

A METHOD FOR TREE-RING ANALYSIS USING *DIVA-GIS* FREEWARE ON SCANNED CORE IMAGES

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ABSTRACT

Tree-ring analysis is a basic technique of paramount importance in forest management, yet it may prove difficult and time-consuming for many slow-growth hardwood tree species. Moreover, it requires the use of specialized tools and proprietary software, which may hinder researchers working with limited budgets. We describe an innovative and inexpensive method using *DIVA-GIS* freeware software to analyze true color high-resolution scanned images of cores previously enhanced with *ImageJ* freeware (*GIS-SDI*), and test its accuracy against the widely-used *LINTAB-TSAPWin*TM and *WinDENDRO*TM methods. For this purpose, *Abies pinsapo* and *Pyrus bourgaeana* increment cores were processed independently using each of the three methods and the results were statistically compared. Dating results were consistent across all three methods, although identifying rings was easier and quicker to perform on the digital images. Using a modern but affordable flatbed scanner to digitize tree cores and the free *DIVA-GIS* software to analyze the scanned digital images proved to be an inexpensive but highly accurate and efficient approach to tree-ring analysis. Furthermore, this method greatly facilitates tree-ring analysis in species with inconspicuous rings, and enables a complete digital record of every core analyzed to be stored.

Keywords: tree-ring analysis, digital image, *DIVA-GIS*, scanned core images, *Pyrus bourgaeana*, freeware software, image analysis.

INTRODUCTION

For decades, image-analysis techniques have been applied to deal with the problem of ring boundary detection in dendrochronology, mainly in the forestry industry. There has also been a boom in the use of these techniques in the fields of forest ecology and conservation biology, with many researchers employing image-analysis techniques as an alternative to traditional methods (Campbell *et al.* 2011). Recent trends in the sustainable management of natural forests to support biodiversity pose new challenges. Currently, there are several crossdating and measurement methods that are applied not only by dendrochronologists but also lay-dendrochronologists in other areas of knowledge where the study of tree rings is

necessary to assess the age and growth of trees or to date events. Thus, the age and growth rate of trees can be determined by dendrochronological methods, which constitute fundamental tools for analyzing the age structure of forests, describing their current status, assessing their stability, and reconstructing their history (Theurillat and Guisan 2001; Worbes 2002).

Although classical dendrochronological techniques continue to be used (Swetnam *et al.* 1985; Schweingruber 1988, 1996), the development of new techniques and equipment has been followed by further advances in methodology, scientific instrumentation, and software (Maxwell *et al.* 2011). Several programs are available to measure tree rings using digital images, differing in cost and capacity (<http://web.utk.edu/~grissino/software.htm>). In practice, the preferred method actually employed varies by country and institution (Thetford *et al.* 1991;

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Soille and Misson 2001; Speer 2010). Among graphical/statistical solutions, one of the most widely-used platforms is the *LINTAB*TM ring measuring station in combination with *TSAP-Win*TM (Rinntech[®] 2010) or *PAST4* (Knibbe 2008). Boundaries between tree rings and the transition from earlywood to latewood cells based on changes in light intensity of an image can be detected using image analysis (Spiecker *et al.* 2000). In this sense, the use of specific software for image analysis in dendrochronological studies is not new (Varem-Sanders and Campbell 1996; Vila-Lameiro and Diaz-Maroto 2007). Indeed, the great potential for tree-ring studies of using digital images from flatbed scanners for automatic and semi-automatic recognition of tree-ring width through specific software has been recognized since the end of the last century. Widely-applied methods include *Addo-X* (Stokes and Smiley 1968), *MacDENDRO* (Guay *et al.* 1990), *DendroScan* (Varem-Sanders and Campbell 1996), *LIGNOVISION*TM (Rinntech[®] 2010), and *WinDENDRO*TM (Regent Instruments Canada Inc. 2009), the latter being especially widely known. *ATRICS* (Levanič 2007), which is not an image analysis program *per se* but an image capture program, running *SigmaScan* (Hietz 2011) software, was developed with the specific purpose of analyzing the growth rings of coniferous species and linking the program for image analysis with Microsoft *Excel* and with the *R* package. However, none of these platforms and programs are easily affordable.

Low-cost imaging equipment could provide tangible benefits to researchers or technicians who cannot afford these imaging systems. Examples include *CDendro/CooRecorder* (*CDendro* (Cybis Elektronik & Data, Saltsjöbaden, Sweden)), which is widely used as a low-cost alternative to *WinDENDRO*TM and is versatile, because it can not only produce ring-width increments from scans, but also other readings such as blue-light measurements and density profiles. For the microscope/computer setup there are free measurement packages such as *PJK* (Lamont-Doherty Earth Observatory 2013) and *Tellervo* (Cornell Tree-Ring Laboratory 2013). And the least expensive and most common linear measuring system currently in use is *Velmex* (Grissino-Mayer *et al.* 1996; Maxwell *et al.* 2011), an open source system.

However, although these programs are the most common, they are not available for all operating systems, not all of them are strictly free, and they need to be supported by other software. In addition, tree-ring width measurements are often duplicated, being repeated both in the wood itself and in the scanned image; measurement in this context is a task that can only be conducted by an expert dendrochronologist with extensive experience in the field. Furthermore, irrespective of the equipment used, working with species whose growth rings are imperceptible, as is the case with many tropical species (Rozendaal and Zuidema 2011) and Mediterranean species (Mitrakos 1980; Cherubini *et al.* 2003) is a complicated and time-consuming task. Unsurprisingly, few papers have been published on growth-ring analysis of Mediterranean species other than conifers (Scarascia-Mugnozza *et al.* 2000; Touchan *et al.* 2005; Copenheaver *et al.* 2010; Tegel *et al.* 2014). In this case, because of the difficulty of accessing such data, some researchers have resorted to combinations of scanned images and other programs that allow dating and measurement of tree rings. Cruz Johnson *et al.* (2007) used *ArcView GIS* software (ESRI 1998) for measuring tree rings on scanned images of cores in order to estimate the volumetric growth of *Notophagus pumilio*. This interesting approach went largely unnoticed and its validity and effectiveness still remain to be tested against any widely-accepted procedure. In addition, *ArcView* and its successor *ArcGIS* (ESRI 2008) are commercial programs requiring a license subject to annual renewal, which render this option less attractive. More recently Aynekulu *et al.* (2009) gave an outline description of a very similar method using the free *GIS* software *ILWIS 3.3* (2005, <http://www.itc.nl/ilwis/>) to analyze images scanned at 600 dpi, highlighting its potential for dendrochronological studies. However, the accuracy of their measurements was not demonstrated and was not compared against other procedures. In this sense, the versatility and potential utility for dating, measuring and analyzing tree rings on scanned images of cores have significantly increased thanks to the improved performance of affordable modern flatbed scanners and the availability of powerful, free computer programs

