Yin Pengxian (Orcid ID: 0000-0003-4433-1664)

A comparison of two centrifuge techniques for constructing vulnerability curves: insight into the 'open-vessel' artifact

Pengxian Yin[a], Feng Meng[b], Qing Liu[a], Rui An[b], Jing Cai[a,*], Guangyuan Du[b,*]

[a] College of Forestry, Northwest A&F University, Yangling, Shaanxi 712100, China

[b] College of Science, Northwest A&F University, Yangling, Shaanxi 712100, China

Correspondence

*Corresponding authors,

e-mails: cjcaijing@163.com;

duguangyuan@aliyun.com

A vulnerability curve (VC) describes the extent of xylem cavitation resistance. Centrifuges have been used to generate VCs for decades via static- and flow-centrifuge methods. Recently, the validity of the centrifuge techniques has been questioned. Researchers have hypothesized that the centrifuge techniques might yield unreliable VCs due to the open-vessel artifact. However, other researchers reject this hypothesis. The focus of the dispute is centred on whether exponential VCs are more reliable when the static-centrifuge method is used than with the flow-centrifuge method. To further test the reliability of the centrifuge technique, two centrifuges were manufactured to simulate the static- and flow-centrifuge methods. VCs of three species with open vessels of known lengths were constructed using the two centrifuges. The results showed that both centrifuge techniques produced invalid VCs for *Robinia* because the water flow through stems under mild tension in centrifuges led to an increasing loss of water conductivity. Additionally, the injection of water in the flow-centrifuge exacerbated the loss of water conductivity. However, both centrifuge techniques yielded reliable VCs for *Prunus*, regardless of the presence of open vessels in the tested samples. We conclude that

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centrifuge techniques can be used in species with open vessels only when the centrifuge produces a VC that matches the bench-dehydration VC.

Abbreviations − BD, bench-dehydration; PLC, percentage loss of hydraulic conductivity; LPFM, low-pressure flow meter; VC, vulnerability curve.

Introduction

There is extensive evidence that tree species with various vascular structures have different vulnerabilities to cavitation events (Wang et al. 2014a). Vulnerability curves (VCs) have been used for many years to quantify the tree cavitation response to drought conditions. The VC displays the percentage loss of hydraulic conductivity (PLC) versus xylem tension experienced during water deficit.

Since 1985, thousands of VCs have been constructed to evaluate the drought resistance of plants (Cochard et al. 2013). Several techniques for measuring VCs have been developed and can be classified based on how cavitation is induced or how hydraulic conductivity is measured (Cochard et al. 2013). Bench-dehydration, air pressurization and centrifugation have been most commonly used to induce cavitation in stems (Alder et al. 1997, Cochard et al. 1992, Ennajeh et al. 2011a, Tyree et al. 1992). Embolism induced via different methods can be detected gravimetrically (Li et al. 2008) or through the Cavitron method (Cochard et al. 2005) by the loss of xylem hydraulic conductivity.

The Cavitron method (also called the flow-centrifuge method and ChinaTron) and the original-rotor centrifuge method (also called the static-centrifuge method and SperryTron method) have been a subject of debate. Cochard et al. (2010) reported that the Cavitron method is subject to an open-vessel artifact. As stem segments are shortened, more vessels are cut open, which leads to a more exponential-shaped curve. In contrast to a sigmoid-shaped curve, an exponential-shaped curve indicates high PLC as soon as the xylem pressure drops

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below zero. It seems unlikely that plants would undergo such high PLC in response to a mild water potential decrease. In recent years, studies have reported on the inaccuracy of the Cavitron methods (Cochard et al. 2010, Choat et al. 2010, Wang et al. 2014a, Martin-StPaul et al. 2014, Torres-Ruiz et al. 2017). However, other researchers have reported that centrifuge curves are not always prone to the open-vessel artifact when the SperryTron method is used (Christman et al. 2012, Sperry et al. 2012, Hacke et al. 2015).

There are several differences between the two types of centrifuge-techniques. In the Cavitron method, the stem is tightly fixed in a narrow and limited slot. The stem ends are enclosed in two cuvettes that are slightly larger than the stem ends. Because the holes are at different heights on the surface of the two cuvettes, different centrifugal forces can be induced at the two ends. Hence, the water level in the upstream cuvette decreases over time in proportion to the water flow. The water flow through the stem can be determined by measuring the distance between the air and water menisci in the cuvettes (Cochard et al. 2005). In the SperryTron method, larger reservoirs are used and stem ends are remote from the reservoir ends. Because the conductivity is not measured and the water levels at the two ends are the same during spinning, there is no water flow through the stems (Sperry et al. 2012).

These variances in the design of the two centrifuge rotors might cause formation of additional types of embolism during spinning. Firstly, the frequent flow induced in the Cavitron rotor might sweep nanoparticles into the stem. These nanoparticles may be air bubbles or solid impurities, which can be filtered out by pit membranes or vessel ends. However, in vessels that have been cut open, solid impurities can easily be swept in and plug a functional vessel. Air bubbles might much more easily cause embolism formation than solid impurities because they can expand in stem vessels in which the pressure is below atmospheric pressure. Air bubbles might also gather and expand in the centre of cut-open vessels by migrating to the site of the most negative tension. Secondly, the cuvettes in the two centrifuge rotors are quite different in size. To observe the meniscus in the Cavitron rotor, cuvettes are much smaller to

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allow them to be tightly fitted. The base area of a common cuvette is usually 1 cm^2, which is 2-3 times larger than the area of a stem section frequently used in a Cavitron. Under this circumstance, a large number of air bubbles can be created by water which hit the meniscus when water is injected via a hypodermic needle during spinning. In the tiny space between the air-filled water and stem ends, air bubbles can be easily swept in and migrate through the open vessels. These bubbles can also expand quickly for the reasons described above. Cuvettes in traditional centrifuge rotors are much larger, and no additional water is injected during spinning, which should reduce the possibility of additional embolism formation.

