1 **ENSURING A REPRESENTATIVE SAMPLE OF EARLYWOOD VESSELS**
2 **FOR DENDROECOLOGICAL STUDIES: AN EXAMPLE FROM TWO**
3 **RING-POROUS SPECIES**

4
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24 **Abstract**

25 The analysis of time-series of wood cell anatomical features (such as the earlywood
26 vessels of ring-porous trees) is a successful approach to understand the effect of
27 environmental factors on tree growth and thus constitutes a valuable source of
28 information about past environmental conditions. However, despite the rising interest in
29 analyzing wood anatomical time-series, little or no attention has been paid to establish
30 an adequate sample of cells in order to minimize the risk of missing a valuable
31 environmental signal.

32 In order to contribute to such methodological bases, this paper is aimed at i) identifying
33 a representative sample of earlywood vessels within a tree which encode the same
34 climatic information and ii) assessing if it is preferable to obtain the sample of vessels
35 along one or two radii. Four individuals of sessile oak (*Quercus petraea* (Mattuschka)
36 Liebl.) and sweet chestnut (*Castanea sativa* Mill.) were harvested and all their
37 earlywood vessel lumina measured along two 40-mm wide radial strips. Measured
38 vessels were stepwise selected while increasing tangential width of the wood section
39 from 1 to 40 mm, analyzing at each step i) the common signal of chronologies and ii)
40 the correlation to the main climatic variables controlling growth. Additionally, both
41 radii in each tree were analyzed together and separately.

42 The results showed that a total tangential width of 10 mm was enough to stabilize the
43 climatic signal, with improvement if it is distributed along two different radii, but a
44 slightly larger tangential width is required to reach an optimal common signal. We
45 suggest that, at least for the case of these two species growing at this specific climatic
46 context, two 5-mm increment cores ensure a representative vessel selection.

47 **Keywords:** Dendrochronology, tree rings, earlywood vessel size, ring-porous,
48 environmental signal, *Castanea sativa* Mill., *Quercus petraea* Mattuschka (Liebl.).

49 **Introduction**

50 Tree growth and consequently wood formation are strongly influenced by the
51 environment (Denn and Dodd 1981), causing variations in wood characteristics that can
52 be analyzed in datable tree rings and provide valuable information about plant
53 functioning and past environmental conditions (e.g. Schweingruber 1996). This
54 information is usually extracted by means of tree-ring proxies such as ring width,
55 maximum density or isotopic content in wood.

56 Recent studies on water conducting elements of hardwoods show that year-to-year
57 variations in their dimensions are capable of encoding valuable ecological information
58 (Eckstein 2004), which varies among species, climatic regions and anatomical features
59 considered. For example, vessel size of different ring-porous oaks (*Quercus* spp.)
60 recorded temperature signals in a dry inneralpine valley (Eilmann et al. 2006) and at
61 their northern distribution limit in Canada (Tardif and Conciatori 2006), but also
62 precipitation under oceanic climate (García-González and Eckstein 2003). Analogously,
63 earlywood vessel size of sweet chestnut (*Castanea sativa* Mill.) registered spring
64 temperature in the southern Swiss Alps (Fonti and García-González 2004).

65 The use of time series of vessel features is becoming more popular (e.g. Pumijumnon
66 and Park 1999, 2001; Corcuera et al. 2004, 2006; Schume et al. 2004; Verheyden et al.
67 2005; Eilmann et al. 2006), but there is no common practice in their sampling
68 procedure. The identification of ecological signals in wood anatomical features also
69 depends on how vessels are sampled within each annual ring. Different studies on water
70 conductive elements differ in the sampling procedures, including the number of radii
71 per tree (one to three), the tangential width of the radii (from 2.4 to 8 mm) and the
72 criteria applied to select the vessels (visual criteria, fixed number, size threshold or *n*-
73 largest vessels; e.g., see methods in Fonti and García-González (2004), Tardif and

74 Conciatori (2006), St. George et al. (2002)). This diversity of procedures causes large
 75 differences in which and how many vessels are considered for the analysis and can
 76 influence the final results of the studies considerably.

