AN ADVANCEMENT IN REMOVING EXTRANEOUS COLOR FROM WOOD FOR LOW-MAGNIFICATION REFLECTED-LIGHT IMAGE ANALYSIS OF CONIFER TREE RINGS

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ABSTRACT

This paper describes the removal of extraneous color from increment cores of conifers prior to reflected-light image analysis of tree rings. Ponderosa pine in central New Mexico was chosen for study. Peroxide bleaching was used as a pretreatment to remove extraneous color and still yield usable wood for image analysis. The cores were bleached in 3% peroxide raised to pH 12 and heated to 60° C, and then they were soaked in 95% ethanol and rinsed in water. The cores were dried slowly to avoid checking or cracking. This treatment removed heartwood color while leaving the wood reasonably sound. Wood reflectance and latewood width were measured using reflected-light image analysis. For dendroclimatic modeling, best-subsets regression was used to determine the strongest predictive model, which was May-September rainfall using latewood reflectance and latewood width. The ability to dendroclimatically model and reconstruct summer precipitation is contingent on having latewood reflectance (density) measurements, and reconstructing summer precipitation in the Southwest will enhance paleoclimatology of the region. Image analysis with reflected white light is thus closer to being more widely applicable in dendrochronology.

Keywords: Wood color, dendrochronology, image analysis, summer precipitation, New Mexico.

INTRODUCTION

This paper describes an advancement for removing extraneous color from increment cores of conifers prior to reflected-light image analysis of tree rings. Image analysis has been used in dendrochronology to obtain proxy data for ring density, especially for the latewood portion of conifer rings (Jagels and Telewski 1990; Sheppard and White 1995; Sheppard et al. 1996). Wood density has played a key role in dendroclimatology (Parker and Henoch 1971; Cleaveland 1986; Briffa et al. 1988; Jacoby et al. 1988), but sample preparation for density analysis can be expensive and/or difficult (Telewski and Jacoby 1987). Image analysis avoids the difficulty and expense of X-ray densitometry of tree rings. The rationale connecting image analysis with density is that high-density latewood appears dark in color whereas low-density latewood appears light in color (Fig. 1). Imaging systems easily detect and quantify this color difference in typical, well-sanded tree-ring specimens.

Unfortunately, extraneous color of wood alters the otherwise straightforward relationship

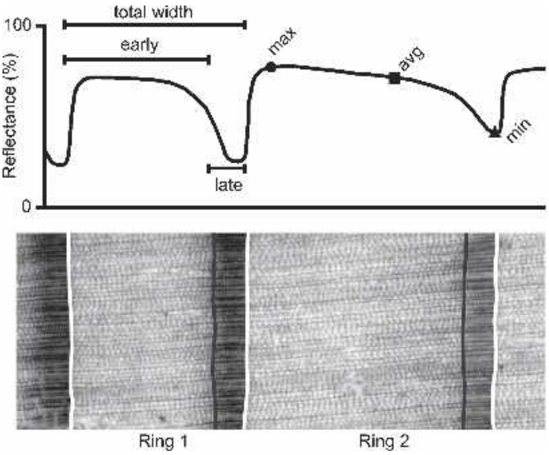

FIG. 1. Two imaged tree rings (bottom) and associated reflectance scan (top). Growth is from left to right. Vertical white lines mark ring boundaries and vertical black lines mark earlywood-latewood transitions. On the reflectance scan, total ring, earlywood, and latewood widths are marked for Ring 1,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

between density and reflectance. Changes in the color of rings, such as during heartwood formation or due to fungal staining (USFS 1983; USFS FPL 1966), are common in pines (*Pinus* spp.) and Douglas-fir (*Pseudotsuga menziesii*), two taxa used extensively in dendrochronology (Grissino-Mayer 1993). Such extraneous color causes non-climatic variation in interannual reflectance of rings. Multi-spectral techniques have been applied to this problem to make extraneous color invisible to the camera (McCarroll et al. 2002), but actually removing extraneous color from tree-ring samples remains to be perfected. Removing extraneous color from wood has been tried using organic solvent extraction, chlorine bleaching, or autoregressive modeling of measurement data, but these techniques have not succeeded fully (Sheppard 1999). Organic solvents do not fully remove heartwood color, and chlorine bleaching renders wood unusable for tree-ring analysis. Autoregressive modeling removes all serial persistence from the data, which is not realistic because environmental factors that limit tree growth typically are autocorrelated to some degree (Wilks 1997).