Because no laboratory has been equipped with both types of centrifuges at the same time, researchers have not been able to compare the VCs provided by the two methods for the same species growing under the same conditions at the same time. Researchers measuring VCs might have to transfer the samples to different laboratories around the world (Torres-Ruiz et al. 2014) or take samples from the same species grown in different places. Neither of these strategies can eliminate the effects of both cavitation fatigue and the environment on the VCs at the same time.

To evaluate the accuracy of the two centrifuge types for measuring VCs, two types of centrifuges (Cavitron and SperryTron) were manufactured by the Xiangyi Company, Hunan, China. Cavitron was based and improved upon the design of Cochard et al. (2005), and SperryTron imitated the original method (Alder et al. 1997), which has two original rotors of different sizes. Using the two centrifuges, we tested the accuracy of the static- and flow-centrifuge techniques. We also designed a series of control experiments to examine species that produced exponentially shaped VCs to seek possible reasons for the open-vessel artifact.

Materials and methods

Plant material

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Experiments were conducted on three tree species during September and October 2017. *Prunus persica* and *Acer mono* were harvested from the south campus of Northwest A&F University in Yangling Shaanxi, China (34°162N 108°42E, 457 m a.s.l.). *Robinia pseudoacacia* stems were harvested from the bank of the Weihe River in Yangling. All experiments were conducted on current-year stems that were harvested in the early morning. Excised branches were longer than 1.3 m, which had been shown to be longer than the maximum vessel length of all species. To prevent further dehydration, excised branches were enclosed in humidified black plastic bags and brought to the laboratory within 20 min. Samples used in the bench-dehydration method were harvested on rainy days so that the high water tension was near zero.

Vessel length measurement and the estimation of the fraction of open vessels

The air injection method was used to measure vessel length. The computation method was as described by Cohen et al. (2003) using a simplified apparatus as modified by Wang et al. (2014a) and Pan et al. (2015). The method involves measuring the pneumatic conductivity under low pressure (< 100 kPa) as stems are progressively cut shorter. The pneumatic conductivity of the cut open vessels to the air (C_N) should be proportional to Q_x (see Eq. 4 in Cohen et al. (2003)), where x is the stem length at which the air flow rate, Q, was measured. Using the theory of Cohen et al. (2003), $C_N = C_0\exp(-\lambda x)$, where C_0 is the limiting pneumatic conductivity as x approaches zero, λ is an extinction coefficient and x is the stem length. The natural log of C versus x was plotted to obtain a slope, λ. According to the theory (Cohen et al. 2003), the mode and mean vessel length are given by $L_{mode} = -1/\lambda$ and $L_{mean} = -2/\lambda$, respectively. The fraction of open vessels in a segment of length x can be computed as $F_x = 1 - ($

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VCs via the bench-dehydration method

VCs determined via the bench-dehydration method were obtained following the method described by Tyree and Dixon (1986) and Sperry et al. (1988). Stems longer than 1.3 m were sampled in the morning on rainy days and then dehydrated on a laboratory bench. Xylem pressures were monitored in the adjacent three leaves in a pressure chamber (model 1505D; PMS Instruments Co, Albany, OR,

USA). PLCs were assessed on three 5-cm segments near the sampled leaves. Before xylem pressure and PLC measurements, the stems were enclosed in a closed black plastic bag to equilibrate for at least 1 h. PLC was calculated as $PLC = 1 - K_h/K_{max}$. The hydraulic conductivity K_h was determined with a low-pressure flow meter (LPFM) immediately after the leaf pressure was measured. K_{max} was obtained after the segments were flushed. An average PLC versus a corresponding average tension was obtained, which is also a point on the VC. At least 20 points were collected that could be evenly scattered to generate a VC.

VCs via the Cavitron method

Measurements of K_h and VCs were carried out using a modified Cochard rotor in a custom-designed centrifuge (Xiangyi, model H2100R) based on the Cavitron centrifuge technique (Alder et al. 1997; Cochard et al. 2005). Design details have been described by Wang et al. (2014b). Briefly, a 27.4 cm stem was flushed under 200 kPa for 30 min to remove embolisms. The solution used for flushing and measuring *Kh* was 10 mM KCl that was degassed and then filtered through a 0.02 μm filter. For species of *Robinia, Prunus* and *Acer*, stems were flushed to remove all embolisms at a pressure of 150 kPa, 200 kPa and 250 kPa for 10 min, 20 min and 30 min, respectively. The pressure and time for flushing had been verified to remove all embolisms in these species (Wang et al. 2014; Wang et al. 2015). During flushing, the temperature was set to 25°C in the Cavitron. Flushed stems with both ends in the cuvettes were mounted in the rotor and then spun at 1000 rpm (tension = 0.083

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MPa) for 10-20 min to stabilize. After stabilization, the K_{max} was measured. Higher spin rates were then gradually applied to increase the tension, and then a series of K_h values were measured sequentially until the PLC was above 98%. More than 6 VCs of each species were fitted to the single or dual Weibull equation described by Cai et al. (2014).

