

**ANALYSIS OF THREE MICROSCOPIC CHARACTERS FOR
SEPARATING THE WOOD OF *PINUS CONTORTA* AND *P. PONDEROSA***

by

Alex C. Wiedenhoeff¹, Regis B. Miller¹ & Terra J. Theim²

SUMMARY

Three microscopic characters were evaluated for the identification of *Pinus contorta* and *Pinus ponderosa*. The tangential diameter of the resin canals, including the epithelium, was compared to the tangential diameter of the entire resin canal complex. The latter measurement was shown to give diagnostic results for these species. Data from the examination of ray composition do not support previously published methods for separating *P. contorta* and *P. ponderosa*. The presence or absence of small elongate crystals in the subsidiary parenchyma of the resin canal complexes was shown to be the most powerful diagnostic character for separating the wood of these species.

Key words: Resin canal, wood anatomy, wood identification, *Pinus contorta*, *Pinus ponderosa*.

INTRODUCTION

In the United States, lodgepole pine (*Pinus contorta* Douglas ex Loudon and *P. contorta* Douglas ex Loudon var. *latifolia* Engelm.) and ponderosa pine (*P. ponderosa* Douglas ex C. Lawson and *P. ponderosa* Douglas ex C. Lawson var. *scopulorum* Engelm.) are the two most commercially important species of the western yellow pines, particularly for the wood preservation industry. Other western species of yellow pines are limited in range, have a poor form, and are generally not cut and marketed for lumber. Current harvest practices tend to mix lodgepole and ponderosa pine in the field or in the mill. However, the ability to separate these woods at the species level is important because their properties and treatability differ. The identification or separation of *P. contorta* and *P. ponderosa* is reported in several textbooks, but the quality and accuracy of the methodology have not been confirmed. Our objective is to establish an accurate and reliable means of separating these species.

Characteristics used in the past to separate lumber of *P. contorta* and *P. ponderosa* relied heavily on the diameter of the material (large versus small) (Hoadley 1990). The

1) USDA Forest Service, Forest Products Laboratory, Madison, WI 53726-2398, U. S. A.*

2) Department of Botany, University of Wisconsin, Madison, WI 53706, U. S. A.

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presence or absence of dimples, most obvious on the split tangential surfaces, was the most common character (Record 1919, 1934; Brown & Panshin 1934, 1940; Phillips 1948; Brown et al. 1949; Kukachka 1960; Panshin & DeZeeuw 1964, 1970, 1980; Core et al. 1979; Hoadley 1990). Kukachka (1960) stated that dimples are smaller and more abundant in lodgepole pine compared to ponderosa pine, whereas the others suggest that dimples are more pronounced or conspicuous in lodgepole pine than in ponderosa pine. Phillips (1948) reported that dimples are infrequent in ponderosa pine.

The present investigation focuses on three microscopic characters to separate the wood of *P. contorta* and *P. ponderosa*. The first and second characters are evaluations of methods published by Panshin and DeZeeuw (1980); the third is a novel character for these species. First, axial resin canals were measured by two methods, and the data were compared to resin canal measurements by Panshin and DeZeeuw. Next, ray composition was assessed in the context of the average number of contiguous rows of ray parenchyma cells in the axial direction (Panshin & DeZeeuw 1980). Finally, resin canal complexes were examined for the presence or absence of small elongate crystals (as in Kellogg et al. 1982; Baas et al. 1986).

MATERIALS AND METHODS

The samples of *Pinus contorta* and *P. ponderosa* from which we obtained data are listed in Table 1. Slides from our slide collection were used when available. All other samples were taken from the MADw or SJRW wood collections; if necessary, the samples were sectioned, stained, and mounted using standard microtechnique protocols. Statistical analyses were performed using an Excel 2000 spreadsheet.