The primary objective of this research was to improve upon wood pretreatment techniques to fully remove extraneous color while preserving usable samples for image analysis. In particular, peroxide bleaching was tested. Upon removing extraneous color, the secondary objective was to further demonstrate the utility of reflected-light image analysis in dendrochronology by analyzing a tree-ring collection of a conifer species commonly used in dendrochronology to fully exploit its climatic potential. In particular, a tree-ring site was chosen in the American Southwest for modeling summer precipitation, a climatic season that is poorly represented in dendroclimatic research.

METHODS

Study site

The study site was chosen to maximize the likelihood of tree-ring growth showing a statistical relationship with summer rainfall. Geographically, the selected site was in central New Mexico (Fig. 2), which is within the United States part of the North American monsoon (Ad-

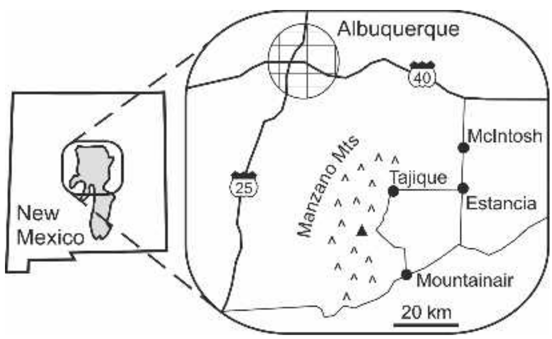

FIG. 2. Map of New Mexico and detail of study area. Filled triangle indicates the study site, and filled circles indicate meteorological stations. The Tajique Canyon tree-ring site reference chronology used to verify crossdating of samples of this current research is located just west of Tajique. Shaded region is New Mexico Climate Division 6 of NOAA.

ams and Comrie 1997). Central New Mexico experiences a summer peak in rainfall, with high monthly totals from June through September, as illustrated with data from NOAA Climate Division 6 (Fig. 3). Interannual variability of rainfall during summer months is low relative to winter months, as indicated by monthly coefficients of variation.

Ponderosa pine (*Pinus ponderosa*) was chosen for study because it is abundant throughout mountainous regions of New Mexico (Burns and Honkala 1990) and is renowned for its sensitivity to precipitation throughout the American Southwest (e.g., Douglass 1920; Grissino-Mayer 1996; Grissino-Mayer et al. 1997; Hildalgo et al. 2001). A suitable stand of trees was found in a flat area at ~2000 masl elevation on the east side of the Manzano Mountains (Fig. 2). The selected stand is homogeneous with respect to tree size, with diameters at breast height ranging from 30 to 40 cm.

Field and laboratory procedures

Field sampling procedures followed typical protocols of dendrochronology (Phipps 1985; Swetnam et al. 1985). For this demonstration study, five trees were sampled, all free of visible injuries. The trees were cored to pith with a 5.1-mm-diameter increment borer. The cores were sanded to expose the transverse surface (Yamaguchi and Brunstein 1991). Samples were visually crossdated by matching patterns of wide and narrow rings across trees (Douglass 1941), and widths of dated rings were measured to ±0.01 mm using a tree-ring increment measuring system (Robinson and Evans 1980). Missing rings were assigned a width of zero. Accuracy of dating and width measurements was checked by cross-correlation analysis (Program COFECHA, Holmes 1983; Grissino-Mayer 2001). Dating accuracy was verified by cross-correlation with a different ponderosa pine chronology from the same area developed independently by others (Grissino-Mayer and Fritts 1997).

