

CHAPTER 5

INFLUENCE OF ENVIRONMENTAL STRESS FACTORS ON STOMATAL FREQUENCY OF FOSSIL *TSUGA HETEROPHYLLA* NEEDLES FROM MOUNT RAINIER (WASHINGTON, USA).

An extremely low number of stomata per mm needle length is encountered between 300 AD and 700 AD in the *Tsuga heterophylla* record at Jay Bath. These low stomatal numbers do not appear to result from extremely high atmospheric CO₂ levels at the time, but do coincide conspicuously with the establishment of the species during a period of major disturbance at the site. The open, exposed setting in the montane environment after this disturbance probably provided highly stressed growth conditions for the pioneering, early-successional *T. heterophylla* trees. Spring water-stress related to prolonged low soil temperatures, would be the most plausible explanation for an acclimational stomatal frequency response to reduced water uptake. Thus, environmental stress factors associated with early-successional montane habitats show the potential to obscure stomatal frequency changes in response to atmospheric CO₂. This complication should be taken into account when selecting leaf material from high-elevation sites for stomatal frequency analysis. Over the past 1200 years, the presence of a stable late-successional forest at Jay Bath, indicates that the latter part of the stomatal record was not influenced by extreme growth conditions and can thus be regarded as a reliable reflection of atmospheric CO₂ levels.

INTRODUCTION

The inverse relation between numbers of leaf stomata and ambient CO₂ enables the reconstruction of past atmospheric CO₂ levels (e.g. Woodward, 1987; Kürschner et al., 1996; Wagner et al., 1996; Royer, 2001; Royer et al., 2001). Stomatal frequency analysis of leaves buried in peat and lake deposits is therefore increasingly applied as a practical tool for detecting and quantifying short-term changes in the Holocene CO₂ regime (e.g. Rundgren & Beerling, 1999; Wagner et al., 1999a; Wagner et al., 2002).

In addition to leaves of angiosperm tree species, well-preserved needles of a variety of conifers may occur abundantly in Holocene deposits. Because of the long-term dominance of conifers in temperate and boreal forest ecosystems, stomatal frequency analysis of conifer needles may widen the spatial coverage of stomata-based CO₂ reconstructions. Analysis of herbarium material and fossil assemblages has demonstrated that species of *Pinus*, *Picea*, *Tsuga*, *Larix* and *Metasequoia* have the capacity to adjust their stomatal numbers to changing CO₂ regimes (Van de Water et al., 1994; Royer et al., 2001; McElwain et al., 2002; Chapter 2). Recently, analysis of a needle record of *Tsuga heterophylla* (western hemlock) from North America confirmed a centennial-scale CO₂ variability between 800 and 2000 AD (Chapter 4). Within uncertainty limits, CO₂ maxima and minima in the stomata-based reconstruction correlate with global temperature changes based on multi-proxy records, long-term changes in North Atlantic sea surface temperature, as well as terrestrial temperature trends on the Northern Hemisphere derived from tree-ring records (Chapter 4).

The 1200-year needle record of *T. heterophylla* was recovered from sediments of Jay Bath, a shallow pond on the southern flank of Mount Rainier (Washington, USA). These sediments contain rich and diversified needle assemblages. The needles can be identified at a species level, and relative-frequency patterns reflect changes in the composition of the conifer-dominant vegetation during the past 6000 years (Dunwiddie, 1986; 1987). Following the apparent dieback of a forest dominated by *Abies amabilis* around 200 AD, rapid expansion of *T. heterophylla* into the area gave rise, after a few centuries, to the formation of the modern forest dominated by *Tsuga mertensiana*, *T. heterophylla* and *A. amabilis*.

Although generally regarded as a shade-tolerant, late-successional species, *T. heterophylla* can be found in all stages of succession. It is an aggressive pioneer because of its quick growth in full overhead light and its ability to survive on a wide variety of seedbed conditions (Packee, 1990). In general, mean stomatal frequency in *T. heterophylla* is not affected significantly by environmental variables other than CO₂ (Chapter 2). However, it should be noted that under suboptimal growth conditions, the effect of CO₂ on stomatal frequency of conifer needles may be obscured by the influence of environmental stress factors. Observations on needles of *Pinus*, *Abies*, and *Picea* along altitudinal gradients (Hultine and Marshall, 2000; Schoettle and Rochelle, 2000; Qiang et al., 2003), suggest that adverse montane growth conditions inhibit formation of needle stomata as an acclimational response to restrict water loss.

At the flanks of Mount Rainier, growth conditions of *T. heterophylla* in open, exposed early-successional habitats are likely to be different from those in the sheltered, late-successional closed forest. In order to assess the reliability of CO₂ reconstructions on the basis of *T. heterophylla* needles, it seems essential to determine whether or not stomatal characteristics are influenced by environmental stress factors associated with early-successional habitats in montane areas. In the present paper we therefore extend the stomatal frequency analysis of the Jay Bath needle assemblages to the onset of the *T. heterophylla* record around 200 AD.

