IMPACT OF AIR POLLUTION ON RING WIDTH AND TRACHEID DIMENSIONS IN ABIES RELIGIOSA IN THE MEXICO CITY BASIN

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SUMMARY

Annual ring width and characteristics of latewood tracheids were characterized for the past 100 years for old trees of Abies religiosa growing in the Desierto de Los Leones within the air-polluted Mexico City basin. Sampled trees had lost nearly 50% of their branches and leaves. Radial variation in most measured characteristics followed typical trends for maturation until the 1970s, when trees were about 70 years old. From that decade onward, there was a continued reduction in annual ring width as well as a reduction in cell wall thickening and tracheid length, but an increase in lumen diameter. These xylem modifications started before the first visual symptoms of leaf damage were detected. We suggest that changes in annual ring width and tracheid size are caused by air pollution rather than by tree age.

Key words: Chronology, forest decline, radial wood variation, tracheid length, wall thickness.

INTRODUCTION

Mexico City is situated in a high semi-closed basin: its mean elevation is 2,200 m, and the surrounding mountains range from 3,200 to more than 5,000 m. Because of this irregular topography and the relatively weak winds, basin ventilation is poor. These geographic conditions contribute to the maintenance of high air pollution levels and reduction of air quality. On days when air movement does occur, the prevailing north winds push air pollutants southward leading to the deposition of high levels of air pollutants in the forested areas (Bravo-Alvarez & Torres-Jardón 2002).

Forest decline was reported for the Mexico City basin in the early 1980s (Vázquez 1987), affecting mainly Abies religiosa and Pinus hartwegii (Vázquez 1987; De Bauer & Krupa 1990; Alvarado et al. 1993). Other species affected by air pollutants to a lesser degree are Pinus montezumae and Prunus serotina (Hernández & De Bauer 1984; De Bauer et al. 1985; Skelly et al. 1997). The forests in the southwestern region of Mexico City basin are reportedly the most severely damaged (De Bauer & Krupa 1990). During 1990–1991, this forest stained more than ten days per year with ozone concentrations greater than 200 ppb for 1 h average (Miller et al. 1994). Other forest sites within the Mexico City basin show different degrees of air pollutant symptoms in several tree species (Hernandez & De Bauer 1984). For example, Alvarado et al. (1993) and Alvarez et al. (1998) reported chlorosis and premature needle senescence in Abies religiosa trees that have had chronic exposure to air pollutants.
Chlorosis and needle loss reduce photosynthesis. In trees, up to 45% of the carbohydrates produced by photosynthesis are allocated to wood production (Creber & Chaloner 1984), and various authors suggested that a reduction in photosynthesis may cause a reduction in cambial activity and a decline in annual ring width (De Kort 1986; Weber & Grulke 1995; Schweingruber & Voronin 1996).

Tree-ring series analysis is a valuable approach to evaluate the effect of atmospheric pollutants on tree growth through time, especially around point sources such as smelters and industrial plants. However, its usefulness is controversial in studies at a regional scale (Innes & Cook 1989). Another method to evaluate the effect of pollutants on tree growth is the study of the anatomical changes within growth rings. Several authors have reported reductions in annual ring width in trees exposed to the aerial pollutants sulphur dioxide, nitrogen oxides, ozone, hydrofluoric acid, and magnesium-dust. These reductions in ring width were correlated with changes in cell size and number (De Kort 1986; Kawakami & Furukawa 1992; Wimmer et al. 1996), as well as with changes at the structural level, e.g., modifications in tracheid shape and cell wall lignification (Nieweglowska-Guzik 1995; Segala Alves 1995; Schmitt et al. 2003).