77 St. George et al. (2002) first dealt with the problematic of vessel sampling for *Quercus*
 78 *macrocarpa* Michx., arguing that 20 vessels per ring (threshold for vessel detection >
 79 $1,200 \mu\text{m}^2$) were enough to provide a good estimate of mean vessel size (i.e. the
 80 standard error of mean stabilized at a low level). However, they did not discuss if this
 81 selection was representative for the expression of the environmental signal. More
 82 recently, a methodological study on earlywood vessels of chestnut (threshold for vessel
 83 detection > $10,000 \mu\text{m}^2$) showed that climate-growth relationships varied considerably
 84 depending on which earlywood vessels were considered for the analysis (García-
 85 González and Fonti 2006). In this particular study, correlations to March temperature
 86 changed from highly significant to non-significant along with the size criteria used to
 87 select the vessels within an 8 mm wide tangential strip. The best result was achieved
 88 when only vessels bearing the same information, e.g., only the earlywood vessels in the
 89 first row, were considered. However, it has not been verified if previous approaches to
 90 select earlywood vessels with the same signal result in a representative sample size.

91 The current study is specifically aimed at identifying the optimal sample of earlywood
 92 vessels to be considered representative to build a time series for each tree. We assume
 93 that vessel sampling is representative when an additional increase of vessels bearing the
 94 same information does not further improve the quality of the chronology (common
 95 signal) or the relationships to climatic variables (climatic signal). In particular, we
 96 progressively increased the tangential width on one or two radii for four individuals of
 97 chestnut and oak, computed chronologies of mean vessel area and compared them to
 98 meteorological data, following the changes in i) the common and ii) climatic signal.

99 **Material and methods**

100 ***Wood material and earlywood vessel survey***

101 The work was carried out on circular stem discs (with a well-centred pith) of 50-year-
102 old dominant stools of chestnut (*Castanea sativa* Mill.) and sessile oak (*Quercus*
103 *petraea* (Mattuschka) Liebl.). Four discs for each species (8 in total) were selected from
104 an oak-chestnut coppice forest from the southern Swiss Alps (Bedigliora, lat. 46°00' N,
105 long. 8°50' E). Stem discs were cut at a height of 50 cm above ground. Cross-sections
106 were sanded to 400-grit, ring widths measured and rings accurately dated. Crossdating
107 was validated using COFECHA (Holmes 1983).

108 The measurement of earlywood vessels was performed on digital images of wood
109 surface along two 40 mm-wide radial strips on each disc (A and B) that were captured
110 with a 4800-dpi resolution scanner (Epson Perfection 4990 Photo, Seiko Epson
111 Corporation, Japan) (Fig. 1). To improve vessel recognition the earlywood vessel
112 lumina were first cleaned with a high-pressure water blast. Afterwards, the strips were
113 coloured black using a marker pen and vessel lumina filled with white chalk powder.
114 Images were semi-automatically analysed using the “Image Pro Plus” digital analysis
115 software (version 1.3 for windows; Media Cybernetics, Inc., Silver Spring, MD, USA).
116 The program was set up with filters (morphological 2x2 squares, 1 pass) and an image
117 enhancer (equalize, best fit) in order to optimize vessel outlines.

118 Earlywood vessels were measured ring by ring from 1975 to 1999 (25 years); rings prior
119 to 1975 were not used to avoid the presence of juvenile wood, which has a strong
120 growth trend with smaller and more numerous vessels (Gasson, 1987; Helinska-
121 Raczkowska, 1994). Earlywood vessels which were misrecognized by the software had
122 to be manually corrected. The lumen cross-sectional area and the position (coordinates)
123 of each vessel were recorded. Only vessels larger than 10,000 μm^2 were considered, so

124 that small vessels produced later in the season, which probably encode a different
125 signal, were not considered into the calculation. This lower limit proved to be successful
126 in previous works (Fonti & García-González, 2004; Fonti et al., 2007), whereby
127 climatic signals in the early season were maximized by the largest vessels (García
128 González & Fonti, 2006).

129 ***Chronology computation and climate-growth relationships***

130 For each ring, we considered two values of mean vessel area (MVA): one for all
131 earlywood vessels and one for only those belonging to the first row. The latter integrate
132 a smaller data set but have surely the same ontogeny, and can sometimes lead to more
133 successful results when comparing to climate (e.g., Fonti et al., 2007; García-González
134 & Fonti, 2006). Average chronologies were built from MVA time series and served to
135 establish climate-growth relationships. The analyses were performed separately for
136 radius A or B (at least 90 degree apart), as well as combining A and B (A+B); in the
137 latter case, both series were averaged into a curve per tree.

