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VARIATIONS OF WOOD $\delta^{13}C$ AND WATER-USE EFFICIENCY OF *ABIES ALBA* DURING THE LAST CENTURY

Didier Bert,[1,3] Steven W. Leavitt,[1] and Jean-Luc Dupouey[2,4]

[1]*Laboratory of Tree-Ring Research, University of Arizona, Tucson, Arizona 85721 USA*
[2]*Ecophysiology Unit, INRA-Nancy, 54280 Champenoux, France*

Abstract. Variations of intrinsic water-use efficiency during the last century were investigated based on analysis of $\delta^{13}C$ in tree rings of *Abies alba* from the Jura Mountains (eastern France). To separate the effects related to the age of the tree at the time the tree ring was formed from effects due to environmental changes, analyzed wood samples were extracted from a very large sample set including different tree ages and calendar dates of wood formation.

For the first 75 yr of the life of *Abies alba*, $\delta^{13}C$ of wood holocellulose increases with the age of the tree from $-24.4‰$ at age 15 to approximately $-22.5‰$ at age 75. Between the ages of 75 and 150 values remain constant at $-22.5‰$. Consequently, the effect of the tree age on isotopic discrimination has to be taken into account in studies on the long-term environmental effects on $\delta^{13}C$ in tree rings.

Divergent trends of $\delta^{13}C$ during the last century were observed between tree rings formed at age 40 and bulk air data. The isotopic discrimination Δ varied insignificantly around a mean of $17.3‰$ between the 1860s and the 1930s. It then decreased to $15.8‰$ from the 1930s to the 1980s. Using these results and classical models of carbon discrimination, we calculated that the intrinsic water-use efficiency (A/g_w, the ratio of CO_2 assimilation rate to stomatal conductance for water vapor), integrated over the year, has increased by 30% between the 1930s and the 1980s. These results, obtained at the level of mature trees, are consistent with the physiological effects of increasing CO_2 concentrations as observed in controlled experiments on young seedlings. They are consistent with the strong increases in radial growth observed for *Abies alba* in western Europe over the past decades. However, other long-term environmental changes such as increasing nitrogen deposition could cause similar effects.

Key words: Abies alba; $^{13}C/^{12}C$ ratio; CO_2 fertilization; dendrochronology; isotopic discrimination; long-term trend; radial growth; stable-carbon isotopes; tree rings; water-use efficiency.

INTRODUCTION

The isotopic composition of tree rings carries an integrated annual record of environmental conditions. This was recognized early by Epstein and Yapp (1976) who found a strong correlation between the Deuterium/Hydrogen ratio in tree-ring cellulose and temperature. Annual variations in the carbon discrimination in tree rings were then also related to temperature and precipitation (Francey and Farquhar 1982, Leavitt and Long 1983). However, models of carbon discrimination during carbon fixation show that C_3 plants are not passive recorders: the carbon isotopic variations are subjected to strong physiological control through leaf gas exchange regulation (Francey and Farquhar 1982). Therefore, records of carbon discrimination in tree-ring cellulose could be used to study past variations of the ecophysiology of trees in reaction to environmental

variations, in addition to the reconstruction of past environments. In this paper, we first present the theoretical background for such studies. We then analyze trends observed for silver fir (*Abies alba* Mill.), a common conifer in European mountains, over the last century.

The isotopic composition of a carbon compound is expressed as $\delta^{13}C$, the proportional deviation of the $^{13}C/^{12}C$ ratio from the internationally accepted Peedee belemnite (PDB) carbonate standard (Craig 1957):

$$\delta^{13}C = \left(\frac{^{13}C/^{12}C_{sample}}{^{13}C/^{12}C_{PDB}} - 1 \right) \times 1000‰. \quad (1)$$