MATERIAL AND METHODS

A 91-cm sediment core containing conifer needles was obtained from Jay Bath, a shallow (about 1.20 m water depth) pond, situated at an altitude of 1311 m on the southern flank of Mount Rainier (Washington, USA; 46°46' N 121°46' W; Fig. 5.1). One-cm thick sediment samples were sieved on a 250µm mesh sieve and the encountered macrofossils were identified [conifer needles were identified at species level (Dunwiddie, 1985), seeds at the genus level] and stored in ethanol. Residues were checked for charcoal fragments and mineral grains.

Needles of *Tsuga heterophylla* were isolated for stomatal analysis. They were bleached with a 4% sodiumhypochloride solution to remove the mesophyll. The remaining cuticle was then stained with safranin and mounted in glycerin jelly on a microscopic slide. Computer-aided measuring of epidermal cell parameters on needle cuticles was performed on a Leica Quantimet 500C/500+ Image Analysis system (Wetzlar, Germany). Stomatal frequency was measured as the number of stomata per millimeter needle length, (TSDL: for analytical details, see Chapter 2). Pore length was measured on 30 stomata per needle at a magnification of ×640.

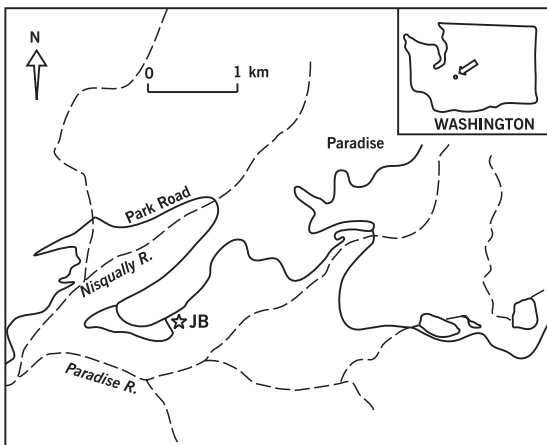


Figure 5.1: Location of Jay Bath (JB) on Mount Rainier, Pierce County, Washington (Reproduced from Dunwiddie, 1986).

Maximum stomatal conductance G_{max} is mainly determined by the density and geometry of stomata and can be calculated as $1/R$ (R = stomatal resistance; Jones, 1995); and according to Parlange and Waggoner (1970):

$$R = 1/nD (d/Bab + \ln(4ab/Ba)) \tag{Eqn. 1}$$

where a (m) represents the major axis radius (PL), b (m) the minor axis radius, D (m^2/s) the diffusive coefficient of CO_2 in air, d (m) length of the diffusive pathway i.e. the depth of the stomatal tube plus the diameter of the substomatal cavity and n the stomatal density (n/m^2).

Age-depth relations for the sediment core were determined by fitting a 4th order polynomial through a series of ten AMS ^{14}C chronologies (calibrated using OxCal 3.8 [Bronk-Ramsey, 1995] and INTCAL98 calibration data [Stuiver et al., 1998]) and two dated tephra layers (Yamaguchi, 1983; Mullineaux, 1996). In this way an average sedimentation rate of one cm per 26 years was obtained for the core (Fig. 5.2).

Student's t-tests and regression analysis was performed using SPSS 10.0 for Windows statistical software (Chicago, Illinois, USA).

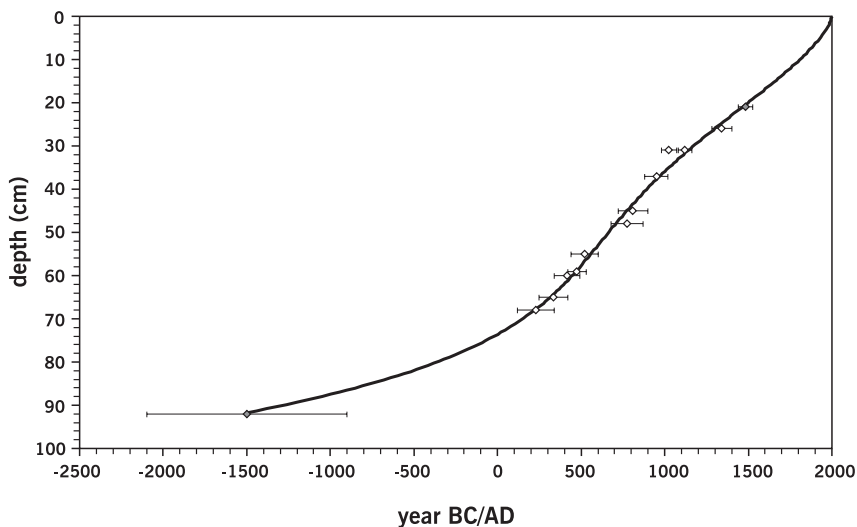


Figure 5.2: Age-depth diagram for Jay Bath sediment core. White diamonds represent AMS dates, converted to calendar age using OxCal 3.8 [Bronk-Ramsey, 1995] and INTCAL98 calibration data [Stuiver et al., 1998]. Error bars indicate 95.4% probability interval. Grey diamonds represent dated tephra layers from nearby Mt. St. Helens (Yamaguchi, 1983; Mullineaux, 1996). Black line is the most likely age-depth model, a 4th order polynomial function [$AGE = -0.00020094 \times (DEPTH)^4 + 0.02951 \times (DEPTH)^3 - 1.33288 \times (DEPTH)^2 - 8.71619 \times (DEPTH) + 2000.22$; $r^2 = 0.9977$].