138 For chronology computation, non-desired growth trends (related to age and forest
139 dynamics) were removed from the MVA individual series by fitting a cubic smoothing
140 spline (32-year stiffness, 50% frequency cutoff) and dividing each value by the
141 function (Cook et al. 1992). The detrended series were then averaged into chronology's
142 growth indices (Fritts 1976). The quality of the chronology, i.e., the quantification of the
143 common signal, was assessed through the mean correlation between trees (Rbt), mean
144 correlation within trees (Rwt), and the standard error of the chronology (SE) (for a more
145 detailed explanation see Briffa and Jones (1992), Fritts (1976) and Wigley et al. (1984)).
146 When combining radii A and B, chronology error was calculated considering their
147 average (A+B) and both separately (A&B), and using the correction for sample size
148 suggested by Briffa and Jones (1992) for more than one radius per tree.

149 Climate-growth relationships were established by means of correlation functions, i.e.,
150 computing Pearson's correlation coefficient between the MVA chronologies and
151 monthly values of mean temperature and total precipitation from previous May to
152 current June. Climatic data came from the nearby weather station of Lugano
153 (MeteoSwiss, Locarno-Monti), located 10 km from the study plot, which has a complete
154 record for the study period.

155 ***Progressive selection of vessels***

156 The first step was to verify if the signal within the earlywood vessels of the four
157 selected trees per species was in agreement with similar studies in the same region
158 (Fonti & García-González, 2004; García-González & Fonti, 2006; Fonti et al., 2007).
159 Then we assessed the influence of sample size on chronology quality and climate-
160 growth relationships by progressively selecting the earlywood vessels according to their
161 tangential position within each radial strip of 40 mm. Computations started with a
162 tangential width of 1 mm, adding progressively the vessels belonging to the following
163 0.5 mm, until the maximum width (40 mm) had been reached (Figure 1); vessels were
164 considered when their centroid fitted within the width frame. After each selection step,
165 new vessel chronologies were computed, assessed for their quality and correlated to
166 climatic data. When combining both radii, the tangential width represents the added
167 width of them (e.g., 5 mm corresponds to 2.5 mm for each radius).
168 Variations in chronology common signal, standard error and correlations to climatic
169 data were tracked along with the tangential width considered. Thus, the assessment of
170 an adequate sample width was evaluated according to how fast the results changed or
171 when they stabilized.

172

173 **Results**

174 *Results using the maximal strip width*

175 On a tangential ring width of 10 mm we counted an average of 49.1 earlywood vessels
176 for chestnut and 41.0 for oak, with about half of them in the first row (24.2 for chestnut,
177 20.1 for oak). Oak vessels are larger than those of chestnut if all earlywood vessels are
178 considered ($49,772 \mu\text{m}^2$ vs. $45,963 \mu\text{m}^2$) but are nearly equal in size for the first row
179 (around $62,000 \mu\text{m}^2$).

180 The main results for the MVA chronologies based on the maximal amount of earlywood
181 vessels considered (i.e., on two 40-mm wide radial strips for each tree) are shown in
182 Figure 2. For chestnut, the signal is better for the first row than for all vessels (higher
183 chronology quality and correlations to climate), whereas the use of all vessels is slightly
184 better for oak. Except for the case “chestnut, all earlywood vessels”, values of Rbt are
185 over 0.50 indicating that the four trees have a strong common signal. This common
186 variation in MVA is highly correlated with climatic records for the period 1975-1999. In
187 particular, the first row of earlywood vessels of chestnut responds negatively to March
188 temperature ($r=-0.40$, $P<0.05$) and is also positively related to precipitation during the
189 previous late summer (July to September, $r=0.54$, $P<0.01$). For oak, all earlywood
190 vessels clearly respond to spring precipitation (March to May, $r=-0.69$, $P<0.001$) and
191 also to previous July temperatures ($r=-0.61$, $P<0.01$).