Annual ring density in conifers has been recognized as one of the most important parameters for dendrochronological studies (Yasue et al. 1996). In conifers, density depends primarily on tracheid dimensions. A change in density may be defined by the ratio between tracheid lumen diameter and tracheid wall thickness (Vaganov 1990). Maximum density is found in the last-formed tracheids in an annual ring. Moreover, changes in the environment would be expected to modify the enlargement of lumen diameter and wall thickness in these last-formed tracheids (Yasue et al. 1996). The purpose of this study was to use the last-formed tracheid features in latewood as an indicator of temporary changes in damaged trees of *Abies religiosa* growing in the Mexico City basin, under chronic exposure to air pollutants.

**MATERIALS AND METHODS**

**Study site and sample trees**

The study area was a natural forest named Desierto de Los Leones (DL), in the southwestern region of the Mexico City basin (19° 19’ N, 99° 20’ W) at an elevation between 2,800 and 3,800 m. Mean annual rainfall is 1,265 mm and mean temperature 10.5 °C. Soils are classified as Eutric Cambisols mixed with Andosol (FAO classification). The main tree components of the coniferous forest are *Abies religiosa*, *Pinus hartwegii*, and species of *Cupressus* (Rzedowski 1978). The dominant species within the studied stands was *Abies religiosa* and herbaceous plants included species of *Senecio*, *Eupathorium*, and *Salvia*. Four stands were selected where no logging activities had taken place since the 1910s, with an elevation between 3,000 and 3,500 m, and slopes > 30°. The stands were located at four corners of a grid, at a distance of 500 m. Five trees were sampled per stand, using only dominant trees that were > 30 m in height. An effort was made to avoid trees with physical defects, or those damaged by fire, insect outbreaks, fungi, or mistletoe. Branch loss percentage (including needle loss) for each sampled tree was determined following the damage scale adapted for *Abies religiosa* by
Alvarado et al. (1993). According to this scale, the crown was divided into sixths, and in each portion color of the foliage (green = 1, other = 0), needle retention (current = 0, year 2 = 2, year 3 = 4, year 4 = 8), and branch retention (0–33% = 0, 33–66% = 2, >66% = 4) were coded. The final score was then evaluated, where 0–10 was interpreted as very severe damage and >40 was very lightly damaged. Stem diameter, damage scale, and chronology length for each tree are given in Table 1. Tree age varied from 86 to 108 years.

Table 1. Stem diameter at 1.3 m height, damage classes, and chronology date for trees of *Abies religiosa* sampled at Desierto de Los Leones. Damage class: moderate (M) = 21–30; severe (S) = 11–20; very severe (VS) = 0–10.

<table>
<thead>
<tr>
<th>Stand number</th>
<th>Tree number</th>
<th>Diameter (cm)</th>
<th>Damage class</th>
<th>Chronology length</th>
</tr>
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<tbody>
<tr>
<td>One</td>
<td>1</td>
<td>46.1</td>
<td>M</td>
<td>1905–1997</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>27.5</td>
<td>S</td>
<td>1894–1997</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>30.2</td>
<td>M</td>
<td>1899–1997</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>26.1</td>
<td>M</td>
<td>1890–1997</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>24.1</td>
<td>S</td>
<td>1889–1997</td>
</tr>
<tr>
<td>Two</td>
<td>6</td>
<td>29.2</td>
<td>VS</td>
<td>1900–1997</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>28.6</td>
<td>VS</td>
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<td>8</td>
<td>27.6</td>
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<td>1911–1997</td>
</tr>
<tr>
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<td>27.3</td>
<td>VS</td>
<td>1908–1997</td>
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<td>25.6</td>
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<td>19.5</td>
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</tr>
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<td></td>
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<td>S</td>
<td>1901–1997</td>
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<td>23.8</td>
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<td>1903–1997</td>
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<tr>
<td></td>
<td>20</td>
<td>21.0</td>
<td>S</td>
<td>1901–1997</td>
</tr>
</tbody>
</table>

