OVERCOMING EXTRANEOUS WOOD COLOR VARIATION DURING LOW-MAGNIFICATION REFLECTED-LIGHT IMAGE ANALYSIS OF CONIFER TREE RINGS

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ABSTRACT

The objective of this study was to test ways of overcoming extraneous wood color variation during low-magnification reflected-light image analysis of conifer tree rings and thereby improve the applicability of reflected-light image analysis in dendrochronology. Increment cores from ponderosa pines exhibiting strong heartwood discoloration were examined using image analysis. The research design included three sample preparation treatments (CONTROL, EXTRACT, or BLEACH) crossed with two dendrochronology treatments (STANDARD or RESIDUAL) crossed with two data treatments (SPLIT at the heartwood-sapwood boundary or left at FULL length) to remove the effects of the extraneous color variation. Using a combination of two ring-brightness variables and total ring width, the climate-ring growth model of the EXTRACT-RESIDUAL-FULL was strongest and explained 31.2% of variation in July–October precipitation of southeastern Arizona. Organic extraction (EXTRACT) was helpful in this study but did not fully remove heartwood discoloration. Weak bleaching (BLEACH) removed extraneous color, including heartwood discoloration, but it also removed the ring-brightness signal related to climate. Removing autocorrelation from brightness variables (RESIDUAL) overcame the problem of extraneous color but also possibly removed environmentally relevant information. Keeping brightness series at full length (FULL) worked satisfactorily. Hopefully, future research can successfully isolate some other bleaching, extraction, and/or staining treatment that removes only extraneous color variation from the wood while retaining environmentally relevant color variation so that low-magnification reflected-light image analysis can be widely applicable in dendrochronological studies of conifers.

Keywords: Dendrochronology, low-magnification reflected-light image analysis, wood color, conifer, bleaching.

INTRODUCTION

Low-magnification reflected-light image analysis of conifer tree rings (Sheppard and Graumlich 1996) allows measurement of several ring brightness and width variables (Fig. 1) and thus enhances dendrochronological research beyond the use of only ring width for various paleoenvironmental applications (Sheppard and White 1995; Sheppard et al. 1996). At low magnification (i.e., the ring level), ring brightness is inversely related to ring density because ring brightness relates directly to the lumen : wall ratio of tracheids (Yanosky and Robinove 1986) and the lumen : wall ratio relates inversely to density (Park and Telewski 1993). Ring density has been used extensively in dendrochronological research (Schweingruber 1990), but X-ray densitometry is expensive and can be difficult to do consistently well (Parker and Meleski 1970). Tracheid morphology measurements using image analysis can also mimic true wood density (Park and Telewski 1993; Evans et al. 1996; Munro et al. 1996), but these applications of image analysis employ relatively higher magnification (i.e., the cell level) and therefore can be more time-consuming than measuring whole rings.

A consistent brightness-density relationship across rings within a dendrochronological sample (a radial file of growth rings) is required for successful application of low-magnification reflected-light image analysis in

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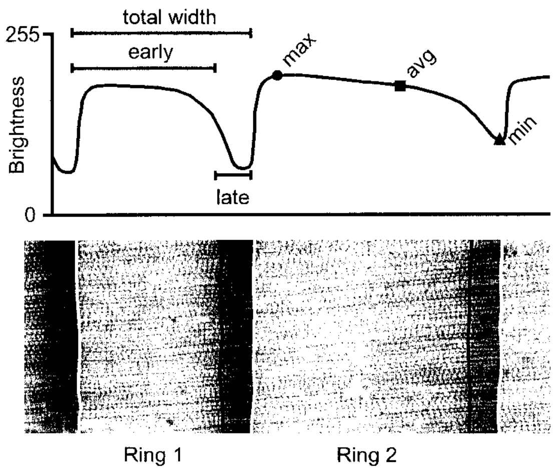

FIG. 1. Two imaged tree rings (bottom) and associated brightness scan (top): On the image, vertical white lines mark ring boundaries and vertical black lines mark earlywood-latewood transitions. On the brightness scan, total ring, earlywood, and latewood widths are marked for Ring 1, and maximum earlywood brightness (circle), average latewood brightness (triangle), and total ring average brightness (square) are marked for Ring 2. Ring 1 has dark latewood with a low minimum latewood brightness while Ring 2 has light latewood with a high minimum latewood brightness. Modified from Sheppard and Graumlich (1996).

