



Is xylem cavitation resistance a relevant criterion for screening drought resistance among *Prunus* species?

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Summary

Fruit trees are likely to suffer from the effects of severe drought in the future; however, sound criteria for evaluating the species' ability to survive these extreme conditions are largely missing. Here, we evaluated the feasibility of using xylem cavitation resistance as a tool for screening *Prunus* species for drought resistance. Ten different *Prunus* species were selected to cover a large range of water requirements, from hydrophilic to xerophilic types. Shoot cavitation resistance was evaluated with the new Cavitron technique. At this inter-specific level, cavitation resistance was related to species drought resistance, with xerophilic species being less vulnerable to cavitation. The Cavitron technique enabled species characterization that required a short time and small amounts of plant material. This technique could be used to evaluate the drought resistance of a limited number of fruit tree genotypes. Genotype screening on a larger scale, however, would likely require another approach. Out of a number of anatomical traits tested, a significant correlation was found between cavitation resistance and inter-vessel wall thickness across species. This anatomical trait is, therefore, suggested as a possible alternative to direct cavitation estimates and could be included as a screening criterion in breeding programs for drought resistance of *Prunus* genotypes.

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Introduction

Drought conditions affect fruit tree production and are expected to become an increasingly severe problem based on future predictions for climate

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change and water availability. The sustainability of fruit production will depend on the identification of genetic material and plant characteristics that will contribute to future selection of drought-resistant fruit tree rootstocks and scions. Until recently, breeding programs for drought resistance focused on traits improving fruit yield under moderate drought conditions. However, during the intense drought episodes that have occurred recently in Europe, irrigation was limited in orchards and nurseries, and trees were subjected to exceptional water stress. This illustrates that the fruit trees may be threatened in the future by very drastic conditions. Tree resilience to drought is therefore an emerging aspect of drought resistance that should now be considered in breeding programs. This will necessitate the identification of new and relevant criteria for selecting more drought-resilient genotypes, as classical characters seem to be poor predictors of drought resilience.

A correlation has been found between drought resistance and xylem vulnerability to cavitation of a number of woody species (Salleo and LoGullo 1993; Cochard et al., 1996; Davis et al., 1998, 2002; Nardini et al., 2000; Vogt, 2001; Cordero and Nilsen, 2002; Sperry and Hacke, 2002; Lo Gullo et al., 2003; Pita et al., 2003; Tyree et al., 2003; Maherali et al., 2004). This suggests that species that are capable of maintaining functional xylem conduits under extreme drought conditions have a higher chance of survival, likely because they are able to extract water from the soil and thereby prevent dehydration of their leaves and meristems.

Determining the parameters of the xylem vulnerability curve (e.g., Alder et al., 1997) has been a tedious and time-consuming task. Therefore, only a small number of samples could have been analyzed and the method could not be applied routinely in selection and breeding programs. Recently, we developed a new measuring technique that makes use of a specially designed measuring rotor attached to a modified centrifuge (Cochard et al., 2005). This new 'Cavitron' technique has a number of advantages over the previous techniques in several key aspects. Unlike the standard technique introduced by Sperry et al. (1988), an entire vulnerability curve can be obtained on one stem segment with the new apparatus (typically a 0.30 m long piece of the tree shoot). This considerably lowers the quantity of plant material needed to record the cavitation resistance of a given genotype. Therefore, this allows the characterization of young plants, a decisive aspect for breeding programs of tree genotypes. The rapidity of the new technique is also a major advantage. The construction of an entire vulnerability curve

required about 1 week for one genotype with the Sperry et al. (1988) technique. With the new technique, this time can be reduced to less than 1 h. A relatively large number of genotypes can now be screened.

In this study, we focused on different species of the genus *Prunus*, which contains many of the most economically important fruit trees such as peaches, plums, apricots, cherries, and almonds. The first objective was to use the Cavitron technique to determine the vulnerability of the shoot of these species to cavitation, and in a number of wild *Prunus* species that could potentially be used in inter-specific crosses to enhance the drought resistance of *Prunus* rootstocks. A second objective was to identify more accessible anatomical traits correlated with cavitation resistance that could be used for screening *Prunus* genotypes on a much larger scale.

