Using the Pneumatic method to estimate embolism resistance in species with long vessels: a commentary on the article "A comparison of five methods to assess embolism resistance in trees"

Luciano Pereira[a,b,*], Paulo R.L. Bittencourt[c,*], Lucy Rowland[c], Mauro Brum[d], Marcela T. Miranda[a], Vinicius S. Pacheco[b], Rafael S. Oliveira[b], Eduardo C. Machado[a], Steven Jansen[e], Rafael V. Ribeiro[a,*]

[a]Laboratory of Plant Physiology "Coaracy M. Franco", Center R&D in Ecophysiology and Biophysics, Agronomic Institute (IAC), Campinas SP, Brazil.
[b]Department of Plant Biology, Institute of Biology, University of Campinas (UNICAMP), Campinas SP, Brazil.
[c] College of Life and Environmental Sciences, University of Exeter, Exeter, United Kingdom.
[d]Department of Ecology and Evolutionary Biology, University of Arizona, Tucson AZ, United States.
[e]Institute of Systematic Botany and Ecology, Ulm University, Ulm, Germany.
*Corresponding authors: biolpereira@gmail.com; paulo09d@gmail.com; rvr@unicamp.br

Comparisons among methods are essential to validate plant traits measured across studies. The paper by Sergent et al. (2020) on potential differences in estimating xylem embolism resistance based on various methods is therefore a welcome contribution. The Bench dehydration (Sperry et al., 1988), Air-pressurization (Cochard et al., 1992), Flow-centrifuge (Cochard, 2002), Microcomputed tomography – MicroCT (Brodersen et al., 2010), and Pneumatic (Pereira et al., 2016) methods were evaluated regarding their reliability. While we do recognize the importance of any validation effort for comparing methods as the leading way to scientific innovativity, a rigorous analysis is a complex task that needs to take into account not only the principle of the method and its correct use, but also inherent intraspecific and interspecific trait variability, something we feel is not fully considered by Sergent et al. (2020).

Sergent et al. (2020) compared the Bench dehydration, MicroCT, and Pneumatic methods using three long vessel species. No method agreed on the estimates of embolism resistance for *Laurus nobilis*, whereas all curves estimated for *Quercus ilex* were similar. On the other hand, the Ψ_{50} value estimated by the Pneumatic method for *Olea europaea* diverged from the estimates of the other methods. We tested this finding here by measuring independently vulnerability curves for *O. europaea*. We used the Pneumatron, an automated tool based on the Pneumatic method (Pereira et al., 2020), to measure embolism vulnerability in one individual of *O. europaea* (Fig.

1). Although the origin of our plant samples was different from Sergent et al. (2020), it is reasonable to compare our curves with this study. In fact, Sergent et al. (2020) assumed a low intraspecific variability and used different plant material for the Bench dehydration, MicroCT, and Pneumatic methods. Our curves produced similar embolism resistance estimates to those obtained by MicroCT and Bench dehydration methods and significantly differed from the Pneumatic curve obtained by Sergent and colleagues. Our Pneumatic Ψ_{50} was -4.18 ± 0.12 MPa and similar to MicroCT Ψ_{50} of -4.4 ± ~0.6 MPa for *O. europaea* (Torres-Ruiz et al., 2014). Unfortunately, the margin of error was not reported for the Ψ_{50} of *O. europaea* based on MicroCT in Sergent et al. (2020) or Torres-Ruiz et al. (2017), where Ψ_{50} was estimated to be -5.36 MPa. In relation to the Bench-dehydration method, Ψ_{50} estimated with the Pneumatic method was slightly higher (-4.18 ± 0.12 *vs.* -5.0 ± 0.3 MPa). More importantly, vulnerability curves obtained with the Pneumatic method were strongly correlated with the ones estimated with the Bench dehydration and Centrifuge methods (Jansen et al., 2020; Pereira et al., 2020, 2016; Zhang et al., 2018) for 18 species, including many long vesseled species, with some of these shown in Figure 1. Based on this, we confirm that the pneumatic method is able to accurately estimate embolism resistance for long-vesselled species.

Although Sergent and colleagues, as well as Zhang et al. (2018), have suggested inconsistencies with the Pneumatic method for tracheid-bearing species, the most important question is why pneumatic measurements are challenging for tracheids with a torus-margo bordered pit structure. Only if we are able to answer this question, the Pneumatic method can be said not to provide a valid approach for conifers, or the method could be modified slightly (such as a reduced vacuum pressure to avoid complete pit aspiration).

What is measured by the Pneumatic method?

