WOOD ANATOMY OF PINUS LONGAEVA (BRISTLECONE PINE) AND THE
SUSTAINED LENGTH-ON-AGE INCREASE OF ITS TRACHEIDS

by

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Summary
Length-on-age curves are presented for tracheids of three stems of bristlecone pine (Pinus longaeva). In the oldest stem tracheid length has steadily increased over the last 2200 years, and there are no signs of a levelling off. In the younger stems, which have the innermost rings dated 1484 and 1445 A.D., it appears that the 'juvenile' phase of steep increase in tracheid length of Pinus longaeva lasts several centuries. The methods of measuring tracheid length from narrow increment cores with a high percentage of damaged tracheids in macerations and in tangential sections using Ladeil's method are compared. The wood anatomy of P. longaeva is described and found very similar to that of P. aristata. Both species differ in minor details from the related P. balfouriana. All three species share minute crystals in the epithelial and sheath cells of the resin ducts.

Key words: Crystals, juvenile wood, Pinus aristata, P. balfouriana, P. longaeva, senescence.

Introduction
Length-on-age variation in tracheids of softwoods and fibres of hardwoods has been of considerable interest ever since Sanio's (1872) classical study of tracheid length of Scotch pine (Pinus sylvestris). The period over which tracheid length increases rapidly is one of the criteria for the definition of juvenile wood and is associated with a number of undesirable wood properties (Brazier, 1985; Fukazawa & Ohtani, 1982; Panshin & De Zeeuw, 1980). Dinwoodie (1961) comprehensively reviewed the older literature, which contains a number of conflicting reports as to whether cell length remains constant after this steep increase or whether there is further variation. On the basis of much original research and the extensive literature on the subject Dinwoodie concluded (l.c.: 130–131): 'It would appear that the maximum tracheid length does not occur till an advanced age (200–300 years) after which there is a period of maximal length before decreasing in extreme old age (400–500 years). In young plantation-grown material there is an apparent maximum tracheid length occurring between 10 and 20 years depending on the species.' This conclusion was based on studies of material with 400–500 years as a maximum age (Bailey, 1958; Bailey & Faull, 1934; Bailey & Tupper, 1918; Gerry, 1916). Older material, however, is not available for most species. Panshin and De Zeeuw (1980) recognised three general types of length-on-age curves for fibres and tracheids: 1) level curves in which length stays constant after the juvenile phase; 2) curves showing continuous increase in cell length from the juvenile zone outward; 3) parabolic curves showing cell length increasing to a maximum followed by a decrease in cell length. These categories are from examples of trees varying in age between 60 and 140 years.

Pinus longaeva D.K. Bailey (1970), the Intermountain or Great Basin bristlecone pine from subalpine altitudes in Utah, Nevada, and central California, can reach an age of almost 5000 years (Currey, 1965; Ferguson, 1968; Fritts, 1976; Schmid & Schmid, 1975). This species was segregated from P. aristata Engelm. (Rocky Mountain or Colorado bristlecone pine) by Bailey in 1970, the latter species being restricted to Colorado, New Mexico, and Arizona. The wood anatomy of P. longaeva has received no special attention, although samples of this species may be at the basis of some of the wood anatomical accounts of P. aristata.

Bristlecone pine is extremely slow growing, producing as many as 75 growth rings per centimetre, each ring averaging 0.1–0.3 mm in our material. The very old ages of the trees and their climatic sensitivity make the species exceptionally valuable for dating purposes. The bristlecone pine-chronology has been extended back more than 8200 years and, in fact, has been used to recalibrate radiocarbon dates (Ferguson, 1968; Fritts, 1976; Schmid & Schmid, 1975; Suess, 1979).

In view of the advanced age bristlecone pine achieves we undertook a study of its tracheid length-on-age variation to establish whether extremely old trees show the classical Sanio curve
or whether signs of senescence as invoked for various 400–500-year-old trees of other species are apparent.

We undertook a comparative wood anatomical study of Pinus longaeva to fill a small gap in the extensive literature on Pinus wood anatomy (Budkevich, 1961; Van der Burgh, 1973; Greguss, 1955, 1972; Greguss & Varga, 1950; Hudson, 1960; Mirov, 1967; Penhallow, 1904, 1907) and to see whether this species differed from its putative allies, Pinus aristata and P. balfouriana S. Wats. (foxtail pine). The latter two species are in the same subsection or group of lower taxonomic rank in most systems of classification of Pinus (cf. Van der Burgh, 1973; Farjon, 1984; Little & Critchfield, 1969; Mirov, 1967). Bailey's (1970) segregation of P. longaeva from P. aristata was recently challenged by Johnson (1976) and Little (1979). The latter reduced P. longaeva to a variety of P. aristata. Hence a wood anatomical comparison was in order in view of this taxonomic controversy.