Material and methods

Plant material

Measurements were conducted on 10 different *Prunus* species. Four wild and six cultivated species were studied. *Prunus padus* L. (Bird Cherry) was collected in a humid area in the Auvergne Volcano Park. *P. avium* (L.) L. (Wild Cherry), and *P. spinosa* L. (Blackthorn) were sampled in a more mesophilic site from the same park. *P. mahaleb* L. (Mahaleb Cherry) was growing in a xerophilic site in the Limagne valley in the vicinity of the INRA-Clermont Campus. *P. cerasifera* Ehrh. (Myrobalan Plum), *P. cerasus* L. (Sour Cherry), *P. persica* (L.) Batsch (Peach), *Prunus domestica* L. (Plum), *P. armeniaca* L. (Apricot), and *P. dulcis* (Mill.) D.A. Webb = *amygdalus* Batsch (Almond) cultivated in the Limagne Valley, were collected from the INRA-Clermont orchards.

One-meter-long branches were sampled between June and July 2006 from the sun-exposed part of different trees for each species and brought to the laboratory in plastic bags (to minimise water loss), where they were analyzed for cavitation resistance on the same day.

Xylem vulnerability to cavitation

Xylem cavitation was assessed with the Cavitron technique (Cochard et al., 2005), a technique derived from the centrifuge method of Alder et al. (1997). The principle of the technique is to use centrifugal force to increase the water tension in a xylem segment and, at the same time, measure the decrease of its hydraulic conductance. The curve of percentage loss of xylem conductance (PLC) versus xylem water tension represents the sample vulnerability to cavitation. Vulnerability curves were determined on four–five different samples for each species. Samples were 0.28 m long and cut in air from the main axis of each branch.

Xylem pressure (P) was first set to a reference pressure (-1 MPa) and the sample maximal conductance (K_{\max}) was determined. The xylem pressure was then set to a more negative pressure and subsequently returned to the reference pressure to determine the new sample conductance K . The sample percent loss of conductance was then computed as $PLC = 100 \times (1 - K/K_{\max})$. The procedure was repeated for more negative pressures (with -0.5 or -1 step increments) until PLC reached at least 95%. Rotor velocity was monitored with an electronic tachymeter (10 rpm resolution) and xylem pressure was adjusted at ca. ± 0.02 MPa. The following sigmoid function was fit to each curve (Pammenter and van der Willigen, 1998):

$$PLC = 100 / (1 + \exp(s/25(P - P_{50}))),$$

where P_{50} is the pressure causing 50 PLC and s is a slope parameter. P_{50} and s values were averaged for each species and an ANOVA model GLM (Statgraphics Plus) was used to compare species.

Wood density

The wood xylem density of all samples analyzed was determined as follows. A 0.03-m-long segment was cut from one end of each sample, its fresh volume was measured by water displacement with an analytical balance, and its dry mass was then determined.

Leaf traits

Ten representative leaves were selected for each species for an estimate of mean leaf area (m^2) and leaf mass per area ($g m^{-2}$). Leaf area was determined with a planimeter (Delta-T, Cambridge, UK) and dry mass was measured on leaves dehydrated 24 h in an oven at $70^\circ C$.

Wood anatomical traits

One representative sample was selected for each species for anatomical observations. Cross-sections were obtained from one sample end and stained with Congo red. Sections were observed with a light microscope (Wild M11, Heerbrugg, Switzerland) equipped with a Camera Lucida and a digital measuring device. The resolution of the system was ca. $0.2 \mu m$. Approximately 60 observations of each of the following parameters were measured: lumen diameter (D) and vessel wall thickness, lumen diameter, and wall thickness of fibres. We also measured the tangential width (or beam) (b) and thickness (t) of bordered pit field walls, i.e., the pitted walls between adjacent vessels. Following Hacke et al. (2001), we derived the vessel hydraulic diameter ($D_h = \sum D^5 / \sum D^4$) and the mechanical strength of the bordered pit fields (t^2/b^2) from these data. Because t^2/b^2 varies greatly with b , we computed this value for $b = D_h$, for b equals the mean diameter of the 10 largest vessels and for $b = 37 \mu m$, the mean vessel diameter across species. Sample anatomical traits were correlated with the P_{50} value of the same sample.