The $\delta^{13}C$ of atmospheric CO_2, δ_a, has a current value of about $-8‰$. During carbon fixation some fractionations associated with physical and enzymatic processes lead organic matter to be ^{13}C depleted in comparison with the air. Hence, plant material $\delta^{13}C$ of C_3 plants, δ_p, ranges from $-22‰$ to $-34‰$. This carbon discrimination by the plant is expressed as:

$$\Delta = \frac{\delta_a - \delta_p}{1 + \delta_p}. \quad (2)$$

This depletion is regulated by the rate at which CO_2 diffuses into the leaf and is fixed by ribulose-1, 5 bisphosphate carboxylase/oxygenase (RuBisCO). Models of discrimination (Farquhar et al. 1982, 1989) show that Δ is related to the ratio between the internal gas phase pressure of CO_2, P_i, and the atmospheric partial pressure, P_a:

$$\Delta = a + (b - a)\frac{P_i}{P_a} - d, \qquad (3)$$

where a is the discrimination due to diffusion of CO_2 in the air (4.4‰), b is the discrimination due to carboxylation by RuBisCO (30‰), and d is a term related to a variety of factors (respiration, liquid-phase diffusion, etc.), often taken as a constant of 1‰ or sometimes neglected. In addition, net photosynthesis, A, measured as CO_2 uptake, and leaf conductance to CO_2, g_c, are linked by Fick's law:

$$A = g_c(P_a - P_i). \qquad (4)$$

Given that g_w, the leaf conductance to water vapor, is 1.6 g_c, and using Eqs. 3 and 4, discrimination can be related to the ratio A/g_w (intrinsic water-use efficiency; Ehleringer et al. 1993) by

$$\Delta = a - d + (b - a)\left[1 - \frac{1.6}{P_a}\left(\frac{A}{g_w}\right)\right]. \qquad (5)$$

This ratio is a component of plant transpiration efficiency, W, the long-term expression of biomass gain with respect to water loss at the whole-plant level (Guehl et al. 1994):

$$W = \frac{A}{g_w}\left(\frac{(1 - \Phi_c)}{v(1 + \Phi_w)}\right) \times \frac{2}{3k}, \qquad (6)$$

where Φ_c is the fraction of carbon lost by respiration, fine root mortality, or exudation, v is the relative water-vapor pressure difference between the leaf and the atmosphere, Φ_w the loss of water not associated with uptake of carbon dioxide, and k the plant carbon concentration.

Using this set of equations, the bio-indicative value of tree-ring $\delta^{13}C$ in environmental studies can be used in different ways. For example, using Eq. 2 and assuming a stable Δ value over the long term, it has been possible to estimate δ_a and thus carbon fluxes at the global level from δ_p measurements (see, e.g., Stuiver 1978, Leavitt and Long 1986). In addition, direct atmospheric measurements of δ_a and indirect long-term estimates from sources other than tree rings (C_4 plants) are now available. Therefore, Eq. 2 can also be used in a reverse mode in order to study long-term changes of Δ and thus of intrinsic water-use efficiency (Eq. 5) from measurements of δ_a and δ_p.

The period of rapid environmental change during the last 100 yr could have promoted a shift in the physiology of trees. Increasing CO_2 has been shown to strongly increase intrinsic water-use efficiency of woody plants under experimental conditions (Easmus and Jarvis 1989, Ceulemans and Mousseau 1994). The retrospective study of $\delta^{13}C$ in tree rings offers the opportunity to scale up these greenhouse observations to the forest level. Moreover, strong increasing trends of growth have been reported for a number of species since the beginning of the century (Becker et al. 1995), and especially for silver fir in western Europe (Becker 1989, Bert 1992). Such tree productivity changes could have been accompanied by changes in water-use efficiency.

However, when dealing with long-term changes of tree physiological functioning as recorded in tree rings, it must be taken into consideration that some of the observed changes are related to the age of the tree, independent of any long-term environmental changes. It has been repeatedly observed that wood formed when the tree was young is significantly more ^{13}C depleted than wood formed at later stages of tree life (Freyer 1979, Francey and Farquhar 1982). This is often referred to as the "age" or "juvenile" effect. Thus, an adequate sampling scheme has to be designed in order to avoid possible confusion between the effects of environmental changes and the effects of tree aging on Δ.

