

Analysis of anatomical and functional traits of xylem in *Humulus lupulus* L. stems

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ABSTRACT

Hop plants have unusually low values of the ratio between internal area of xylem conducts and leaf area, indicating that their xylem conducts solutes very efficiently. In the present study we analyzed the structure and function of xylem in stems of field-grown hop plants. Vessels of secondary xylem (SX) were more potent to conduct solutes compared to primary xylem (PRX) as they were wider (maximal/mean diameter of SX vessels in basal stem segments was on average 209/73 μm compared to 82/40 μm in PRX) and longer (up to 75 cm, compared up to 30 cm in PRX). The contribution of PRX to total Kh of the segment was on average $45.0 \pm 29.9\%$ in apical, but only $1.8 \pm 0.4\%$ in basal stem segments with well differentiated SX. We discuss differences and non-linear relationship between measured hydraulic conductivity (Kh) and Kh calculated from vessel diameters and suggest a simplified approach suitable for routine evaluation of theoretical Kh of hop cultivars.

Keywords: Hagen-Poiseuille equation; Huber value; hydraulic conductivity; vessel diameter; vessel length

Significant reduction of precipitations and their highly non-equal distribution throughout the growth seasons was observed recently in regions where hop is grown in the Czech Republic (Trnka et al. 2009). It has an overall negative effect on hop growth and can cause high interannual fluctuations in hop productivity (Kopecký and Ježek 2008). Water availability thus represents recently emerging serious problem for hop production in the Czech Republic.

Hop is a climbing species with up to 12 m long but thin stems supporting relatively large leaf area. Sufficient supply of water to the leaves requires high transport capacity per stem cross sectional area. Unfortunately, very limited information is available about both structural and functional properties of xylem transport system in hop plants (Fric 1991). The knowledge of structural properties of xylem, namely number of conduits and their dimensions would be however very useful in the estimation of maximum water transport rates or vulnerability of conduits to cavitation, i.e. disruption of continuous water columns in conduits, that leads to the restriction of the transport (Sperry et al. 2006). These charac-

teristics, still unknown for hop plants, contribute to the response of this crop to drought.

In this paper we aimed to provide detailed quantitative information about structure of xylem in hop stems. We also linked information about xylem structure with functional measurements of stem conductivity and discuss specific structural and functional traits of water conducting pathway in hop stems.

MATERIALS AND METHODS

Hop plants of Saaz aroma variety (*Oswald clone* 31) that just reached the top of the trellis (approx. 7 m above soil level – growth stage BBCH 37–38; Rossbauer et al. 1995) were sampled on the experimental farm of the Hop research institute in Žatec, Czech Republic. After removing all leaves, three stem segments were sampled from each of eight plants examined, apical (6 to 4 m above soil), middle (4–2 m), and basal (2–0.5 m). Each segment was used for measurement of hydraulic conductivity

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(Kh) and analysis of xylem anatomy in the upper end of each segment, from which theoretical Kh was calculated. Hydraulic conductivity of stem xylem was measured as flow rate of ultrafiltered (membrane filters with 0.2 µm pores) 10 mmol/L KCl solution through the stem segments (longer than 1 m) under stable pressure gradient (Sperry et al. 1988). The flow rate was determined by directing the solution flow to a precise analytical balance and data collected with custom-made data acquisition software in connected computer. Internal diameters of xylem vessels in stems were measured on digital microphotographs of cross hand sections using image analysis software Image Tool v. 3.0 (Health Science Center, University of Texas, San Antonio, USA). The calculation of predicted Kh was based on the Hagen-Poiseuille equation:

$$K_{\text{capillary}} = r^4 \pi / 8 \eta$$

Where: r is the capillary radius and η is dynamic viscosity of the liquid (Tyree et al. 1994).