for image analysis (e.g. *ImageJ* 2009) and Geographical Information Systems.

In this paper we describe and test the accuracy of the *GIS-SDI* method, an easy, innovative and inexpensive tool for the analysis and measurement of growth rings on digital images of tree cores. To evaluate the performance of this method, an analysis was conducted comparing it with both the *LINTAB-TSAPWin*TM and *WinDENDRO*TM methods.

METHODS

Tree-Ring Data

Eight increment cores from live Iberian wild pear (*Pyrus bourgaeana* (D.)) and eight from Spanish fir (*Abies pinsapo* (B.)) trees were obtained from two collections of the Ecology and Forestry Departments (University of Cordoba), respectively. The increment cores of *P. bourgaeana* were collected from different populations in Sierra Morena (37°53'53"N; 4°58'5"W; Cordoba-Ciudad Real, Spain) and Doñana National Park (37°2'0"N; 6°24'28"W, Huelva-Seville-Cadiz, Spain) (Arenas-Castro 2012). Those of *A. pinsapo* came from Sierra de Grazalema (36°46'07"N; 5°25'51"W, Cadiz-Malaga, Spain) and Sierra de las Nieves (36°41'00"N; 5°00'00"W, Malaga, Spain). The only exclusion criterion for the selected cores was that they should have no deformities or scars that would prevent the identification of the rings. All the samples were dried, mounted and sanded according to standard dendrochronological techniques for further treatment (Stokes and Smiley 1968). Progressively finer grades of sandpaper (60–1200 grit cm⁻²) were used to prepare the surface of the increment cores. This ensured a smooth, flat and transverse surface, facilitating the visual identification of the rings. Then, each increment core was scanned using the method set out below. Although *A. pinsapo* cores show clear and easily-identifiable tree rings, those of *P. bourgaeana* cores are much less conspicuous and therefore more difficult to identify. Consequently, each digital image was subsequently enhanced by adjusting brightness and contrast (Schneider *et al.* 2012).

Study Design

The basic study design consisted of analyzing the same increment cores and their digital images across three different methods of tree-ring analysis. The digital images were subsequently analyzed with *DIVA-GIS*, a free computer program originally devised for mapping and geographic data analysis (Hijmans *et al.* 2004). For simplicity, this procedure will be referred to as the *GIS-SDI* method hereafter. The performance and reliability of this method was tested against the widely-used *LINTAB-TSAPWin*TM platform as well as the *WinDENDRO*TM method. Ring dating and measurement of the sample cores was carried out with the three methods independently, and covered the same span of years (1978–2007). Therefore, because the number of dated series per species was eight, the total rings in all series were 240 for *A. pinsapo* and 240 for *P. bourgaeana*. Ring dating, measurement and crossdating of the sample cores scanned were carried out by the same technician (with four years' experience). To ensure as much independence as possible, the original tags of the cores were hidden, different provisional codes were assigned and later the same cores were dated and measured at random (without following a fixed order) on different dates, using the three different methods separately. Dating was performed following standard methods (Stokes and Smiley 1968; Swetnam *et al.* 1985), with reference to the most external ring formed before the date of core extraction. Only for the comparison between methods, and not as a step in the method process, a fine center line was drawn in pencil along each core, across the 1978–2007 rings. This line was later used as a guide for assessing the consistency of annual ring-width measurement between the three methods. A useful feature of some of the commercial methods for analyzing tree rings is that they allow a segmented radial path across the scanned image to be easily drawn. However, because the focus of this study was to compare a new method to other commercial methods, we had the center line follow the straight path across the core, instead of following the ring curvature by the rays of the wood.

Description of the Methods Used

LINTAB-TSAPWinTM Package

LINTABTM is widely used for measuring tree rings, because it provides high precision (maximum resolution: 0.001 mm) and a reliable measurement process. Its properties and advantages make it very versatile, and it is thus used in a wide range of applications, such as dendrochronology, stem analysis and ecological and geo-morphological analyses. Together, *TSAP-WinTM* and *LINTABTM* constitute a strong combination with *TSAP-WinTM* offering a powerful software platform for tree-ring analysis. *TSAP-WinTM* allows measurement and evaluation of tree-ring sequences, providing a large number of graphic and statistical functions, as well as database links to facilitate data management. Both are available separately or as a package. More information about the tree-ring analysis procedure with the *LINTAB-TSAPWinTM* package can be found at <http://www.rinntech.com>. For this work, a *Leica S6D* binocular microscope equipped with the *L2 Leica* cold light source was used to identify rings and measure annual growth in each core using the *LINTABTM-5* station, with a resolution of 0.01 mm. The data were automatically stored with *TSAPWinTM* in *.rwl format.