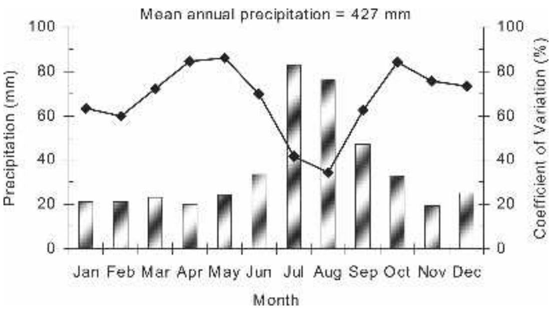

FIG. 3. Monthly precipitation patterns for New Mexico Climate Division 6 for the period 1895–2005. Bars indicate average monthly totals, and filled squares indicate coefficients of variation, that is, standard deviation divided by the mean (Sokal and Rohlf 1981).

Peroxide bleaching pretreatment

Each tree had heartwood color to at least some degree (Fig. 4a). To remove this color and still preserve sound wood, the cores were bleached in 3% peroxide (H_2O_2) raised to pH 12 by adding NaOH and heated to 60° C. Bleaching was done for several hours in a Pyrex pan that was sealed to prevent evaporation. After bleaching, the cores were soaked for two hours in 95% ethanol and then rinsed briefly in water. The cores were air-dried slowly to avoid checking or cracking that can occur when wood is dried quickly (Laanterä et al. 1993). For this, the cores were stored in waxed-paper straws with minimal air circulation. The cores took a few days to dry fully, at which time they were glued into protective mounts and resanded.

Image analysis of tree rings

Wood reflectance and latewood width were measured using reflected-light image analysis. Images of rings were captured using a digital camera (Sony X700) with a zoom microscope (Nikon SMZ-U), and width and reflectance values for each ring were extracted from images (Sheppard and Graumlich 1996). Whenever the zoom magnification of the microscope was changed to accommodate very wide or very narrow rings, the camera shutter and/or gain settings were adjusted to return the system effectively back to its original optical configuration, allowing legitimate comparison of reflectance values for all rings (Sheppard and Singavarapu 2006).

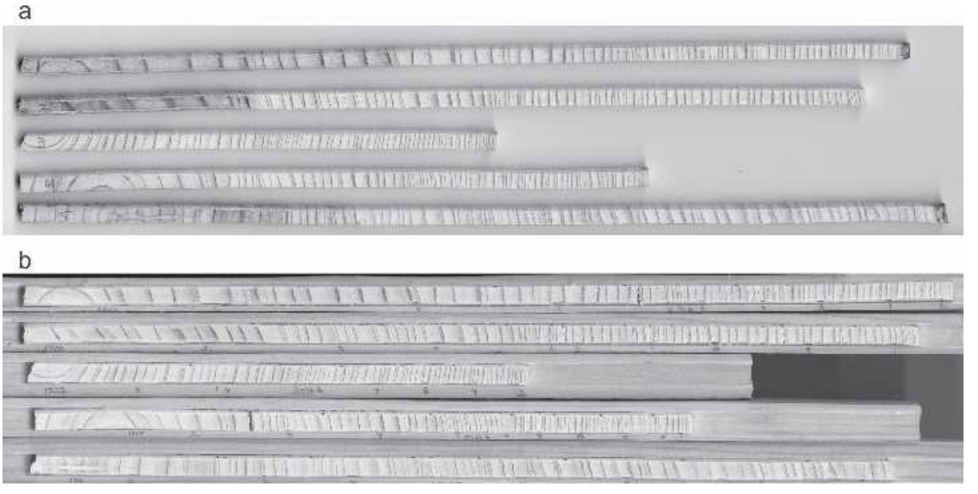

FIG. 4. Ponderosa pine cores (a) prior to peroxide bleaching pretreatment and (b) after bleaching. Direction of tree growth is from left to right.

Quantitative analysis

For each measured ring, latewood width as a percentage of total ring width was calculated. For missing rings, values for latewood percentage and latewood reflectance were set to the average for the entire core, not to zero.

