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

Paul R. Sheppard

Laboratory of Tree-Ring Research The University of Arizona Tucson, AZ 85721

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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 climatering 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.

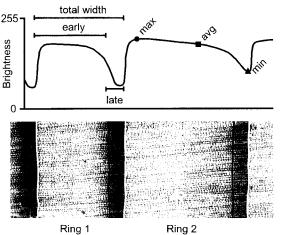
Keywords: Dendrochronology, low-magnification reflected-light image analysis, wood color, conifer, bleaching.

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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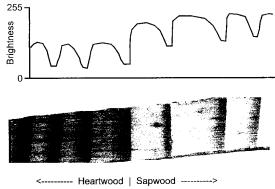


FIG. 2. Heartwood-sapwood rings (bottom) and associated brightness scan (top).

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).

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 lastformed 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 CON-TROL cores were imaged, they were bleached for 2 h at 70°C in a 0.11-M solution of NaClO₂ with glacial acetic acid (Leavitt and Danzer 1993). Bleached cores were then dried and reimaged (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 leastsquares 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).

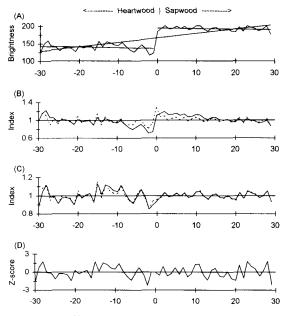


FIG. 3. Effect of heartwood discoloration: (A) Raw earlywood maximum brightness with trends lines, (B) standard (solid line) and residual (dashed line) indices after removing the full-strength trend, (C) standard (solid line) and residual (dashed line) indices after removing split-length trends, and (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 splitlength 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

 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 SPLIT Data	FULL Data SPLIT Data	FULL Data SPLIT Data
RESIDUAL Chronology	FULL Data SPLIT Data	FULL Data SPLIT Data	FULL Data SPLIT Data

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.

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

		STANDARD chronology	/ treatment		
		Regression coefficients ^a			
	Ring brightness			-	
Chemical treatment	Earlywood maximum	Latewood minimum	Total ring width	R^2 adj ^b (%)	R ² pred ^c (%)
CONTROL	-0.001	+0.001	+0.001	22.6	17.3
EXTRACT	-0.001	+0.001	+0.001	22.9	16.5
BLEACH	-0.104	+0.003	+0.002	11.7	4.2
		RESIDUAL chronology	treatment		
		Regression coefficients ^a		_	
	Ring brightness				
Chemical treatment	Earlywood minimum	Latewood maximum	Total ring width	R^2 adj ^b (%)	R ² pred ^c (%)
CONTROL	-0.001	+0.005	+0.001	26.1	21.9
EXTRACT	-0.001	+0.007	+0.001	26.6	21.8
BLEACH	-0.021	+0.022	+0.004	12.0	5.5

 TABLE 2.
 Climate-ring growth models using SPLIT data treatment: Climate variable is July–October precipitation from 1906–1989, with 1983 deleted as an extreme outlier, averaged for Tucson and Willcox.

^a Sign is direction of slope of coefficient and number is its significance level.

^b Coefficient of determination, adjusted for loss of degrees of freedom (Draper and Smith 1981) ^c Prediction coefficient of determination (Michaelsen 1987).

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checked for all monthly and various seasonal temperature averages and precipitation totals. Ring-growth variables that were not highly cross-correlated as well as their 1-year forward and backward lagged series were included in this best-subset regression modeling.

Best-subset climate-ring growth models were identified for all treatment combinations with optimum tradeoff between explained variance and number of included variables using Mallows Cp statistic (Draper and Smith 1981). Climate-ring growth models were evaluated by comparing adjusted R^2 values (indicating percent variance shared by the tree-ring data and climate, Draper and Smith 1981), prediction R^2 values (indicating the true reconstruction skill of a model, Michaelsen 1987; Haston and Michaelsen 1994), and the significance of predictor coefficients. Residuals of all models were checked for normality, correlation with predicted values, autocorrelation, and influence (Draper and Smith 1981).

RESULTS

For the six SPLIT data sets, the strongest climate-ring growth model associated July– October precipitation with earlywood maximum and latewood minimum brightness and total ring width (Table 2). The 1983 observation was deleted as an outlier due to an extreme storm over southern Arizona during October. In all treatment combinations, earlywood maximum brightness had a negative coefficient, while latewood minimum brightness and total ring width had positive coefficients. BLEACH models had very low adjusted R^2 and prediction R^2 values and thus were drastically weaker than CONTROL or EXTRACT. RESIDUAL models were stronger than STANDARD, and of the RESIDUAL models, EXTRACT was slightly stronger than CON-TROL. Thus, of the six SPLIT data sets in this research design, the EXTRACT-RESIDUAL treatment combination had the strongest climate-ring growth model.

