

Inherent variability in structural and functional traits of xylem among three hop varieties

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ABSTRACT

Intervarietal differences in xylem structure could significantly affect water transport as well as vulnerability to cavitations and thus crop yield. Evaluation of crop varieties with respect to the specific traits can be thus helpful in breeding and agricultural practice. In the present study we compared basic anatomical traits (vessel length and diameter) as well as theoretical and measured hydraulic conductivities (K) of xylem in stems of three hop varieties (Agnus, Saaz hop – Oswald's clone 31, Vital). There were no statistically significant intervarietal differences in measured K (overall mean $1.68 \times 10^{-6} \text{ m}^4/\text{MPa/s}$), the ratio between K and theoretical conductivity (mean = 0.194), as well as vessel diameter and vessel length distributions. The only noticeable difference was in the distribution of vessels with the diameter $\geq 100 \mu\text{m}$ which differed in Vital compared to Agnus or Oswald's clone 31 (Kruskal-Wallis test, $P < 0.001$). Our results thus indicate highly conservative nature of xylem basic functional and anatomical characteristics in hop varieties studied.

Keywords: *Humulus lupulus*; hydraulic conductivity; vessel diameter; vessel length

Delivery of water from roots to leaves through the xylem is critically important for maintenance of high yield of agricultural plants. This long-distance water transport is significantly affected by anatomical traits of xylem conduits. Dimensions, shape and arrangement of xylem conduits influence both transport efficiency (i.e., the ability to transport water with minimal resistance) and safety (i.e., the ability to prevent embolism), as previously found in many studies (Sperry et al. 2005, Wheeler et al. 2005, Hacke et al. 2006). Confident determination of structural properties of xylem conduits (e.g. length, diameter) can be useful for estimation of their hydraulic resistance to sap flow (Sperry et al. 2005, Christman and Sperry 2010) as well as vulnerability to dysfunction due to cavitation (Tyree and Sperry 1989, Hacke and Sperry 2001).

Anatomical structure of xylem belongs to well genetically fixed traits and, thus, large differences exist among plant species (Wheeler et al. 2005, Choat et al. 2007). However, little is known about intraspecific variability of the xylem traits. Results of the comparative study with various peach rootstocks showed a positive correlation between mean diameter of xylem vessels in stems and vigour of the plant (Tombesi et al. 2011). Architecture of xylem may be also related to plant water use as found in two cultivars of grapevine. Cultivar Grenache with higher hydraulic conductivity of xylem showed little changes in leaf water potential during the day in contrast to cultivar Syrah with lower hydraulic conductivity (Schulz 2003). These few examples illustrate that structure of xylem plays an important role in plant water relations and can be potentially

used for identification of plants with different physiological response to the environment.

Structure of xylem in stems of hop (*Humulus lupulus* L.) plants is typical for climbing plants. Small number of long vessels (up to 80 cm) with large inner diameters (on average 209 μm) provides high transport capacity for sufficient supply of water to the large leaf area (Gloser et al. 2011). Such large conduits are very efficient in water transport but may be more vulnerable to cavitation compared to smaller vessels that are abundant in vascular bundles. Analysis of a relationship between structure and functioning of xylem in hop stems with respect to the potential differences among hop varieties is still missing, although it may be useful for selection and breeding of new drought-tolerant varieties. In this paper we aimed to compare basic traits of xylem in stems of three varieties of hop grown commercially in the Czech Republic. Previous experiments showed significant differences in plant water relations and response to drought among these varieties (Gloser et al. unpublished). We hypothesized that significant differences in xylem structure exist among hop varieties and these may potentially contribute to previously observed differences in function.

MATERIAL AND METHODS

Three varieties of hop (*Humulus lupulus* L.) were used: traditional Saaz hop (Osvald's clone 31 – OK31), Agnus, and Vital. Plants at growth stage BBCH 37–38 (Rossbauer et al. 1995) were sampled in July 2011 in hop gardens of the Hop Research Institute Co., Ltd., near Stekník, Czech Republic. One stem segment ranging from 0.5 to 3 m above soil was sampled from each of five selected plants for each variety. After removing all leaves, the cut petioles were sealed with glue (Superbond Loctite 409, Loctite Corp., Rocky Hill, USA). Segments were transported to the laboratory in plastic bag with moist paper tissue.