All series were standardized using conservative detrending techniques. For total ring width and latewood width, trend lines were estimated as modified negative exponential curves or straight lines, a common approach used for standardizing ring-width variables (Fritts et al. 1969). Latewood percentage and latewood reflectance do not necessarily trend up or down through time or with tree age, so their trend lines were estimated more flexibly with cubic smoothing splines with a 50% cutoff at 100 years (Cook and Peters 1981). Measurement series for each variable were divided by their fitted trend lines to create dimensionless index series that were then averaged together into a site chronology (Fritts 1976).

Dendroclimatic modeling

Divisional climate data were used for dendroclimatic modeling rather than individual station data because divisional data smooth out spatial variability between stations (Blasing et al. 1981), which can be especially high for summer rainfall (Bradfield 1971). The tree-ring site is located within New Mexico Climate Division 6 (Fig. 2). Because the tree-ring dataset includes multiple chronologies, as opposed to total ring width alone, the bivariate correlation analysis for months and seasons that is often done in dendroclimatology (Blasing et al. 1984) was replaced with best-subsets regressions (Draper and Smith 1981). For each month beginning with July of the previous year and extending to December of the current year, multivariate models between precipitation and the four tree-ring chronologies were evaluated for adjusted R^2 and Mallows Cp. Beyond this preliminary testing at the monthly scale, logical seasons of rainfall were also evaluated. The model with the strongest season based on adjusted R^2 was then verified using the prediction sum of squares statistic (PRESS R^2, Haston and Michaelsen 1984; Michaelsen 1987). Residuals of the chosen model were checked for normality, correlation with predicted values, autocorrelation, and influence (Draper and Smith 1981).

RESULTS

The total ring-width chronology for the Manzano Mountains correlates significantly (r =

+0.70, n = 57, t = 9.5, p < 0.001) with a ponderosa pine chronology from nearby Tajique Canyon (Fig. 2). This confirms the accuracy of the crossdating of the Manzano Mountain tree-ring samples.

Effect of peroxide bleaching treatment

Peroxide bleaching removed heartwood color while leaving the wood reasonably sound. Bleached cores were relatively uniform in color from pith to bark (Fig. 4b), and they could still be sanded to provide a usable transverse surface. Interannual variation in latewood reflectance was still present in the cores after bleaching. Some cracking and checking of the wood occurred during drying, but not to the extent that image analysis was difficult or impossible.

Multiple tree-ring chronologies from one site

All four ring-growth variables have high-frequency signals held in common across trees, as indicated by the average interseries cross-correlation between individual index series and their respective chronologies (Table 1). Total ring width has an especially strong signal, which is typical for mid-elevation pines of the Southwest (Schulman 1956). The latewood width signal is weaker than total ring width but still strong, and latewood percentage is slightly weaker still. Latewood reflectance is weakest of the four variables, but the average interseries correlation of individual index series with their chronology is still moderately strong at +0.54, indicating an acceptable signal.

Average series-length standard deviations are similar for the width variables, but standard deviation is considerably lower for reflectance (Table 1), indicating reduced interannual variability in reflectance. Average first-order autocorrelation is similar for the width and reflectance variables, but it is nonexistent for latewood percentage.

The chronologies of these four variables correlate with one another to varying degrees (Table 2). Latewood percentage and latewood reflectance correlate weakly with each other as well as with the width variable. Total ring width and latewood width correlate strongly with one another, and therefore it would not be appropriate to include both of these width variables as candidate predictors in a regression model. After excluding total ring width, the other three variables can be used as independent predictors in a multivariate regression analysis with climate as the dependent variable (Ostrom 1990).

Dendroclimatic modeling of two independent seasons of precipitation

Without using total ring width as a predictor, no single month of precipitation is modeled very strongly by the other tree-ring variables (Fig. 5). However, various multi-month seasons of summer rainfall are modeled strongly enough to have adjusted R^2 values greater than 20%. The strongest model is May-September rainfall using latewood reflectance and latewood width, with an adjusted R^2 of 25% and a PRESS R^2 of 22%.