**Preparation of samples and measurements**

We removed two increment cores per tree, avoiding the slope to eliminate the occurrence of compression wood. They were taken at 1.3 m above ground with a 12-mm diameter increment borer, and were 30–45 cm long. One core was mounted and prepared following standard dendrochronological techniques (Stokes & Smiley 1968). The radial increments were cross-dated by the skeleton plotting method (Stokes & Smiley 1968) and then statistically cross-checked with the COFECHA program (Holmes 1983) for detection of false or missing rings. The tree-ring chronology was calculated by taking the mean of tree-ring series and was plotted on a natural log scale. We then removed the latewood from 4-year increments and macerated them using a hydrogen peroxide...
and acetic acid solution (1:1) for 48 h at 80 °C (Berlyn & Miksche 1976). When macerations were ready, tracheids were washed and mounted on temporary slides.

The second core was labeled and fixed in formaldehyde-acetic acid-ethanol (Berlyn & Miksche 1976). After 24 h, the core was stored in a solution of glycerin-ethanol-water (1:1:1) to avoid drying. In the laboratory, the core was cut transversely with a twin-bladed saw and cross-dated. The core was then divided into segments 3 mm wide which were then transverse-sectioned 30 μm thick with a sliding microtome. These sections were stained with safranin and mounted with synthetic resin.

For the transverse sections of each ring we chose the two radial rows of the last-formed tracheids (Fig. 1) to lessen the within-growth-ring variation (Vysotskaya & Vaganov 1989). We made 25 measurements per ring for each of the following tracheid features: tangential diameter, tangential lumen diameter, and single cell-wall thickness. With the macerated samples, we measured the tracheid length of 50 cells per 4-year increment. The measurements for each character within each ring were performed with an image analyzer Image-Pro Plus, version 3.01 (Media Cybernetics 1997) using a Hitachi KP-D51 video camera and both compound and dissecting microscopes.

![Fig. 1. Light micrograph showing the last-formed cell of an annual ring of Abies religiosa. The rectangle indicates the cell measured.](image)

**Statistical analyses**

We obtained basic statistical values from univariate analyses, then constructed curves of mean values to assess radial variation. Each ring was represented by the mean of 25 or 50 measurements. After log-transforming the means per annual ring, we conducted a variance analysis for unbalanced design using the general linear model for the means of each decade asking whether statistically significant differences existed for the anatomical characters among decades (1960s, 1970s, 1980s, and 1990s). Differences among means of the four decades were compared and segregated with a Tukey test (p < 0.05). Statistical analyses were performed with SAS software (SAS Institute 1989).
RESULTS

The chronologies of the *Abies religiosa* tree showed a reduction in ring width in the 1930s and the 1970s. The reduction during the 1930s was temporary, because trees recovered their growth ring width, whereas trees showed a clear and permanent growth reduction in the years 1975–76 (Fig. 2). This last reduction was simultaneous with a decrease in tracheid wall thickness and length (Fig. 3c, d).

Latewood tangential diameter and lumen diameter showed slight fluctuations and a progressive increase from pith to bark. There was a decrease during the 1950s (Fig. 3a, b). Latewood tangential cell wall thickness showed an increase near the pith, and a decrease during the 1930s and 1970s, similar to annual ring width. Cell wall thickness recovered from the depression in the 1930s, but not from the depression in the 1970s (Fig. 3c). Tracheid length increased from the pith toward bark until the 1970s, after which it decreased (Fig. 3d).

Variance analysis showed significant differences among decades for tangential lumen diameter ($F = 17.06, \text{df} = 3, p < 0.001, n = 740$), tangential wall thickness ($F = 68.52, \text{df} = 3, p < 0.0001, n = 740$), and length ($F = 5.45, \text{df} = 3, p < 0.001, n = 200$) of latewood tracheids (Table 2). Tukey multiple comparison analyses showed significant differences only between some decades (Table 2). No decadal differences were detected for tracheid tangential diameter ($F = 0.42, \text{df} = 3, p > 0.06, n = 740$).