WinDENDROTM

Unlike the *LINTAB-TSAPWinTM* package, *WinDENDROTM* is an image analysis system for tree-ring analysis. In contrast to manual measurement systems, *WinDENDROTM* can work either directly with images obtained from a scanner or by opening a digitized image file, offering numerous advantages such as productivity gain, operator comfort and image archiving with or without their analysis, among others. In addition, this system is a versatile method because it allows tree-ring analysis from disks of wood, cores and images produced by digital X-ray systems. This method was specifically designed for dendrochronologists looking for a precise and efficient way to analyze tree rings. The use of *WinDENDROTM* is quite straightforward and is well described elsewhere (Regent Instruments Canada Inc. 2009). Among the various versions of this software, the regular *WinDENDROTM* 2009c version was selected as

best meeting the requirements of the study. According to dendrochronologists, if the distance between rings is less than 1 mm, as is frequently the case with non-coniferous species, a resolution of more than 400 dpi is required, and high-resolution images are normally used (Guay *et al.* 1992). Thus, because the distance between rings in *P. bourgaeana* was found to be less than 2 mm, software default settings were retained for the narrow rings function. *WinDENDROTM* is usually purchased along with a *LA2400* scanner, which has an optical resolution of 2400 dpi and a minimum pixel size of 0.011 mm. We wanted to assess to what extent a modern but affordable flatbed scanner like the *HP Scanjet G4050* could rival the *LA2400* scanner for ring measurement with *WinDENDROTM*. To this end, a sample of five cores was processed, chosen randomly from all cores, using both scanning devices prior to independently measuring the rings from the 1978–2007 period with *WinDENDROTM*. Because of the ease of identifying tree rings on *A. pinsapo* cores without resorting to scanned images, only increment cores of *P. bourgaeana* were assessed. Measurements made on digital images acquired with the *LA2400* scanner were compared with those obtained with the inexpensive *HP Scanjet* by means of a paired T-test (Zar 1999).

GIS-SDI Method

All cores of each species were simultaneously scanned with an *HP Scanjet G4050* to obtain an A4-size (210 × 297 mm) true-color digital image (red, green and blue channels) of 2400 dpi in uncompressed *tiff* format, with file sizes ranging from 60 to 80 MB. This resolution equates to 944.88 points per cm, so each pixel measures 0.0105 mm. Although the scan is capable of providing an optical resolution of 4800 dpi and 16 bits per channel, we determined that 2400 dpi was enough for our purposes. The digital image obtained was enhanced with the open source, public domain *ImageJ* software (Rasband 1997–2009) by adjusting its brightness and contrast, as well as its color balance if necessary. This fast and easy procedure greatly improves the ability to discriminate imperceptible tree rings in difficult species like *P. bourgaeana* (Figure 1; Supplementary Material). For convenience, the image of each

core was cropped from the A4-size image and saved as an independent file.

Each digital image was subsequently analyzed with free *DIVA-GISv.7* software (Hijmans *et al.* 2004). First, the digital image was imported to a new *GIS* project and then geo-referenced. Geo-referencing is a key step, required to set the scale of the image for later measurements. We used the *Georeference Image tool* (Figure 2a of Supplementary Material) for this task. With *Georeference Image*, a file called “world file” is created and linked to the image file. The process is straightforward and enables a relationship to be established between the coordinate system of the original image in pixels (rows and columns) and the actual coordinates in millimeters, giving the actual size of the scanned area (A4: 210 × 297 mm). A new image set to scale is obtained, allowing highly accurate measurements using the *GIS* tools. After geo-referencing the digital image, we added a thin line coinciding with the penciled guideline on the original core, analogous to the “path line” of *WinDENDRO*TM. This step was carried out only for the purposes of comparing the methods.

From the different types of vector data that are needed to work with *GIS* (point, polyline and polygon), we generated a point-type layer containing points drawn to precisely mark the intersections of the guideline with the growth rings. These points would be later used as landmarks for ring measurements. Finally, a new polyline-type layer was generated and superimposed on the previous layers (Figure 2b of Supplementary Material). This shape includes line segments drawn along the guideline between pairs of consecutive landmark points. This procedure allowed a better comparison of the measurements obtained with *DIVA-GIS* with *LINTAB-TSAPWin*TM and *WinDENDRO*TM. However, *DIVA-GIS* can draw polylines of any type and inclination on core images, automatically recording their dimensions. The polyline-type layer has an associated table of attributes, collating relevant information on each element of the layer and its properties. The table fields include: core number, number of annual radii, year in which each ring was formed and length of the radius (in mm). The table of attributes can easily be exported to *Excel* and other software

packages for further analysis. *DIVA-GIS* allows the insertion of tags that facilitate the tasks of dating and measurement (Figure 2c of Supplementary Material).

STATISTICAL ANALYSIS AND COMPARISON

Taking the null hypothesis of there being no differences between ring measurements made with the *GIS-SDI* method on the one hand, and either *LINTAB-TSAPWin*TM or *WinDENDRO*TM on the other, we predicted that measurements should be indistinguishable between methods. Statistical analyses were carried out separately for each species (*A. pinsapo* and *P. bourgaeana*). Independent analyses were carried out to test the *GIS-SDI* versus the *LINTAB-TSAPWin*TM measurements and subsequently the *GIS-SDI* versus the *WinDENDRO*TM measurements.

Crossdating

In accordance with crossdating techniques (Grissino-Mayer 2001), comparisons between methods and species were carried out on the same set of cores using output tables from *COFECHA* 6.06P (Grissino-Mayer 2001; Holmes 2000). For this purpose we focused on the main outputs of *COFECHA*, namely (1) tree-ring series correlations with the master chronology, (2) mean sensitivity (Cook and Briffa 1990; Fritts 1976), a measure of the variation between consecutive years (*i.e.* variability at inter-annual time scales) ranged between 0 (least sensitive) and 1 (most sensitive), and thus an indication of the limitations, presumably climate-related, to growth within the environment, and (3) number of flagged segments (year sections of an individual tree-ring series with low correlations to the master chronology). By default, *COFECHA* sets displaced segments of 50 years with 25-year overlap. Because *P. bourgaeana* is not a long-lived species, we configured the settings of *COFECHA* to analyze displaced segments of 20 years with 10-year overlap. For further explanation of *COFECHA* results, see Grissino-Mayer (2001).

Table 1. Mean ring size \pm 1 SD (mm) by species and method of tree-ring analysis.