Results

The vulnerability curves established with the Cavitron technique are shown in Figure 1 for the 10 different *Prunus* species. Curves had a typical 's' shape and were well fitted with the sigmoid function ($R^2 > 0.99$). Cavitation resistance varied significantly among *Prunus* species (Figure 2). The xylem pressure P_{50} corresponding to 50 percent loss of conductance ranged from -3.5 MPa for the most vulnerable species (*P. padus*) to -6.3 MPa for the least vulnerable one (*P. cerasifera*) (Figure 2). Species having more negative P_{50} values exhibited significantly lower slopes as well (Figure 3), but the relation was weak ($R^2 = 0.41$, $P = 0.04$). Species were classified into three groups according to their P_{50} values: cavitation vulnerable species with P_{50}

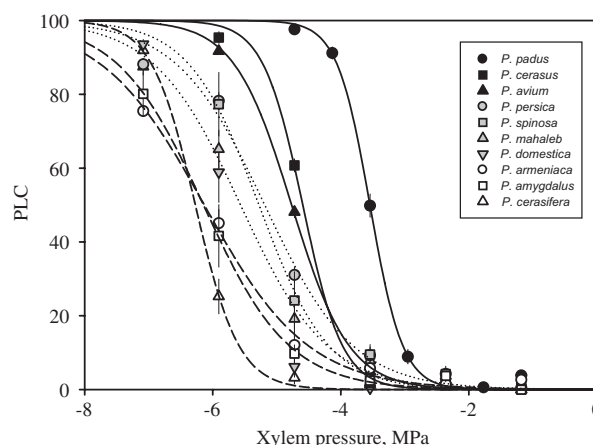


Figure 1. Xylem vulnerability curves of the shoots of 10 *Prunus* species. Lines are best logistic fits and error bars represent one SE.

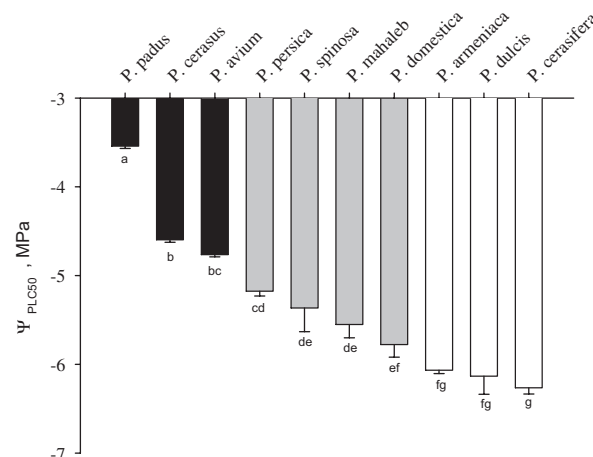


Figure 2. Mean xylem pressures causing 50 percent loss of xylem conductance (P_{50}) for 10 *Prunus* species. Error bars represent one SE. Species not sharing a letter in common are significantly different at $P = 0.05$.

above -5 MPa (*P. padus*, *P. avium*, *P. cerasus*); resistant species with P_{50} values below -6 MPa (*P. dulcis*, *P. armeniaca*, *P. cerasifera*); and intermediate species (*P. spinosa*, *P. domestica*, *P. persica*, *P. mahaleb*).

Significant differences were found between species in the different microscopic and macroscopic traits measured. However, very few were correlated with cavitation resistance (Table 1). For instance, vessel wall thickness was fairly constant across species and did not vary with vessel diameter (Figure 4). Fiber wall thickness exhibited more variation across species but was also not

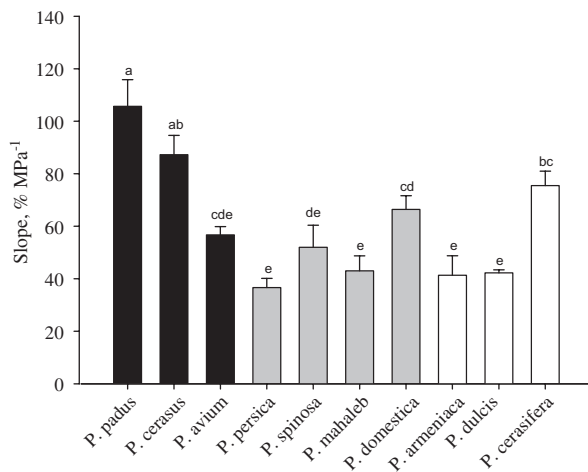


Figure 3. Mean slope parameter of the vulnerability curve for 10 *Prunus* species. Error bars represent one SE. Species not sharing a letter in common are significantly different at $P = 0.05$.