Each stem segment was trimmed about 50 cm on both ends to the final length approximately 1.5 m and used for measurements of hydraulic conductivity (K), vessel diameters and lengths.

Each stem segment was flushed with filtered 10 mmol KCl solution at 110 kPa for 30 min to eliminate embolism. K was measured as flow rate of KCl solution under a stable pressure gradient as described in Glaser et al. (2011).

After measurement of K , hand cross sections were made on the distal (outflow) end of each segment. The sections were stained with a 1% phloroglucinol solution in acidified ethanol and photographed bright field microscopy. Lumen area, major and minor axes of xylem vessels were measured on photographs using ImageTool v. 3 software (Health Science Center, University of Texas, San Antonio, USA).

Theoretical hydraulic conductivity (K_T) was calculated using two models that differed in assumed shape of vessel cross-sectional area. If the circular shape of vessel cross-sectional area was assumed, the calculation was based on original Hagen-Poiseuille (HP) equation (Tyree and Zimmermann 2002).

If the elliptic shape of vessel cross-sectional area was assumed, the K_T was calculated as (Tyree and Zimmermann 2002):

$$K_T = (\pi/64\eta) [(a^3 \times b^3)/(a^2 + b^2)]$$

Where: a and b – major and minor axis lengths, and η – dynamic viscosity of water 1×10^{-8} MPa/s.

Two component silicone QSil 218 (ACC silicones, Bridgewater, UK) with dissolved fluorescent dye Tinopal OB (Ciba AG, Basel, Switzerland) was injected in stem segment as described previously (Gloser et al. 2011). Number of silicone-filled vessels was counted on microphotographs (fluorescence microscope Olympus BX 51, filter set U-MWBV2, Olympus E-410 digital camera, Olympus Corp., Tokyo, Japan) of hand cross sections prepared in 5 cm intervals from the point of silicone injection. Distribution of vessel lengths was calculated using the exponential decay function (Cohen et al. 2003, Sperry et al. 2005). This function is based on declining number of filled conduits (N_L) with distance (L) from the silicone injection surface:

$$N_L = N_0 e^{(-kL)}$$

Where: N_0 – number of vessels at the injection surface, and k – extinction coefficient.

Two additional parameters describing vessel length distribution were calculated: the most common vessel length L_0 (Cohen et al. 2003) and the minimal length of 1% of longest vessels L_{max} (Nijssse 2004).

Statistica v. 10 (StatSoft Inc., Tulsa, USA) was used for statistical analyses. Prior to parametric ANOVA, homogeneity of variances was tested by combined Cochran-Hartley-Bartlett test and normality of the residuals by Shapiro-Wilk test.

Table 1. Measured hydraulic conductivity (K) and theoretical hydraulic conductivity (K_T) assuming either circular or elliptic shape of vessels cross-sectional area in stems of three varieties of hop plants

Variety	Measured K		Circle model K_T		Ellipse model K_T	
	mean	SE	mean	SE	mean	SE
Agnus	1.39E-6	1.90E-7	8.44E-6	1.81E-6	8.63E-6	1.82E-6
Saaz hop (OK31)	1.86E-6	9.53E-8	8.19E-6	5.77E-7	8.37E-6	6.10E-7
Vital	1.79E-6	3.16E-7	1.03E-5	4.01E-7	1.08E-5	4.35E-7

All conductivity values are given in $m^4/MPa/s$; $n = 5$

The other specific statistical tests are indicated directly in the text. The results are presented as mean (or the other statistics) \pm standard error (n , number of replicates), until stated otherwise.

RESULTS

Hydraulic conductivity. The values of measured K were on average $18.0 \pm 2.5\%$ ($n = 5$) of K_T calcu-