Method	<i>A. pinsapo</i>			<i>P. bourgaeana</i>		
	Mean	SD	n	Mean	SD	n
<i>GIS-SDI</i>	1.88	0.9	80	1.36	1.18	80
<i>LINTAB-TSAPWin</i> TM	1.87	0.9	80	1.45	1.16	80
<i>WinDENDRO</i> TM	1.88	0.9	80	1.37	1.18	80

Statistical Analysis

Table 1 shows descriptive statistical data classified by species and methods of analysis. Through the measurements of tree-ring widths, Pearson's *r* was used to calculate correlation coefficients between methods. *SMATR* vers. 2.0 was used to fit bivariate lines to the data using the standardized major axis (*SMA*) regression technique (Falster *et al.* 2006; Warton *et al.* 2006). When measurements achieved with *GIS-SDI* are equal to those obtained with *LINTAB-TSAPWin*TM, the slope of the regression and the intercept should be similar to 1 and 0, respectively. In addition, the null hypothesis that the mean difference between pairs of measurements would be zero was tested using the paired T-test, each pair of measurements having been made on the same core and ring by both methods. Finally, a *Bland-Altman Plot* was used to analyze and illustrate the agreement between two different sets of measurements (Bland and Altman 1986, 1999), namely the *GIS-SDI* vs. the *LINTAB-TSAPWin*TM measurements and the *GIS-SDI* vs. the *WinDENDRO*TM measurements. In the *Bland-Altman Plot* the differences between each pair of measurements is plotted against their respective mean values. The mean difference between the two datasets and the limits of agreement (mean \pm 1.96 SD) are also plotted.

RESULTS

Crossdating

The output tables from *COFECHA* reveal slight differences in the accuracy of crossdating across species and methods (Table 2). Tree-ring width measurements carried out on *A. pinsapo* had higher series intercorrelations than measurements made on *P. bourgaeana*, regardless of the crossdating method. The short length of series made the dating and measuring process of growth tree rings easier. In this respect, there were no cases of missing ring measurements. However, turning to the number of flagged segments, there were great differences between species, but not between methods. Although there were no flags in the case of *A. pinsapo*, using any method, the number of flags for measurements made on cores of *P. bourgaeana* was similar, regardless of the method used. These discrepancies could be the result of the difficulty of identifying tree rings in cores of *P. bourgaeana*. But in this case, there were no significant differences between methods. With regard to the variation between consecutive years, values of mean sensitivity were higher for *P. bourgaeana*, which could indicate the strength of dependence on a limiting factor (climatic, biotic, management, and others) having high-frequency variance.

Statistical Analysis of the Measurements

The identification of growth rings of *A. pinsapo* was relatively easy. In contrast, the identification of *P. bourgaeana* rings was more complicated, because the rings are imperceptible and unclear. Although this task was difficult and time-consuming using the *LINTAB-TSAPWin*TM station and *WinDENDRO*TM images, it was easier

Table 2. *COFECHA* results from crossdating groups (species and methods).

	<i>A. pinsapo</i>			<i>P. bourgaeana</i>		
	<i>LINTAB-TSAPWin</i> TM	<i>WinDENDRO</i> TM	<i>GIS-SDI</i>	<i>LINTAB-TSAPWin</i> TM	<i>WinDENDRO</i> TM	<i>GIS-SDI</i>
Series intercorrelation	0.738	0.732	0.739	0.523	0.521	0.521
Average mean sensitivity	0.174	0.189	0.171	0.241	0.249	0.247
Flagged segments	0	0	0	3	2	2

Table 3. Pearson's r coefficient for *GIS-SDI* vs. *LINTAB-TSAPWin*TM and *GIS-SDI* vs. *WinDENDRO*TM.

	<i>GIS-SDI</i>					
	<i>A. pinsapo</i>			<i>P. bourgaeana</i>		
	r	p	n	r	p	n
<i>LINTAB-TSAPWin</i> TM	0.998	<0.001	240	0.975	<0.001	240
<i>WinDENDRO</i> TM	0.999	<0.001	240	0.999	<0.001	240

to perform using the *DIVA-GIS* tools on the digitally enhanced images of the cores. However, the main question was whether *GIS* analysis of digital images of cores would provide consistent results, in agreement with those obtained with *LINTAB-TSAPWin*TM and *WinDENDRO*TM. The graphs of Figure 3 (in Supplementary Material) show the annual radial increments for the series of 30 years (1978–2007) measured by the three methods for each core. In all cases, tree-ring measurements of the sample cores made with *GIS-SDI* almost exactly matched those of *LINTAB-TSAPWin*TM and *WinDENDRO*TM. Overall, tree-ring measurements obtained with *GIS-SDI* were highly correlated with those obtained using the *LINTAB-TSAPWin*TM and *WinDENDRO*TM methods (Table 3).

GIS-SDI vs. *LINTAB-TSAPWin*TM Comparison

Outcomes of linear regression analysis (*SMATR*) showed a strong and significant fit between the measurements made with *GIS-SDI* and those made with *LINTAB-TSAPWin*TM (Table 4 and Figure 1a-b). In both species the slope did not differ from 1 and the intercept did not differ from 0, therefore measurements achieved with *GIS-SDI* are equal to those obtained

with *LINTAB-TSAPWin*TM. In addition, for both *A. pinsapo* and *P. bourgaeana*, results of paired T-tests showed no significant differences in measurements between the *GIS-SDI* and the *LINTAB-TSAPWin*TM methods (Table 5).

The *Bland-Altman Plot* (Figure 2) showed a high level of agreement between the *GIS-SDI* and *LINTAB-TSAPWin*TM measurements of both *A. pinsapo* and *P. bourgaeana* growth rings. The differences between pairs of measurements made with the two methods were near zero, independently of the values of their mean differences, and located within the agreement limits (mean difference \pm 1.96 SD).