Axial resin canal complexes

Resin canals were measured using a projecting microscope, digitizing tablet, and accompanying computer software for electronic data capture (Quirk 1981). Terminology in Wiedenhoef and Miller (2002) was followed for resin canal complexes. The distinction between the axial resin canal complex and the resin canal itself was paramount to our methods. We measured the tangential diameter of axial resin canals inclusive of the epithelium (Fig. 1A, B) and the tangential diameter of the entire axial resin canal complex (Fig. 1C, D). In this paper we refer to the former method as the epithelial method (EM) and the latter as the resin canal complex method (RCCM).

For RCCM, all axial resin canal complexes on a given transverse section were measured. For EM, at least 20 canals were measured per sample; not all canals were measurable due to tear-out of the epithelium and subsidiary cells of the resin canal complex (see Wiedenhoef & Miller 2002). The minimum, mean, and maximum measurements and standard deviation for the mean were calculated for each specimen. For each species, the means from individual specimens were averaged to give average mean diameter values. Analyses were performed in this fashion to lend equal weight to each sample rather than biasing for a sample with more axial resin canal complexes. The use of an arbitrary cut-off (e.g., measurement of 25 canals) was also avoided so that each sample was as well represented as possible in the generation of its individual mean value.

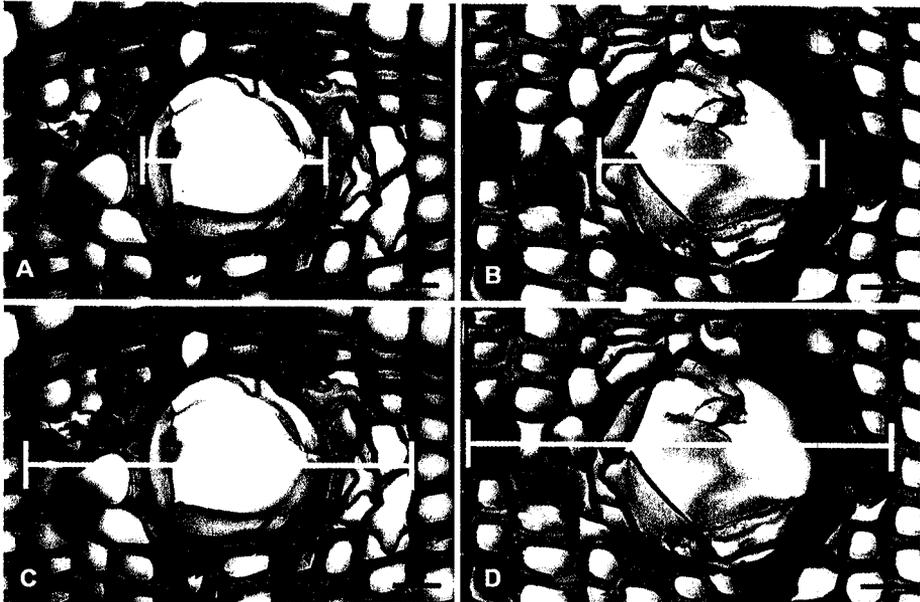


Fig. 1. Transverse sections of *Pinus contorta* (A, C) and *Pinus ponderosa* (B, D). – A & B: epithelial method (EM). – C & D: resin canal complex method (RCCM). – Scale bars = 25 μ m.

In many cases, RCCM and EM data were not collected for each sample. The EM data were collected several years earlier in the initial phases of this project, and those data are included for comparison with the RCCM data. We sampled broadly for the remainder of the study to reduce any impact of local similarities among neighboring stands of trees.

Ray composition

Ray composition was assessed by collecting data from all unique rays on a radial section observed with a light microscope. No method was suggested in the original work citing this method (Panshin & DeZeeuw 1980). Consequently, the number of contiguous upper marginal ray tracheids was tallied, then the ray parenchyma cells, and finally the lower marginal ray tracheids (Fig. 2A). A feature noted in the collection of these data was the presence of interspersed ray tracheids in the body of some rays (Fig. 2B). Because rays with these ray tracheids seemed likely to have fewer contiguous ray parenchyma cells, the number of rays with interspersed ray tracheids was counted. Fusiform rays were excluded from this analysis, as were any rays that appeared to terminate with ray parenchyma cells at the margins; such rays were assumed to be out of plane.