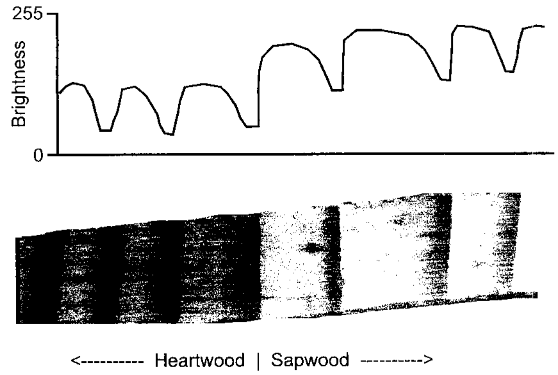

FIG. 2. Heartwood-sapwood rings (bottom) and associated brightness scan (top).

dendrochronological research (Sheppard and Graumlich 1996). Unfortunately, many species exhibit wood color variation that occurs after rings are formed and therefore is extraneous to wood density and to environmental conditions at the time of ring formation. When extraneous color variation occurs in conifer growth rings, the brightness-density relationship is not consistent within a sample and thus a primary requirement for applying low-magnification reflected-light image analysis in dendrochronological research is not met (Telewski and Jacoby 1987; Yanosky et al. 1987). A reflected-light imaging system that detects environmentally relevant ring-brightness variation could certainly detect extraneous color variation (Fig. 2), which would be statistical noise in later analyses.

A notable type of extraneous wood color variation in many conifer species is heartwood discoloration. Extraneous color variation also results from fungal staining (Kreber and Byrne 1994) and compartmentalization of wounds (Shigo 1985). Conifer species that exhibit pronounced heartwood discoloration include pines (*Pinus* L.), which comprise the majority of dendrochronological collections (Grissino-Mayer 1993). Given that ring density of pines has been used for reconstructing past climate (Cleaveland 1986), it is logical to apply image analysis to pine species. Thus, for low-magnification reflected-light image analysis to be widely applicable in dendrochronological research, the problem of extraneous wood color variation must be overcome, either by chemically removing it from wood itself or by mathematically removing its effects from measured data (Yanosky et al. 1987).

The objective of this study was to test ways of overcoming extraneous wood color variation during low-magnification reflected-light image analysis of conifer tree rings and thereby improve the applicability of this technique in dendrochronology. Samples from pines exhibiting strong heartwood discoloration were examined using image analysis. To overcome extraneous color variation, various combinations of chemical preparation, chronology type, and data format strategies were tested. The effectiveness of the treatment combinations was evaluated by comparing climate-ring growth models using ring brightness.

METHODS

Field sampling and crossdating

In October 1992, increment cores were collected from 18 ponderosa pines (*Pinus ponderosa* Dougl. Ex Laws.) growing at 2,350 m elevation on the southwest slope of Mica Mountain, southeastern Arizona (32°12'N, 110°33'W, 2,627 m elevation). Mostly standard field techniques for dendroclimatological research were employed (Schweingruber et al. 1990), except that a total of four cores were collected per tree, i.e., two cores each from two opposing radii, to have enough samples to test chemical preparation treatments for removing extraneous color variation from wood.

The cores were air-dried and sanded according to standard dendrochronological procedures (Phipps 1985; Stokes and Smiley 1968). The rings were crossdated by matching patterns of relatively wide and narrow rings across samples to identify and compensate for possible missing or intra-annual rings (Douglass 1941). With 1992 as the date of the last-formed ring of each sample, the calendar year of formation was assigned to each annual tree ring.

Chemical preparation, chronology, and data treatments

CONTROL, BLEACH, and EXTRACT chemical preparation.—Three chemical preparation treatments were tested for removing extraneous color from wood. One pair of cores from opposing radii of each sampled tree was imaged (Fig. 1; Sheppard and Graumlich 1996) prior to any chemical treatment (henceforth referred to as CONTROL). After CONTROL cores were imaged, they were bleached for 2 h at 70°C in a 0.11-M solution of $NaClO_2$ with glacial acetic acid (Leavitt and Danzer 1993). Bleached cores were then dried and re-imaged (henceforth referred to as BLEACH). The second pair of cores of each tree was extracted for 4 h in a 50:50 ethanol : toluene mixture, followed by 4 h in ethanol and an additional hour in distilled water (Park et al. 1992). Each solvent was alternately vaporized and distilled in a Soxhlet extraction apparatus. Extracted cores were then dried and imaged (henceforth referred to as EXTRACT).

STANDARD and RESIDUAL brightness chronologies.—Series-length trends were removed from brightness series by dividing measurements by fitted values from straight lines estimated using ordinary least-squares regression. This detrending step resulted in dimensionless index series that were averaged together into a standard chronology for each variable (Fritts 1976; henceforth referred to as STANDARD). Similarly, series-length trends were removed from ring-width series by dividing measurements by fitted values from either modified negative-exponential curves or straight lines estimated using iterative least-squares regression (Fritts 1976). Additionally, to eliminate potential effects of extraneous color variation from standard brightness chronologies, autocorrelation was modeled out of each STANDARD brightness index series and the resultant residual index series were averaged together into residual brightness chronologies (Cook 1985; henceforth referred to as RESIDUAL).