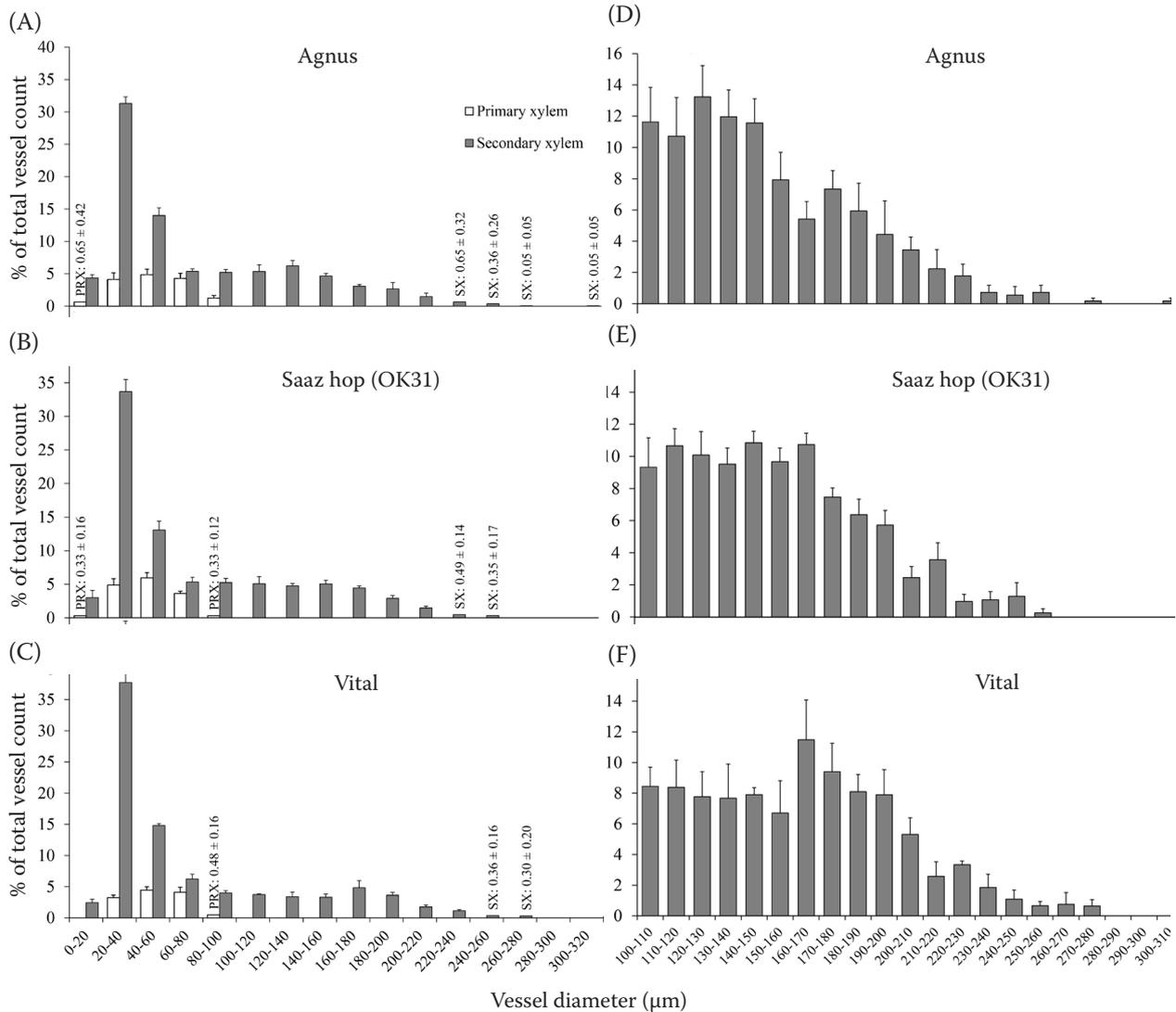


Figure 1. Frequency distribution of primary (PRX) and secondary (SX) xylem vessels in stem segments of three hop varieties. Data shown for all vessels (A–C) and in detail for vessels with i.d. greater than 100 μm (D–F). Means \pm SE, $n = 5$

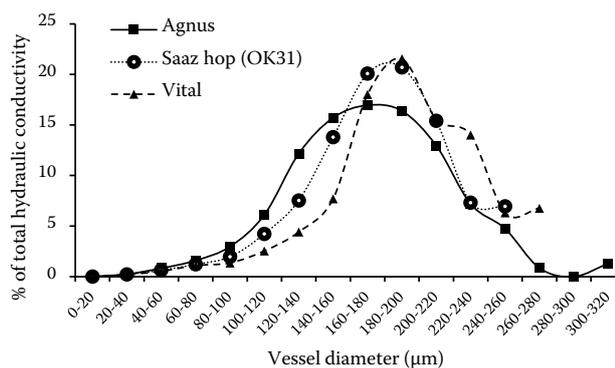


Figure 2. Relative contribution of vessels of different internal diameters to theoretical hydraulic conductivity of stem segments of three hop varieties. Values assuming circular shape of vessel cross-sectional area were calculated from the HP equation and connected with trend line. Means \pm SE, $n = 5$