Crystals

The presence or absence of crystals in the axial resin canal complexes was determined by polarized light microscopy of each plane of section, but longitudinal sections

Table 1. Characteristics of *Pinus contorta* and *P. ponderosa* as measured by resin canal complex method (RCCM) and epithelial method (EM).

Species and accession no.	Collector and location	Crys- tals ^a	Canals (no.)	RCCM		EM		Ray comp ^b
				Mean diam. (μm)	Std dev. (μm)	Mean diam. (μm)	Std dev. (μm)	
<i>Pinus contorta</i>								
MADw 122	Maddox & Bishop: Canada	p	24	168	26	–	–	–
MADw 125	Wilson: CA	p	36	135	38	–	–	–
MADw 816	Knapp: B.C.	p	106	166	36	–	–	–
MADw 6018	Montana	p	171	173	43	–	–	–
MADw 6020	Montana	p	86	141	32	–	–	–
MADw 6022	Montana	p	36	136	26	–	–	–
MADw 6023	Montana	p	87	123	23	–	–	–
MADw 6024	Montana	p	155	167	45	–	–	–
MADw 6211	Koehler: CO	p	36	158	32	–	–	–
MADw 6310	Koehler: CA	p	26	145	25	–	–	–
MADw 6312	Koehler: CA	p	111	173	37	–	–	–
MADw 8887	Becraft & Jahn: ID	p	72	179	37	–	–	–
MADw 8923 ^c	Fritz: CA	p	84	142	31	–	–	–
MADw 9191	Cockrell: CA	p	129	143	36	–	–	M
MADw 9200	Preston: CO	p	143	173	40	–	–	–
MADw 15575	Wyoming	p	134	154	33	–	–	–
MADw 15576	Wyoming	p	74	110	22	–	–	–
MADw 15578	Wyoming	p	41	144	38	–	–	–
MADw 17981	Scotland	p	86	170	36	–	–	–
MADw 44007	South Dakota	p	33	165	33	88	17	–
MADw 44008	South Dakota	p	32	133	23	81	13	–
MADw 44009	South Dakota	p	41	148	28	67	17	–
MADw 44010	South Dakota	p	50	147	30	79	15	–
MADw 44011	South Dakota	p	30	161	38	75	10	–
MADw 44014	South Dakota	p	27	132	29	76	14	M
MADw 44015	South Dakota	p	65	120	31	99	17	M
MADw 44016	South Dakota	p	42	128	30	89	13	M
MADw 44017	South Dakota	p	44	114	33	96	14	M
MADw 44018	South Dakota	p	46	162	28	98	15	M
SJRw 11355	United States	p	98	145	31	–	–	–
SJRw 26890	Standley: OR	p	86	177	52	–	–	–
SJRw 40039	Cockrell: U.S.	p	41	155	28	–	–	M
SJRw 40040	Cockrell: U.S.	p	77	134	34	90	15	M
SJRw 40496	Proctor: ID	p	39	160	48	–	–	–
SJRw 47014 ^c	Fawcett & Carl- son: ID	p	89	162	44	–	–	–
SJRw 47784	West: U.S.	p	55	132	29	–	–	–
SJRw 49172	Knapp: B.C.	p	82	156	31	–	–	–
SJRw 49173 ^c	Becraft & Jahn: ID	p	65	163	35	–	–	–
SJRw 49174 ^c	Fritz: CA	p	40	161	33	–	–	–
SJRw 49175 ^c	Cockrell: CA	p	95	140	30	–	–	–
SJRw 49176 ^c	Preston: CO	p	51	140	30	–	–	–