related to cavitation resistance (Figure 5). Wood density and leaf traits were very poorly correlated with cavitation resistance ($r^2 < 0.4$). However, the thickness of the wall between two adjacent vessels (t , walls with bordered pit fields) differed significantly across *Prunus* species and consistently exhibited higher values for more cavitation-resistant ones. t increased with vessel lumen diameter (Figure 6), but high correlations with cavitation resistance were found both with the overall mean t value, and with the t value of the largest vessels (Figure 7). The correlation with inter-vessel wall mechanical strength (t^2/b^2) was less significant

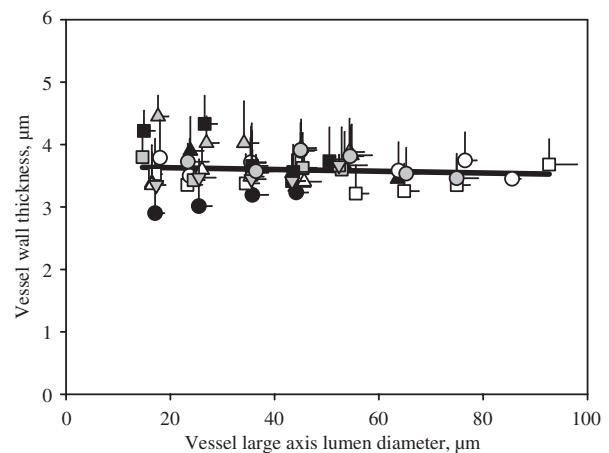


Figure 4. Xylem vessel wall thickness versus large axis lumen diameter of these vessels. Error bars represent one SD. Species symbols are as in Figure 1.

Table 1. Correlation analysis between xylem cavitation (P_{50}) and different macroscopic or microscopic traits

Traits	r	F	P
Inter-vessel wall thickness (10 biggest vessels)	-0.94	56.6	<0.0001
Inter-vessel wall thickness (total vessels)	-0.90	32.8	0.0004
Inter-vessel wall thickness (37 μm vessels)	-0.90	34.4	0.0004
Inter-vessel wall strength (37 μm vessels)	-0.87	25.1	0.001
Fiber lumen diameter	0.65	5.9	0.04
Inter-vessel wall strength (total vessels)	-0.62	5.1	0.05
Vessel wall strength (10 biggest vessels)	0.62	4.9	0.06
Inter-vessel wall strength (10 biggest vessels)	-0.62	4.9	0.06
Leaf mass area	-0.61	4.7	0.06
Vessel diameter	-0.61	4.7	0.06
Fiber apparent density	-0.51	2.8	0.14
Wood density	-0.38	1.3	0.28
Fiber wall strength	-0.35	1.1	0.32
Inter-vessel wall strength (at hydraulic diameter)	-0.26	0.6	0.46
Vessel wall thickness (total vessels)	-0.26	0.6	0.47
Fiber wall thickness	-0.24	0.5	0.51
Leaf area	0.22	0.4	0.55

Traits with significant correlations ($P < 0.05$) are shown in bold.

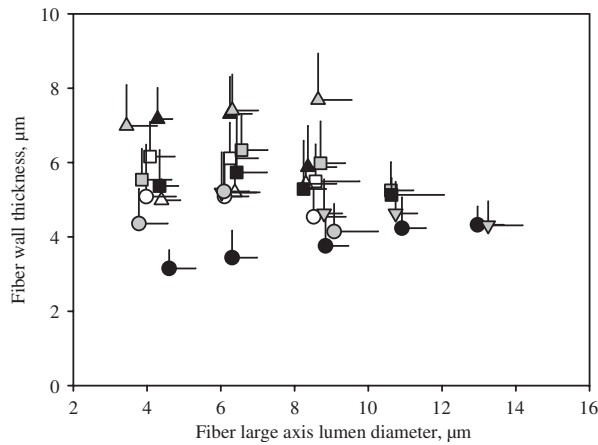


Figure 5. Fiber wall thickness versus fiber large axis lumen diameter. Error bars represent one SD. Species symbols are as in Figure 1.