lated using the circle model in Agnus variety, $22.9 \pm 0.9\%$ ($n = 5$) in OK31, and $17.3 \pm 3.1\%$ ($n = 5$) in Vital variety (Table 1). The differences among these means were not statistically significant ($F = 1.68$; $P = 0.23$). The corresponding values comparing the measured K and the K_T calculated using the ellipse model were $17.5 \pm 2.5\%$ ($n = 5$) for Agnus, $22.4 \pm 1.0\%$ ($n = 5$) for OK31, and $16.6 \pm 2.9\%$ ($n = 5$) for Vital; the differences among these means were not statistically significant again ($F = 1.88$; $P = 0.19$). The values of K_T calculated for all three varieties using the ellipse model were on average $97.2 \pm 0.4\%$ ($n = 15$) of corresponding K_T values calculated using the circle model; the differences between varieties were not significant ($F = 1.52$; $P = 0.26$).

Vessel diameter. We observed huge differences between distributions of inner diameters (i.d.) of primary (PRX) and secondary (SX) xylem vessels in all three varieties (Figures 1A–C). The distributions of PRX vessel diameters were in all three varieties almost perfectly symmetrical, but platykurtic (skewness 0.10 ± 0.12 , kurtosis -0.89 ± 0.27 , $n = 335$ for Agnus; corresponding values were 0.20 ± 0.14 , -0.73 ± 0.28 , $n = 312$ for OK31 and 0.01 ± 0.15 , -0.86 ± 0.30 , $n = 266$ for Vital). There were no statistical differences between mean values of PRX vessels i.d. in Agnus ($52.0 \pm 1.1 \mu\text{m}$) and Vital ($52.8 \pm 1.0 \mu\text{m}$), but in OK31 they were significantly narrower ($48.5 \pm 0.9 \mu\text{m}$; one-way ANOVA, $F = 4.90$, $P = 0.008$, followed by Scheffé_{0.05} test). The distributions of SX vessel diameters were in all three varieties skewed to the

right (skewness 1.08 ± 0.06 , kurtosis 0.23 ± 0.11 , $n = 1,854$ for Agnus; corresponding values were 1.04 ± 0.06 , -0.07 ± 0.12 , $n = 1,752$ for OK31 and 1.27 ± 0.06 , 0.45 ± 0.11 , $n = 1,901$ for Vital). When treated with non-parametric Kruskal-Wallis test, the differences in the distributions of SX vessels i.d. between the varieties were not statistically significant ($H = 1.46$; $P = 0.48$).

However, despite the narrow vessels (i.d. $< 100 \mu\text{m}$) were abundant, their contribution to the total K_T was low ($5.6 \pm 1.3\%$, $n = 5$ in Agnus, $4.0 \pm 0.5\%$, $n = 5$ in OK31, and $3.2 \pm 0.3\%$, $n = 5$ in Vital; Figure 2). For that reason, we tested the differences in distributions of the wide vessels diameters (i.d. $\geq 100 \mu\text{m}$) in the varieties studied (Kruskal-Wallis test, $H = 38.3$; $P < 0.001$). While there was no statistical