VCs via the SperryTron method

A modified centrifuge, SperryTron, with two different-sized rotors (30 cm and 15 cm) was constructed based on the traditional centrifuge technique (Alder et al. 1997, Li et al. 2008). Stems were processed in the same way as described above. Stems were then assembled in the relevant rotor with two ends dipped in custom-built reservoirs. Water was placed in the bottom of the L-shaped reservoirs first so that it would reach the lateral part of the reservoirs during spinning, which ensured that the stem ends would be submerged in water during spinning. The desired negative pressure was induced by adjusting the spinning speed, which was maintained for 15 min under each tension. After spinning, stems were removed from the SperryTron. The conductivity, K_h, was then measured by LPFM. It is important to note that stems may absorb water after spinning, which induces a small negative flow also called background flow. Therefore, the flow measured in LPFM would be much lower than the net flow. To derive the net flow, the background flow should be measured under a non-pressurized flow (zero head flow; see Sperry et al. (2012) for a full explanation). The net flow is the measured flow minus the background flow. At given pressures, a series of PLCs can be obtained and a VC can be generated. To obtain a full VC, 6 stems per species were used. VCs were fitted in the Cavitron method.

Control experiments to verify the formation of additional embolism induced in with exponential VCs

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Robinia stem segments 27.4 cm long were spun under a low tension (spinning at 1000 rpm, tension = 0.083 MPa) to determine the maximum conductivity. Then, the spin speed was increased to 3363 rpm (tension = 1 MPa) and a series of conductivities were monitored at 2-3 min intervals. Before each measurement, water was supplemented to the upstream reservoirs by a needle during spinning. After 1 h, the stems were removed from the centrifuge and immersed in water for further measurements (described below).

The samples were immersed in water for at least 1 h to relax the tension to atmospheric conditions to avoid the cutting artifact (Wheeler et al. 2013). To test the distribution of embolisms, the 27.4 cm stems were cut into 5 segments of equal length. Embolism was quantified by PLC measured by LPFM, which was calculated as $PLC = 1 - K_h/K_{max}$, where K_h is the conductivity of the cut segment. K_{max} could be obtained after the segments were flushed. The PLCs of each segment position were averaged from 6 replicates using different stems.

To test whether water flow during centrifugation in the SperryTron induced additional embolism, 14.4-cm *Robinia* stems were used, which have the most open vessels (approximately 53%). All stems were flushed with filtered 10 mM KCl for 10 min under 150 kPa. Before centrifugation, the maximum conductivity was measured. In the control experiment, the water levels in the two relevant reservoirs were equal. Because the two ends of the stem immersed in water inside the reservoirs were slightly different in diameter, this difference was also considered. The amount of water to be placed in the reservoirs was calculated, then accurately added through a pipette. In another experiment, 2 g of water was

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added to the basal-end reservoir than to the distal-end reservoir. Six stems were used in each experiment. Stems in all groups were tested under different centrifugation tensions of 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 4.0 MPa for 15 min. After the centrifugation, PLCs were quantified using LPFM and the PLCs of each group were compared.

Results

Vessel length and the fraction of open vessels

The mean vessel lengths of *Acer*, *Prunus*, and *Robinia* were 4.47 ± 0.32 cm, 12.87 ± 0.39 cm, 18.14 ± 1.51 cm, respectively. The maximum vessel lengths of *Acer*, *Prunus*, and *Robinia* were 17.0 ± 0.5 cm (Yin and Cai 2018), 44.6 ± 0.8 cm, 51.6 ± 1.7 cm, respectively. Segments of 27.4 cm were used for the larger rotor of the SperryTron and Cavitron. The fractions of open vessels in the 27.4-cm segments for *Acer*, *Prunus* and *Robinia* were 0, 7.44, and 19.61%, respectively. Segments of 14.4 cm were used for the smaller SperryTron rotor. The fractions of open vessels of the 14.4-cm segments for *Acer, Prunus* and *Robinia* were 1.18, 34.56, and 52.89%, respectively (Table 1).

VCs obtained by bench-dehydration

The bench-dehydration VCs of the three species were typically sigmoidal (Fig. 1), and these were used to evaluate the VCs yielded by the centrifuges. The bench-dehydration VCs showed a safe range before the PLC increased rapidly. The results indicated that the vulnerability differs among the three species. *Acer* displayed the highest absolute P_{12}, P_{50} and P_{88} (Table 2). *Robinia* displayed the lowest absolute P_{50} and P_{88}, which means that *Robinia* was the most vulnerable species. Although samples were harvested on steadily rainy days, *Prunus* still exhibited small embolisms under near-zero tensions. Comparing the bench-dehydration VCs of *Prunus* and *Robinia* described in previous studies (Cochard et al. 2010, Wang et al. 2014a), we found that the VCs in the two species were very similar.

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Although the sampling sites of *Prunus* and the sampling time of *Robinia* were different from those described in the previous studies, they had similar VCs.

VCs obtained by centrifuge-based methods

The results showed that the VCs of the two centrifuge-based methods were similar in shape (Fig. 2). In the *Acer* results, the shape of the VCs of the 27.4-cm and 14.4-cm segments measured by the SperryTron were similar to the bench-dehydration VC. P_{12} in SperryTron (27.4 cm) was lower than P_{12} in Cavitron (27.4 cm), whereas P_{88} was higher, and P_{50} showed no significant difference. For *Prunus*, the shapes of the VCs from all measurements by Cavitron and SperryTron method were almost the same. When the stems of *Prunus* were shortened to 14.4 cm, the resulting VC was slightly shifted relative to the bench-dehydration VC. There were no significant differences found in P_{12}, P_{50} or P_{88} for *Prunus* (Table 2). For *Robinia*, all the centrifuge VCs showed exponential curves. VCs measured by Cavitron method resulted in the most vulnerable curves, followed by the VCs of SperryTron for 14.4-cm stems and those of SperryTron for 27.4-cm stems.