192 *Variation in the quality of the chronology*

193 The variations of common signal along with the considered tangential width of the
194 wood section are shown in Figure 3. In all cases, the pattern is initially characterized by
195 a clear ascending trend which stabilizes at 10-15 mm, becoming very close to the value
196 observed when both 40-mm strips are entirely used; at that point, an additional increase
197 in tangential width results in an irrelevant improvement of the correlations (Rbt and

198 Rwt). For example, if both radii are used (A+B), 80% of the maximum value of Rbt is
199 attained at 7 mm (all vessels) and 9 mm (first row) for oak, and at 12 mm (all vessels)
200 and 7 mm (first row) for chestnut. The trend is similar regardless of considering a single
201 radius (A or B) or both (A+B), but values are higher and stabilize slightly earlier (at a
202 lower tangential width) for the latter. There is an important intra-tree variability of
203 earlywood vessel size as indicated by values of mean correlation within trees (Rwt) that
204 are not clearly higher than those of Rbt.

205 Likewise common signal, the reduction of the standard error of the chronology (Figure
206 4) is more efficient when increasing a small tangential width, but a sample size over 10-
207 15 mm hardly contributes to decrease the error. When using only one radius (A or B),
208 chronology error is considerably higher but, for the case of two radii, there is no
209 improvement if processing them separately to compute the error (after Briffa and Jones
210 (1992)) instead of combining them, despite the increase in the number of time series (8
211 vs. 4). This result confirms the important variation within trees and the considerable
212 reduction of error by averaging two different radii.

213 ***Variation in the climatic signal***

214 The correlations between MVA chronologies and climatic factors have a similar trend to
215 the common signal, but a smaller tangential width of only a few millimeters is sufficient
216 to stabilize the results in this case. Figure 5 shows three examples for the variation of
217 significant ($P < 0.05$ to $P < 0.01$) climatic factors in spring as tangential width increases.

218 In general, the main factors controlling growth are significant from the early beginning
219 (i.e., for only 2-3 mm). For a radial strip around 5 mm (the width of a standard
220 increment core), correlations are very similar to those with the whole 40-mm strip. In
221 general two radii provide a better result than a single one (especially at small tangential

widths), but differences between A and B are negligible and either of them is able to identify climate-growth relationships unequivocally.

Discussion

The assessment of an adequate sample size is a very important aspect in tree-ring research. For this, several statistic parameters were proposed and are widely accepted by the dendrochronological community (e.g. Wigley et al., 1984 or Briffa and Jones, 1992).

Upon these bases, some attempts are occasionally made to optimize sample size for some specific objectives (e.g., Leavitt and Long, 1984; Mäkinen and Vanninen, 1999).

Such methodological works, which usually include only a few trees at specific areas, can provide useful procedures for other researches working on the same field. In the present study, although it had to be limited to only four trees per species and short chronologies of 25 years, the results were highly consistent with similar works for the same species and within the same climatic context (Fonti and García-González, 2004, García-González and Fonti, 2006, Fonti et al. 2007), confirming that the material was representative for the prevailing environmental conditions. Therefore, some indications can be drawn to help with other studies on the earlywood vessels of ring-porous trees, since it demonstrates the importance of an adequate vessel sample.

It has been shown that when enlarging tangential width, both common and climatic signals initially increase rapidly while standard error decreases and they become stable afterwards, though at a different sample size. Values of common signal stabilize at earliest for a total tangential width of 7-12 mm over two radii. This fact explains why common signal in the present study (based on the whole 40 mm strip at two radii) is higher than previously observed for MVA (e.g., García-González and Eckstein (2003) or Tardif and Conciatori (2006)) reported values of Rbt ranging from 0.2 to 0.4 for longer time periods); but similar when tangential width is reduced to that of 1-2

248 increment cores. Contrary to the common signal, the main climatic signals can be
249 established at a narrower tangential width. A unique core of 5 mm is sufficient to detect
250 a relationship between vessel size and climate, but does not correctly characterize the
251 common signal. This is why very low common signals in previous works (Fonti and
252 García-González, 2004; Fonti et al., 2007) led to satisfactory results when chronologies
253 were compared to climate. The required tangential width to stabilize the signal when
254 only the first row of earlywood is considered is similar to that for all vessels, despite its
255 lower vessel number. These results show how a vessel sample based on a minimum
256 number disregarding for their radial position into the ring can result in an under-
257 expression of the signal (García-González and Fonti 2006). Therefore, a ‘minimum
258 tangential width’ should be considered rather than a ‘minimum number of vessels’ for a
259 representative sampling.