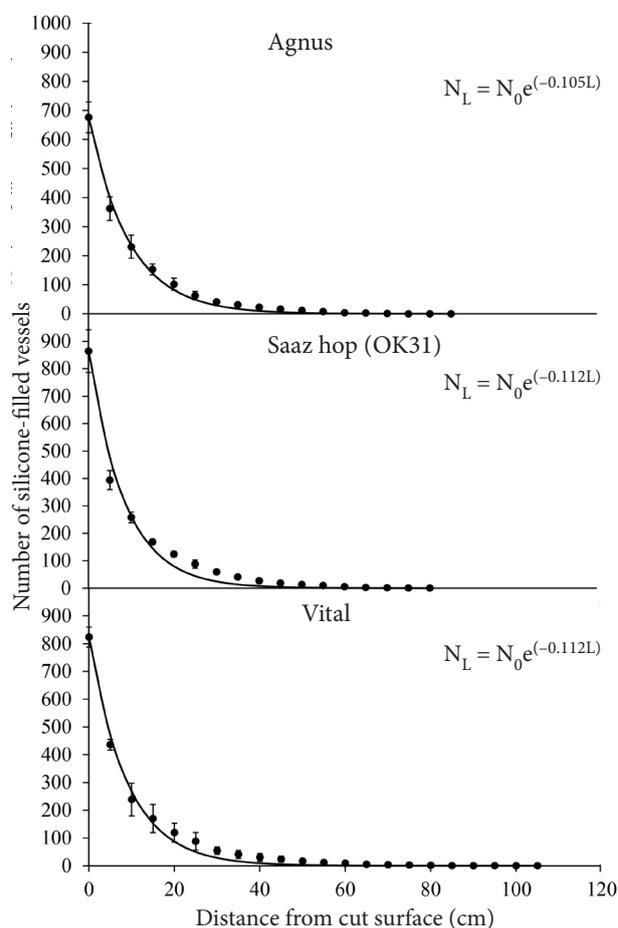


Figure 3. The number of silicone filled vessels as function of increasing distance from the point of the silicone injection in three varieties of hop plants (circles). Fitted exponential function was based on number of filled conduits (N_L) and distance (L) from the silicone injection point. N_0 was the number of vessels at the injection surface. Shown means \pm SD, $n = 5$

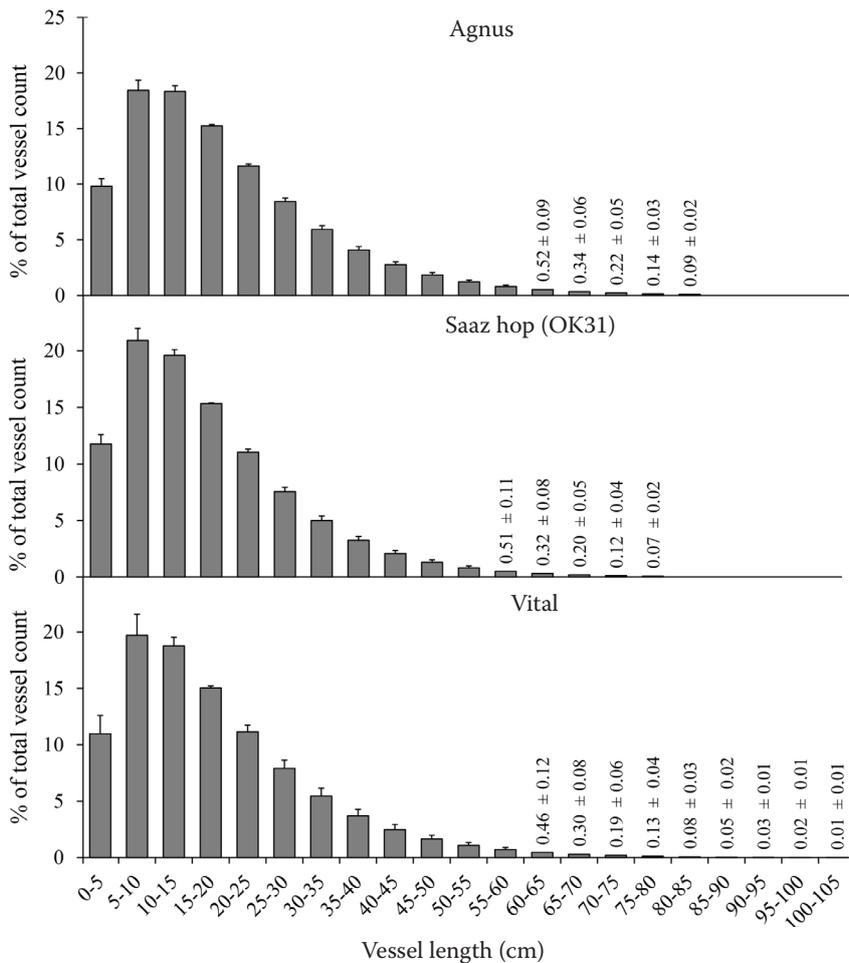


Figure 4. Frequency distribution of the length of xylem vessels in stem segments of three hop varieties. Means ± SE, $n = 5$

difference among the distributions of wide vessels diameters in Agnus and OK31, the distribution in Vital was significantly different (post hoc z-tests, $P < 0.001$; Figures 1D–F).