TABLE 1. *Tree-ring descriptive statistics for ponderosa pine samples from the Manzano Mountains, New Mexico. In all cases, n = 5 trees.*

Variable	Average interseries correlation of index series with chronology	Average standard deviation of index series	Average first-order autocorrelation of index series
Total ring width	+0.88	0.51	0.44
Latewood width	+0.78	0.57	0.34
Latewood percentage	+0.73	0.50	0.00
Latewood reflectance	+0.54	0.10	0.31

TABLE 2. *Cross-correlations of tree-ring chronologies of ponderosa pine samples from the Manzano Mountains, New Mexico. Number in parentheses is p-value for significance.*

	Latewood width	Latewood percentage	Latewood reflectance
Total ring width	+0.80 (<0.001)	−0.33 (0.002)	−0.14 (0.18)
Latewood width		+0.16 (0.13)	−0.06 (0.57)
Latewood percentage			+0.06 (0.60)

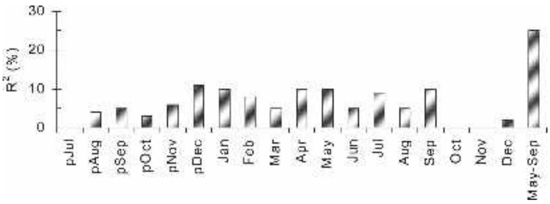

FIG. 5. "Correlagram" for precipitation using latewood width, latewood percentage, and latewood reflectance but not total ring width. Climate data are from New Mexico Climate Division 6. R^2 values show percent shared variation between precipitation and tree-ring data.

This model is significant (F = 15.9, residual error df = 86, $p < 0.001$), with both coefficients being significantly different from zero. After scaling the variance of the predicted series to equal that of the actual series, predicted values match actual values reasonably well (Fig. 6). At the decadal scale, both series show low values during the 1940s–1950s as well as high values during the 1920s–1930s and the 1980s–1990s. Both series record the current drought. Residuals from this model are normal, not autocorrelated, and not correlated with predictors or the predicted values.

By comparison, May-September rainfall is not modeled as well using only total ring width or only latewood ring width. Both of these ring-width models have an adjusted R^2 of only 16%. The ability to dendroclimatically model and reconstruct summer precipitation at this site is contingent on having latewood reflectance (density) measurements.

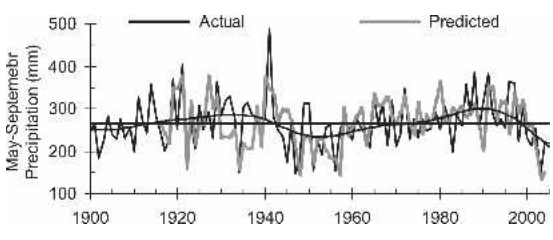

FIG. 6. Time series of actual (dark line) and predicted (gray line) precipitation totals for summer (May-September). Predicted values are modeled using latewood reflectance and latewood width. Smooth line is the cubic smoothing spine with a 50% cutoff at 30 years (Cook and Peters 1981). Straight reference line is series-length average.

DISCUSSION

Wood color

Extractives can accumulate in the lumina of cells or as infiltrants into cell walls (Bowyer et al. 2003). Removing extractives from lumina involves dissolving extractives with a solvent introduced into the specimen, whereas removing extractives from cell walls requires a penetrating solvent. For solid wood, such as an increment core, the rate-limiting step in extraction is diffusion or bulk flow of the solvent through the wood. In standard increment cores, complete infiltration of solvents into cells is unlikely due to blockage by air bubbles trapped within cells. Without displacing air, solvents cannot fully infiltrate the wood. Even with vacuum treatment to displace air and allow full infiltration, solvents in the vicinity of extractives become saturated because bulk flow within the core ceases when full infiltration is achieved. At that point, the rate-limiting step for removing extractives is the liquid diffusion rate of extractives in the solvent.

Removing extractives from cell lumina does not ensure removal of extractives from cell walls, even when solvents are used for long periods. Extractives within cell-wall matrices are often less likely to undergo solvation than are lumen-resident extractives. In general wood chemistry, this problem can be overcome by grinding solid wood into small particles to increase surface area before exposing the particles to warmed organic solvents in a Soxhlet apparatus (Rowell et al. 2005). However, grinding wood is clearly inappropriate for tree-ring analysis. Given that the intent of this study is the efficient image analysis of tree rings, lengthy extraction times and/or mechanical breaking of specimens will not be helpful.