The Pneumatic method measures the amount of gas that can be sucked from desiccating plant xylem. Gas diffusion kinetics of pneumatic measurements rely on Fick's law for gas diffusion and the ideal gas law, considering that the pressure change is measured during the gas extraction. Also, partitioning of gas concentration between liquid and gas phases, as described by Henry's law, may explain a small part of the total amount of gas discharged (Melvin Tyree, personal communication). Thus, the Pneumatic method quantifies embolism resistance based on gas extraction from intact and embolised conduits that are connected via interconduit pit membranes with the cut open vessels to which the Pneumatic apparatus is attached (Jansen et al., 2020). Obviously, this method represents a non-hydraulic approach and is comparable with other methods that directly quantify embolism formation by either volume or area. Depending on the species, it may not be directly related to loss of conductivity (Venturas et al., 2019) and this could be caused by xylem pressure heterogeneity among large and small vessels (Bouda et al., 2019). Vulnerabilty curves obtained by methods based on different principles measure different responses to dehydration. For this reason, the Pneumatic method should be comparable with other non-hydraulic methods such as the MicroCT, Optical, and Acoustic methods. Also, practice with available methods is a key factor and may justify contrasting results obtained by different research groups using the same method. The Pneumatic method is relatively new (Pereira et al., 2016) and common mistakes are possible when the method is applied by new users, such as incorrect or no adjustment of the discharge tube volume when measuring plant species with a variable xylem anatomy. Since the ideal gas law is used for gas quantification, the discharge tube volume is crucial for precise measurements (see discussion in Pereira et al. (2020) and Jansen et al. (2020)). Another essential aspect includes correct measurements of the minimum (GD_{min}) and maximum (GD_{max}) amount of gas discharged, because the percentage of gas discharged (PGD) is determined by these two reference points. Therefore, stable measurements of GD_{min} and GD_{max} should be taken. Moreover, accurate measurements of GD_{min} and GD_{max} are less straightforward with the manual Pneumatic apparatus than the Pneumatron (Pereira et al. 2016). For this reason, we encourage users to use the automated Pneumatron (Pereira et al., 2020), which guarantees correct measurements of GD_{min} and GD_{max} due to the high temporal resolution, and clearly allows interbranch and interspecific comparisons (Fig. 1).

The Pneumatic method – due to its simplicity to collect and analyze data – provides a straightforward technique, not only for enhancing our understanding of embolism resistance in highly diverse biomes (e.g. Barros et al., 2019; Bittencourt et al., 2020; Brum et al., 2019; Lima et al., 2018; Oliveira et al., 2019), but also to evaluate embolism resistance in breeding programs that aim to select crosses/hybrids with interesting hydraulic traits and high resistance to embolism (Jansen et al., 2020). Because pneumatic measurements rely on gas diffusion, this method provides also novel approaches to study embolism spreading and air-seeding, which remain one the most important shortcomings in our understanding of plant water transport under negative pressure.

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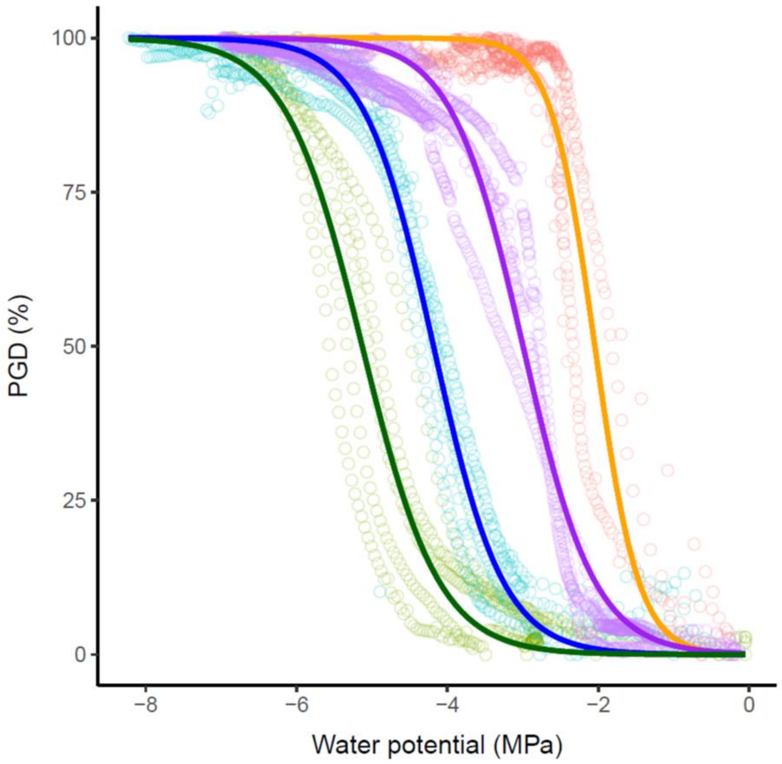

Fig. 1 – Percentage of gas discharged (PGD) as a function of xylem water potential measured in terminal branches of species with long vessels: *Citrus sinensis* (Ψ_{50} = -1.44 ± 0.55, *n* = 4, orange), *Schinus terebinthifolius* (Ψ_{50} = -2.09 ± 0.09, *n* = 4, purple), *Olea europaea* (Ψ_{50} = -4.18 ± 0.12 MPa, *n* = 5, blue), and *Eucalyptus camaldulensis* (Ψ_{50} = -4.17 ± 0.58, *n* = 5, green). Data from Pereira et al. (2020), except for *Olea europaea*. PGD was automatically measured every 15 min and the xylem water potential was measured five times during the branch dehydration of *O. europaea*, using a pressure chamber. Then, the water potential was estimated for every 15 min considering a linear decrease between consecutive measurements, as done by Pereira et al. (2020). We used terminal branches longer than 1 m with a similar canopy position to avoid variability. All samples were collected before 7:00 am to avoid dehydration.