continued →

Table 1 continued

Species and accession no.	Collector and location	Crystals ^a	Canals (no.)	RCCM		EM		Ray comp ^b
				Mean diam. (μm)	Std dev. (μm)	Mean diam. (μm)	Std dev. (μm)	
<i>Pinus ponderosa</i>								
MADw 25	Sudworth: CA	a	46	290	99	–	–	–
MADw 42	Sudworth OR	a	132	212	44	–	–	–
MADw 43	Phillips: NM	a	54	179	35	–	–	–
MADw 48	Sudworth: MT	a	91	208	41	–	–	–
MADw 50	Forester: AZ	a	79	241	64	–	–	–
MADw 51	California	a	25	299	73	–	–	–
MADw 57	Gaskill: ID	a	80	249	65	–	–	–
MADw 847	Marckworth: WA, US	a	35	219	52	–	–	–
MADw 880	Cockrell: CA	a	60	239	68	–	–	–
MADw 6329	Koehler: MT	a	59	217	43	–	–	–
MADw 8817	Osborn: AZ	a	102	213	43	–	–	–
MADw 9021	Preston: CO	a	63	204	51	–	–	–
MADw 9450	Fritz: CA	a	54	211	45	–	–	–
MADw 17386	South Dakota	a	55	209	57	–	–	M
MADw 44001	United States	a	33	181	38	–	–	–
MADw 44002	United States	a	28	239	56	89	14	M
MADw 44003	United States	a	36	154	38	111	14	M
MADw 44004	United States	a	31	161	34	98	17	M
MADw 44005	United States	a	31	210	51	98	16	M
MADw 44006	United States	a	59	135	24	77	14	M
MADw 44012	United States	a	34	198	28	102	19	M
MADw 44020	United States	a	44	227	52	112	80	M
MADw 44021	United States	a	42	124	24	91	39	M
MADw 44022	United States	a	38	117	23	109	15	M
MADw 44025	United States	a	35	211	54	92	16	M
MADw 44026	United States	a	65	212	50	128	22	M
MADw 44027	United States	a	58	182	40	100	15	M
MADw 44028	United States	a	45	177	28	93	13	M
MADw 44029	United States	a	53	171	40	107	18	M
SJRw 11389	United States	a	29	141	29	–	–	M
SJRw 45933 ^d	South Dakota	a	87	187	51	–	–	M
SJRw 47026	Faucett & Peader: ID	a	42	197	47	–	–	–
SJRw 47038	Osborn: AZ	a	92	192	45	–	–	–
SJRw 49198	Becraft & Jahn: ID	a	96	212	52	–	–	–
SJRw 49199	Fritz: CA	a	35	254	60	–	–	–
SJRw 49200	Little & Wadsworth: AZ	a	70	238	62	–	–	–
SJRw 49201	Cockrell: CA	a	62	195	63	–	–	–
SJRw 49202	Cockrell: CA	a	41	230	43	–	–	–
SJRw 49203	Marckworth: WA	a	90	213	66	–	–	–
SJRw 49204	Cockrell: CA	a	47	236	61	–	–	–
SJRw 49205 ^d	Preston: CO	a	99	208	50	–	–	–
SJRw 52842	South Dakota	a	56	258	59	–	–	–

^a p = crystals present; a = crystals absent.
^b – = not measured; M = measured.
^c *Pinus contorta* var. *latifolia*
^d *Pinus ponderosa* var. *scopulorum*

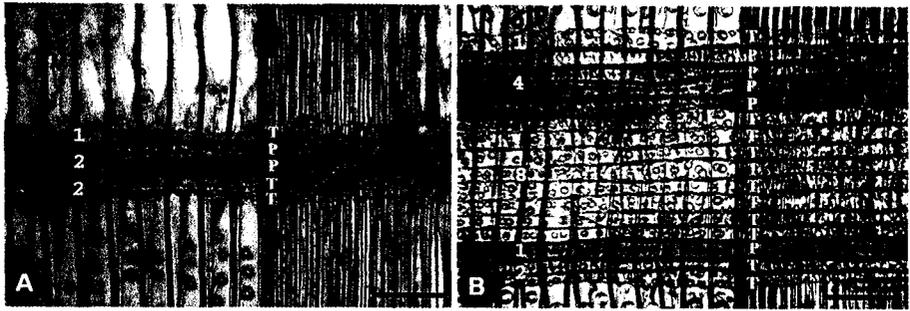


Fig. 2. Radial sections of *Pinus contorta* (MADw 6019). – A: Normal ray with only marginal tracheids (T) and parenchyma cells (P). – B: ray with both marginal and interspersed tracheids (T) and parenchyma cells (P). Numerals indicate the number of contiguous rows of each cell type. – Scale bars = 100 μm .