Separate set of six plants was used for determination of xylem vessel lengths. Stem segments approx 3 m long from the middle of the plant height were sampled and used for the determination. Stems were flushed with ultrafiltered distilled water (membrane filters with 0.2 µm pores) to remove embolism and injected with two component silicone QSil 218 (ACC silicones, Bridgwater, UK) with dissolved fluorescent dye Tinopal OB (Ciba AG, Basel, Switzerland) at the 0.04% concentration. Earlier works indicated that the silicone does not penetrate pit membranes and fills the vessel completely (Wheeler et al. 2005). Calculation of the distribution of the vessel length was based on the double-difference algorithm (Tyree and Zimmermann 2002).

Leaf area of plants was determined from dry mass of leaf lamina using the ratio between leaf area (measured by image analysis) and corresponding dry mass determined in smaller sub-samples separately for leaves in various parts of the canopy. Leaf specific conductivity was determined as the ratio between measured Kh and leaf area distal from the segment (Ewers et al. 1989). Huber value was calculated as ratio of conducting xylem area per supported leaf area (Tyree and Ewers 1991).

Before the treatment of the data with analyses of variance, homogeneity of variances was tested using combined Cochran-Hartley-Bartlett test and normality of the residuals was checked using normal probability plot. Post-hoc $LSD_{0.05}$ test was used to identify differences between the group means. Software package Statistica release 9 (StatSoft Inc., Tulsa, USA) was used for all statistical treatments.

RESULTS

Transverse sections of hop internodes showed a typical dicotyledonous stem structure (Figure 1). There were conspicuous differences among basal, middle, and apical stem segments of hop plants namely in the differentiation of secondary xylem vessels and predicted hydraulic conductivities. As the consequence, the frequency distributions of primary and secondary xylem vessels in accordance to their inside diameters were quite different. While the diameter of primary xylem vessels only rarely exceeded 100 µm (less than 0.2% of primary xylem vessels count in apical segments, none in middle or basal segments), on average 16.1% of secondary vessels count in middle segments and 19.2% in basal segments had the diameter above this value. Although low in count, these wide vessels contributed to 60.5% of total predicted Kh in apical segments, 91.6% in middle and 95.1% in basal segments. The significance of primary xylem in hydraulic conductivity of the stem declined rapidly basipetally along the stem axis. While in apical stem segments the vessels of primary xylem contributed to the total predicted Kh by 45.0% (SD = 29.9%, $n = 8$), in middle and basal segments the corresponding values were 4.4% (SD = 1.1%, $n = 8$) and 1.8% (SD = 0.4%, $n = 8$), respectively. If the differences in predicted Kh of primary xylem in apical, middle, and basal segments were tested using repeated measures ANOVA followed by $LSD_{0.05}$ test, there were no differences between means of apical ($9.10 \times 10^{-8} \text{ m}^4/\text{MPa/s}$) and middle stem segments ($9.08 \times 10^{-8} \text{ m}^4/\text{MPa/s}$), both these being however different from basal segments ($5.92 \times 10^{-8} \text{ m}^4/\text{MPa/s}$).

The vessels of primary and secondary xylem differed not only in diameter, but also in their lengths (Figure 2). While primary xylem vessels were only rarely longer than 30 cm (on average only 0.05% of primary vessels count), 10.5% of secondary conduits were longer than 30 cm with the longest vessels up to 75 cm long. The mean areas of both primary and secondary vessels increased with the distance reached by silicone containing fluorescent dye from proximal end of stem segment (Figure 3A). The long secondary xylem vessels of high inside diameter were, however, very low in count, on average less than 10 conduits per stem segment (Figure 3B).

The predicted and measured hydraulic conductivities of apical, middle and basal segments of hop stems correlated well (Spearman $r = 0.84$, $P < 0.001$), but this relationship was clearly non-