The effect of open vessels on the shape of VCs

The results above revealed that the effect of open vessels on VCs differs among species. The VC of the 14.4-cm *Acer* segment was more vulnerable than that of the 27.4-cm segment as measured by SperryTron, but the fractions of open vessels in both 14.4-cm and 27.4-cm segments were extremely low (1.18 and 0%, respectively). For *Prunus*, the faction of open vessels in the 14.4-cm segment was 27.12% higher than that of the 27.4-cm segment. However, the 14.4-cm segment VC was less vulnerable. For *Robinia*, the more open vessels that were present, the more vulnerable the VCs were.

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PLCs change in 1 h under 1 MPa centrifugation with a series of water injections

The tension was increased to 1 MPa after K_{max} was measured at a low tension of 0.083 MPa in the Cavitron, which caused the conductivity to decrease by 29.48-52.17%, with an average of 41.77 ± 3.07%. In the subsequent hour, PLCs continued to increase during water-injection and measurements. The lowest growth was 13.19%, and the highest was an increase of 26.57%, with an average increase of 20.48 ± 1.67% (Fig. 3).

Distribution of PLCs along the stem

It has been reported based on theoretical analysis that the most negative tension occurs in the centre of the rotor and the tension increases along the axis of rotation (Cai and Tyree, 2010). Embolism formed in the stem should be proportional to the tension. Our results indicated that PLCs were not evenly distributed along the stem. The PLCs distributed at five cut segments from the basal end to the distal end were 62.11 ± 9.23%, 53.02 ±4.91%, 41.22 ± 6.50%, 26.19 ± 5.51% and 24.10 ± 6.06%. The greatest PLC occurred at the basal end, and PLCs decreased from the basal end to the distal end.

The effect of water difference on PLCs in stems centrifuged in a SperryTron

Controlling the water difference between the two relative cuvettes induces water flow during centrifugation. PLCs were significantly different at 0.5 and 1.0 MPa, with higher PLCs under the control of a 2 g water difference than with a 0 g water difference. However, PLCs were not significantly different under the remaining tensions (Fig. 5). These results suggest that water flow under mild tension induced slightly higher embolism formation. However, the effect of water flow on embolism formation can be ignored under more negative tensions. In

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all cases, the VCs of *Robinia* via SperryTron were inaccurate regardless of whether water flow was induced or not.

Discussion

It has long been debated whether centrifuge techniques are reliable for measuring long-vessel species, especially for the centrifuge with original rotor. Researchers have come to a consensus that the Cavitron method is not appropriate to measure VCs in long-vessel species (Cochard et al. 2010, Choat et al. 2010, Wang et al. 2014a, Torres-Ruiz et al. 2017). The factor thought to be responsible for this inaccuracy is called the open-vessel artifact. As the stems are cut to a short length, intact long vessels are cut open, which then do not have vessel ends to filter tiny particles. These tiny particles may lead to bubble nucleation, and the bubbles can grow rapidly and migrate to the middle of the stem to embolize the intact vessel. However, other researchers have reported that centrifuge curves are not always prone to the open-vessel artifact when the SperryTron method is used because these centrifuge curves match the bench-dehydration curves in their studies (Christman et al. 2012, Sperry et al. 2012, Hacke et al. 2015).

In the present study, the bench-dehydration curves from the three species were sigmoidal, and two (*Prunus*, *Robinia*) were similar to results reported by Cochard et al. (2010) and Wang et al. (2014a) using the same method. Therefore, we used the bench-dehydration curves as reference VCs in the current research.

Are all centrifuges and species subject to the open-vessel artifact?

Among the three species, despite the sigmoidal VCs obtained by all methods for *Acer*, it was worth to note three characteristics in terms of the shape of VCs. The first characteristic was that the VC of 14.4 cm segment was more vulnerable than the VC of 27.4 cm segment

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produced by the SperryTron method, with the P_{50} decreased from 4.66 MPa to 4.01 MPa (Fig. 2). The fractions of open vessels in both 14.4 cm and 27.4 cm segments were extremely low (1.18 and 0%, respectively), which seemed unlikely to generate the significant change for P_{50}. However, the ratio of open-to-centre vessels (vessels which are cut open from the end to the centre of the stem) increased to 16.8% in segment of 14.4 cm, but it was still quite low in the segment of 27.4 cm (1.6%). The sharp increase of the fraction of open to centre vessels in segment of 14.4 cm might account for the more vulnerable VC. In addition, it was much faster for embolism to spread and reach equilibrium in a shorter segment.

The second characteristic was that the slope of VC produced by the Cavitron method was much steeper than the VCs produced by other methods. The reason might be that the embolism induced by Cavitron method was accumulated by cyclic water injection through the whole measurement. While in a SperryTron, the segment experienced the cycle of mounting, spinning and removing, leading to a simultaneous change of the bubble pressure inside the embolized vessels from the desired tension induced via centrifugation to the atmosphere, which might lead to the recovery of the conductivity (Wang et al. 2015) and account for the gentle slope of VC produced by SperryTron.

The third characteristic was that the PLCs of *Acer* in SperryTron method was increased earlier than in Cavitron method, especially under tensions from 2.0 MPa to 4.7 MPa (Fig. 2). It indicated that a part of xylem conduits in *Acer* could not tolerate drastic change of tensions during the measurement with SperryTron method, while the increase of tension was consecutive and mild in Cavitron. In addition, frequent flow induced in Cavitron might act as a disincentive to the embolism formation under mild tensions.

The VCs of *Prunus* measured in different ways were most similar to each other. We found no significant differences in P_{50} or P_{88} between the Cavitron and SperryTron curves. Although there were 27.12% more open vessels in the 14.4 cm segments than in the 27.4 cm segments, the VCs generated with 14.4 cm segments in the SperryTron showed that they were slightly

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more resistant to cavitation. When 27.4 cm segments were used to generate VCs, the results obtained by the two centrifuge techniques were almost the same. Compared with the same species used by Cochard et al. (2010), we found that the VCs of *Prunus* generated by bench-dehydration were similar. However, Cochard et al. concluded that the segment length of *Prunus* changed the shaped and the P_{50} of VCs, in contrast with our results.