GIS-SDI vs. *WinDENDRO*TM Comparison

The results clearly show that digital images of the *P. bourgaeana* sample cores independently acquired with the *LA2400* scanner and the affordable *HP Scanjet G4050* both provided very similar results when analyzed with *WinDENDRO*TM (paired T-test, $t = -1.487$, $df = 149$, $p = 0.382$; *HP Scanjet* $\bar{x} = 1.425$, $\sigma = 1.011$, $n = 150$; *RegentLA2400* scanner $\bar{x} = 1.395$, $\sigma = 1.102$, $n = 150$).

In both species the linear regression between *GIS-SDI* and *WinDENDRO*TM measurements of annual rings displayed a neat fit, as shown in Table 4 and Figure 1c-d. In neither case did the slope differ from 1, or the intercept from 0 (Table 4); therefore, measurements achieved with *GIS-SDI* are equivalent to those obtained with *WinDENDRO*TM. In addition, the observed small differences in ring measurements between methods were not statistically significant (Table 5), in either species. In terms of the *Bland-Altman Plot*, there was a high level of agreement

Table 4. Linear regression analysis (*SMATR*) for *GIS-SDI* vs. *LINTAB-TSAPWin*TM and *GIS-SDI* vs. *WinDENDRO*TM methods.

	<i>GIS-SDI</i> vs. <i>LINTAB-TSAPWin</i> TM										
	R ²	p	n	Hb	F	p	df	Ha	T	p	df
<i>A. pinsapo</i>	0.997	<0.001	240	1	2.712	0.14	239	0	0.029	0.977	239
<i>P. bourgaeana</i>	0.952	<0.001	240	1	0.621	0.432	239	0	3.912	0.419	239
	<i>GIS-SDI</i> vs. <i>WinDENDRO</i> TM										
	R ²	p	n	Hb	F	p	df	Ha	T	p	df
<i>A. pinsapo</i>	0.998	<0.001	240	1	0.388	0.534	239	0	0.007	0.974	239
<i>P. bourgaeana</i>	0.999	<0.001	240	1	3.324	0.170	239	0	0.242	0.809	239

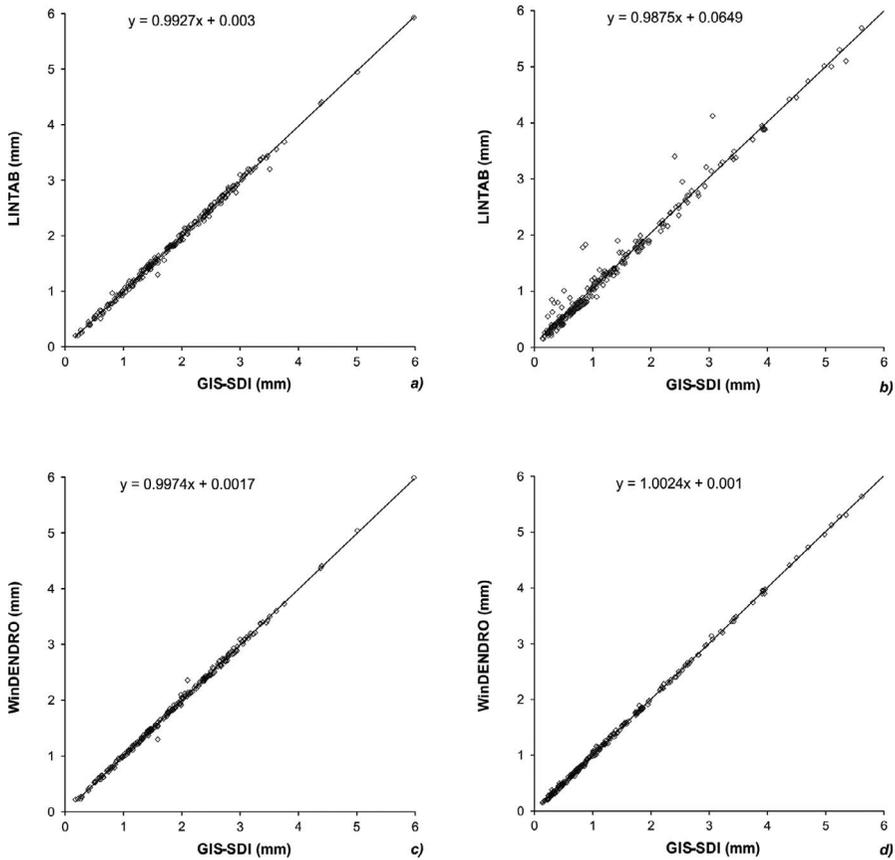


Figure 1. Linear regression between *GIS-SDI*, *LINTAB-TSAPWin*TM and *WinDENDRO*TM methods for tree-ring measurements of *A. pinsapo* (a-c) and *P. bourgaeana* (b-d).

between the two methods both for *A. pinsapo* and for *P. bourgaeana* (Figure 3).

DISCUSSION

The results show that measures of annual rings in cores of *A. pinsapo* and *P. bourgaeana* made with the *GIS-SDI* method were nearly identical to those achieved with *WinDENDRO*TM, and both closely

followed those obtained with *LINTAB-TSAPWin*TM. The results of the tests carried out to compare the performance of the *GIS-SDI* method against the *LINTAB-TSAPWin*TM and the *WinDENDRO*TM methods for identifying, dating and measuring tree rings coincided, indicating the validity of the *GIS-SDI* approach. High correlations and strong agreements were found in tree-ring measurements for the two species studied, regardless of the method analyzed. Although the correlation between all methods for the analysis of cores of *A. pinsapo* was very high, the slight differences between *LINTAB-TSAPWin*TM and *GIS-SDI* for cores of *P. bourgaeana* could be caused by the difficulty of identifying the growth rings in the original cores of this species. In hardwood species, the transition or color change from earlywood to latewood shows less contrast than in conifers, and the superimposed and very narrow rings are

Table 5. Paired T-tests *GIS-SDI* vs. *LINTAB-TSAPWin*TM and *GIS-SDI* vs. *WinDENDRO*TM.