RESULTS

Stomatal frequency record

TSDL and pore length measurements, calculated maximum stomatal conductance are summarized in Appendix A2. TSDL ranges between 159 and 266 stomata per millimeter needle length (Fig. 5.3A). Stomatal frequency (TSDL) shows centennial scale variations along the depth of the core. Extremely low TSDL values occur over the last 200 years and between 300 and 700 AD.

Average pore length ranges from 24.8 to 31.2 μm (Fig. 5.3B). TSDL and pore length are overall negatively correlated ($\text{PL} = -0.0275 \times \text{TSDL} + 33.74$; $r^2 = 0.2375$).

The calculated maximum stomatal conductance (G_{max}) varied between 8.5 and 15.1 mm s^{-1} (Fig. 5.3C). The periods before 750 AD and after 1800 AD are characterized by low G_{max} .

CO₂ reconstruction

By using the relation between stomatal density per mm needle length and atmospheric CO₂ mixing ratios as quantified in a training set over the last century, stomatal frequency fluctuations of *T. heterophylla* needles from Jay Bath can be converted to CO₂ levels (Fig. 5.4). The calculated CO₂ record starts with values around 300 ppmv between 200 and 300 AD, but rises drastically up to 390 ppmv within 100 years. Over the next 300 years CO₂ levels decline again to values that strongly fluctuate around the average pre-industrial level of 280–290 ppmv. A centennial-scale CO₂ variability is punctuated by minima centred around 860, 1150, 1600, and 1800 AD, and maxima around 1000, 1300, and 1700 AD. After 1850 AD there is a sharp rise from 280 ppmv to a modern CO₂ value of 370 ppmv (see also Chapter 4).

Macrofossil record

Figure 5.5 shows the presence of needles of selected conifer species in the Jay Bath core since the deposition of the Mt. St Helens Y-ash layer at approximately 1400 yr BC. Accessory elements (not depicted) are *Chamaecyparis nootkatensis* and *Pinus monticola*. Relative frequencies are expressed as a percentages of the total needle sum (see Dunwiddie, 1986). An important change in the composition of needle assemblages takes place between 200 and 300 AD, when dominance of needles of *Abies amabilis* is rapidly taken over by needles of *Abies procera* and *Tsuga heterophylla*. There are no large fragments of charcoal in the investigated samples; coarse mineral grains occur in the 200–300 AD interval.

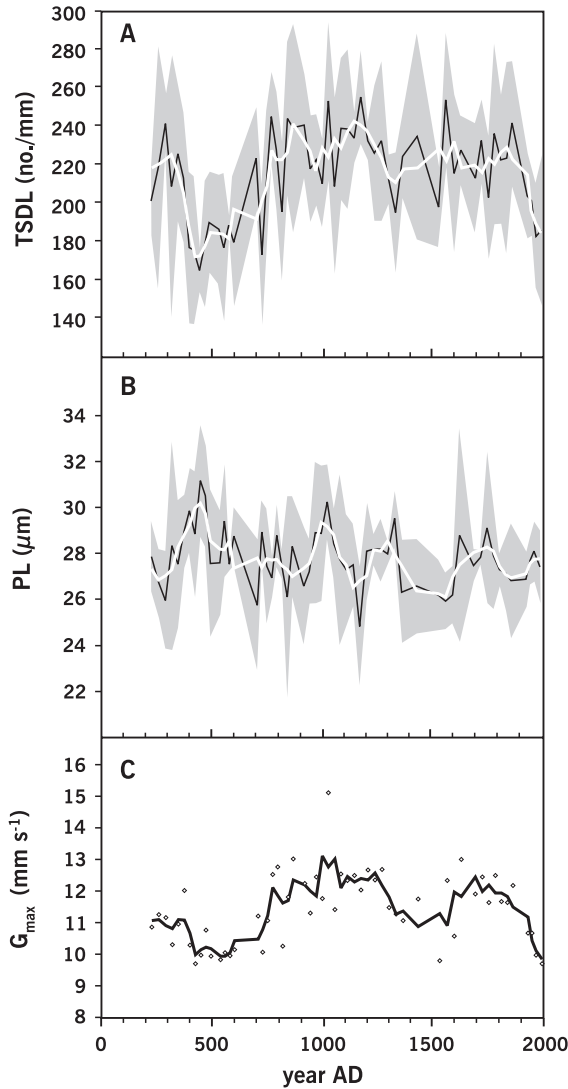


Figure 5.3: **A:** Stomatal density per mm needle length (TSDL, in n/mm) of *Tsuga heterophylla* needles from Jay Bath (Washington, USA). Black line represents mean values of 3–5 needles per depth interval. The grey band indicates ± 1 se. The white line represents a 3 point moving average. **B:** Pore length (PL in μm) of *Tsuga heterophylla* needles from Jay Bath (Washington, USA). Black line represents mean values of 3–5 needles per depth interval. The grey band indicates ± 1 se. The white line represents a 3 point moving average. **C:** Estimated maximum stomatal conductance (G_{max} in $mm s^{-1}$) based on TSDL and PL measurements of figure 5.2, using equation 1 ($G = 1/R$; $R = 1/nD (d/\pi ab + \ln(4ab/\pi a))$; $a = PL$ (m); b was not directly measured, but calculated as $b = 0.109 \times a + 5.3184 \times 10^{-6}$ (measured in 50 stomata), D (m^2/s) is the diffusive coefficient of CO_2 in air ($1.47 \times 10^{-5} m^2 s^{-1}$ at $20^\circ C$ and 101.3 kPa), d (m) is the length of the diffusive pathway (not measured, but estimated as