Compared to removing extractives from cores with organic solvents (Sheppard 1999), peroxide was more successful at removing color differences between heartwood and sapwood. Peroxide bleaching represents a conceptually different approach than organic solvents to the problem of extractive color in wood. The perhydroxyl ion produced by the breakdown of peroxide attacks chromophores in extractives, thus reducing or eliminating their effect on wood reflectance and leaving only cell-wall thickness as the source for reflectance. The perhydroxyl ion also attacks carbonyl structures in lignin, some of which are chromophores that affect pulp brightness in paper (Andrews and Singh 1979). Removing color from cell walls would be undesirable, because it would artificially increase reflectance. This was avoided by optimizing bleaching time and pH to control the effect of peroxide on the wood itself.

By altering the structure of lignin, peroxide bleaching not only changes the color and reflectance of wood, but it also reduces the stiffness and general strength of the core. Loss of wood strength is consistent with the use of peroxide bleaching as a partial delignifier. As lignin is broken down, cellulose and hemicelloloses are made more accessible and thus are more likely to be leached out in pretreatment. Hemicelluloses are soluble at a pH of 12, and thus their extraction is likely (Rowell et al. 2005).

It is also likely that the increased permeability during bleaching leads to more efficient bulk flow and fluid exchange within cores. The combination of partial delignification and greater potential leaching of holocellulose can easily lead to highly damaged cores. For specimens with reduced strength and awkward aspect ratios relative to primary directions in wood (such as increment cores, where the long axis of the core is perpendicular to the grain of the wood), controlling the drying rate is critical. Cracks and checks introduced through rapid drying could cause the specimen to fragment and thus reduce the applicability of image analysis.

A solvent dehydration method to control the removal of water from the core (and thus the rate of shrinkage) minimizes the mechanical stresses that build up as wood dries. Wood is hygroscopic and anisotropic and therefore gains and loses moisture in response to environmental conditions, changing shape unevenly as it does so (Bowyer et al. 2003). Moving quickly from the fully hydrated state (fiber saturation, over 25% moisture content), such as during peroxide bleaching, down to the approximately 6% moisture content that is typical for dried increment core specimens, would cause the wood to shrink quickly and thus increase stress within cores. By soaking in ethanol, the cores went from fully saturated with water (with an arbitrary swelling capacity of 1.0) to nearly fully saturated with ethanol (arbitrary swelling capacity of roughly 0.75 relative to water), during which some shrinkage took place during the process of solvent replacement (Mantanis et al. 1994a, b). Following the ethanol soak with a water rinse caused cores to dry more slowly, with water on only the outside of the cores and ethanol within. Surface tension of ethanol is roughly 31% that of water, and its comparatively lower ability to form hydrogen bonds with wood makes ethanol drying a more gradual process. Wrapping cores in waxed-paper straws further controlled this drying process by slowing the rate at which the cores could come to equilibrium moisture content with the environment.

Drying cores with a graded ethanol series would result in even more gradual and controlled shrinkage than an abrupt plunge from aqueous peroxide to 95% ethanol. Though such care was not needed for specimens in this study, for highly degraded, over-bleached cores from a different site, a 30-50-70-95-100% ethanol series was followed by a solvent transition from 100% ethanol to a series of ethanol-toluene mixtures to 100% toluene (arbitrary swelling capacity of 0.18, relative to water, Mantanis et al. 1994a, b). The cores were then allowed to dry to approximately 10% moisture content, and no cracking, fragmentation, or deformation occurred. Though more time-consuming, a stepwise series such as this should maintain the structural integrity of the cores even after harsh chemical treatment, resulting in little or no cracking or checking of the wood.