provided better viewing (Kellogg et al. 1982). Crystals were considered present if they were observed in more than one cell and were visible under polarized light (Fig. 3). Determination of the types of cells in which the crystals were found was made by careful examination of median longitudinal sections of resin canal complexes. For general data collection and routine observation, however, no effort was made to determine the precise cells in which the crystals occurred. When slides had few or no resin canals

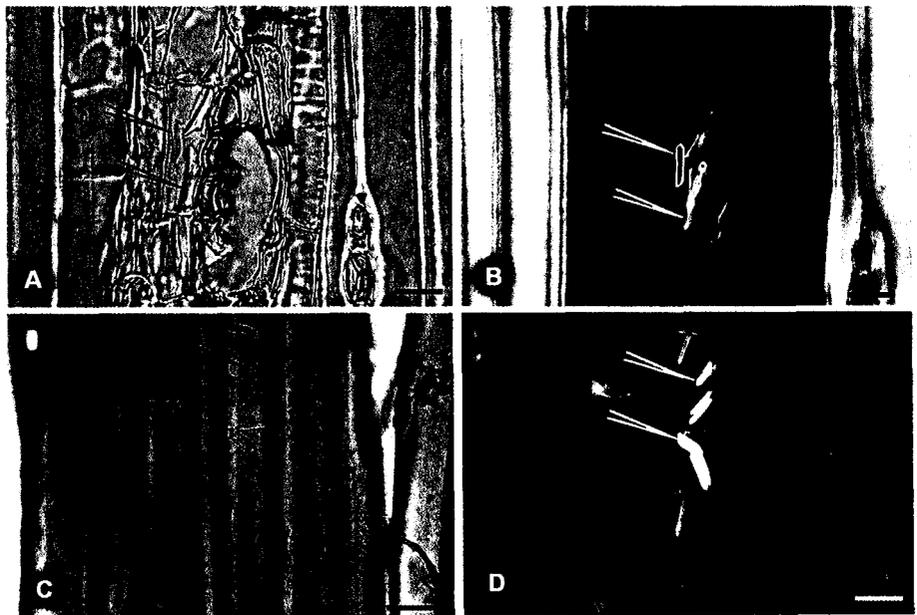


Fig. 3. Tangential sections of axial resin canal complexes in *Pinus contorta* (MADw 6021). – A & C: subsidiary parenchyma cells with crystals (arrows) in normal light. – B & D: same fields of view as A and C in polarized light; crystals much more easily viewed. – Scale bars = 25 μm .

or where the presence or absence of crystals was equivocal, free-hand radial sections were mounted in glycerin-ethanol (1 : 1) and those slides were analyzed in a similar fashion. No efforts were made to avoid or prefer sapwood or heartwood.

RESULTS

We did not find any noticeable anatomical differences between the varieties within each species for any of the data measured. As a result, we report the data at the species level and do not consider each variety separately. Varieties are footnoted in Table 1.

Axial resin canal complexes

The specimens measured by each method and the number of resin canal complexes measured by RCCM are shown in Table 1, along with mean values and standard deviations for both EM and RCCM for each specimen. The average mean RCCM and EM diameters and standard deviations for each species are shown in Table 2, as are the ranges of the means. For *P. contorta* and *P. ponderosa*, the average mean RCCM diameters differed by 56 μm , whereas the average mean EM diameters differed by 15 μm . For RCCM, there was a 33% overlap in the ranges of means for the two species and for EM, a 36% overlap.

Table 2. Tangential resin canal diameters of *Pinus contorta* and *P. ponderosa* by RCCM and EM.