Material and Methods

Several increment cores, previously used for dendrochronological studies at the Laboratory of Tree-ring Research in Tucson, Arizona, were kindly made available by Dr. Donald A. Graybill. The cores were taken in 1979 from trees in the Methuselah Walk locality (37° 26' N, 118° 10' W, 2900 m elevation) in the Inyo National Forest of central eastern California. All cores are from climatically sensitive trees growing in a small protected valley with a north-north eastern exposure. The cores measure about 4 mm in diameter, and about 0.5 mm less in the vertical direction because they had been sanded down on one side to optimally visualise the annual rings.

We selected three cores for determining length-on-age variation in tracheid length: 1) from a tree over 2200 years old, with the innermost ring dated 215 B.C. but not yet reaching the pith area, approximately 100–200 years missing (code TRL 79-125); 2) from a tree about 500 years old, with the innermost ring dated 1484 A.D., and probably five rings distant from the pith (TRL 79-130A); 3) from a tree nearly 600 years old, with the innermost ring dated 1445 A.D., with about 20–50 annual rings missing from the pith area (TRL 79-129B). The last core had been taken through the whole stem and included sapwood from 1893–98 to 1979 at both ends of the core so that two length-on-age curves could be constructed. In addition, we sampled the most recently formed rings of three other cores (TRL 79-96A, 96B, 126A) varying in age from about 480 to 1400 years.

Tracheid length was determined in two ways: 1) Fifty tracheids per sample point were measured in the usual way with a calibrated ocular micrometer from macerations obtained via Franklin's method. Care was taken to avoid extremely narrow latewood tracheids formed in the outermost cell layers of the growth ring. Due to the extremely narrow rings of Pinus longaeva we considered further precautions to eliminate within-ring variation in tracheid length impractical. Sample points included single rings or, in case of extremely narrow rings, two to three rings at intervals of 10, 50, or 100 years depending on the expected amount of increase in tracheid length over short intervals. 2) Using tangential sections and the method of Ladell (1959), which was recently revived by Wilkins and Bamber (1983), tracheid tips were counted among 300–400 tracheids in a single direction between two lines at a distance of 1 mm. Average tracheid length was calculated as follows:

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\text{length} = \frac{\text{number of tracheids} \times \text{distance between lines}}{\text{number of tracheid tips}}
\]

Instead of using lines on a projection screen, as recommended by Ladell, we used the outer lines of an ocular grid micrometer to delimit the counting area. We applied Ladell's method to a smaller number of sample points in the cores than with the maceration technique. Yet, use of Ladell's method was essential in view of the small diameter of the cores, which with the maceration technique would bias measurement of unbroken tracheids toward shorter ones.

Macerations were mounted in glycerin jelly, tangential sections directly in Karo (commercial light corn syrup). Longitudinal shrinkage is negligible in both media.

For the comparative wood anatomy study additional samples were sectioned of P. longaeva (White Mountains, California: *Mirov s.n., collected 1948—mature, undated specimen with sapwood attached, P. aristata (San Francisco Peak, Arizona: *Robinson s.n.—mature sample from fast growing tree; Boulder County, Colorado: Hufford s.n.—3.5 cm branch from young tree), and P. balfouriana (California: D. K. Bailey s.n., *CA 34—slow-growing specimen; Sierra Nevada, California, *CA 35—fast-growing specimen). The asterisked samples are from the collection of the Forest Products Laboratory, Richmond, California.

Results and Discussion

Differences in determinations of tracheid length using macerations or Ladell's method

Figure 1 plots all determinations of tracheid length with Ladell's method against average length values obtained by direct measurement.
in macerations of directly adjacent annual rings. Except for one sampling point all values with Ladell's method are consistently higher than those of macerated tracheids. Moreover, the differences become greater with increasing tracheid length. This is according to expectations. In samples with an average tracheid length of c. 3000 μm, maximum tracheid length is about 4000 μm. In cores of less than 4 mm axial diameter it is impossible to preserve these tracheids. Anyway, the chance for tracheids to be broken in these narrow cores increases with length, leaving a relatively higher proportion of shorter, unbroken tracheids likely to be selected for measurement.

The results of Figure 1 validate the use of Ladell's method in our material. Thus values of average length of tracheids measured from macerations are (0—7—20(−23)% below the presumably realistic values obtained with Ladell's method. However, we do not share the enthusiasm of Ladell (1959) and Wilkins and Bamber (1983) for its ease and efficiency. In a wood with fairly narrow tracheids like bristlecone pine, it is quite easy to miss tracheid tips at low power magnification, the latter being a necessary condition for sufficient line distance, which again is a prerequisite for accuracy of the method. For our material a reproducible Ladell determination of tracheid length took at least as much time as the conventional eye-straining measurement of 50 tracheids in macerations. We can, however, confirm the accuracy of the Ladell method: measurement of 50 macerated tracheids from the large wood sample (Mirow s.n.) yielded an average value of 2940 μm, which matched very closely the Ladell value of 2960 μm from tangential sections.