Because the coefficients for earlywood maximum brightness were negative while those for latewood minimum brightness were positive (Table 2), a new brightness variable called "difference" was derived by subtracting latewood brightness from earlywood brightness. For the EXTRACT-RESIDUAL-SPLIT treatment combination, best-subset regression was redone using brightness differ-

TABLE 3. Climate-ring growth models using difference and total ring average brightness: Brightness chronologies are from the EXTRACT-RESIDUAL data set. Climate variable is July–October precipitation from 1906–1989, with 1983 deleted as an extreme outlier, averaged for Tucson and Willcox.

	Regression coefficients ^a			·· ···································	
- Data treatment	Ring brightness			-	
	Earlywood-latewood difference	Total ring average	Total ring width	R^2 adj ^b (%)	R^2 pred ^c (%)
SPLIT	-0.001	-0.067	+0.001	29.9	25.4
FULL	-0.001	-0.041	+0.001	31.2	27.4

^a Sign is direction of slope of coefficient and number is its significance level.

^b Coefficient of determination, adjusted for loss of degrees of freedom (Draper and Smith 1981). ^c Predicition coefficient of determination (Michaelsen 1987).

ence in place of earlywood maximum and latewood minimum brightness. The strongest climate-ring growth model associated July– October precipitation with difference, total ring brightness, and total ring width (Table 3), and this model was slightly stronger than that using earlywood maximum and latewood minimum brightness with total ring width (Table 2). The coefficients for difference and for total ring brightness were negative.

Using brightness difference, total ring brightness, and total ring width from the EX-TRACT-RESIDUAL data set, the FULL model was slightly stronger than the SPLIT model (Table 3). The EXTRACT-RESIDUAL-FULL model explained 31.2% of variation in July– October precipitation, and its prediction R^2 was only slightly lower than its adjusted R^2 . This model matched actual values particularly well for 1910–1930 and 1950–1970 (Fig. 4). The high-frequency match was relatively poor

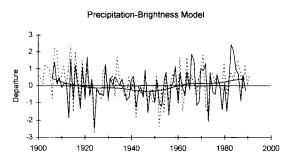


FIG. 4. Mica Mountain climate-ring growth model: Actual with cubic smoothing spline (both solid lines) and predicted (dashed line) July–October precipitation departures, relative to the mean of the entire time period, using EXTRACT-RESIDUAL-FULL treatment combination.

for 1930–1950 and the 1980s, but the model correctly matched the below average mean and variance of actual July–October precipitation for 1930–1950.

DISCUSSION

Overcoming extraneous color

Chemical treatment.—Chemical treatments that remove extraneous color variation must not affect the dendroclimatologically relevant color variation of earlywood and latewood brightness, which is due primarily to differences in the light scattering power of the wood itself (Wilcox 1975). When done too much, bleaching can reduce wood to cellulose by removing most mobile components as well as tannins, which darken the color of wood tissue (Chattaway 1952), and lignin (Leavitt and Danzer 1993). In this study, BLEACH removed extraneous color, including heartwood discoloration, but it also removed the ringbrightness signal that relates to July-October precipitation (Table 2) in spite of the fact that the cores were bleached for only 2 h in a weak bleaching solution. Thus, BLEACH did not overcome the problem of extraneous color variation, and for bleaching to be effective, some other bleaching agent and/or treatment will be necessary. Perhaps uniform staining of bleached wood can recover environmentally sensitive ring-brightness signals (Jagels and Telewski 1990).

BLEACH had additional adverse side effects. Its effectiveness varied across samples (Leavitt and Danzer 1993), and it would be

difficult to know *a priori* what the correct bleaching treatment should be for any particular sample. Its effectiveness also varied within each core, both between heartwood and sapwood rings and between the surface and interior of the core. BLEACH cores were brittle and contained numerous hairline fractures, making them difficult to handle and measure using low-magnification reflected-light image analysis.

Many extractable wood materials, e.g., polyphenolic compounds, darken the color of wood (Hillis 1968, 1971), but in this study EXTRACT did not greatly enhance the brightness signal that relates to July-October precipitation (Table 2). Organic extraction primarily removes resins from wood (Mutton 1962), but resins do not cause severe extraneous color variation such as heartwood discoloration. Given that EXTRACT climate-ring growth models were only slightly stronger than CONTROL, the EXTRACT treatment did not overcome the problem of extraneous color variation in this study, probably because the Mica Mountain ponderosa pine cores were not particularly resinous. In dendrochronological research of species that are more resinous, e.g., bristlecone pine (Pinus longeava D.K. Bailey) and foxtail pine (Pinus balfouriana Grev. and Balf.), organic extraction to remove resins might more substantially enhance the ring-brightness signal for low-magnification reflected-light image analysis.