In this study we report long-term changes in intrinsic water-use efficiency during the last 130 yr for silver fir, a species that shows a strong increasing growth trend during this period of time. We also try to separate the effects of the age of the tree on carbon discrimination, which could mask the environmental effects. To this end, we devise an appropriate methodology.

MATERIALS AND METHODS

The distinction between the effects of tree age and the effects of environmental changes on long-term $^{13}C/^{12}C$ variations was made as follows. (1) At the life-span scale, we compared the $\delta^{13}C$ in tree rings with the same formation date, but from trees of different ages. In this manner, the effects of long-term environmental changes are discarded and tree-age effects maximized. (2) At the century scale, we analyzed rings formed in trees with the same age, but at different dates during the 20th century. In this latter case, tree-age effects were removed and the effects of environmental changes over time, referred to later as "date" effects, were maximized. Such a design requires a very large sampling basis that includes trees of various ages.

We used cores collected for a previous dendroecological study (Bert 1993) involving many trees and sites in the calcareous Jura Mountains, at the border between France and Switzerland. Within a large area (230 km long and 50 km wide) 208 pure stands of silver fir were selected in order to cover the whole ecological variation and a large range of stand ages. The site elevations ranged from 550 to 1350 m and the precipitation from 1200 to 2500 mm/yr. Two different types of stands were sampled: selection forest and even-aged high forest. Six dominant silver firs were chosen at each
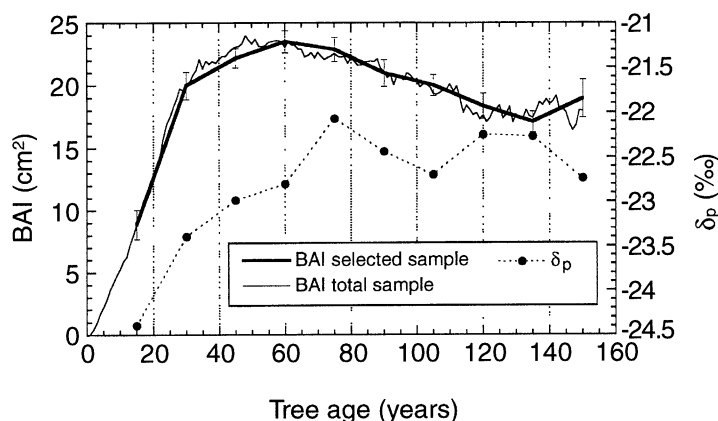
FIG. 1.   Mean basal area increment (BAI) of the total initial set of tree rings (BAI total sample), mean BAI of tree rings analyzed for stable-carbon isotope content in the present work (BAI selected sample), and wood $\delta^{13}C$ (δ_p) of *Abies alba* during the period 1940–1988 according to the age of the tree. The mean BAI curve of the initial set was obtained from the rings of 380 trees, on average, for each year. Each δ_p value was measured on holocellulose from a pooled batch of five rings from 10 trees. The error bars give the 95% confidence interval for each BAI mean.

site and cored to the pith at breast height. The 1248 corresponding cores were analyzed using standard dendrochronological procedures (see Bert 1993 for a detailed description); 115 170 ring widths were measured then used to calculate basal area increment (BAI) variations since 1800.

Tree-age effect on δ_p

In order to avoid the long-term trends due to environmental effects, only tree rings formed between 1940 and 1988 were investigated for this study. Ten 15-yr age classes were chosen: 15, 30 . . . 135, and 150 yr old. All trees, from the total sample of 1248 trees, which reached any of these ages between 1940 and 1988, were examined. For each of these age classes, 10 trees were selected for δ_p measures if they showed a BAI curve close to the mean BAI curve of all the trees. Bias of δ_p measurements was limited by the following constraints: (1) The possible effect of particular years was limited by pooling the studied tree ring with its two younger and its two older neighboring tree rings; (2) in order to discard any remnant date effects, the 10 chosen trees reached the studied age at different dates distributed between 1940 and 1988; and (3) similarly, the site effects were averaged by choosing the 10 trees at different locations throughout the study area. For each tree, the selected tree-ring pentad was set by counting from the pith, then excised from the core with a razor blade. For each studied age, the 10 tree-ring pentads were ground together and one δ_p value was measured.