0.1 mm) and n the stomatal density (n/m^2). Because in conifers stomata are not distributed uniformly on the leaf, TSDL is expressed per mm needle length. Needles of *Tsuga heterophylla* are ± 2 mm in width, so n (n/m^2) was calculated as $TSDL$ (n/mm^{-1}) $\times 0.5$ mm^{-1} (needles per mm) $\times 10^6$. White diamonds are average conductance per depth (3–5 needles), black line is a 3 point moving average. Calculated G_{max} is in the same order of magnitude as measured G_{max} of *T. heterophylla* (1.08 mm s^{-1} ; Korol, 2001). The deviation between estimated and measured values may be attributed to underestimation of d (length of the stomatal pathway), partial or patchy closure of stomata during the measurements, the additional resistance of the boundary layer, which was not included in the estimations, and probably also the difference in altitude and environment between Jay Bath and the experimental forest sites in Montana and Idaho.

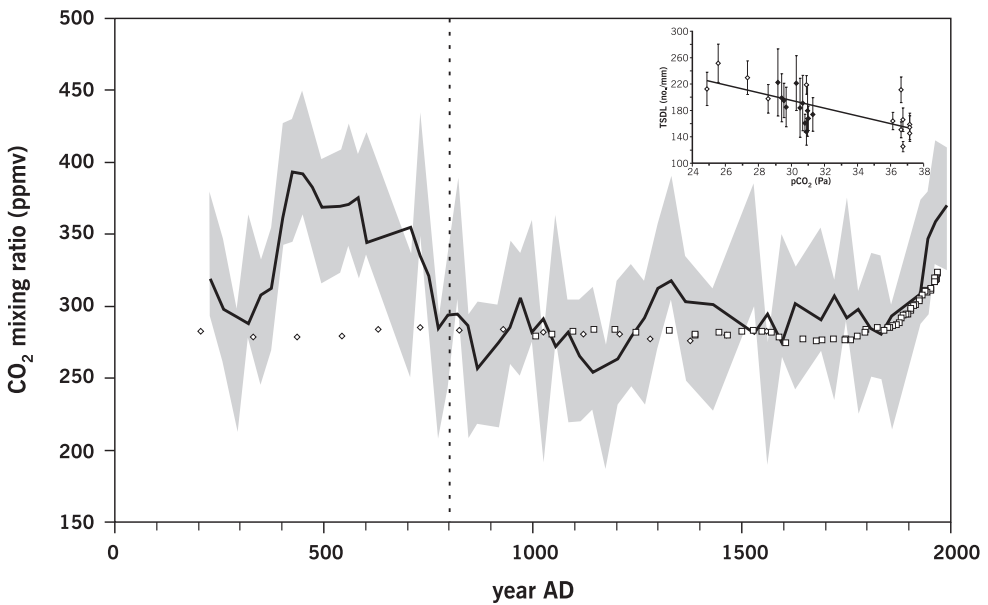
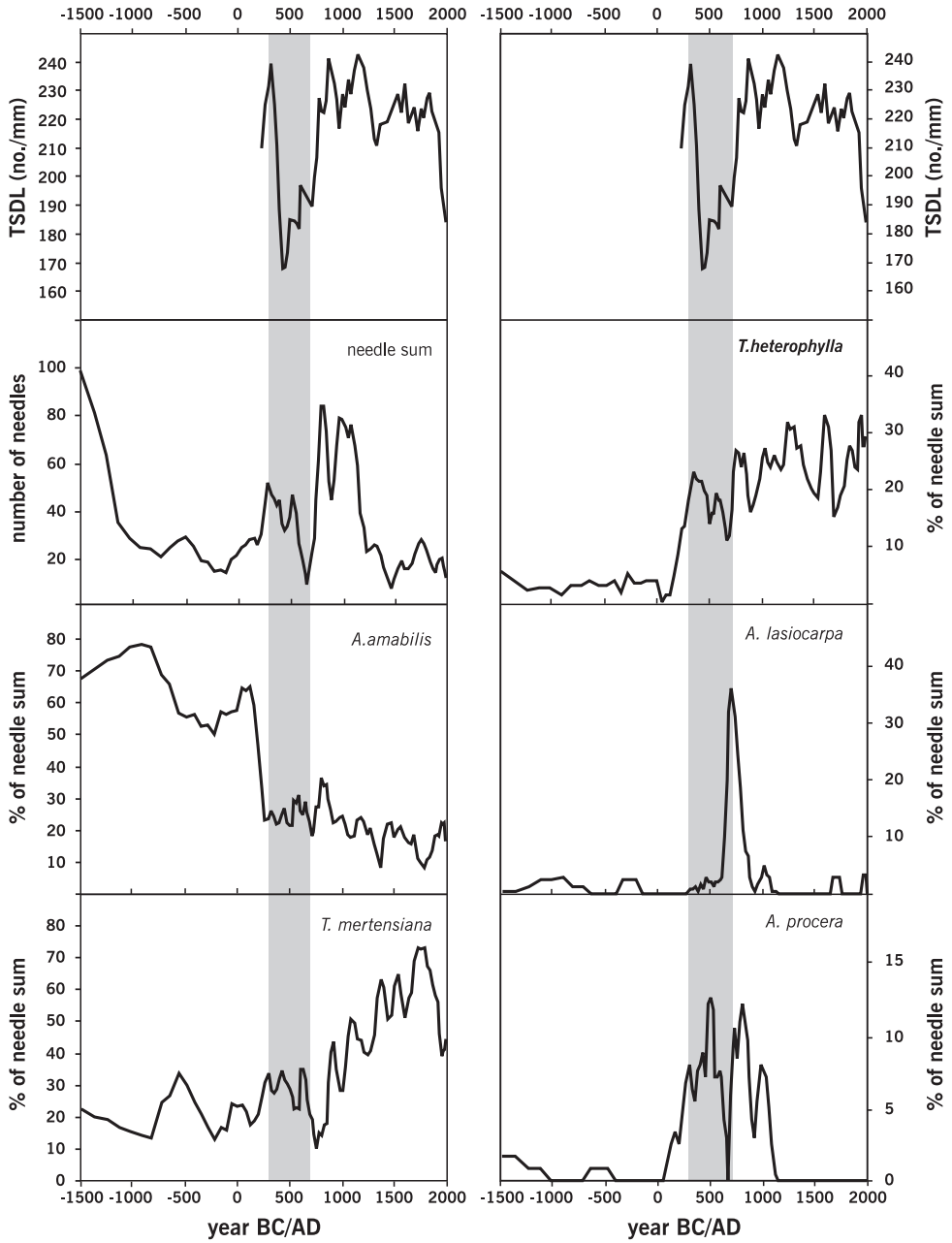