	<i>GIS-SDI</i>					
	<i>A. pinsapo</i>			<i>P. bourgaeana</i>		
	t	df	p	t	df	p
<i>LINTAB-TSAPWin</i> TM	-3.345	239	0.297	5.023	239	0.432
<i>WinDENDRO</i> TM	-1.28	239	0.201	2.47	239	0.314

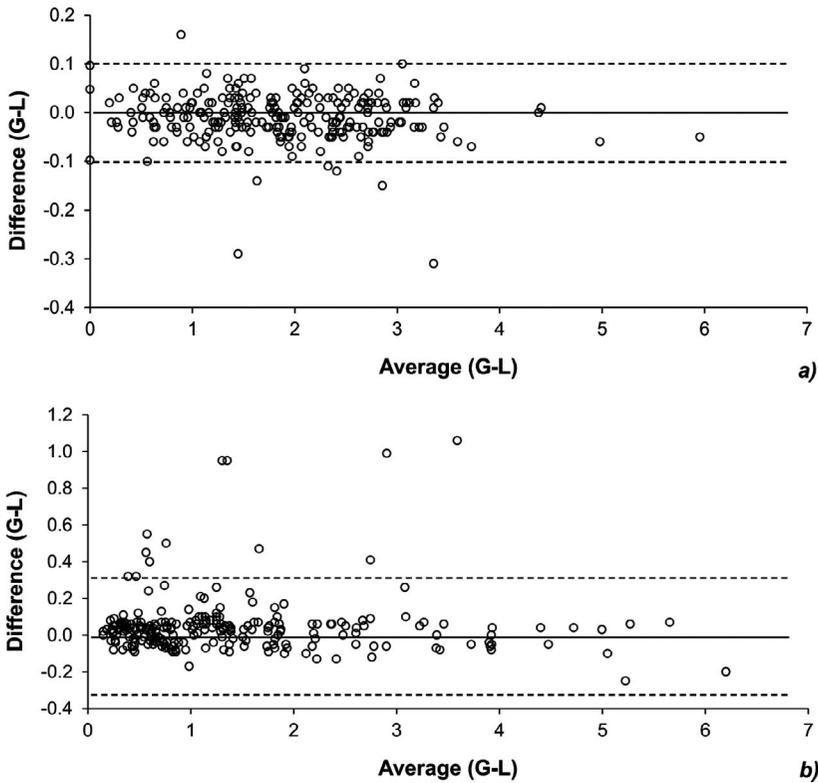


Figure 2. A Bland-Altman Plot to assess agreement between GIS-SDI (G) and LINTAB-TSAPWin™ (L) ring measurements of *A. pinsapo* (a) and *P. bourgaeana* (b).

intensified (Thetford *et al.* 1991). In terms of the COFECHA output, small differences in the accuracy of crossdating across species and methods were found. Measurements on cores of *A. pinsapo* had higher series of intercorrelations than measurements made on cores of *P. bourgaeana*, regardless of the crossdating method. However, the number of flagged segments for measurements made on cores of *P. bourgaeana* was similar regardless of the method used, whereas in the case of *A. pinsapo* there were no flags for any method. Although the short length of series made the dating and measuring process of tree rings easier, these discrepancies could be the result of the difficulty of identifying tree rings in cores of *P. bourgaeana*.

However, digital images of cores scanned in true color and high-resolution (2400 dpi), using an affordable flatbed scanner such as *HP Scanjet G4050* or similar, are a good alternative for measuring annual radial increments, particularly for the study of species in which the identification

of growth rings is problematic. This approach is supported by the results of the comparison between both scanners (*HP Scanjet G4050* and *LA2400*). After analyzing a sample of cores of *P. bourgaeana*, no significant differences were found between scanners. In addition, the identification of the unclear rings of *P. bourgaeana* was easier using the scanned images than using the LINTAB™ platform. Growth rings of *P. bourgaeana* are imperceptible, and its identification using the *Leica S6D* binocular microscope attached to the LINTAB station was a complicated and time-consuming task. In contrast, digital images in true color and high-resolution of the cores greatly facilitated the task. The use of digital images is therefore clearly advantageous when working with species like *P. bourgaeana* that exhibit barely-visible rings (Hietz 2011).

On the other hand, although LINTAB-TSAPWin™ may be the fastest way to measure tree rings on prepared cores directly at the

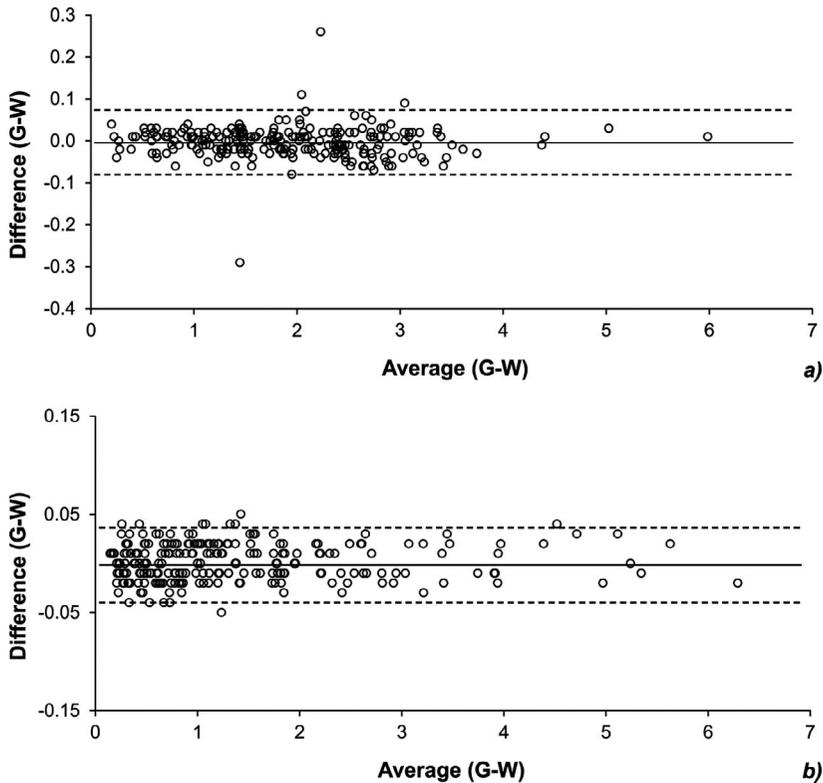


Figure 3. The Bland-Altman Plot of agreement between *GIS-SDI* (G) and *WinDENDRO*[™] (W) measurements for both *A. pinsapo* (a) and *P. bourgaeana* (b).