However, the results were quite different with *Robinia*, in which the mean vessel length was 5 cm longer than that of *Prunus*. The open vessels of *Robinia* were 52.89 and 19.61% for the 14.4 cm and 27.4 cm segments, respectively. The centrifuge VCs of *Robinia* were typically exponential, which differed the most from the sigmoidal bench-dehydration VCs among the species tested. This finding is consistent with the results reported by Wang et al. (2014a). VCs of the shorter segments showed that they were more vulnerable, which may have been caused by more open vesselsthan in longer segments. All the findings from *Robinia* with the centrifuge techniques seem to be consistent with the open-vessel hypothesis (Choat et al. 2010, Cochard et al. 2010, Torres-Ruiz et al. 2014, Choat et al. 2016).

From the above argument, we conclude that not all species with a large number of open vessels will exhibit an exponential VC according to the centrifuge technique. If a species generates an exponential VC, both centrifuge techniques will reveal this pattern.

Could the open-vessel artifact in have been induced by water flow during centrifugation?

Researchers have raised two possible explanations for the open-vessel artifact. Some researchers have speculated that air bubbles can be induced in the cuvettes at the beginning of the spinning or from injected water during conductivity measurements. These nanobubbles could be easily swept into the open vessels, which simultaneously drain water (Choat et al. 2010, Wang et al. 2014a). Another explanation is that bubble nuclei can be induced by

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flushing the sample or by flow measurement perfusion of open vessels because open vessels cannot filter the nuclei through pit membranes (Rockwell et al. 2014). It is likely that these nuclei cause bubble expansion that can block a vessel under low tension. Flow in a stem in a Cavitron is frequently induced for the measurement of conductivity. In contrast, flow in the SperryTron rotor can be much lower because the water level at the two ends of the stem will rapidly reach equilibrium, and there is no additional water being injected into the cuvettes. These considerations raise the question as to whether an exponential curve could account for the water flow during centrifugation.

After a series of control experiments, we concluded that continuous injection of water decreased the water conductivity. This effect may occur because the injection of water stimulates the formation of air bubbles, which continuously migrate into the middle part of the stem section contributing to embolism formation. Additional evidence for this process is that the distribution of PLC did not match the theoretical expectation that the highest level of embolism would occur in the middle of the stem, where the tension is the most negative (Alder et al. 1997, Wang et al. 2014a). Our results are consistent with those of Martin-StPaul et al. (2014), who observed that the highest embolism exists at the water injection end, where the nanobubbles were produced. These nanobubbles migrate in the direction of the flow, resulting in a gradual step-down trend along the stem.

Our results indicated that the open-vessel artifact may also exist in the SperryTron method. Regardless of whether the water flow was controlled, PLC increased rapidly even under mild tension (Fig. 5). Rockwell (2014) reported that both flow measurement perfusion and flushing induced bubble nuclei in the vessels, even if the stem was not flushed, and bubble nuclei from the reservoirs could still invade the xylem driven by buoyancy during spinning. Indeed, our results in Fig. 5 support this hypothesis. We should also note that flow will inevitably induce more bubble nuclei, which exacerbates embolism formation in open vessels under mild tension. When higher tension is applied, even a small number of nuclei can produce the same

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embolisms due to their rapid growth and expansion.

Why was not subject to the open-vessel artifact?

We argue above that the open-vessel artifact is related to the species rather than the centrifuge method. *Prunus* segments 14.4 cm in length possess 34.56% open vessels, or even up to 69.22% if open-to-centre vessels are included. However, we did not observe a sharp rise in PLC under mild tension, and we did not find that the 14.4 cm segments were more vulnerable than the 27.4 cm segments. Therefore, we conclude that occurrence of the open-vessel artifact is dependent on the species. Open vessels are considered an important factor accounting for the exponential curve (Cochard et al. 2010), but not all long-vessel species that have open vessels in centrifuge samples produce an exponential curve. With the development of techniques applied to plant hydraulics, new insights have been obtained in understanding the mechanisms of embolism formation, including the characteristics of the hydrophobic surfaces of the conduits, xylem surfactants, etc. (Jansen and Schenk 2015, Schenk et al. 2017). Further insight into the mechanisms of the open-vessel effect may be obtained in future research.

Conclusions

Our study aimed to compare the static-centrifuge method and the flow-centrifuge method in the production of VCs to conclude on the long-lasting debate on whether one, both or none of these techniques are reliable. We showed that more than in the techniques, the unreliability lies in the sample species itself. We confirmed that centrifuge-based techniques (Cavitron and SperryTron method) may give inaccurate VCs for *Robinia* with long vessels. The injection of water in the Cavitron method exacerbated the loss of water conductivity due to the hypothesis of the 'open vessel' artifact. However, centrifuge techniques yielded reliable VCs for *Prunus*, regardless of the presence of open vessels. Further investigation is needed to resolve how

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species like *Prunus* avoid the 'open vessel' artefact produced by the centrifuge-based techniques.

Author contributions

P.Y., J.C. and G.D. designed the experiments. P.Y., F.M., Q.L. and R.A. performed the experiments. P.Y. and Q.L. analysed the data and wrote the article. J.C. and G.D. revised the article.

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Table 1. The mean vessel length, maximum vessel length and the fraction of open vessels in 14.4 and 27.4 cm segments of three species. L_v is the mean vessel length given by the mean ± SE. ML_v is the maximum vessel length given by the mean ± SE. $F_{ov\text{-}14.4}$ and $F_{ov\text{-}27.4}$ are the fraction of open vessels in segments of 14.4 and 27.4 cm, respectively.