Vessel length distribution. The extinction coefficients k , calculated by a direct non-linear solution using least squares method for data from each stem segment separately (Figure 3), were not statistically different between the varieties tested (one-way ANOVA; $F = 1.02$; $P = 0.39$). In Agnus, the mean k value was 0.105 ± 0.004 ($n = 5$), in OK31 0.119 ± 0.005 ($n = 5$), and in Vital 0.112 ± 0.010 ($n = 5$). Using the extinction coefficients

for individual stems, the frequency distributions were calculated (Figure 4) as well as L_0 and L_{max} (Table 2). There were no statistical differences among varieties tested in both L_0 and L_{max} (one-way ANOVA, $F = 1.21$; $P = 0.33$; $n = 5$).

DISCUSSION

Intraspecific comparisons of the xylem structure among different crop varieties should carefully respect the marked changes that the xylem tissue undergoes during ontogenesis (Gloser et al. 2011)

Table 2. Values of the most common vessel length (L_0) and the length of the longest 1% of vessels (L_{max}) in stems of three hop varieties in cm. Means ± SE, $n = 5$

Variety	L_0		L_{max}	
	mean	SE	mean	SE
Agnus	9.6	0.4	44.1	1.8
Saaz hop (OK31)	8.5	0.4	39.1	1.8
Vital	9.2	0.7	42.2	3.1

and due to variation of environmental conditions (Plavcová and Hacke 2012). Varietal comparison in this study used samples from the same part of the stems from plants at the same ontogenetic stage. Varieties also grew on adjacent experimental fields with similar microclimatic and edaphic conditions and identical agricultural treatments. Despite this careful sample selection, there were only marginal differences between three hop varieties tested in the basic morphometrics of xylem vessels. Mean i.d. of PRX vessels was approx. 8% lower in OK31 variety (48.5 μm) compared to Agnus (52 μm) or Vital (52.8 μm), but considering very low (< 6%) contribution of both PRX and SX vessels with i.d. < 100 μm to the overall stem K , this difference is negligible. More noticeable and important effect on K can be expected if distribution of wide vessels would differ, because K grows with fourth power of the vessel diameter according the HP equation. This was found for SX vessels wider than 100 μm , as their distribution in Vital differed from Agnus and OK31. There were no differences among the varieties as regards the ratio between measured and theoretically calculated K , as well as the vessels length distribution assessed using the exponential decay function. Our results thus imply that the basic characteristics of xylem were very conservative among the hop varieties tested.

There is increasing evidence that measured K is commonly less than half of the K_T , calculated from vessel lumen dimensions assuming either circular or elliptical shape of the vessel lumen (Lewis and Boose 1995). In this study the measured K varied between 13% and 28% of K_T calculated assuming circular shape of the lumen. These results are very similar to those found in a previous study on hop stems (Gloser et al. 2011). The resistance of end walls is considered as the most significant factor contributing to the difference between theoretical and measured K (Sperry et al. 2005). Stems with longer vessels contain smaller number of end walls per stem unit length and, hence, fewer obstacles to water flow. This implies that in hop varieties with longer vessels lower difference between theoretical and measured K could be observed. In our study there were no statistically significant differences between varieties in the vessel length as assessed by the extinction coefficients (k) from the exponential decay function. This function was chosen for fitting the measured data preferentially to the double-difference algorithm (Tyree and Zimmermann 2002) because calculation of a single

parameter (k) allowed simple direct comparison of varieties as regard the length of their vessels. The exponential decay function apparently underestimated the frequencies of vessels in the length interval approx. 5–60 cm (Figure 3), but as this overestimation was found in all three varieties, the varietal comparison drawn from the k values was not violated.

The results of this study show that the basic functional and anatomical features of the xylem in stems of three hop varieties were surprisingly conservative. Thus the vulnerability to drought-induced embolism, although not measured in this study, can be expected similar for the varieties as it was found that positively correlated to vessel length and diameter (Tyree et al. 1994, Hacke et al. 2006). Considering the increasing evidence about the plasticity of xylem characteristics as affected by various factors like nutrient availability and drought (Awad et. al. 2010, Plavcová and Hacke 2012), much attention should be paid to the xylem formation and functioning under extreme climatic conditions (namely drought) in future studies.

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