SPLIT and FULL brightness data.—Even after autoregressive modeling, it is possible for residual brightness index series to retain artifacts of extraneous color variation. As an example, ring brightness of 30 rings prior to and after the heartwood-sapwood boundary of a representative sample clearly shows the effects of heartwood discoloration (Fig. 3A). If the series is kept at its full length, the series-length trend is positive through time (Fig. 3A), and the standard and residual index series exhibit extraneous trends and/or spikes at the boundary (Fig. 3B). If the brightness series is split into two at the heartwood-sapwood boundary, then the series-length trends are relatively flat through time (Fig. 3A), and the standard and residual index series show no extraneous trends and/or spikes at the boundary (Fig. 3C). Both index series show high variance for heartwood rings versus low variance for sapwood rings, which can be corrected by normalizing each series (Fig. 3D).

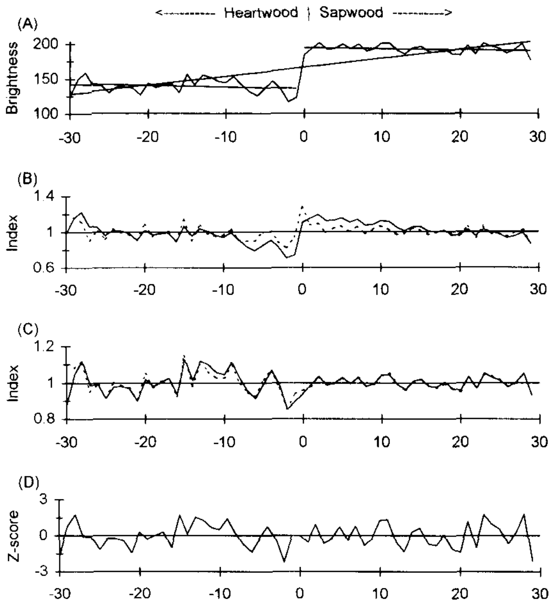

FIG. 3. Effect of heartwood discoloration: (A) Raw earlywood maximum brightness with trends lines, (B) standard (solid line) and residual (dashed line) indices after removing the full-strength trend, (C) standard (solid line) and residual (dashed line) indices after removing split-length trends, and (D) normalized z-scores after removing split-length trends. For all time series, x-axis units are years relative to the heartwood-sapwood boundary, with 30 years measured before and after the boundary.

Accordingly, each ring-brightness series was split into two parts at the observed heartwood-sapwood boundary, and series-length trends were removed. The standard index series were then normalized. The two split-length index series were reattached into one series for every core, and the split-length index series were averaged into chronologies. These series are henceforth referred to as SPLIT to distinguish them from the FULL treatment, which used the full-length time series as normal.

Thus, to test ways of overcoming the effects of extraneous color on ring brightness, this research design had three sample preparation treatments (CONTROL, EXTRACT, or BLEACH) crossed with two chronology treatments (STANDARD or RESIDUAL) crossed with two data treatments (SPLIT or FULL), for a total of 12 treatment combinations (Table 1). However, because of the potential drawback of the FULL data treatment (Fig. 3), this analysis was initially restricted to the six SPLIT treatment combinations. Only the strongest climate-ring growth model from the SPLIT treatment combinations was compared to its conjugate model from the FULL data treatment.

TABLE 1. *Experimental research design to test ways of overcoming extraneous color variation.*

	CONTROL Chemical preparation	BLEACH Chemical preparation	EXTRACT Chemical preparation
STANDARD Chronology	FULL Data	FULL Data	FULL Data
	SPLIT Data	SPLIT Data	SPLIT Data
RESIDUAL Chronology	FULL Data	FULL Data	FULL Data
	SPLIT Data	SPLIT Data	SPLIT Data

Quality control of dating and measurements

After measuring ring width and brightness, all measurement series were checked for dating and/or measuring errors by cross-correlating prewhitened series with their respective mean-value series (Holmes 1983). The dating of the ring-width chronology was verified by cross-correlation with other ponderosa pine ring-width chronologies made from earlier collections at Mica Mountain (Grissino-Mayer and Fritts 1997).

Modeling of climate with ring growth

To model climate with ring growth, weather records extending from 1906 to 1989 of the Historical Climatology Network (Karl et al. 1990) stations of Tucson, Arizona (32°16′N, 111°00′W, 788 m elevation, 40 km west of and 1,839 m lower than Mica Mountain) and Willcox, Arizona (32°18′N, 109°51′W, 1,273 m, 68 km east of and 1,354 m lower than Mica Mountain) were merged. Using best-subset regression (Draper and Smith 1981) with ring width and brightness as candidate predictors, climate-ring growth relationships were