Summer precipitation of central New Mexico

Dendroclimatic modeling for New Mexico specifically and the Southwest in general has focused primarily on winter or full year precipitation (e.g., D'Arrigo and Jacoby 1991; Grissino-Mayer 1996; Ni et al. 2002). The ability to model and reconstruct summer precipitation in the Southwest would enhance paleoclimatology of the region. Summer monsoonal rains are critical to many ecosystem and socioeconomic functions, including: (1) occurrence, timing, intensity, and extent of wildfires during the summer monsoon (Grissino-Mayer and Swetnam 2000), (2) abundance of warm-season grasses and forbs that rely on summer moisture (Wright and Bailey 1982), (3) phenology of Southwestern tree species (Meko and Baisan 2001), (4) understanding and possibly mitigating effects of flooding associated with strong monsoon rain events (Berg et al. 2000), and (5) success of livestock and agricultural industries that are dependent on reservoirs being replenished during summer months (Jurwitz 1953; Cox 1988, Liverman 1999; Eakin and Conley 2002).

It might be questioned if a dendroclimatic model with an R^2 of 25% is sufficiently strong to merit interest in climatological research. Summer precipitation in the Southwest can be quite heterogeneous spatially (Merideth 2001). For example, four individual meteorological stations within New Mexico Climate Division 6 (Fig. 2) have an average shared variation for May-September rainfall of only 43% (Table 3). Thus, the R^2 of 25% for the dendroclimatic model of May-September rainfall using latewood reflectance and latewood width accounts for about 58% of the variation shared in common by the meteorological stations themselves.

Compared to summer rainfall, dendroclimatic models of winter or full water year rainfall in central New Mexico are better in absolute terms. Winter rainfall over the Southwest comes as large frontal storms originating from the Pacific Ocean (Sheppard et al. 2002), so winter rainfall is homogeneous over New Mexico. The same four meteorological stations of New Mexico Climate Division 6 have an average shared variation for prior September-May rainfall of 75% (Table 3). A dendroclimatic model of prior September-May rainfall using total ring width has an R^2 of 41%, or about 55% of the signal shared in common by the stations themselves. This is approximately the same relative level of statistical performance shown by the dendroclimatic modeling of summer precipitation. In other words, dendroclimatic modeling of summer precipitation is about as strong as the more well-known modeling of winter precipitation when both models are standardized by the amounts of common signal held across different meteorological stations. It would appear that 25% shared variation in a dendroclimatic model is currently about as good as can be expected for summer rainfall in central New Mexico.

CONCLUSIONS

Peroxide bleaching of increment cores followed by slow drying works as a pretreatment for removing extraneous wood color while still leaving usable cores for tree-ring analysis. Bleached cores are uniform in color from pith to bark and are not unduly cracked or checked.

TABLE 3. *Amount of shared variation (R^2, %) in summer and winter rainfall seasons of selected meteorological stations of New Mexico Climate Division 6 (Fig. 2).*

	Summer (May–September)		
	McIntosh 1932–1976	Mountainair 1932–2005	Tajique 1932–1976
Estancia 1937–2005	46	48	61
McIntosh 1932–1976		21	42
Mountainair 1932–2005			41
Average R^2 for summer = 43%			
	Winter (prior September–May)		
	McIntosh 1932–1976	Mountainair 1932–2005	Tajique 1932–1976
Estancia 1937–2005	89	77	68
McIntosh 1932–1976		75	73
Mountainair 1932–2005			69
Average R^2 for winter = 75%			

Extraneous wood color should no longer impede the use of reflected-light image analysis of tree rings.

As an example of reflected-light image analysis of conifers, ponderosa pine of central New Mexico is encouraging. Latewood reflectance and latewood width are useful for reconstructing summer precipitation, a climate feature that is underrepresented in dendroclimatology of the Southwest because it is not reconstructed well using total ring width alone. Summer rainfall is important to many modern-day stakeholders in the Southwest and it undoubtedly affected past societies (Bradfield 1971), so improved understanding of its long-term variability is important. Total ring width is still useful for reconstructing winter or full water year rainfall, so now with reflected-light image analysis of conifer rings, both modes of rainfall of the American Southwest can be reconstructed. Additionally, the ability to measure ring reflectance should enhance dendroclimatology of other settings (e.g., Conkey 1988; Hughes et al. 1994; Rigling et al. 2001) as well as other wood science research (McMillin 1982).

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