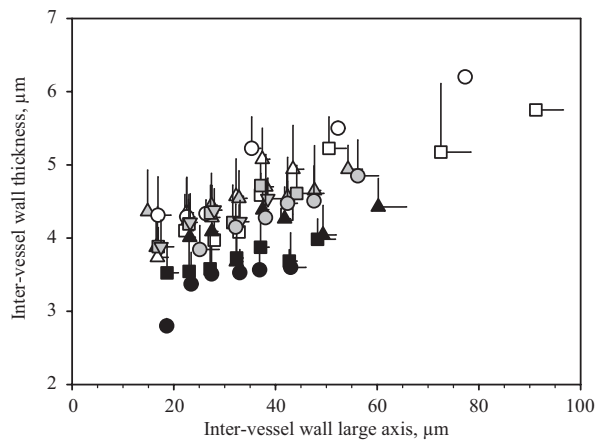


Figure 6. Thickness of the wall between adjacent xylem conduits versus the width of the wall. Error bars represent one SD. Cavitation-resistant species (open symbols) have thicker walls. Species symbols are as in Figure 1.

except when species were compared at a mean wall width of 37 μm (Figure 8).

Discussion

To the best of our knowledge, cavitation resistance has not yet been documented for the *Prunus* species included in this study. In a previous study (Améglio et al., 1998), we used the air injection method to induce cavitation in peach tree. The results with this technique were different ($P_{50} = -3.0$ MPa compared with -5.2 MPa in this study) and inconsistent with embolism levels measured on dehydrating trees. Further, we reported a complete stomatal closure at -3.5 MPa,

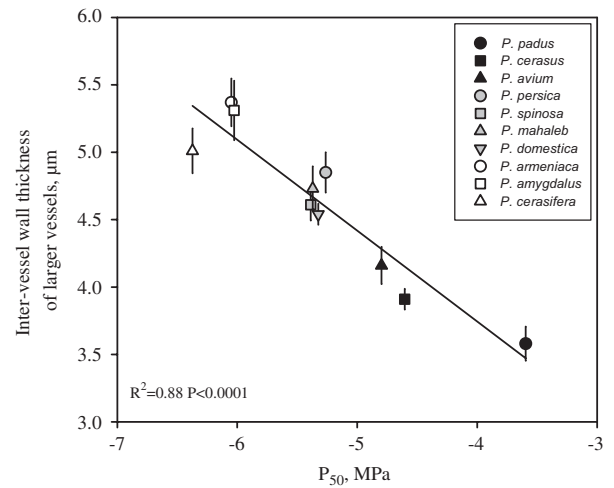


Figure 7. Inter-specific correlation between inter-vessel wall thickness and the xylem pressure causing 50% loss of the hydraulic conductance (P_{50}). The relation is shown for the largest vessels in each species. Error bars represent one SE. The more cavitation-resistant species (open symbols) exhibit thicker walls.

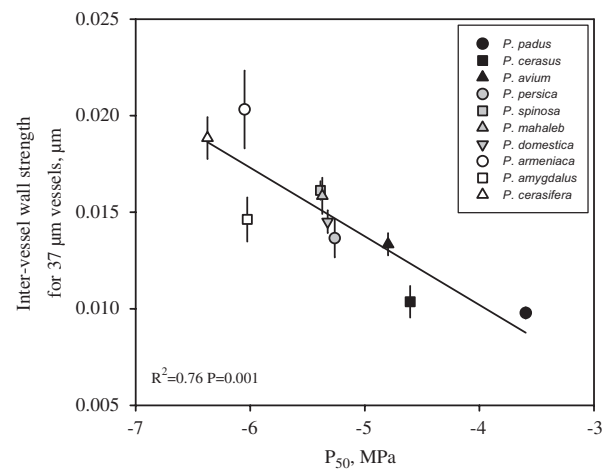


Figure 8. Inter-specific correlation between inter-vessel wall mechanical strength and the xylem pressure causing 50% loss of hydraulic conductance (P_{50}). Wall strength is computed as the square of the ratio between wall thickness and wall width. The relation is shown for wall having a width of 37 μm. Error bars represent one SE. The more cavitation-resistant species (open symbols) exhibit stiffer walls.

which is consistent with the onset of cavitation reported in this study.

