Maherali et al., 2004). However, the difference between irrigating to maintain
grapevines at or above −0.5 MPa compared with at or above −1.7 MPa would drastically alter water use in vineyards. In a multi-yr field experiment with various rates of applied irrigation water as a function of % crop evapotranspiration (ETc), Williams et al. (2010) demonstrated that 140% ETc is required to maintain midday mean $\Psi_{\text{leaf}} > -0.9$ MPa (c. -0.6 MPa $\Psi_{\text{stem}}$), while a 20% ETc irrigation treatment maintained $\Psi_{\text{leaf}} > -1.4$ MPa (c. -1.0 MPa $\Psi_{\text{stem}}$). Hence, seven times more irrigation water was required for relatively small changes in $\Psi_{\text{leaf}}$. If growers are led to believe that all grapevines lose c. 90% of their conductive capacity by $\Psi_{\text{stem}}$ = -1.0 MPa (as suggested Jacobsen & Pratt, 2012 and other studies cited within), overwatering of the crop is guaranteed to occur. Water-use efficiency is of particular concern since grapevines are most commonly grown in habitats that chronically suffer from water scarcity (e.g. Mediterranean climate of the western USA).

Conclusions

The data summarized here show that PLC measurements performed on V. vinifera grapevine stems immediately upon removal from the NMR are remarkably consistent with HRCT, field measurements, dehydration techniques, and air injection on long segments data for this variety. The only outliers inconsistent with this trend are the vulnerability curves generated using the short rotor (14.5 cm) centrifuge and air injection on short segments (Choat et al., 2010). There is now considerable debate over the impact of vessel length on vulnerability curves generated by centrifugal force (Cochard et al., 2010; Sperry et al., 2012). The cause of differences between various methods, different xylem anatomies, and versions of the centrifuge technique remains to be elucidated. We would not expect the data presented by Jacobsen and Pratt (2012: Fig. 1) to resolve this issue as both sets of samples (i.e. 14.5 vs 27.1 cm) still contained open conduits. Choat et al. (2010) suggested that for species/organisms with exceptionally long conduits (i.e. grapevines or roots) caution needs to be taken when deciding on the most appropriate method for generating vulnerability curves. We felt this was important given the proliferation of studies in this area in recent years. However, we again strongly emphasize that the centrifuge method works well for the vast majority of species. In agreement with Sperry et al. (2012), we feel that vulnerability curve methods should be validated with measurements of native embolism in intact plants using advanced visualization techniques or field dry-down conditions (Chardonnay field data presented in Fig. 2 of Choat et al., 2010). Additionally, promise may lie in developing a centrifuge with a longer rotor (1 m) that would ensure very few open vessels were present in samples from long-vasseled species.

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