measurement stage, this method is very time-consuming if rings are liable to be indistinct and data acquisition is difficult to check or repeat, making it necessary to compare and re-measure the original cores (Levanič 2007). Applied to the case of *P. bourgaeana*, the difficulties in identifying the rings of this species became a serious obstacle when analyses of cores were carried out, and could account for some small differences observed between the *LINTAB-TSAPWin*[™] and both the *WinDENDRO*[™] and *GIS-SDI* measurements. These two latter methods, used in conjunction with digital images, seem to provide more consistent measurements. Moreover, the analysis of digital images of cores with *GIS-SDI* produces a complete record of each core, including the high-resolution image (expandable to a convenient size), the point-type layer containing the reference points in the rings used to take ring measurements, plus labels indicating the year of ring formation, the polyline-type layer containing the segments

measuring the annual radial increments and the corresponding associated data table, all of this through the *DIVA-GIS* package. Keeping a collection of digitized cores and the corresponding *GIS* layers and tables has great additional advantages over a conventional collection of tree cores, such as ease of storage, handling and transportation. All these data can be quickly and easily reviewed and may also be easily shared online with other researchers. Therefore, the *GIS-SDI* method greatly increases the potential for collaboration.

CONCLUSIONS

In short, the analysis of true color high-resolution scanned images of tree cores using *GIS-SDI* is an inexpensive but highly efficient alternative approach to tree-ring analysis. This method provides high accuracy in ring measurement. Furthermore, it greatly facilitates ring analysis in species with inconspicuous rings and

allows the maintenance of a complete digital record of every core analyzed, which can be easily shared online.

Supplementary Material is available at <http://www.treeringsociety.org/TRBTRR/TRBTRR.htm>

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REFERENCES CITED

- Arenas-Castro, S., 2012. Análisis de la estructura de una población de Pirúetano (*Pyrus bourgaeana*) basado en técnicas de Teledetección y SIG (Analysis of the structure of a population of wild pear (*Pyrus bourgaeana*, D) based on remote sensing and GIS techniques). Thesis, University of Cordoba, Spain (in Spanish). <http://hdl.handle.net/10396/7832>.
- Aynekulu, E., M. Denich, and B. Neuwirth, 2009. Applicability of GIS in Dendrochronology. Association of American Geographers Annual Meeting, Las Vegas, NV, USA, 22–27 March 2009.
- Bland, J. M., and D. G. Altman, 1986. Statistical method for assessing agreement between two methods of clinical measurement. *The Lancet* 1:307–310.
- Bland, J. M., and D. G. Altman, 1999. Measuring agreement in method comparison studies. *Statistical Methods in Medical Research* 8:135–160.
- Campbell, R., D. McCarroll, I. Robertson, N. J. Loader, H. Grudd, and B. Gunnarson, 2011. Blue intensity in *Pinus sylvestris* tree rings: A manual for a new palaeoclimate proxy. *Tree-Ring Research* 67:127–134.
- Cherubini, P., B. L. Gartner, R. Tognetti, O. U. Braker, W. Schoch, and J. L. Innes, 2003. Identification, measurement and interpretation of tree rings in woody species from Mediterranean climates. *Biological Reviews of the Cambridge Philosophical Society* 78:119–148.
- Cook, E. R., and K. R. Briffa, 1990. A comparison of some tree-ring standardization methods. In *Methods of Dendrochronology: Applications in the Environmental Sciences*, edited by E. R. Cook, and L. A. Kairiukstis, pp. 153–162. Kluwer, Dordrecht.
- Copenheaver, C. A., H. Gartner, I. Schafer, F. P. Vaccari, and P. Cherubini, 2010. Drought-triggered false ring formation in a Mediterranean shrub. *Botany-Botanique* 88:545–555.
- Cruz Johnson, P., P. Honeyman Lucchini, A. Pezo Correa, and C. Schulze del Canto, 2007. Growing analysis of old lenga trees (*Nothofagus pumilio*) in forests at XII Region, Chile. *Bosque* 28:18–24.
- ESRI, Environmental Systems Research Institute, 1998. *ArcView GIS*. Environmental Systems Research Institute, Redlands, CA, USA. <http://www.resources.esri.com>.
- ESRI, Environmental Systems Research Institute, 2008. *ArcGIS 9.3 Resource Center*. Environmental Systems Research Institute, Redlands, CA, USA. <http://www.resources.esri.com>.
- Falster, D. S., D. I. Warton, and I. J. Wright, 2006. *SMATR: Standardized Major Axis Tests and Routines, vers. 2.0*. <http://www.bio.mq.edu.au/ecology/SMATR/>.
- Fritts, H. C., 1976. *Tree Rings and Climate*. Academic Press, London, UK; 567 pp.
- Grissino-Mayer, H. D., R. Holmes, and H. C. Fritts, 1996. *International Tree-Ring Data Bank Program Library, Version 2.0 User's Manual*, University of Arizona, Tucson; 106 pp.
- Grissino-Mayer, H. D., 2001. Evaluating crossdating accuracy: a manual and tutorial for the computer program COFECHA. *Tree-Ring Research* 57:205–221.
- Guay, R., R. Gagnon, and H. Morin, 1990. MacDENDRO, a new automatic and interactive tree-ring measurement system based on image processing. In *Tree Rings and Environment: Proceedings of the International Dendrochronological Symposium* (LUNDQUA Report 34), pp. 128–131. Ystad, Sweden.
- Guay, R., R. Gagnon, and H. Morin, 1992. A new automatic and interactive tree-ring measurement system based on a line scan camera. *Forestry Chronicle* 68:138–141.
- Hietz, P., 2011. A simple program to measure and analyse tree rings using Excel, R and SigmaScan. *Dendrochronologia* 29: 245–250.
- Hijmans, R. J., L. Guarino, C. Bussink, P. Mathur, M. Cruz, I. Barrantes, and E. Rojas, 2004. *DIVA-GIS, v. 4. A geographic information system for the analysis of biodiversity data*. <http://www.diva-gis.org/>.
- Holmes, R., 2000. *Dendrochronology Program Library*. University of Arizona, Tucson.
- ITC (University of Twente, Faculty of Geo-Information Science and Earth Observation), 2005. The Integrated Land and Water Information System (ILWIS) remote sensing and GIS software. <http://www.itc.nl/ilwis/>.
- Knibbe, B., 2008. *PAST4 – Personal Analysis System for Treering Research version 4.5*. Instruction Manual, SCIEM/ Bernhard Knibbe, Vienna; 140 pp.
- Levanič, T., 2007. ATRICS – A new system for image acquisition in Dendrochronology. *Tree-Ring Research* 63: 117–122.
- Maxwell, R. S., J. A. Wixom, and A. E. Hessler, 2011. A comparison of two techniques for measuring and crossdating tree rings. *Dendrochronologia* 29:237–243.