Long-term changes of δ_p

Only tree rings that formed at the tree age of 40 yr were selected in this second part of the work in order to remove any tree-age effect. Trees from extreme ecological conditions (driest or wettest soils, lowest or highest elevations, etc.) were discarded from the sample to avoid any site effects. Thirteen decades were studied (1860–1869, 1870–1879 . . . 1980–1988, noted 1860, 1870 . . . 1980 hereafter). For each of these time periods, 10 trees that reached 40 during the time period

were selected, and 5-yr tree-ring groups of 38 to 42 yr old were extracted from the cores. The 10 tree-ring pentads were ground together and one δ_p value measured for each date. The two types of forest management (selection forests and even-aged forests) revealed different increasing BAI long-term trends (see Fig. 2). Therefore, the previous sampling process was repeated for both, and two time series of δ_p were measured separately.

Stable-carbon isotope content measurements

Holocellulose was extracted from tree-ring samples before carbon isotope analyses were conducted in order to avoid the effects of radial translocation of carbon or varying proportion of wood compounds. All the wood was ground in a mill and sequentially treated in a soxhlet, first with 2:1 toluene : ethanol, then 100% ethanol (Leavitt and Danzer 1993). The treated wood was then boiled in deionized water and bleached to holocellulose in a 70°C acetic acid acidified solution to which sodium chlorite was added to decompose the lignin. Conversion of the holocellulose to CO_2 was accomplished by dry combustion at 520°C in sealed, evacuated Pyrex tubes containing precombusted CuO as the oxygen source (Sofer 1980). Next, the CO_2 was separated from the other combustion products by cryogenic distillation and the $^{13}C/^{12}C$ ratio was measured with a Micromass 602C mass spectrometer. The results were expressed as δ_p (‰) with respect to the PDB standard.

The reproducibility of measurements was assessed using the holocellulose of a white spruce (*Picea glauca* [Moench] Voss) from Alaska. This laboratory standard was combusted with each batch of nine samples of holocellulose (2.5 mg). Precision from combustion of holocellulose through mass spectrometer measurement inclusive was 0.24‰ (standard deviation of 26 standard replications).

Calculation of Δ, P_i, and intrinsic water-use efficiency

Δ, P_i, and A/g_w were calculated using Eqs. 2, 3, and 5, respectively. The numeric values attributed to δ_p

FIG. 2. Mean BAI (basal area increment) of the 40-yr-old tree rings used for δ_p analyses according to date for two types of forest stands (30–50 tree rings for each mean). The error bars give the 95% confidence interval of the mean. The series are significantly different at the 5% level when the error bars are separated at a given date.
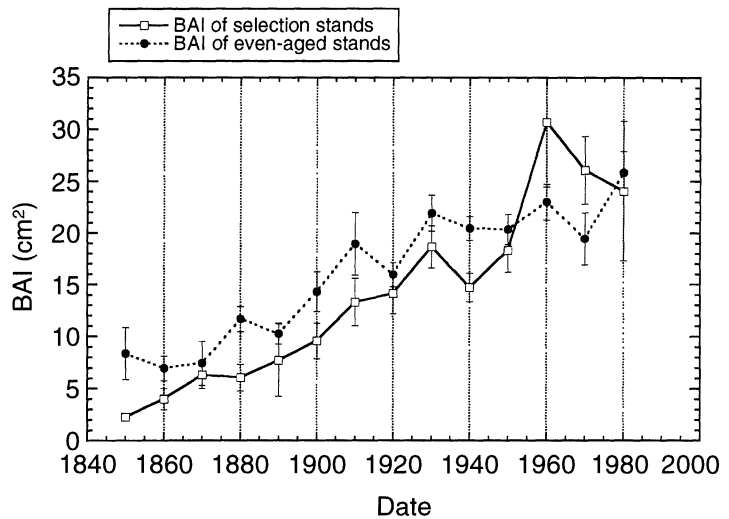
were the measured wood cellulose $\delta^{13}C$. Theoretically, they should have been the $\delta^{13}C$ values of the total pool of carbon fixed during photosynthesis. The assumption is often made that whole-tissue $\delta^{13}C$ of leaves reflects well this total carbon pool. Total or cellulose wood $\delta^{13}C$ values are usually reported as 1.5–3.5‰ less negative than the corresponding leaf tissue $\delta^{13}C$ (Leavitt and Long 1982, Ehleringer 1991, Yoder et al. 1994). However, we did not correct our calculations for this systematic difference because it only produces changes in absolute values of Δ, P_i/P_a, and A/g_w, but does not affect their relative variations over time.