**Length-on-age curves**

Figures 2 and 3 summarise the length-on-age variation in tracheids of three trees of *Pinus*
Fig. 3. Length-on-age variation in tracheids in two opposite radii of a single core of *Pinus longaeva*. Solid lines: curves fitted for averages derived from measuring macerated tracheids; broken lines: curves fitted for calculated values using Ladell’s method; circles: ‘left hand’ (L) radius; squares: ‘right hand’ (R) radius.

*longaeva*. The straight lines for the oldest tree and the curves for the two younger trees have been intuitively estimated as a linking of points in chronological order would give rather irregular graphs.

The most striking result is the continued increase in tracheid length in the c. 2200-year-old tree (Fig. 2). Even at the time of sampling in 1979 maximum tracheid length does not seem to have been reached. The oldest part of the core at 215 B.C. is some distance removed from the pith and in our data there is no sign of the juvenile phase with a steep increase of tracheid length. The irregularities between 215 B.C. and 100 B.C. may be due to the vicinity of a knot or to local damage to the cambium. Around 150 B.C. there is a crack with strongly discoloured wood that is associated with unexpected extremes in low and high average tracheid length at 160 B.C. and 140–110 B.C. Shortening of fibres or tracheids as a response to injury has been invoked by various authors, notably Bailey and Tupper (1918), to account for sudden drops in length-on-age curves. In the early part of this increment core we also found many compression wood tracheids, which might account for part of the irregularities.

In the other two trees, of which the innermost rings in the cores were closer to the pith, the length-on-age curves show a prolonged phase of steep increase in tracheid length lasting from about 250 up to 500 years. For one of the radii of the core presented in Figure 3 it is not even clear whether the phase of strong length increase is already over at the time of sampling in 1979. Figure 3 also shows considerable differences in the curves for the radii of opposite direction. Unfortunately information of the cardinal direction in which the core was taken is not available, but it would seem plausible that the lower curves for the ‘left hand’ part of the core would represent the north side and the higher curves the south side of the tree, in accordance with Liese and Dadsell’s (1959) findings that south-facing stem sectors have longer wood elements than north-facing ones. Growth rates in both sides of the stem were about the same throughout the life span of the tree. The drop in tracheid length at the end of one radius (Fig. 3) remains unexplained.

The lower levels of average tracheid length at the stem periphery of the c. 500-year-old tree (Figs. 2 & 3) and of the additionally sampled sapwood of the other cores (Ladell values of 2710 and 2890 µm for two cores of a c. 1500-year-old tree and 3030 µm for a 550-year-old tree) as compared with the values for the periphery of the c. 2200-year-old tree could be interpreted by considering these stems as still ‘too young’ to have reached their maximum tracheid length. However, this conjecture is not really justified since many factors are known to affect the level of length-on-age curves between trees and within individual trees (for reviews, cf. Dinwoodie, 1961, and Panshin & DeZeeuw, 1980).

Fig. 4–7. *Pinus longaeva*. – 4: Transverse section; x 115. – 5: Tangential longitudinal section through earlywood; x 115. – 6: Radial longitudinal section, showing ray parenchyma, ray tracheids and axial tracheids. Tangential pits in latewood tracheids arrowed; x 280. – 7: Tangential longitudinal slanting section through resin duct showing minute crystals; x 400 (polarised light).
The absence of any decline of tracheid length in the 2200-year-old tree is remarkable in view of the generalisation that decline in length occurs after a certain age (Dinwoodie, 1961; Panshin & De Zeeuw, 1980), apparently in association with senescence. Although there are no arguments to reverse this statement and to consider the sustained increase of tracheid length in old bristlecone pines as a sign of vigour, this phenomenon certainly promotes *P. longaeva*, whose tracheids have been referred to as 'the oldest living things on earth', to a record-breaking species in wood anatomy. The same might apply to the prolonged duration of the juvenile phase as judged from steep increase in tracheid length in the two trees over periods of 2.5 to 5 centuries.