Chronology treatment.—Removing autocorrelation from ring-brightness variables overcame the problem of extraneous color variation in that climate-ring growth models using RESIDUAL brightness chronologies were all stronger than those using STANDARD chronologies (Table 2). However, it is mathematically difficult for autoregressive modeling to differentiate autocorrelation due to extraneous color variation from that due to other factors. Consequently, an adverse side effect of autoregressive modeling is the possibility of losing environmentally relevant information. Low-frequency variation of ring density has been associated with climate or other environmental factors in past research (Briffa et al. 1992; Stahle et al. 1992), so losing that information by autoregressive modeling could limit the interpretability of ring-brightness data.

Data length treatment.—Splitting brightness series at the heartwood-sapwood boundary prior to analysis theoretically overcame the problem of extraneous color variation (Fig. 3). This data treatment uses the same rationale used for splitting ring-width series into segments of widely different mean values for trees exhibiting abrupt changes in ring width (Blasing et al. 1983). A drawback of splitting series is that it adds yet more steps to a dataanalysis process that is already complex.

While it is potentially risky not to split brightness series when analyzing samples with strong heartwood discoloration, keeping brightness series at full length worked satisfactorily in this study (Table 3). It is unlikely that heartwood-sapwood boundaries would be synchronous across trees of a noncommercial forest. If extraneous color variation is not synchronous across trees, then averaging index series of a well-replicated tree-ring collection should eliminate the effects of that variation from final chronologies. This would be especially true when using a robust averaging process (Cook 1985), which excludes far-outlying values from the calculation.

Climate-ring growth relationship using ring brightness

This study confirmed results relating density or tracheid morphology of conifers to moisture availability for semiarid Southwest tree-ring sites generally (Cleaveland 1986) and for Mica Mountain specifically (Park 1990). Given that the growing season for ponderosa pine of southern Arizona begins by May and extends to at least late September (Baisan and Swetnam 1994), the inverse relationship of earlywood brightness to July–October precipitation (Table 2) is physiologically reasonable. By July, earlywood tracheids probably are fully expanded but still thickening their cell walls. Above average July–October soil mois-

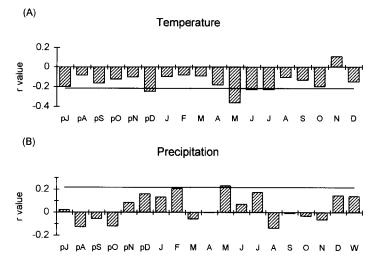


FIG. 5. Mica mountain climate-total ring width correlations: Product moment r values for total ring width of Mica Mountain ponderosa pine with (A) temperature and (B) precipitation recorded at Tucson, Arizona. X-axes are months from prior-year July to current-year December; the W for precipitation indicates winter (October–April). For all correlations, the time period was 1906–1989 without 1983, and values exceeding ± 0.217 are significant at the 0.05 level.

ture should lead to above average cell-wall thickening of earlywood cells, which would make earlywood denser and therefore darker. Conversely, below average July–October soil moisture should lead to below average cellwall thickening of earlywood cells, which would make earlywood less dense and brighter.

The positive relationship of latewood brightness to July–October precipitation (Table 2) is also physiologically reasonable. Latewood tracheids probably are not fully expanded even as late as September. Above average July–October soil moisture should lead to above average expansion of latewood cells, which would make latewood less dense and therefore brighter. Conversely, below average July–October soil moisture should lead to below average expansion of latewood cells, which would make latewood denser and darker.

By extension of that logic, above average July–October precipitation should reduce the difference between earlywood and latewood brightness, which was evident in this study (Table 3). It is difficult to confidently infer wide applicability of the difference in brightness to dendrochronology as there is little prior dendrochronological research in which climate has been modeled using the difference between earlywood and latewood density or brightness. An advantage of analyzing the difference between earlywood and latewood brightness is that it combines information of those two variables into one and allows for other brightness and/or width variables to enter into multivariate climate-ring growth models and thereby improve their predictive ability. The opportunity to derive new variables and enhance the complexity of analyses is, in general, a positive side effect of applying image analysis in dendrochronology (Jagels and Telewski 1990).

Lastly, without brightness variables, climate-ring growth modeling in this study using ponderosa pine of Mica Mountain was relatively weak. For example, ring width by itself did not correlate to any monthly or seasonal climate variable strongly enough to warrant a dendrochronological reconstruction for paleoclimatological analysis (Fig. 5). Thus, for some dendroclimatological studies it would be useful, if not imperative, to have brightness, tracheid morphology, or true density measurements.

CONCLUSIONS

One solution to overcoming extraneous wood color is to analytically remove its effects from measured brightness data using autoregressive modeling, but such an approach may also remove other low-frequency variation that might be environmentally relevant. Another approach is to chemically remove the extraneous color variation from the actual wood, but this also has difficulties. Extraction treatments would work only if extractable compounds were the primary cause of wood discoloration. Bleaching treatments would work only if they removed most of the extraneous wood color variation and preserved the ringbrightness variation that relates to climate. Hopefully future cross-disciplinary research on this problem, involving dendrochronologists and wood chemists, will isolate some combination of bleaching, extraction, and/or staining that removes only extraneous color variation while retaining environmentally relevant color variation in the wood.

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