$\delta^{13}C$ values of the air (δ_a) are needed in Eq. 2 for the calculation of Δ. During the last two centuries, δ_a continuously decreased because of the increasing release of ^{13}C-depleted CO_2 in the atmosphere from fossil fuel consumption and deforestation. We gathered published values of δ_a from ice-core measurements (Friedli et al. 1986) and direct atmospheric monitoring (Keeling et al. 1979, Keeling et al. 1984, Friedli et al. 1987, Keeling et al. 1989, Leavitt and Long 1989a) or inferred from C_4 plants (Marino and McElroy 1991). A cubic spline function was fitted to these values using a single knot at 1942 (Fig. 3) and subsequently used in Eq. 2.

The CO_2 concentration in the atmosphere (P_a) is needed in Eqs. 3 and 5. We also fitted a cubic spline function using a single knot at 1959 (Fig. 5) to values of P_a collected from ice-core data (Neftel et al. 1985, Raynaud and Barnola 1985, Friedli et al. 1986, Pearman et al. 1986, Staffelbach et al. 1991) and direct atmospheric measurements (Keeling et al. 1979, 1984, 1989, Mook et al. 1983, Fraser et al. 1986, Friedli et al. 1987, Leavitt and Long 1989a, Keeling and Whorf 1991).

Finally, the significance of observed trends in time or age was tested by t tests applied to the slope of

simple linear regression lines fitted to the observed values, with the null hypothesis being a zero slope.

RESULTS AND DISCUSSION

Tree-age effect on δ_p

Fig. 1 shows the variations of BAI and δ_p as a function of the age of the tree. Basal area increments display a classical pattern of variation with age: after a sharp rise up to age 50, they slowly and regularly decrease. δ_p values steadily increase from $-24.4‰$ to $-22.5‰$ between the ages of 15 and 75 at breast height. The slope of the simple regression is $+0.035‰/yr$ ($n = 5$, $P = 0.006$; $r^2 = 0.92$). Between the ages of 75 and 150, δ_p is nearly constant at approximately $-22.5‰$. The slightly decreasing slope is not significant ($n = 6$, $P = 0.332$). A similar pattern of increasing δ_p values with tree age has already been reported for silver fir (Lipp et al. 1991). However, in these previous observations, the effect of tree age was confounded with the date effect, since tree rings analyzed at different tree ages had also formed at different dates. We confirm the existence of an "age" trend for silver fir, independent of any long-term environmental changes.

Variations of δ_p with tree age have been attributed either to changes of external environmental factors associated with tree height, canopy closure, and stand level competition, e.g., atmospheric concentration of respired CO_2 from the soil and the plant canopy (Schleser and Jayasekara 1985), available soil water and light regime, or to internal age-related physiological factors (Francey 1981, Francey and Farquhar 1982).