<table>
<thead>
<tr>
<th>Features</th>
<th>1960s</th>
<th>1970s</th>
<th>1980s</th>
<th>1990s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latewood diameter (μm)</td>
<td>$47.5 \pm 0.4^a$</td>
<td>$47.9 \pm 0.6^a$</td>
<td>$47.9 \pm 0.6^a$</td>
<td>$47.9 \pm 0.5^a$</td>
</tr>
<tr>
<td>Latewood lumen diameter (μm)</td>
<td>$33.6 \pm 0.4^a$</td>
<td>$34.1 \pm 0.4^a$</td>
<td>$35.4 \pm 0.5^a$</td>
<td>$37.5 \pm 0.5^b$</td>
</tr>
<tr>
<td>Latewood wall thickness (μm)</td>
<td>$6.9 \pm 0.05^a$</td>
<td>$6.8 \pm 0.08^a$</td>
<td>$6.2 \pm 0.08^b$</td>
<td>$5.1 \pm 0.07^c$</td>
</tr>
<tr>
<td>Length (mm)</td>
<td>$4.2 \pm 0.05^a$</td>
<td>$4.3 \pm 0.05^a$</td>
<td>$4.1 \pm 0.06^a$</td>
<td>$3.6 \pm 0.08^b$</td>
</tr>
</tbody>
</table>
Fig. 3. Mean tracheid dimensions in the latewood of damage trees of *Abies religiosa* growing in the air-polluted forests of Desierto de Los Leones. – **a**: Tangential diameter (μm). – **b**: Tangential lumen diameter (μm). – **c**: Wall thickness (μm). – **d**: Length (mm).
DISCUSSION

The ring width decrease detected in this study was consistent with observations reported for *Abies religiosa* (Alvarado et al. 1993; Watmough & Hutchinson 1999) and *Pinus hartwegii* (Alarcón et al. 1993) growing within the Mexico City basin forests. Using dendrochronological analyses, Alarcón et al. (1993) attributed the tree growth reduction during the 1930s to a period of low precipitation in the Mexico City basin. However, they did not find any correlation between ring width and precipitation in the 1970s. Reduction in radial growth in polluted areas has been detected in several sites (Evertsen et al. 1986; Dünisch et al. 1996), but this feature alone cannot be used to demonstrate the effect of air pollution on tree growth at a regional scale (Peterson & Arbaugh 1988; Van den Brakel & Visser 1996) because at this scale the effect of pollution is confounded with the climatic signal extractable from tree rings.

The decrease in ring width associated with damaged trees in declining forests has been reported in numerous studies (De Kort 1986; Jagels 1986; Eckstein et al. 1989; Innes & Cook 1989). For some species annual ring width reduction has been observed before foliar symptoms are visible (Stoikov et al. 1994), in contrast to the effects detected when biological agents are involved (Krause & Hubert 1995). In *Abies religiosa* trees, the first foliage symptoms were detected during 1983 at the same site (Vázquez 1987). Nevertheless, the chronologies reported here show that ring width reduction started ten years earlier, similar to the situation reported by Stoikov et al. (1994) for *A. alba*. Our results also support the assertions of Alvarado et al. (1993) that individual trees in pollution-impacted sites remain for longer periods in the same size class. Although the chronology showed several peaks of growth within the last decades, these peaks are associated with a high natural self-thinning in the forest during the early 1980s.

Curves of radial variation for wood anatomical characters can be divided into two phases. The initial or juvenile phase located near the pith is identified as the zone in which tracheid dimensions tended to increase, and is associated with vascular cambium maturation. The second phase corresponds to the stabilized growth period in which the vascular cambium is mature, and the anatomical features attain a relatively stable size. The behavior of tracheid characters observed for *Abies religiosa* trees followed a pattern similar to that reported for *Abies pindrow*, *Pinus ponderosa*, and *Pinus strobus* (reviewed in Panshin & DeZeeuw 1980). The tangential diameter and tangential lumen diameter showed pith-to-bark fluctuations. However, tracheids maintained a slight diameter increase from pith to bark. Increases in tangential tracheid diameter with tree age has been reported by several authors (Bannan 1944; Larson 1969; Terrazas 1991). Because of the decline in cell wall thickness, especially in the last two decades, the ratio of tangential lumen diameter increases in that period.