**Wood anatomical description of Pinus longaeva (Figs. 4–7)**

Growth rings typically very narrow, 2–20 (mostly 5–8) cells wide. Transition from earlywood to latewood only apparent by radial flattening of last one or two layers of tracheids. Tracheids c. 30 μm (15–45 μm) in tangential diameter, with bordered pits in one row in the radial walls, tangential pits smaller, confined to the last row of tracheids of each growth ring and very abundant in the walls separating latewood from subsequently formed earlywood. Crossfield pitting half-bordered to almost simple, typically pinoïd with 4 (1–5) pits per crossfield, occasionally tending to piceoid with slit-like, extended apertures. Axial parenchyma, except for the sheath cells around the resin ducts, absent. Rays uniseriate and fusiform. The uniseriate rays 5–8 (1–24) cells high, 30–40 per tangential sq. mm.; the fusiform rays with a multiseriate central portion containing a resin duct each, 0.5–3 per tangential sq. mm. Ray parenchyma with nodular end walls and thick, usually densely pitted lateral walls. Ray tracheids in one or two marginal rows, sometimes interspersed, absent from some of the rays, with typically non-dentate walls, but sometimes with weak wall thickenings. Some of the low rays entirely composed of ray tracheids. Axial resin ducts solitary or in tangential pairs, with moderately thick-walled epithelium surrounded by a mostly aliform parenchymatous sheath, un lignified in sapwood, lignified in heartwood. Some of the sheath cells even weakly sclerotic. Intercellular spaces often conspicuous between sheath cells. Tracheids around resin ducts sometimes with horizontal division walls, which contain bordered pits or not. Ducts sometimes occluded by thin-walled tylosoids. Epithelium and surrounding sheath parenchyma cells often containing minute, mostly elongate to styloïd crystals (c. 1–3 by 2–10 μm). Resinous contents abundant in axial tracheids and ray cells of the heartwood. Trabeulae occasionally present.

**Comparison of Pinus longaeva with P. aristata and P. balfouriana**

In all respects the wood anatomy of *P. longaeva* is similar to that of *P. aristata*. This is borne out not only by a comparison with data from the literature (Budkevich, 1961; Van der Burgh, 1973; Greguss, 1955; Greguss & Varga, 1950; Hudson, 1960; Mirov, 1967; Penhallow, 1904, 1907), but also by our comparison with several samples of *P. aristata*, which appeared to share characters such as crystal-liferous resin duct cells, smooth to weakly thickened ray tracheid walls, occasional tendency towards piceoid crossfield pitting, and overlap in all quantitative characters not commonly recorded in the literature. *P. balfouriana* differs from both *P. aristata* and *P. longaeva* in a more conspicuous dentation (albeit rather fine) of the ray tracheid walls and a stronger tendency towards piceoid crossfield pitting. In one of our samples (CA 35) this was the dominant type; in another (CA 34) the pinoid type was more common, but piceoid pits were more in evidence than in any of the samples of *P. aristata* or *P. longaeva*. On the other hand, *P. balfouriana* shares many characters with the other two species, including the occurrence of crystals in the epithelial and sheath cells of the resin ducts and the occasional sclerification of sheath cells. Crystals are here reported for the first time in these three species (Fig. 7). They are of the same type as those found to be highly diagnostic for *P. flexilis* and *P. albicaulis* by Kellogg et al. (1982), who were, to our knowledge, the first to report them in *Pinus*. This second report of crystals in three other species of pine suggests that they may occur more commonly in the genus.

The above comparison confirms the close affinities of *P. longaeva* and *P. aristata*. From wood anatomy alone no evidence can be derived to segregate the two species, but it should be kept in mind that many morphologically distinct species of *Pinus* cannot be separated on the basis of wood anatomy. Therefore, we advise against the use of the wood anatomical similarity of *P. longaeva* and *P. aristata* in support of combining these species again, as suggested by Johnson (1976) and Little (1979); after all, detailed morphological and phytochemical studies have demonstrated that Bailey's (1970) recognition of the segregate *P. longaeva* is valid (Zavarin et al., 1976; Zavarin & Snajberk, 1973), although hybridisation between the species is possible (Critchfield, 1977), and although
chemically intermediate populations occur (Zavarin et al., 1972).

The wood anatomical differences between *P. balfouriana* and the previous two species are by themselves not sufficient to justify the recognition of a subsection (also including *P. krempfii* from Indochnia) separate from *P. arista tata* as suggested by Van der Burgh (1973). This subsection, *Balfourianae*, would moreover be wood anatomically quite heterogeneous, because *P. krempfii* has smooth ray tracheid walls and larger pinoid crossfield pits in a much lower number per crossfield than *P. balfouriana* (cf. Greguss, 1972). A more recent classification by Farjon (1984), as influenced by Van der Burgh, suggesting a separate subsection, *Aristatae*, for *P. arista tata* and treating *P. balfouriana* and *P. longaeva* together in a subsection *Balfourianae* (*P. krempfii* being placed in its own subsection, *Krempfiana*) is also in conflict with the wood anatomical evidence. Our data are more in accord with Little and Critchfield's (1969) treatment of *P. arista tata* and *P. balfouriana* in the same subsection, *Balfourianae*, along with the later described *P. longaeva*. The close affinities of the latter species with subspecies *austrina* of *P. balfouriana* have moreover clearly been demonstrated by Mastrogiuseppe and Mastrogiu-seppe (1980).

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References