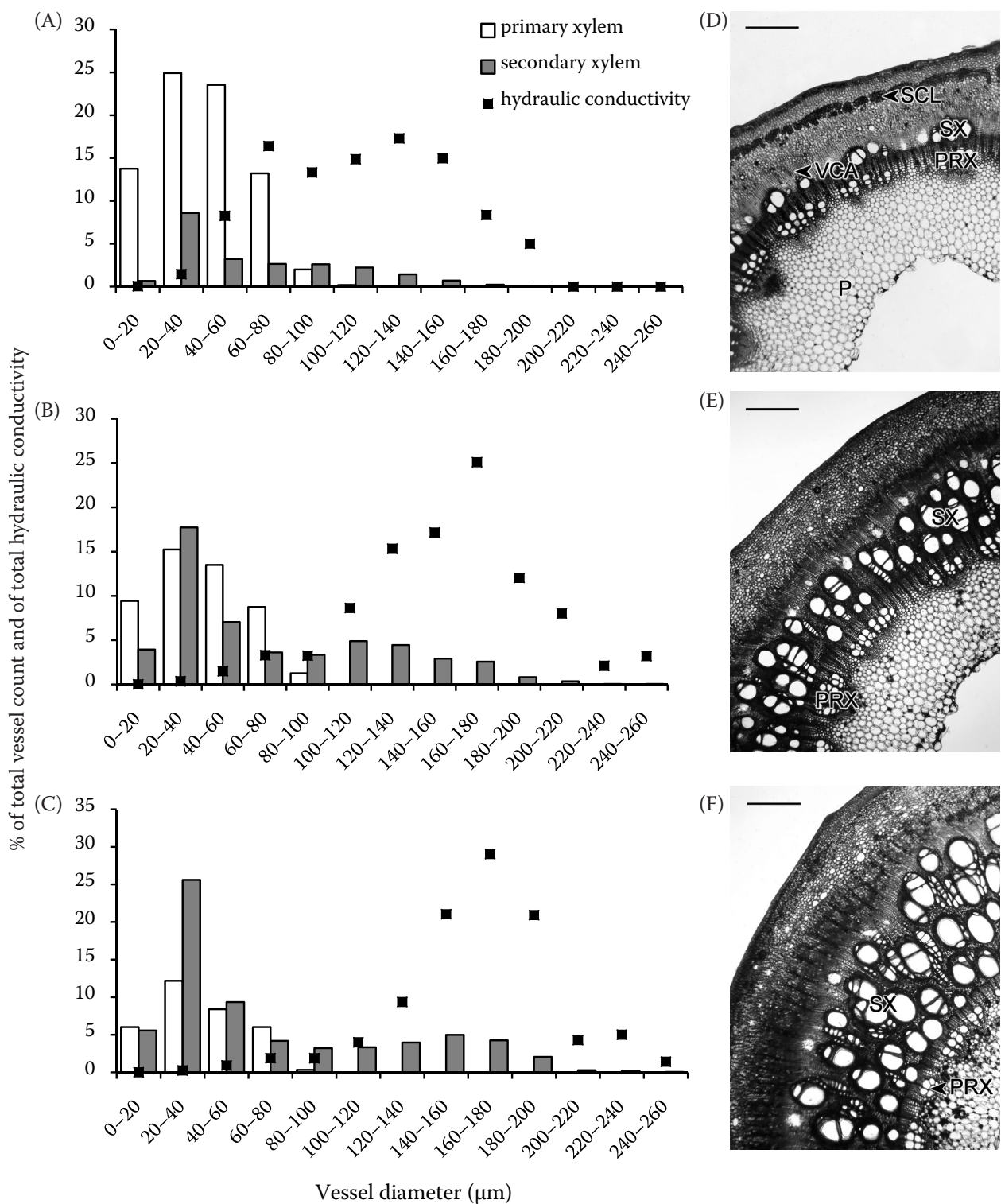


Figure 1. Frequency distribution of primary and secondary xylem vessels and their contribution to total predicted hydraulic conductivity in accordance to their internal diameter in apical (A), middle (B) and basal (C) hop stem segments. Means were calculated from 8 replicates. Inset microphotographs show transverse hand sections of hop internodes at the upper ends of apical (D), middle (E), and basal (F) stem segments. P – pith; PRX – primary xylem; SCL – sclerenchyma; SX – secondary xylem; VCA – vascular cambium. Bars represent 500 μm

linear (Figure 4). For the apical stem segments, in which the vessels of primary xylem contributed to the total predicted Kh more than by 10%, the measured Kh values reached on average 13.6%