Cell wall thickness and tracheid length declined progressively in the 1970s. Although the four decades analyzed corresponded to the second phase of the radial variation curves, statistically significant differences existed only between 1990s and the other three decades. This pattern is contrary to the typical mature wood curves in which tracheid dimensions tend to conserve or slightly increase their size.

Attention has rarely been given to wall thickness, but reports exist on the reduction in latewood density in some species related to forest decline. Moreover, tracheid wall...
thickness has a strong influence on wood density (Eckstein et al. 1989). Baucker et al. (1996) and Schweingruber and Voronin (1996) report a scarce accumulation of cell wall in pollution-damaged trees of *Picea abies* and *P. obovata*, respectively. Venogupal and Krishnamurthy (1987) indicated that development of tracheary elements is related strongly to hormonal metabolism, but cell-wall accumulation is mostly associated with the concentration of carbohydrates in the storage tissue. However, we observed no carbohydrate storage in the wood parenchyma cells of the studied trees throughout the year (Bernal-Salazar unpubl. data). These observations are consistent with the shortage of carbohydrates reported in other pollution-damaged tree species (Gregory et al. 1986; Torelli et al. 1986). We suggest that this carbohydrate shortage was one of the possible causes that contribute to the development of thinner tracheid walls in damaged trees of *Abies religiosa*.

The trees in this study were 100 years old, and yet they can live to 300 years. Therefore we suggest that the decrease in tracheid dimensions was associated with environmental stress, and not tree age. Size reduction of tracheary elements after exposure to air pollution is well known (Kawakami & Furakawa 1992; De Kort 1986, 1990; Baucker et al. 1996). A reduction in tracheid length is caused by a decrease in the length of fusiform initial cells and in intrusive growth, both of which can be caused by a change in plant growth regulators near the vascular cambium (Kurczynska et al. 1998).

Tracheid differentiation is a response to the basipetal and acropetal transport of carbohydrates and growth hormones, which are produced in actively growing needles, meristems of tree crown, and root system. Availability of carbohydrates and plant growth regulators, which are produced within the tree crown and root system, will regulate tracheid differentiation (Larson 1994). Consequently, crown loss may directly modify tracheid features in wood (Larson 1969; Roberts et al. 1988; Aloni 1992). Within the last three decades, *Abies religiosa* trees reduced their crown by nearly 50%, and the chronic exposure to air pollutants damaged palisade cells in their leaves reducing their photosynthetic capacity (Alvarez et al. 1998). One of the possible consequences of this depletion in stem photosynthate accumulation is the change in cambial activity and its derivatives as reported in *Pinus strobus* and *Abies alba* (Bartholomay et al. 1997; Schmitt et al. 2003). Studies on this mechanism are currently under progress.

**CONCLUSIONS**

Tracheid characteristics in latewood: tangential diameter, tangential lumen diameter, wall thickness, and length for *Abies religiosa* showed a tendency to increase with age from pith to bark. However, starting in the 1970s, there was a significant reduction in wall thickness, tracheid length and annual ring width. Tracheid modifications were probably related not to age, but to stress caused by air pollution, especially ozone, the most phytotoxic pollutant occurring at high levels southwest of the Mexico City basin. Tracheid modifications were observable before the first visual symptoms of tree decline for the area were detected. The chronic exposure of *Abies religiosa* trees to atmospheric pollutants during the last decades may have modified different physiological and biochemical pathways, and their effects have probably changed the tracheid size progressively.
ACKNOWLEDGEMENTS

The senior author thanks Consejo Nacional de Ciencia y Tecnología (CONACYT, No. 114367) for a scholarship and the authorities of Mexico City for allowing to conduct this research in Desierto de Los Leones.

REFERENCES