260 Although common and climatic signals are reliable when both radii are considered, the
261 use of a single radius can affect common signal, mainly for narrow tangential width (< 5
262 mm), but has less impact on the identification of climate-growth relationships.
263 Consequently, within-tree variability needs to be minimized to improve chronology
264 quality, done in this work by averaging the MVA series of both radii. If otherwise all
265 their vessels are pooled for each ring and MVA calculated afterwards, differences in
266 common signal are irrelevant (data not shown). On the other hand, correlations to
267 climate do not differ significantly if vessels are measured on radius A or B, nor do they
268 deviate from the average of both radii (A+B).

269 The results of this paper showed that an adequate sample size can be established to
270 optimize the amount of earlywood vessels to be measured. However, this procedure
271 depends on the type of material available. The highest confidence would be gained for
272 cross sections or 12 mm increment cores, from which two strips of about 10 mm would

273 be optimal. Choosing a single strip would be expected to lead also to satisfactory
274 results, but common signal would not be maximized. Unfortunately, cross sections are
275 usually not available, and 12 mm cores cannot be extracted in very hard woods, but it is
276 still possible to succeed with 5-mm cores provided that at least two cores are used per
277 tree. Although one single core might be enough for climate-growth relationships, this is
278 required to correctly assess the quality of the chronology. Finally, these working
279 recommendations should be verified for other species and climatic contexts, since the
280 establishment of a representative sample size or earlywood vessels would facilitate their
281 use in tree-ring research and thus yield more ecological information.

282

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344

345 **Figures**

346

347

348 **Fig. 1: Example of *Quercus petraea* cross-section image used for calculations.** Size
349 and position of earlywood vessels are surveyed year by year along a radial strip. Time
350 series of mean vessel size are repeatedly computed by progressively enlarging the width
351 of the radial strip so that an increasing number of vessels are included into the
352 calculations. Vessels within the frame correspond to first row vessels of 1995 annual
353 ring; vessel numbers indicate object labels during analysis.

355

356 **Fig. 2: Simple correlations between earlywood mean vessel area and monthly**
357 **climatic factors for the period 1975-1999.** Data refer to vessel measurements
358 performed on two radial strips of 40 mm in each tree. Graphs are presented separately
359 for all earlywood vessels and for vessels belonging to the first row only. Horizontal
360 lines indicate the significance level ($P < 0.01$ and $P < 0.05$). Chronology quality is also
361 shown (Rbt = correlation between trees; EPS = expressed population signal; %Var =
362 variance in the first eigenvector of a principal component analysis on the individual
363 series). EPS and %Var are provided only for comparison with other chronologies.
364

a) Chestnut, all vessels

b) Oak, all vessels

c) Chestnut, first row

d) Oak, first row

365

366 **Fig. 3: Variation of common signal (mean correlation between trees) with**
 367 **gradually increasing tangential width.** Data refer to measurements performed for both
 368 all vessels and the first row of earlywood vessels. Results are presented for both species
 369 and for radius A and B as well as for the composite of both radii (A+B). Plain line refers
 370 to mean correlation within trees (Rwt).
 371

a) Chestnut, all vessels

b) Oak, all vessels

c) Chestnut, first row

d) Oak, first row

372

373 **Fig. 4: Evolution of the standard error of chronology with gradually increasing**
 374 **tangential width.** Data refer to measurements performed for both all vessels and the
 375 first row of earlywood vessels. Results are presented for both species and when using
 376 one (A or B) radius or both; for two radii, standard error is presented for the average
 377 (A+B) and when processing both radii separately (A&B).
 378

a) Chestnut, all vessels

b) Oak, all vessels

c) Chestnut, first row

d) Oak, first row

379

380 Fig. 5: Examples of variation in growth-climate relationships with gradually
 381 increasing tangential width. Results are presented for radius A and B as well as for the
 382 composite of both radii (A+B).

383 a) Chestnut, first row and March temperature

384 b) Oak, all vessels and March to May precipitation

385 c) Oak, first row and March to April precipitation