Which method to assess embolism resistance?
A comparison of 4 techniques on 5 native Patagonian species

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Introduction

- ❑ **Vulnerability curve**, the relationship between the embolism estimate and the xylem potential, describes plant vulnerability to drought induced cavitation, a crucial trait that correlates with several plant functions and adaptive strategies.
- ❑ Several techniques have been developed to build VC [1], among them :
  - ✓ The *classic bench dehydration* (bench) consists in measuring concurrently water potential and the loss of hydraulic conductance at different dehydration steps on samples freely dehydrating in the air.
  - ✓ The *pneumatic bench dehydration* (pneumatic) consists in assessing concurrently water potential and an estimate of air volume (AV) that progresses into a branch while it dehydrates in the air.
  - ✓ The *air injection* method consist in applying positive pressures to induce embolism and concurrently measuring the loss of hydraulic conductance.
  - ✓ The *Cavitron* uses centrifugation to generate negative pressure and embolism. Conductivity is assess during the spinning procedure thanks to a hydrostatic gradient.
- ❑ Previous studies showed that methods to perform VC can be subjected to important artifacts that generally underestimate cavitation resistance [1]. It is important to provide rigorous comparison of Bench, Cavitron and Air Injection. The pneumatic still needs independent evaluation.

OBJECTIVE : Comparing four methods to assess vulnerability to cavitation on six native Patagonian species

Material, methods and checkings

❑ Plant materials size and sampling :

Measurements were performed on the same 5 individuals per species during the same month. Samples were collected in the same area at max (50 km from Bariloche, Argentina)

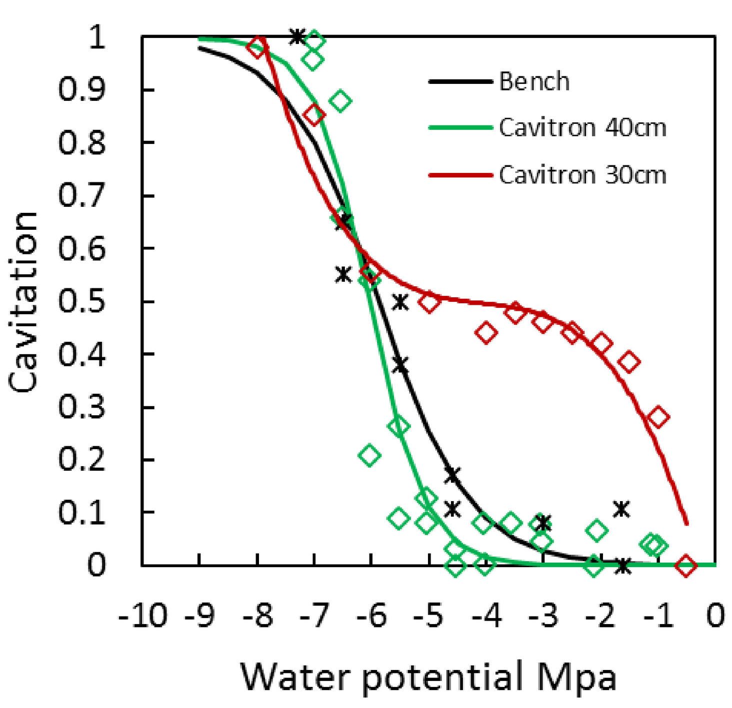
Species	*Nothofagus antartica*	*Nothofagus pumilio*	*Lomatia hirsuta*	*Maytenus boria*	*Austrocedrus chilensis*
Local name	Ñire	Lenga	Radal	Maiten	Cipres
Vessel length	19	16	12	22	Tracheids

❑ VC construction methods :

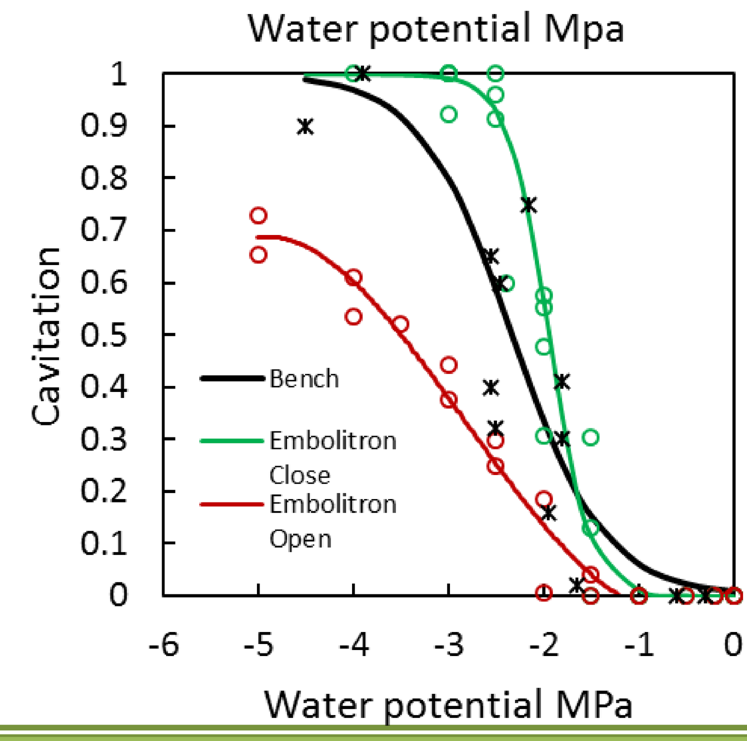
➢ The Bench Dehydration was applied to angiosperms by taking all precautions [ref]. For conifer PLC was measured gravimetrically on different samples of a same branch.