Species	L_v, cm	ML_v, cm	$F_{ov\text{-}27.4}$, %	$F_{ov\text{-}14.4}$, %
Acer	4.47 ± 0.32	17.0 ± 0.5	0.00	1.18
Prunus	12.87 ± 0.39	44.6 ± 0.8	7.44	34.56
Robinia	18.14 ± 1.54	51.6 ± 1.7	19.61	52.89

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Table 2. Means and SE of the absolute pressure P_{12}, P_{50}, P_{88} (in MPa) of the three species computed with four methods (BD, bench-dehydration; Cavitron; SperryTron-27.4, segment length was 27.4 cm; SperryTron-14.4, segment length was 14.4 cm). The maximum absolute pressure at each level is indicated in bold. Significant differences are indicated by different letters (Student's t-test ± d 0.05) between the two methods by considering all samples.

Species	PLC	BD	Cavitron	SperryTron (27.4)	SperryTron (14.4)
Acer	P_{12}	3.14	**3.96 ± 0.08a**	2.93 ± 0.24b	2.75 ± 0.22b
	P_{50}	**4.98**	4.75 ± 0.15a	4.66 ± 0.14a	4.01 ± 0.12b
	P_{88}	**6.49**	5.44 ± 0.10a	6.41 ± 0.14b	5.26 ± 0.13a
Prunus	P_{12}	1.41	0.79 ± 0.13a	0.85 ± 0.05a	**1.48 ± 0.11b**
	P_{50}	**4.07**	3.81 ± 0.19a	3.77 ± 0.20a	3.91 ± 0.08a
	P_{88}	**5.45**	5.32 ± 0.06a	5.44 ± 0.23a	5.31± 0.06a
Robinia	P_{12}	**2.11**	0.22 ± 0.04a	0.43 ± 0.04b	0.32 ± 0.03ab
	P_{50}	**3.34**	0.43 ± 0.02a	0.96 ± 0.02b	0.73 ± 0.05c
	P_{88}	**4.07**	2.49 ± 0.01a	3.75 ± 0.17b	1.78 ± 0.16c

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Figure legends

Fig. 1. Xylem vulnerability to cavitation of three tree species via the bench-dehydration method. Closed symbols represent native or dehydrated embolisms under relative tension. The black lines are the VCs computed with a single or dual Weibull fit.

Fig. 2. VCs of three species plotted with three techniques. The black lines with triangles are VCs obtained via the Cavitron technique; lines with diamonds are the VCs obtained via the SperryTron technique generated with 27.4 cm segments; lines with squares are the VCs obtained via the SperryTron technique generated with 14.4 cm segments. The black lines with no symbols are the VCs generated via the bench-dehydration methods.

Fig. 3. The changes in PLCs in six stems over 1 h under a tension of 1 MPa in the Cavitron system. Twenty-two measurements are listed in the figure. Black solids are the average PLCs of six points from different stems. Error bars represent standard errors of six points.

Fig. 4. The distribution of PLCs in different parts of the stem centrifuged for 1 h under 1 MPa in the Cavitron. Numbers 1 to 5 represent different segments from the basal end to the distal end. Each bar represents the average PLC computed from six segments. Error bars are the standard error (n = 6). Different letters indicate significant differences at $P<0.05$ based on a *t*-test.

Fig. 5. VCs of *Robinia* via the SperryTron method under different experimental conditions. The solid squares are the PLCs generated with 2 g water difference in the relative reservoirs in the SperryTron system. Open squares indicate the PLCs generated with the same water level in the relative reservoirs in the SperryTron. Each point with error bars corresponds to the average of six points with standard error. The symbols * and ** indicate that the means are significantly different from the PLCs under the same tension at $P<0.05$ and 0.01, respectively.

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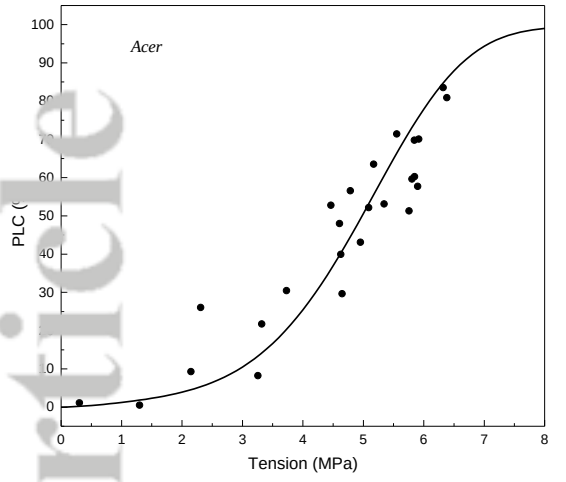