Cavitation resistance differed significantly across the *Prunus* species we evaluated in the present study. Compared with other woody species, *Prunus* are relatively resistant to cavitation (Maherali et al., 2004), although substantial variation exists across species. Species habitat preferences were consistent with their vulnerability to cavitation.

For instance, the most vulnerable species (*P. padus*) was the only species clearly restricted to wet habitats, while more resistant species such as *P. dulcis*, *P. armeniaca*, *P. cerasifera*, or *P. mahaleb* were clearly associated with drier Mediterranean climates. In this study, the native species were sampled in their natural habitats, and were therefore exposed to contrasting growth conditions. An acclimation of the hydraulic traits to these conditions is possible (Ladjal et al., 2005). The results of this study await confirmation from a common garden experiment. With this possible restriction, our data would confirm the hypothesis that cavitation resistance correlates with drought resilience across species and may thus be used as a screening criterion for this character.

The new Cavitron technique was successful in discerning differences in cavitation resistance among *Prunus* species. As samples were cut in air, the longest vessels were probably not conducting water. This may have biased the vulnerability curves towards more negative values, as longer and bigger vessels tend to be more vulnerable to cavitation within a sample (Lo Gullo et al., 1995). However, xylem anatomy was relatively homogeneous across species; thus, if there was a bias with the technique, it most likely affected all species in the same manner. Establishing the vulnerability curve of one sample with the Cavitron typically took half an hour. Because of inherent variation between samples, four–five replicates were necessary in order to ascertain statistically significant differences across species. This means that no more than two species (or two genotypes) could be evaluated during a working day with this technique. This is considerably faster than previous techniques. It could be possible to determine the PLC value at one single pressure (typically -5 MPa) instead of constructing a whole curve, but this may only double the number of samples a day. The method is thus appropriate to characterize the drought performance of identified genotypes with high agricultural value. However, it remains unrealistic to use this technique in progeny or QTL analyses when hundreds of genotypes must be evaluated. Therefore, large-scale screening for cavitation resistance is probably conceivable only with correlated and more accessible parameters.

Among the different microscopic and macroscopic traits we measured, very few were correlated with xylem cavitation. The most accessible parameters (such as leaf traits or vessel diameter) were poorly or negatively correlated with P_{50} (the more cavitation-resistant *Prunus* species had larger vessels). Wood density was not correlated with P_{50} in this study, contrary to recent reports (Hacke et al., 2001;

Jacobsen et al., 2005). The better correlation in these studies was likely due to a much larger range of P_{50} values across species. The possible presence of reaction wood in our samples may also explain this result. Nevertheless, this result suggests that the use of wood density as an indicator of cavitation resistance across *Prunus* species is not realistic. However, good correlations exist between cavitation resistance and the anatomy of walls between adjacent vessels among *Prunus* species. Hacke et al. (2001) suggested that vessel wall reinforcement is required for cavitation resistance in order to prevent wall implosion when xylem pressure is very negative. Our study confirms this finding. Theoretically, the best correlation should be found between P_{50} and $(t^2/b^2)_h$, the wall reinforcement at the mean vessel hydraulic diameter. Here, the best correlation was found with $(t^2/b^2)_{37}$, with the wall reinforcement for vessel wall having $37\ \mu\text{m}$ width. This discrepancy might be due to the fact that larger vessels were not conductive in our samples. However, an even better correlation was found between P_{50} and the wall thickness of the largest vessels. This correlation is interesting for practical considerations. Indeed, (t^2/b^2) values vary considerably between vessels of different size and a correct estimate of $(t^2/b^2)_h$ is difficult. Measuring t values only for the largest vessels is much more feasible and may easily be programmed with digital image analysis software.

In conclusion, cavitation resistance shows substantial variation across *Prunus* species and is consistent with the variation in species water requirements. The Cavitron technique is successful in discerning differences among species, and requires a relatively short time and only a small amount of plant material. It may be a valuable tool for evaluating the drought resistance of a moderate number of genotypes. However, screening of hundreds of genotypes with this technique is unrealistic. The thickness of the wall between adjacent vessels was the only anatomic parameter highly correlated with cavitation resistance and it is conceivable to use this trait in screening programs for *Prunus* drought resistance. Differences at the species level may be too coarse for agricultural purposes. For such applications, the main aim is to find the more resistant genotypes within the species, comparing different cultivars/lines/accessions. It remains to be established that differences in cavitation resistance are discernable at this level.

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