➢ The Pneumatic was applied on samples at least 3 times larger than maximum vessel length. Air volume was repeatedly measured along with water potential during branch dessication [2]

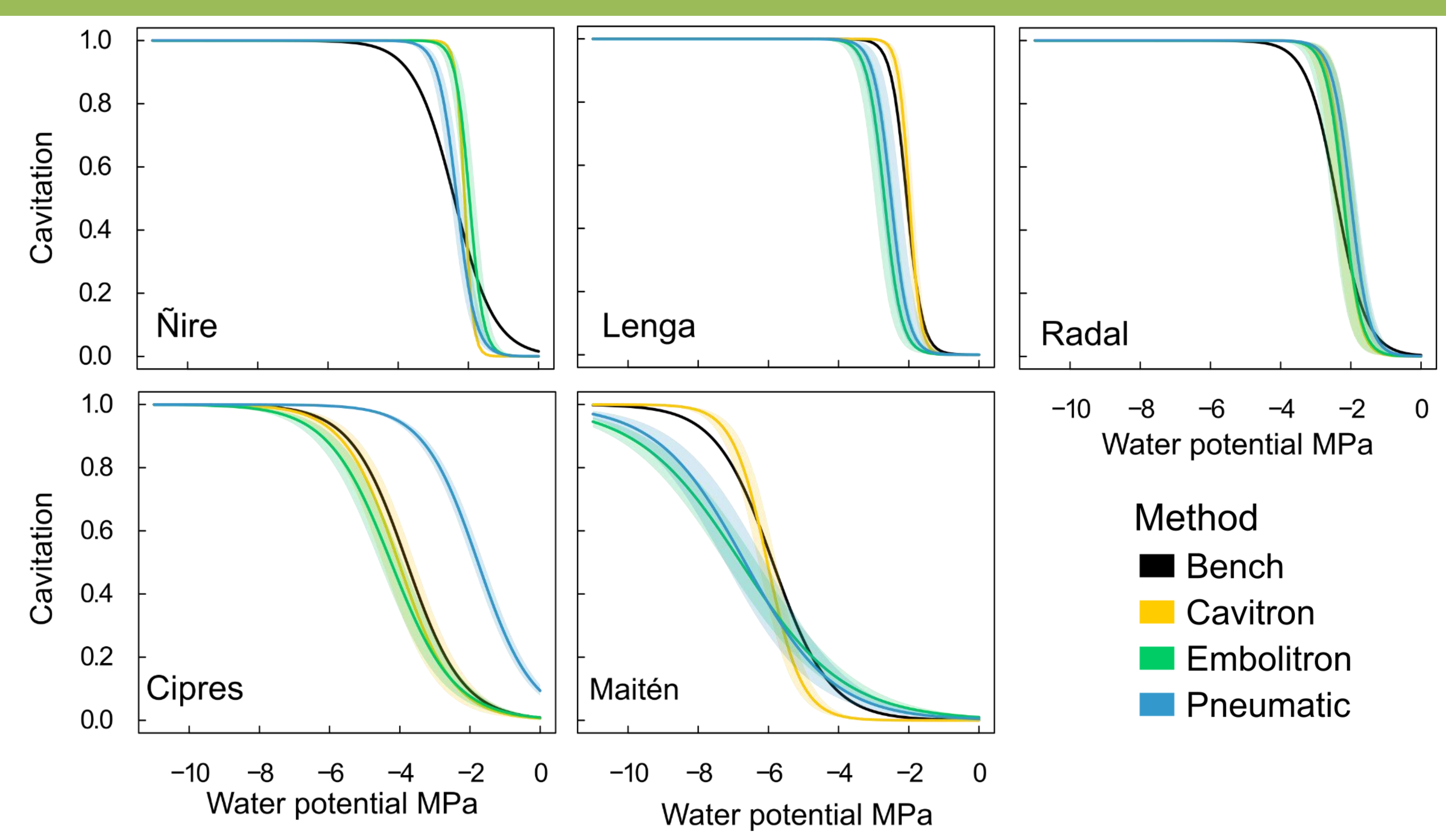
➢ The Cavitron was applied with two different rotor size. For *Maitenus*, species with longest vessel we report doubtfull shapes (R or double sigmoids) that could be corrected by using longer rotors. Other species showed similar results with the two rotor sizes.