(SD = 2.5%, $n = 5$) of corresponding predicted Kh value, whereas for middle segment it was 16.5% (SD = 8.5%, $n = 8$) and for basal segments 25.8% of predicted Kh (SD = 9.4%, $n = 7$); only the differ-

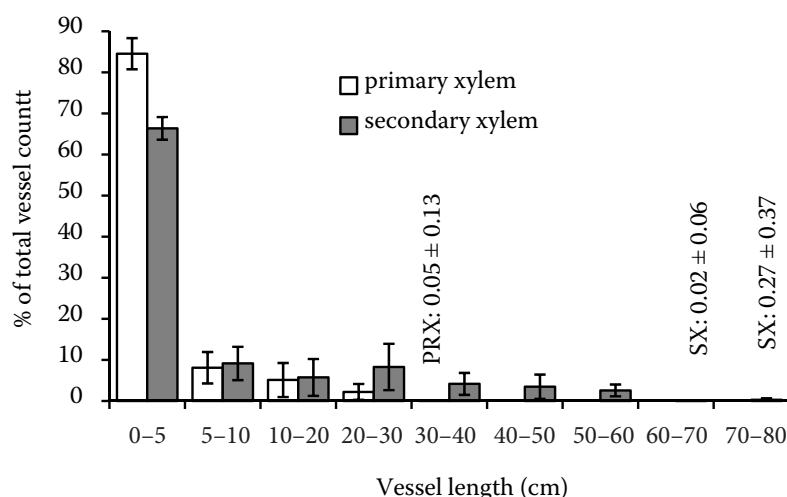


Figure 2. Frequency distribution of primary (PRX) and secondary xylem (SX) vessel in accordance to their lengths. Means \pm standard deviations were calculated from six replicates

ence between the mean value of basal and apical segments was statistically significant (repeated measures ANOVA followed by $LSD_{0.05}$ test).

DISCUSSION

In our experiments we not only observed but with a help of modern methods of image analysis also quantified basic anatomical characteristics of xylem that determine high hydraulic conductivity of hop stems. As expected, there was strong gradient of secondary xylem differentiation along the stem axis. The tracheary elements of secondary xylem were wider (Figure 1) and longer (Figure 2) than elements of primary xylem and thus more potent to conduct solutes. The dimensions of vessels in vines are generally considered to be the greatest in plant kingdom (Tyree and Zimmermann 2002). Ewers et al. (1991) found in survey of 33 species of climbing plants the average maximum vessel

dimensions to be 207 μm for inside diameter and 1.46 m for length. Our data (maximal inside diameter of vessels was on average 209 μm) fit well with these observations and show that hop plants utilize these wide conduits. The vessels were, however, substantially shorter than in woody lianas with the maximum length only 75 cm.

The contribution of primary xylem to the hydraulic conductivity of whole stem segment decreased substantially in basipetal direction. In the apical segments this contribution was on average 45% but only 4.4% in the middle and 1.8% in the basal segments. These values were, however, calculated using Hagen-Poiseuille equation using only conduit diameter, not its length as predictive variable. As the consequence, the contribution of primary xylem to the total stem conductivity was overestimated. Considering the fact that detailed quantification of predicted K_h of primary xylem is very laborious, we propose a simplified approach for future characterization of hop cultivars in their xylem traits:

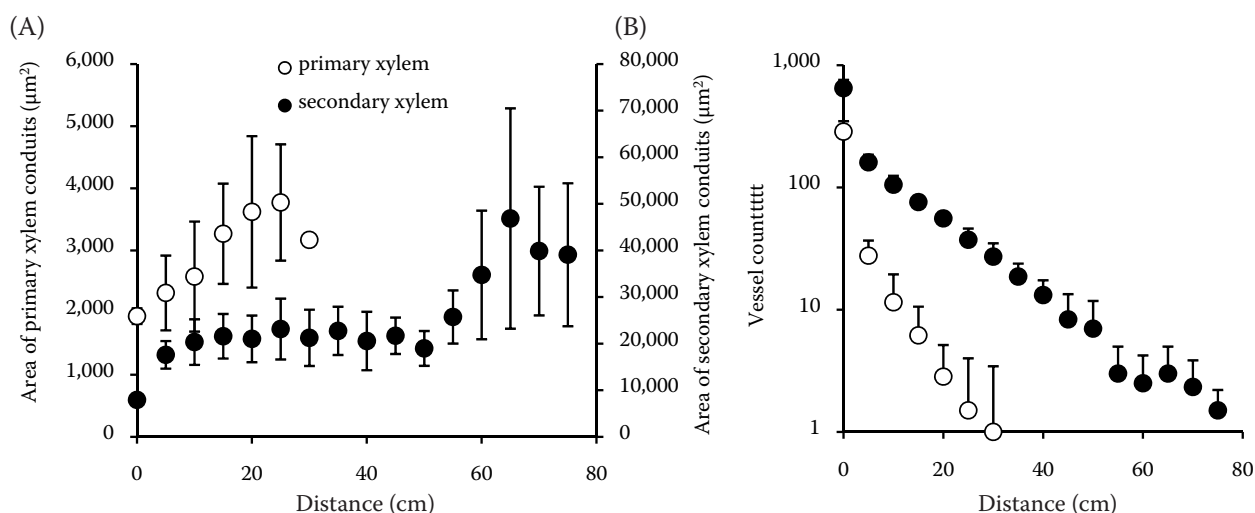


Figure 3. Mean vessel area (A) and count of primary and secondary vessels (B) filled with silicone containing fluorescent dye in relation to the distance from the proximal end of stem segment. The spread of the silicone was restricted by end walls of vessels. Means \pm standard deviations were calculated from six replicates

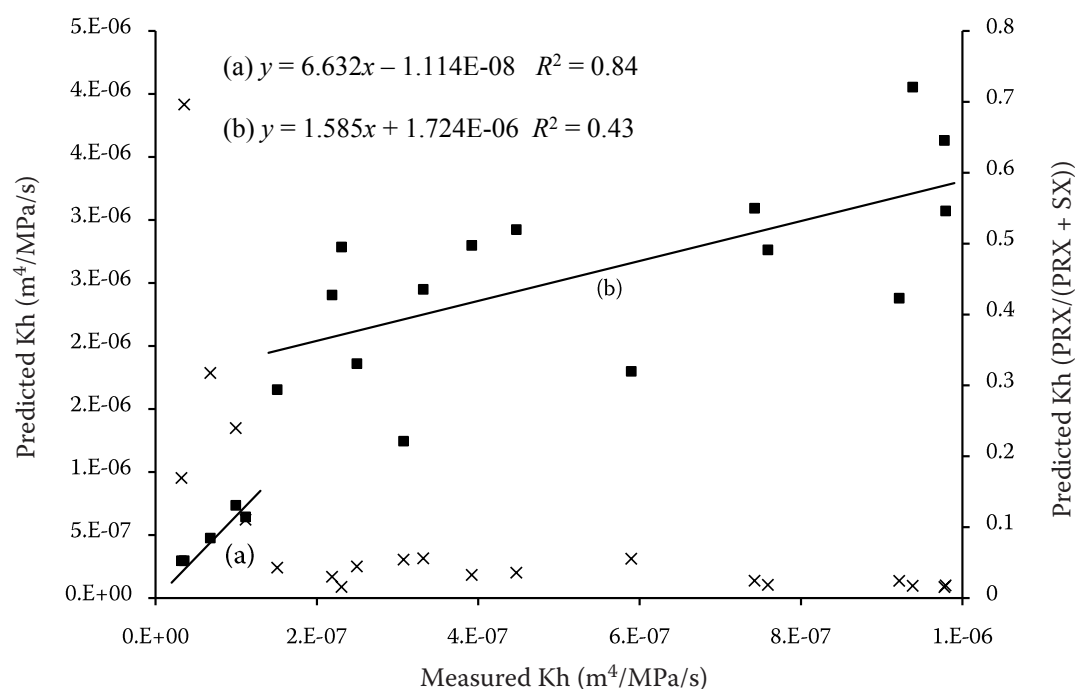


Figure 4. Relationship between predicted and measured hydraulic conductivity (Kh; squares) of apical (a) and middle or basal (b) segments of hop plants. The proportion of Kh attributable to the primary xylem (PRX) from Kh of the whole stem segment (PRX + SX) is plotted against secondary y-axis (multiplication sign)