Figure 5.4: Reconstruction of paleo-atmospheric CO_2 levels when stomatal frequency of fossil needles is converted to CO_2 mixing ratios using the relation between CO_2 and TSDL as quantified in the training set. Black line represents a 3 point running average based on 3–5 needles per depth. Grey area indicates the RMSE in the calibration. White diamonds are data measured in the Taylor Dome ice core (Indermühle et al., 1999); white squares CO_2 measurements from the Law Dome ice-core (Etheridge et al., 1996). Inset: Training set of TSDL response of *Tsuga heterophylla* needles from the Pacific Northwest region to CO_2 changes over the past century (Chapter 4).



DISCUSSION

The sharp rise in the stomata-based CO₂ curve after 1850 AD corresponds to the industrial CO₂ increase apparent in instrumental records (Keeling and Whorf, 2002) and shallow ice-cores (Neftel et al., 1985). This correspondence corroborates the reliability of the use of *T. heterophylla* needles in the reconstruction of past atmospheric CO₂ levels. The reconstructed fluctuations over the preceding 1200 years of the record could be linked to climate changes in this time period (Chapter 4) and have been reproduced for a significant part in a data set based on oak leaves from the Netherlands (Van Hoof, in prep.).

When going further back in time, due to extremely low stomatal frequency values in *T. heterophylla* needles, the reconstructed curve would suggest a major CO₂ excursion of about 100 ppmv between 300 and 750 AD. In the following paragraphs the reality of this excursion is discussed in relation to other CO₂ proxy records, documented global climate change, local CO₂ production, regional climate variation, and local stand dynamics.

CO₂ data from ice cores

Figure 5.4 shows the atmospheric CO₂ curve inferred from *T. heterophylla* needles plotted together with CO₂ data derived from the Antarctic Law Dome and Taylor Dome ice-cores (Etheridge et al., 1996; Indermühle et al., 1999a). Although centennial-scale CO₂ fluctuations of the last millennium are not apparent in the ice-core data, a mean value of about 280 ppmv in the stomata-based reconstruction corresponds to the long-term pre-industrial record from Antarctic ice. The absence of centennial-scale fluctuations in the ice-core reconstructions may be explained by varying age distributions of the air in the bubbles related to the enclosure time in the firn-ice transition zone (Schwander, 1996; Spahni, et al., 2003) and/or post-depositional physicochemical reactions in the ice that may increase as well as decrease the CO₂ concentration in air bubbles (Anklin et al., 1995; Stauffer and Tschumi 2000).

In contrast to the last millennium, CO₂ reconstructions before 800 AD show a marked discrepancy between ice-core data and the needle-based record. Whereas ice cores continue to show CO₂ values of about 280 ppmv, stomata-based CO₂ levels seem to be consistently well above 300 ppmv during a period of 400 years. The magnitude of the CO₂ excursion (in-

Figure 5.5: Abundance of selected conifer species in the sediment core from Jay Bath. Values on y-axis are the percentage of needle equivalents of the selected species [entire needles, or combinations of top, midsection and base approximately equalling a needle in length (Dunwiddie 1986)] relative to the total needle sum. Three point running means are shown to facilitate comparison to the fossil needle record of Dunwiddie (1986), which was based on 2.5 cm slices of sediments. Because needles of *C. nootkatensis* and *P. monticola* break up in small fragments and are thus difficult to express in needle equivalents, these species were not included in the figure. Depicted at the top of both columns is the 3 point running average of the TSDL record. Grey area indicates the period of abnormally low stomatal numbers.

crease of 100 ppmv to a maximum level of almost 400 ppmv), as well as the exceptional high rate of the increase (about 10 ppmv / 10 years, which is twice the rate of the anthropogenic CO₂ increase during the past 200 years), question the authenticity of this CO₂ event.