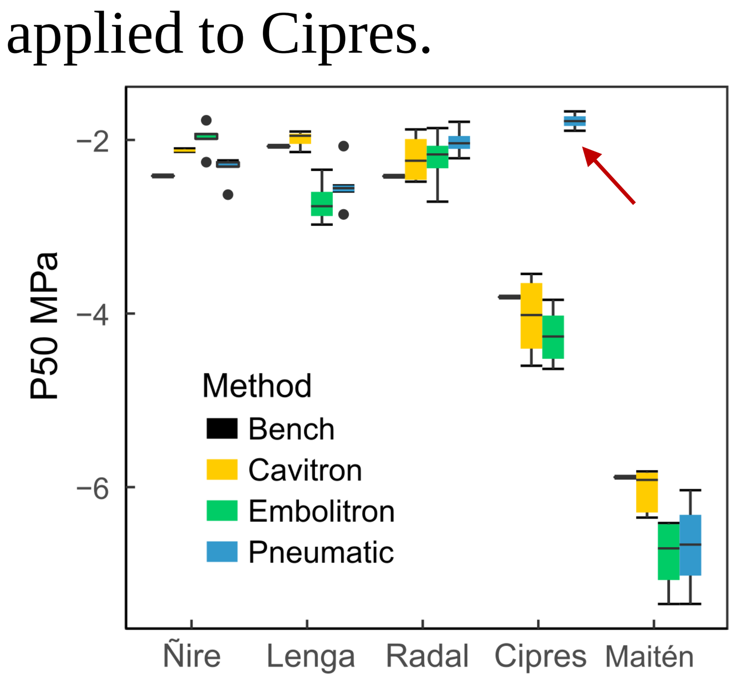
➢ The Air injection was applied with the semi-automatized device called *Embolitron*. It was necessary to position the chamber on the middle of the sample and to use samples longer than maximum vessel length with tiny diameters. Otherwise a strong overestimation of embolism resistance was obtained. This is probably due to a vessel length-type artifact.



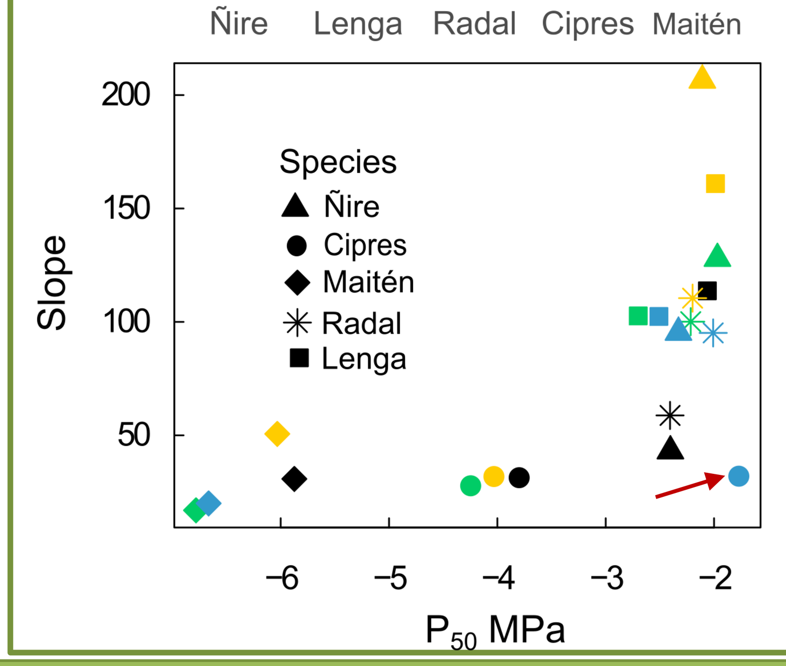
Results



For each species the fitted vulnerability curve and confident interval (95%) are represented for all methods. The confidence interval could not be computed for the bench. The plot evidence similar pattern for all methods for a given species except for the pneumatic method applied to Cipres.



For each species the distribution of individual P_{50} are shown for all methods with different colors. The outlier obtained with the pneumatic on cipres is indicated by an arrow.



Relationship between *Slope* and P_{50} of the vulnerability curve among species obtained with each method. The outlier obtained with the pneumatic on cipres is indicated by an arrow.

Discussion & Conclusion

- ❑ After ensuring there was no open-vessels artifact in *Cavitron* and *Embolitron* measurements, all methods yielded similar ranking of embolism resistance among angiosperm species and consistent pattern of slope vs P_{50} for angiosperm.
- ❑ Open-vessel artifact can lead to an overestimation of embolism resistance in *Embolitron*
- ❑ For Cipres all methods yielded similar vulnerability curves, except the Pneumatic which strongly underestimated of P_{50} qnd overestimqted the *slope*.
- ❑ For some species with high P_{50} significant differences was punctually obtained (up to 30% for Lenga)

References

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[2] Pereira, L., Oliveira, R.S., Pereira, L., Bittencourt, P.R.L., Oliveira, R.S., Junior, M.B.M., *et al.* (2016). Plant pneumatics : stem air flow is related to embolism – new perspectives on methods in plant hydraulics Methods Plant pneumatics : stem air flow is related to embolism – new perspectives on methods in plant hydraulics. *New Phytol.*, 357–370

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