Species	Average mean diameter (μm)	Standard deviation (μm)	Range of mean diameters (μm)
Resin canal complex method (RCCM)			
<i>P. contorta</i>	150	18	110–179
<i>P. ponderosa</i>	206	39	117–299
			33% overlap
Epithelial method (EM)			
<i>P. contorta</i>	86	10	67–99
<i>P. ponderosa</i>	101	13	71–128
			36% overlap

Ray composition

The data on ray composition are shown in Table 3. In normal rays, the average number of contiguous ray parenchyma cells in the axial direction was the same in both species. Rays with interspersed ray tracheids averaged fewer contiguous ray parenchyma cells, but neither the average number of contiguous ray parenchyma cells nor the proportion of these rays to normal rays was significantly different ($p = 0.92$ and $p = 0.063$, respectively, by two-sided t-test) between the species.

Crystals

All 41 samples of *P. ponderosa* examined lacked elongate crystals in the subsidiary parenchyma (Table 1), whereas all 40 samples of *P. contorta* had elongate crystals in the subsidiary parenchyma ($p \ll 0.0001$, chi-squared test) (Fig. 3). Elongate styloid

Table 3. Ray composition data for *Pinus contorta* and *P. ponderosa*.

Ray composition	Average number of contiguous ray parenchyma cells	
	<i>Pinus contorta</i>	<i>Pinus ponderosa</i>
Normal rays	2.6	2.6
Rays with interspersed ray tracheids	1.9	1.6
All rays	2.4	2.3
Percentage of rays with interspersed ray tracheids	18%	17%

crystals occurred singly or with other crystals in the subsidiary parenchyma cells and possibly in the epithelium. They were approximately 8 to 12 μm long and 2 to 1 μm wide. We also observed radial resin canal complexes in several samples of each species, and the presence or absence of crystals paralleled the presence or absence of axial canal complexes.

DISCUSSION

Axial resin canal complexes

Kellogg et al. (1982) measured resin canal diameter by the epithelial method (EM) in their study of western white pines of North America. Their data suggested that within the western white pines, resin canal diameter measured by EM is not a diagnostic character for wood identification.

For hand lens or microscopic identification, many authors (Brown & Panshin 1934, 1940; Brown et al. 1949; Greguss 1955; Panshin & DeZeeuw 1964, 1970, 1980; Core et al. 1979; Hoadley 1990) suggested the size of the resin canals as a diagnostic feature. but others (Record 1919; Phillips 1948; Kukachka 1960) did not mention resin canal size. Only Greguss (1955) and the textbook series (Brown & Panshin 1934, 1940; Brown et al. 1949; Panshin & DeZeeuw 1964, 1970, 1980) reported actual values for average tangential diameters, but the method of canal measurement is unclear or inconsistent.

For *P. contorta*, Panshin and DeZeeuw (1980) reported resin canal diameters of SO to 90 μm , with a maximum diameter of 110 μm . These values are quite different from our RCCM average mean value of 150 μm and maximum mean of 179 μm , but close to our EM average mean value of 86 μm and maximum mean of 99 μm . For *P. ponderosa*, Panshin and DeZeeuw (1980) reported resin canal diameters of 160 to 185 μm , with a maximum diameter of 230 μm . Again, these values are lower than our RCCM values (average mean diameter of 206 μm and maximum mean of 299 μm) and closer to our EM values (average mean diameter value of 101 μm and maximum mean diameter of 128 μm). These results suggest that Panshin and DeZeeuw did not measure the entirety of the resin canal complex but rather only the canal and two framing epithelial cells. This is particularly clear in the data for *P. contorta*. Apparently, these authors did not measure the equivalent of the resin canal complex, even though they define the epithelium as consisting of one to several layers of cells in thickness (Panshin & DeZeeuw 1980).