Global temperature records

Reconstructed CO₂ fluctuations over the last 1200 years in the Jay Bath record correlate broadly with global temperature changes based on multi-proxy records (Mann and Jones, 2003), most remarkably in the timing of the warm periods and the CO₂ maxima around 1000 and 1300 AD (Fig. 5.6A; see also Chapter 4). Within uncertainty limits, changes in North Atlantic Ocean sea surface temperature as recorded offshore West Africa (DeMenocal et al., 2000) and North America (Cronin et al., 2003) are synchronous with CO₂ maxima and minima in the stomata-based record (Chapter 4). This also applies to terrestrial temperature trends on the Northern Hemisphere derived from tree-ring records (Briffa, 2000; Esper et al., 2002; Chapter 4). These correlations suggest that CO₂ fluctuations over the last millennium at least partly originated from temperature-driven changes in CO₂ flux between ocean surface waters and atmosphere (Chapter 4).

A prolonged period with elevated CO₂ levels between 300 and 750 AD, on the other hand, would not match reconstructed temperature trends. Although there is some evidence for relatively high Southern Hemisphere temperatures between 500 and 750 AD (Mann and Jones, 2003), the global record shows no indication of pronounced warming during this period (Fig. 5.6A).

Since the reconstructed enhanced CO₂ levels between 300 and 750 AD are incongruent with global climate changes, the extremely low stomatal frequency of *T. heterophylla* in this period is unlikely to reflect pronounced changes in the global atmospheric CO₂ regime.

Local volcanic CO₂ production

Because Mount Rainier is a currently active volcano, volcanically produced CO₂ could potentially be responsible for enhanced CO₂ levels in the Jay Bath area. It is known in other parts of the world, that plants can be exposed to natural elevated CO₂ concentrations, due to their proximity to CO₂-emitting springs and vents. Such sites contain local communities that may have persisted for generations at extreme CO₂ levels (reported values as high as 35,000 ppmv). However, stomatal frequency responses are not always apparent (Miglietta and Raschi, 1993; Tognetti et al., 2000). As a result of the non-linear nature of the relation between stomatal frequency and atmospheric CO₂ many species may already have reached their response limit at present-day CO₂ levels, so that further CO₂ increase cannot be detected on the basis of stomatal frequency analysis (Kürschner et al., 1997). On the other hand, there are also species in which excessive CO₂ has caused significant reduction of the stomatal frequency (Fernandez et al., 1998; Tognetti et al., 2000).

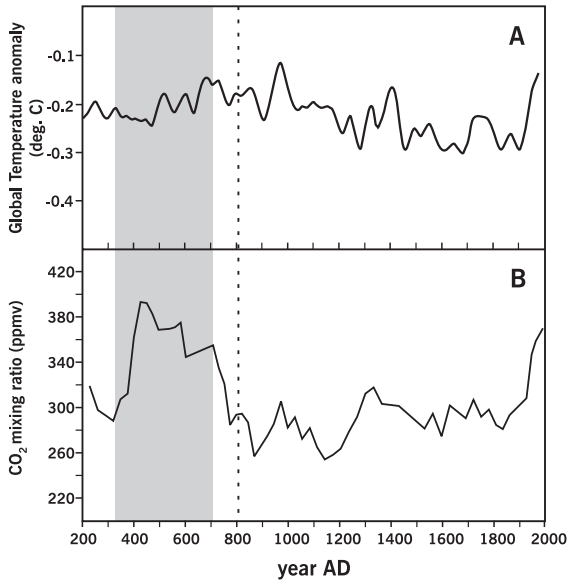


Figure 5.6: **A:** Global mean temperature anomaly from multi-proxy records based on 1961–1990 reference period (Mann and Jones, 2003) **B:** Reconstruction of paleo-atmospheric CO₂ levels based on stomatal frequency analysis of fossil *Tsuga heterophylla* needles. Black line represents a 3 point running average based on 3–5 needles per depth.

The question whether or not stomatal frequency data from Jay Bath could have been influenced by local CO₂ emissions is discussed in Chapter 6 on the basis of carbon-isotope data from the studied sediment core. Over the past two millennia, there are no signals of excess volcanogenic CO₂ in the $\delta^{13}\text{C}$ record of organic matter. Moreover, no indication of a volcanic imprint on the ¹⁴C measurements was found, while incorporation of significant amounts of volcanogenic CO₂ during photosynthesis would have caused an artificial aging in the order of at least a thousand years. Thus, interpretations of the stomatal frequency record from Jay Bath are not compromised by effects of Mount Rainier volcanic activity.

Regional climate variation

The expansion of *T. heterophylla* on Mount Rainier might have been associated with an increase in summer temperatures and/or a longer growing season. However, conifer taxa like *T. mertensiana*, adapted to colder/drier conditions, do not show a concurrent decline (Dunwiddie, 1986). The measured increase in temperature of 1 °C during the first half of the last century did not affect stomatal numbers of *T. heterophylla* (Chapter 2).