if apical stem segments where secondary xylem is still poorly differentiated would be of interest, the hydraulic conductivity of primary xylem should be determined and the value obtained used for middle or basal segments without re-assessment. We found that hydraulic conductivity of primary xylem was lower in basal compared to middle or apical segments (Table 1), however, the differences were negligible in context of total hydraulic conductivity of stem with well differentiated secondary xylem. When we compared hydraulic conductivity of the widest secondary vessel to Kh of the whole primary xylem, we found that it equals on average to 54.5% (range 27.5–130.4%, $n = 8$) of Kh calculated for all primary tracheary elements in middle and 92.1% (range 59.8–145.9%, $n = 8$) in basal stem segments. Hence, correct and precise calculation of Kh of secondary xylem is much more important for the prediction of the total Kh from anatomical traits than including primary xylem in the calculation. Future methodological efforts should be also focused on improvement of precise identification of truly functional vessels in vascular bundles.

The values of measured Kh were on average only 14–26% of theoretically predicted Kh values based on Hagen-Poiseuille equation for ideal capillary. Similar results were found also in other studies (Ewers et al. 1989, Martre et al. 2000). There are

three main factors that contribute to this discrepancy. First, the shape of vessels can deviate from ideal capillary considerably, second the length of single vessel is limited and its end with pit pores represents a serious restriction for sap flow, third some mature vessels may be non-functional (e.g. blocked by tyloses). Predicted and practically measured Kh were well correlated, but not in linear relationship. A similar pattern between predicted and measured hydraulic conductivities (Figure 4) was previously observed (Ewers et al. 1989); however, the authors did not provide any explanation. We suggest a positive correlation between xylem vessel diameter and vessel length for explanation. We observed substantial increase in mean vessel area of both primary and secondary xylem filled with silicone with growing distance from the point of silicone injection (Figure 3). The vessel end walls, which decrease vessel conductivity in angiosperms considerably due to presence of uniformly microporous pit membranes (Pittermann et al. 2005), are thus less frequent per length of stem segment in wide vessels. The ratio of measured Kh to predicted Kh is, therefore, higher in stem segment with well developed secondary xylem with wide and long vessels.

The functional properties of xylem were previously studied in vines and other plant species, namely trees (Ewers et al. 1989, Nijssen et al. 2001,

Sperry et al. 2006). This study provides, to our knowledge, the first detailed information about xylem anatomy and function in stem of hop plants. The capacity and efficiency of xylem transport in plant stems can be characterized by Huber values (HV), which typically range from 10^{-3} (m^{-2} of xylem per m^{-2} of leaves) for some angiosperms to 10^{-5} for tropical lianas (Tyree and Zimmermann 2002). The values of HV our hop plants were in the range from 2.5×10^{-6} to 7×10^{-7} . Such an extremely low HV clearly shows that hop stems belong to the most efficient in conducting water among all plant groups. On the other side, the large leaf area supported by relatively small amount of conducting tissues represents a potentially dangerous configuration since the cavitation of small number of vessels can have large impact on total transport capacity of the stem. The justification of the relationship between the size of xylem conduits and their sensitivity to cavitation is an important next step in this research.

Results of our work confirmed that methodology frequently used for evaluation of properties of xylem in trees and other woody species can be also applied on hop stems. Knowledge of basic parameters that determine hydraulic conductivity of xylem can be used in the future for predictions of water conducting abilities between different cultivars. They could be also useful, in context with other physiological properties, for predictions of plant vulnerability to water deficiency in environment.

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