The difference between measuring *P. contorta* and *P. ponderosa* by EM and RCCM is shown in Table 2. The ranges of mean values for the two methods overlap to a similar degree (33% and 36% for EM and RCCM, respectively), but we contend that EM is inferior to RCCM for separating the species. For both methods, it is feasible to separate *P. ponderosa* only on the basis of its larger canals; that is, only the diameter of large resin canals should be used as a positive character for *P. ponderosa*,

The EM identifies any specimen with an average canal diameter greater than 100 μm as *P. ponderosa*. In our study, only 46% of *P. ponderosa* specimens measured had average canal diameters greater than 100 μm . This means that in a group of *P. ponderosa* specimens, only half will be positively identified as that species by EM. The RCCM identifies any specimen with an average resin canal complex diameter greater than 180 μm as *P. ponderosa*. This correctly identified 78% of the specimens we studied. Thus, for *P. ponderosa*, only 1 specimen in 2 can be identified by EM, whereas almost 4 specimens in 5 can be identified by RCCM.

In addition, RCCM is preferred to EM from a microtechnical and practical standpoint because the epithelium and some subsidiary cells are often torn in the sectioning process. The RCCM can be employed in these cases because it is a measure of the entire resin canal complex, the boundary of which remains intact in all but the poorest sections. It is also important to note that as few as 10 measurements per specimen are adequate to generate a meaningful average diameter for a specimen (data not shown).

Our results indicate that EM should not be used to separate *P. contorta* and *P. ponderosa* on the basis of resin canal diameter. The most reliable diagnostic difference between the sizes of axial resin canals of these two species is the tangential width of the axial resin canal complex, as measured by RCCM.

Ray composition

Panshin and DeZeeuw's (1980) method of using the number of contiguous rows of ray parenchyma cells to separate *P. contorta* and *P. ponderosa* (fewer than three rows for *P. contorta*, more than three rows for *P. ponderosa*) does not bear scientific scrutiny. Previous editions of that text offered conflicting data for this character; none of the versions is supported by our data. As Table 3 shows, neither species averaged more than three contiguous ray parenchyma cells in the axial direction, even in rays that lacked interspersed tracheids. Therefore, this method is not recommended for separating the woods of *P. contorta* and *P. ponderosa*.

Table 3 also displays the average proportion of rays with interspersed ray tracheids for each species. The proportion of rays with interspersed ray tracheids to normal rays did not vary significantly between the species ($p = 0.063$, two-sided t-test). The relative proportion of rays with interspersed ray tracheids is not a recommended character for separating *P. contorta* and *P. ponderosa*.

Crystals

In measuring resin canal lumina, Kellogg et al. (1982) reported the presence of small crystals in the epithelial cells (*sensu lato*) of certain western white pine species. Their study is the only known publication on the diagnostic significance of such crystals in

pinus, though Fahn (1979) found a crystal in a so-called sheath cell in one figure and Baas et al. (1986) reported crystals in *P. longaeva*, *P. balfouriana*, and *P. aristata*. Wood identification, plant anatomy, and wood anatomy texts (Greguss 1955; Jane 1970; Esau 1977; Grosser 1977; Schweingruber 1978; Core et al. 1979; Panshin & DeZeeuw 1980; Wilson & White 1986; Hoadley 1990; Dickison 2000) do not mention crystals, and with the exception of Fahn (1979) and Baas et al. (1986), these crystals seem to be unutilized in wood identification or even unknown.

The characteristic presence of elongate (styloid) crystals in the subsidiary parenchyma of *P. contorta* and characteristic lack of such crystals in *P. ponderosa* is the most powerful anatomical method for distinguishing the two species. These crystals are very small and apparently share a similar refractive index with other resin canal complex contents, which makes the use of polarizing optics a necessity for accurate identification.

Wood anatomical key

The following wood anatomical key can be used to separate *P. contorta* and *P. ponderosa*:

- Small crystals in cells of resin canal complex; average tangential diameter of resin canal complex not greater than 180 μm *Pinus contorta*
- Small crystals absent from cells of resin canal complex; average tangential diameter of resin canal complex greater than 180 μm *Pinus ponderosa*

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