Also pollen records from Mount Rainier do not show any significant vegetation changes that may reflect drastic changes in temperature or precipitation regimes during the last two millennia (Dunwiddie, 1986). It is unlikely, therefore, that low stomatal frequency of *T. heterophylla* between 300 and 750 AD could be the result of regional climate variation. Moreover, it should be noted that in experimental studies stomatal indices were not influenced by temperature (Reddy et al., 1998), or only after extreme increase that could not occur in nature (Wagner, 1998).

Local Stand Dynamics

Ecology of conifers on Mount Rainier

Jay Bath is located in the upper range of the *Abies amabilis* vegetation zone (Dunwiddie, 1986). In this zone, ranging from 900-1600 m altitude at Mount Rainier, late-successional forests are currently dominated by *A. amabilis* and *Tsuga mertensiana* as shade-tolerant competitors (Franklin and Dyrness, 1973). *Tsuga heterophylla* is very shade-tolerant as well and generally regarded as a late-successional element. However, the species also grows fast in full overhead light and can thus be present in both early and late successional stages (Fischer and Bradley, 1987; Packee, 1990).

Abies lasiocarpa is prominent in late-successional stages together with *T. mertensiana* in the *T. mertensiana* vegetation zone at higher elevations, because it is very tolerant of harsh conditions with short growing seasons, summer frost and heavy snowpacks at higher elevations. In lower elevation settings in the Cascade Range, such as Jay Bath, *A. lasiocarpa* can chiefly be found as a shade-intolerant species in early successions, invading disturbed sites (Franklin and Mitchell, 1967; Franklin and Dyrness, 1973). In temperate, high moisture conifer forests, *Abies procera* is dominant in early conifer successions, as it is unable to grow under closed canopies (Franklin and Dyrness, 1973; Stewart, 1986).

Late Holocene vegetation at Jay Bath

In contrast to pollen records that reveal regional vegetation history, macrofossil records reflect changes in the local vegetation around the pond. The relative abundance of needles of the different species in the sediment provides a reliable estimate of their respective basal area at the locality (Dunwiddie, 1987).

Vegetation development at Jay Bath since 4000 BC based on pollen and needle records has previously been described by Dunwiddie (1986). In the period after the deposition of the basal lahar (volcanic mudflow), an early successional vegetation consisting of *Pinus* spp., *Pseudotsuga menziesii*, *Abies lasiocarpa*, and *A. procera* occupied the newly formed landscape. *Abies amabilis* gradually became more prominent, but the continued presence of these disturbance-tolerant species, typical of early successions, suggest that the vegetation at the stand was subjected to frequent fires under a warmer and/or more arid climatic regime, as supported by regular charcoal finds.

The macrofossil record in the present study (Fig 5.5), starting immediately after deposition of the Y-tephra at approximately 1400 BC, is in very good agreement with Dunwiddie's (1986) results, but has a higher resolution and enhanced chronological accuracy. After the Y-tephra deposition, the needle record reflects the presence of a late-successional forest dominated by *A. amabilis* and *T. mertensiana* and without any disturbance-tolerant species. Before 200 AD *T. heterophylla* is sparsely present. But following a significant decline of *A. amabilis*, the element becomes prominent at the site. The concomitant expansion of *Abies procera* and, subsequently, *A. lasiocarpa* in the record between 300 and 1000 AD indicates forest succession after a major disturbance. Consequently, the initial expansion of *T.*

heterophylla can be related to its pioneering abilities in an open, early-successional habitat (Packee, 1990). After this period, the lack of disturbance-related tree species reflects highly stable late-successional habitats over the last 1300 years up to the present-day forest of *T. mertensiana*, *T. heterophylla*, *A. amabilis*, and *Chamaecyparis nootkatensis* (the latter species is not included in the macrofossil diagram).

Timing and nature of the disturbance

Disturbance, reflected by the decline of *A. amabilis* and the proliferation of *A. procera*, becomes apparent between 200 and 300 AD. Establishment of *A. procera* requires major stand openings. The trees generally live for 400–600 years, occasionally persisting under closed canopies, where they are unable to regenerate (Franklin and Dyrness, 1973). An open stand structure probably prevailed at Jay Bath until 600–700 AD. *A. lasiocarpa* reaches its peak between 600 and 800 AD. These trees are much slower growing and probably did not reach sufficient height to contribute significantly to the needle record until they had reached an age of about 100 years (Alexander et al., 1984), which suggests that the species established at the site around 500 AD. Because *A. lasiocarpa* trees usually die before they are 250–400 years old (Alexander, 1987), no more seedlings germinated after around 700 AD.

A. lasiocarpa is very tolerant of short growing seasons, frosts and heavy snowpack, and seeds need overwintering in or under snow to germinate. The inability of *A. lasiocarpa* seedlings to survive after 700 AD could be related to amelioration of harsh, exposed local conditions by forest closure at the stand, enabling *T. heterophylla*, *T. mertensiana*, and *A. amabilis* to outcompete *A. lasiocarpa*. The timing of reconstructed presence of *A. procera* and *A. lasiocarpa* at Jay Bath indicate that open conditions prevailed at Jay Bath after the disturbance at around 300 AD, and that a closed forest stand was not re-established until 700 AD. Open conditions at the site are confirmed by the presence of coarse-grained mineral material in the sediment at the time of initial vegetation change.