- Mitrakos, K., 1980. A theory for Mediterranean plant-life. *Acta Oecologica/Oecologia Plantarum* 3:245–252.
- Rasband, W., 1997–2009. ImageJ 1.34s. National Institutes of Health USA, <http://rsb.info.nih.gov/ij/>.
- Regent Instruments Canada Inc., 2009. WINDENDRO for Tree-ring Analysis. Quebec, Canada.
- Rinntech®, 2010. LIGNOVISION™. <http://www.rinntech.com>, Heidelberg, Germany.
- Rinntech®, 2010. LINTAB™: Tree-ring station measurement. <http://www.rinntech.com>, Heidelberg, Germany
- Rinntech®, 2010. TSAP-WIN™: Time series analysis and presentation for dendrochronology and related applications. <http://www.rinntech.com>, Heidelberg, Germany.
- Rozendaal, D. M. A., and P. A. Zuidema, 2011. Dendroecology in the tropics: a review. *Trees-Structure and Function* 25:3–16.
- Scarascia-Mugnozza, G., H. Oswald, P. Piussi, and K. Radoglou, 2000. Forests of the Mediterranean region: Gaps in knowledge and research needs. *Forest Ecology and Management* 132:97–109.
- Schneider, C. A., W. S. Rasband, and K. W. Eliceiri, 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* 9:671–675.
- Schweingruber, F. H., 1988. *Tree Rings. Basics and Applications in Dendrochronology*. D. Reidel Publishing Company, Dordrecht, Netherlands; 292 pp.
- Schweingruber, F. H., 1996. *Tree Rings and Environment: Dendroecology*. Berne, Switzerland; 609 pp.
- Soille, P., and L. Misson, 2001. Tree ring area measurements using morphological image analysis. *Canadian Journal of Forest Research* 31:1074–1083.
- Speer, J. H., 2010. *Fundamentals of Tree Ring Research*. University of Arizona Press, Tucson.
- Spiecker, H., M. G. Schinker, J. Hansen, Y.-I. Park, T. Ebding, and W. Döll, 2000. Cell structure in tree rings: Novel methods for preparation and image analysis of large cross sections. *IAWA J* 21:361–373.
- Stokes, M. A., and T. L. Smiley, 1968. *An Introduction to Tree-Ring Dating*. University of Chicago Press, Chicago; 73 pp.
- Swetnam, T., M. Thompson, and E. Sutherland, 1985. *Using Dendrochronology to Measure Radial Growth of Defoliated Trees, Agricultural Handbook No. 639*. U.S. Department of Agriculture, Washington, DC, USA; 39 pp.
- Tegel, W., A. Seim, D. Hakelberg, S. Hoffmann, M. Panev, T. Westphal, and U. Büntgen, 2014. A recent growth increase of European beech (*Fagus sylvatica* L.) at its Mediterranean distribution limit contradicts drought stress. *European Journal of Forest Research* 133:61–71.
- Thetford, R. D., R. D. D'Arrigo, and G. C. Jacoby, 1991. An image-analysis system for determining densitometric and ring-width time-series. *Canadian Journal of Forest Research* 21:1544–1549.
- Theurillat, J. P., and A. Guisan, 2001. Potential impact of climate change on vegetation in the European Alps: A review. *Climatic Change* 50:77–109.
- Touchan, R., E. Xoplaki, G. Funkhouser, J. Luterbacher, M. K. Hughes, N. Erkan, U. Akkemik, and J. Stephan, 2005. Reconstructions of spring/summer precipitation for the Eastern Mediterranean from tree-ring widths and its connection to large-scale atmospheric circulation. *Climate Dynamics* 25:75–98.
- Varem-Sanders, T. M. L., and I. D. Campbell, 1996. *DendroScan: A Tree-Ring Width and Density Measurement System. Special Report*. Canadian Forest Service, The Northern Forestry Centre Book 10, Edmonton, Alberta.
- Vila-Lameiro, P., and I. J. Diaz-Maroto, 2007. Use of digital images in computer-assisted dendrochronological analysis of deciduous hardwoods. *Wood Research* 52:15–31.
- Warton, D. I., I. J. Wright, D. S. Falster, and M. Westoby, 2006. Bivariate line-fitting methods for allometry. *Biological Reviews of the Cambridge Philosophical Society* 81:259–291.
- Worbes, M., 2002. One hundred years of tree-ring research in the tropics – A brief history and an outlook to future challenges. *Dendrochronologia* 20:217–231.
- Zar, J. H., 1999. *Biostatistical Analysis. 4th ed.* Prentice Hall, NJ; 929 pp.

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