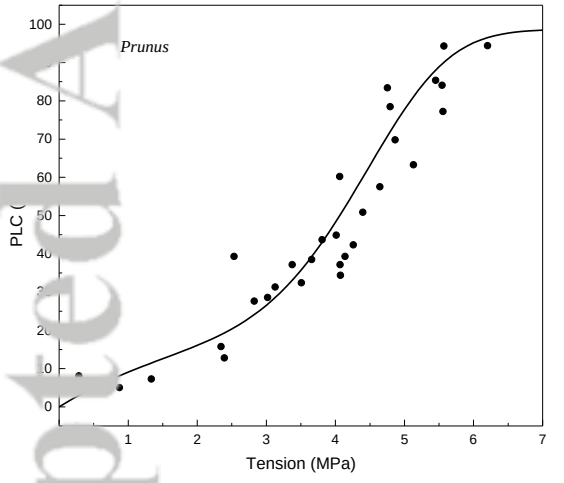

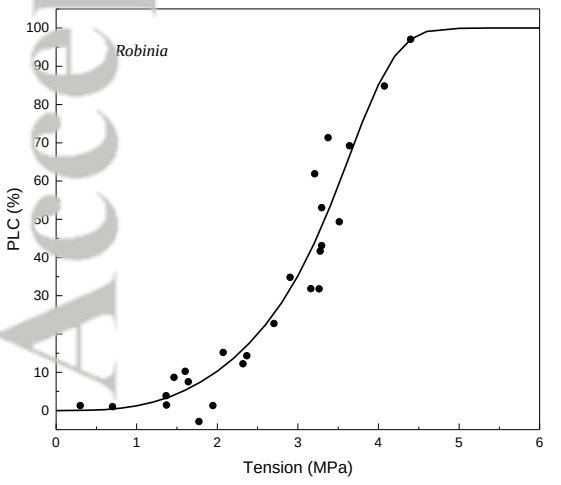

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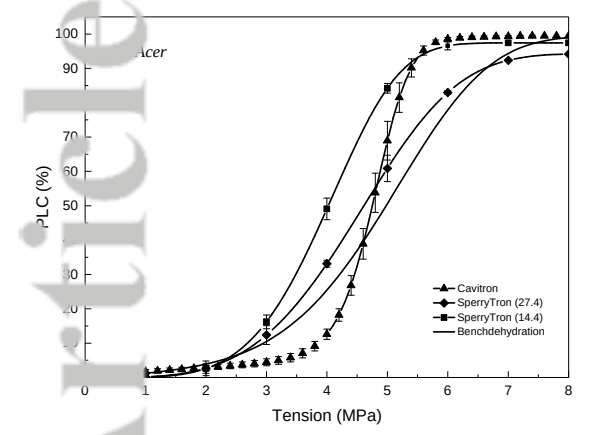

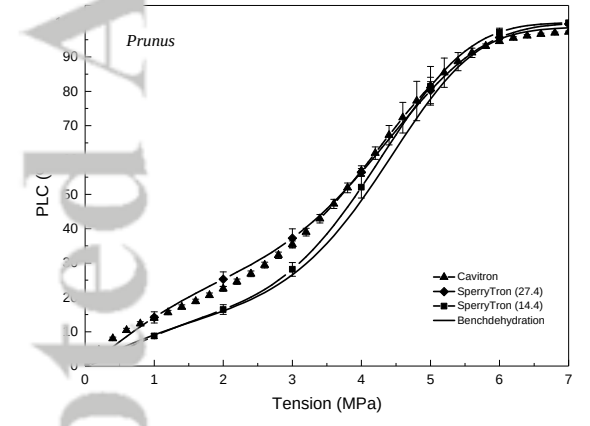

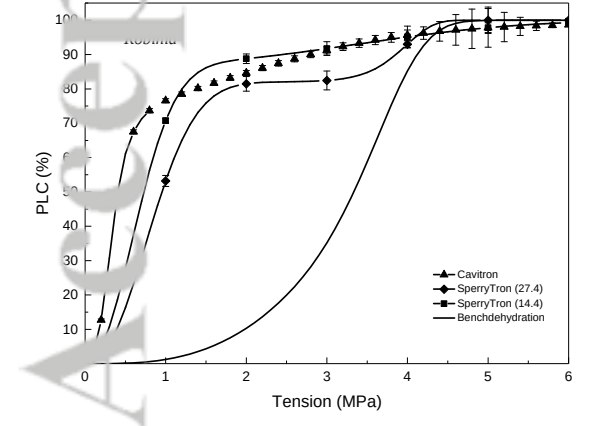

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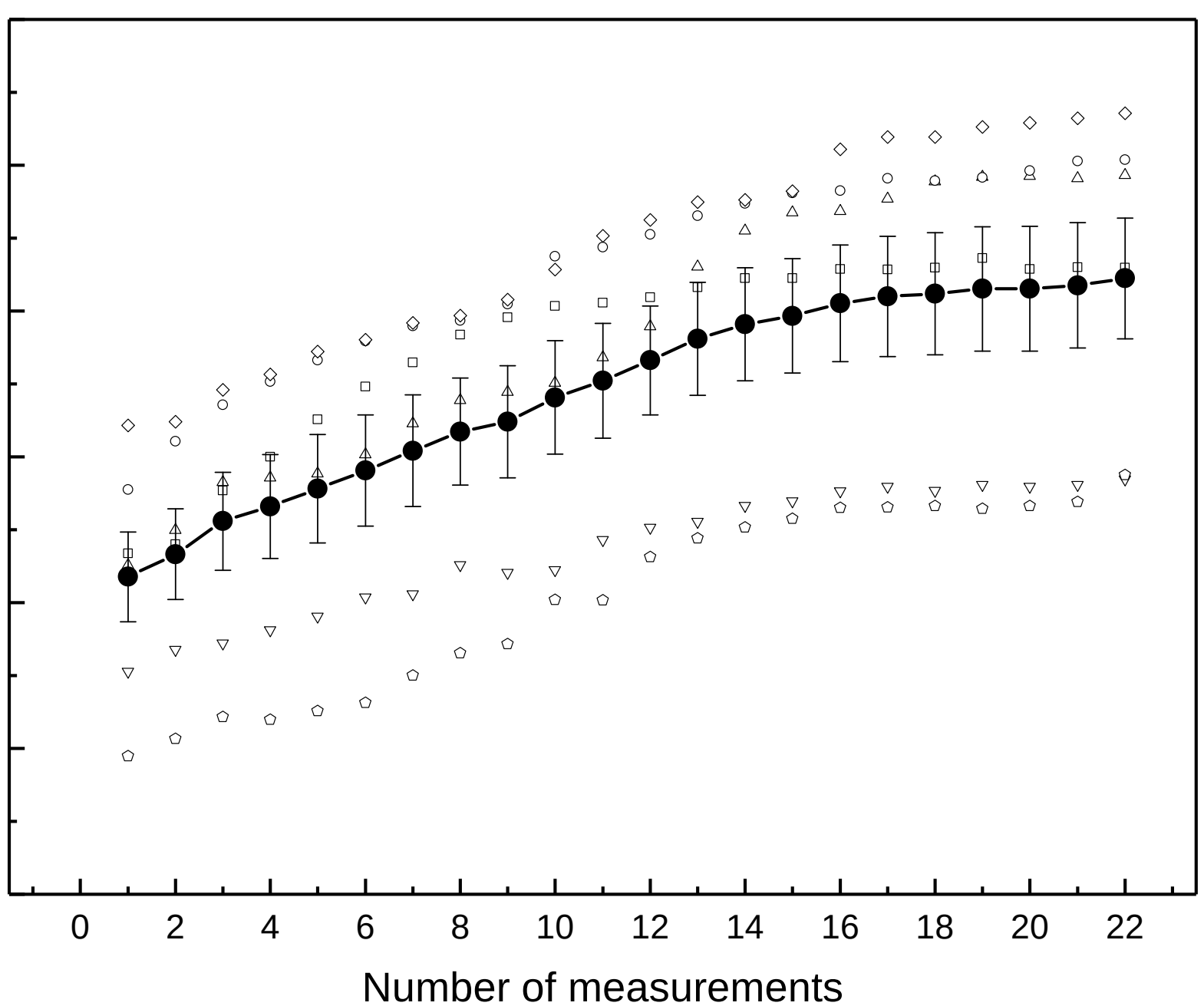