Various types of disturbances may affect forest composition on Mount Rainier. Large catastrophic fire is the main large-scale disturbance factor. In general, fires occur once every 400–500 years (Hemstrom and Franklin, 1982). Included in estimates of the fire regime are fires set by Indians. Indian tribes inhabited the surrounding area for the last 10,000 years, using fire to concentrate game, increase berry harvest and improve hunting visibility and grazing. Such fires could have reached Mount Rainier, but there is some doubt whether man-made fire was important in the western Cascades (Hemstrom and Franklin, 1982). European settlers did not arrive until the 19th century.

No large charcoal fragments evidencing local fires have been found in the sediment record after the Y-ash. Absence of fire may be due to the cooler and moister climate compared to that of the pre-Y ash period (Dunwiddie, 1986). Other important forest disturbances that could have affected local stand structure, include avalanches, lahar flows, windthrow and

pathogens such as insects or woodrot (Hemstrom and Franklin, 1982). Core data from Jay Bath exclude avalanches or lahars. Because of the apparently selective dieback of *Abies amabilis*, pathogens could be a realistic option.

Effect of post-disturbance conditions on stomatal numbers of T. heterophylla

Although the exact nature of the disturbance can not be established, the local vegetation record clearly shows that *T. heterophylla* initially became prominent in an early-successional open habitat. The period of renewed forest establishment coincides with lower stomatal numbers on fossil *T. heterophylla* needles (indicated by the grey area in Fig 5.5). As soon as a stable, late-successional forest stand was present again, stomatal numbers reach their average values over the past 1200 years. It may therefore be hypothesized that the lower stomatal numbers were a response to growth in open, exposed conditions instead of a sheltered closed forest.

In the central Rocky Mountains (Hultine and Marshall, 2000; Schoettle and Rochelle, 2000), and the Qilian Mountains in China (Qiang et al., 2003) it was demonstrated that the stomatal frequency of *Pinus flexilis*, *P. contorta*, *Abies lasiocarpa* and *Picea crassifolia* is influenced by suboptimal growth conditions. Stomatal frequency decreases towards the upper elevational limits of the species. In *P. crassifolia* the decrease, concerning both stomatal density and number of stomatal rows, follows an expected increase that corresponds to decreasing CO₂ partial pressure (Qiang et al., 2003). The observed decrease has been related to adverse growing conditions that characterize high altitudes, more particularly to water stress (Schoettle and Rochelle, 2000).

Open habitats have a much more extreme growing environment than closed forest stands, with higher temperature gradients, lower soil temperatures, a more severe frost regime, lower humidity and no shelter from high (UV)-irradiance (Tucker et al., 1987; Man and Lieffers, 1999). Although influence of factors such as UV-stress and wind stress can not be ruled out, water stress related to prolonged chilling of root systems seems to be the most prominent environmental stress factor that could explain low stomatal frequency and low stomatal conductance of *T. heterophylla*, when growing in open montane habitats. Boreal and montane conifers are particularly sensitive to adverse effects of low (< 8°C) soil temperature on tree water uptake. Growth-chamber and field experiments with *Picea engelmannii* (De Lucia, 1986) and *Pinus sylvestris* (Mellander, 2003; Strand et al., 2002) indicate that root chilling will reduce water uptake and net photosynthesis in the critical transition of winter dormancy to the growing season. Prolonged exposure of roots to soil temperatures between 0°C and 1°C strongly reduces stomatal conductance.

In the Mount Rainier region, low soil temperature in the early growing season is probably the main limitation for conifer growth in open early-successional habitats. Mount Rainier is known for heavy snowfall (Graumlich and Brubaker; 1986). In an open vegetation on the southern flank of the mountain, snowpack cover is anticipated to continue into the active growth phase of plants. In order to successfully compete in such habitats, trees

should be equipped with a plastic phenotype capable of adjusting stomatal numbers to prevent excess water loss during the spring. The fact that *T. heterophylla* is competitive in both early- and late-successional habitats is consistent with the wide physiological plasticity of the species.

CONCLUSIONS

The extremely low number of stomata per mm needle length in the *Tsuga heterophylla* record at Jay Bath between 300 and 700 AD does not appear to result from extremely high atmospheric CO₂ levels at the time, but coincides with the establishment of the species during a period of major disturbance at the site. The open, exposed setting after this disturbance probably provided highly stressed growth conditions for pioneering, early-successional *T. heterophylla* trees. Spring water-stress related to low soil temperature, would be the most plausible explanation for an acclimational stomatal frequency response to reduced water uptake.

Thus, environmental stress factors associated with early-successional montane habitats show the potential to obscure stomatal frequency changes in response to atmospheric CO₂. This complication should be taken into account when selecting leaf material from high-elevation sites for stomatal frequency analysis. It is essential to concomitantly analyze the local successional forest developments that correspond to a montane leaf record. For the Jay Bath record, the late-successional closed forest, prevalent at the site from 800 AD until present, indicates that stomatal numbers of *Tsuga heterophylla* over the past 1200 years are not affected by these extreme growth conditions, and can be relied upon to reflect atmospheric CO₂ changes.