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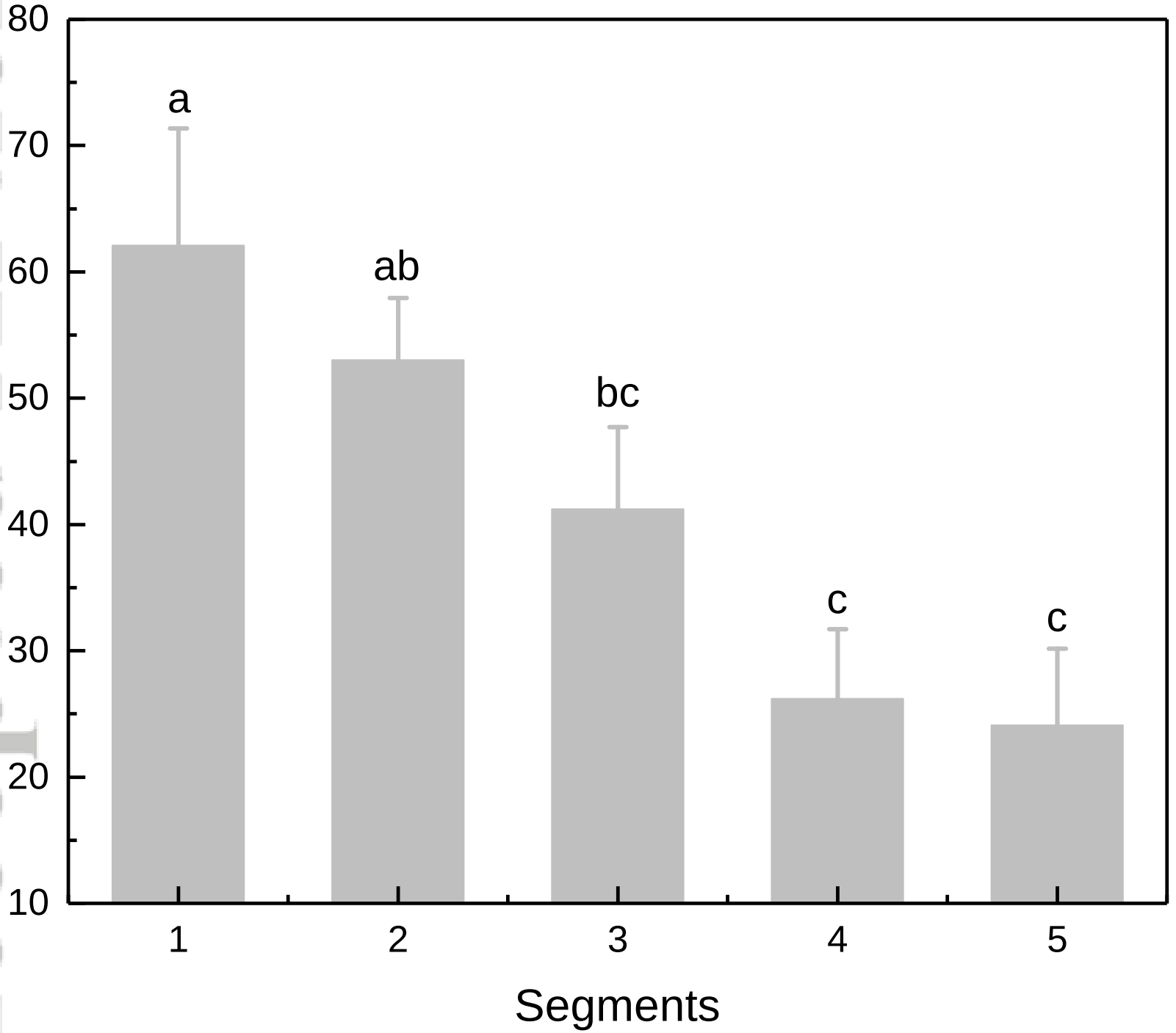

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