Near the ground, the atmosphere is usually ^{13}C depleted due to soil respiration. Because of the shade-tolerant behavior of silver fir, young trees grow under the canopy of the mother trees for $\approx 50–60$ yr during the regeneration process. Thus, they probably reassimilate a significant part of this respired CO_2, with a
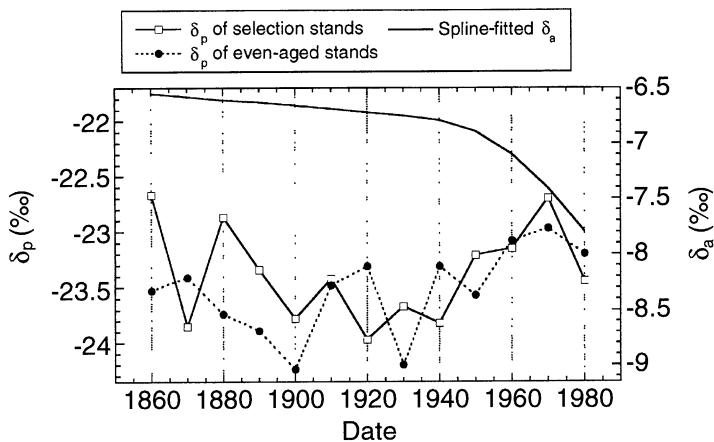
FIG. 3. Plot of δ_p of 40-yr-old tree rings sampled in even-aged and selection stands (left axis) and $\delta^{13}C$ of atmospheric CO_2 (δ_a, right axis) according to the date. Each δ_p value was measured from a pooled batch of 30–50 tree rings, after holocellulose extraction. The δ_a curve is a cubic spline fitted to published values of atmospheric $\delta^{13}C$.

low δ_a retained under the canopy. Few direct measurements of δ_a gradients within and under canopies are available (see Broadmeadow and Griffiths 1993 for a synthesis). Within a temperate Norway spruce stand, which is structurally similar to our stands, values of δ_a were constant for the photosynthetically active portion of the canopy and only decreased by 0.5‰ close to the ground (Broadmeadow and Griffiths 1993). Steeper gradients, up to 2.5‰, were observed in tropical forests (Sternberg et al. 1989). Thus, the increasing height of young silver fir in a ^{13}C-depleted atmosphere could contribute to the observed age-related δ_p trend.

Two other environmental factors display similar vertical gradients within closed stands, which could explain the decreasing P_i/P_a ratio as the tree ages and grows, i.e., leaf-to-air vapor pressure difference and photosynthetic photon flux density. (1) High vapor pressure deficit near the top of the canopy could cause lower P_i and greater δ_p. Older trees are more likely to experience such drier conditions than younger trees growing in lower strata. (2) In the forest, effects of irradiance are often confounded with those of vapor pressure deficit: shading of young trees under the canopy of adult trees could also result in higher ratios of intercellular to ambient CO_2 (Francey and Farquhar 1982, Farquhar et al. 1989). Finally, CO_2 concentration also varies within stands, according to location, type of forest, hour of day, and meteorological conditions. However, the air is only slightly depleted under the canopy (by 4 µL/L in Francey and Farquhar 1982, by 30 µL/L in summer in Bazzaz and Williams 1991) and enriched by ≈ 20–50 µL/L only close to the ground (Bazzaz and Williams 1991, Broadmeadow et al. 1992). Since a CO_2 enrichment likely promotes a decrease of P_i/P_a, such CO_2 gradients probably do not explain our observed δ_p trend.

Besides the influence of external environmental factors, some studies stress the role of internal age-related physiological factors. Between the ages of 40 and 250, the δ_p of *Pinus ponderosa* wood changes from $-25.8‰$ to $-24.5‰$ (Yoder et al. 1994). Any microclimatic or

environmental gradient was ruled out because the studied stand was very open. The loss of hydraulic conductance in long, i.e., old branches was presumed to be the regulating mechanism of decreasing stomatal conductance with age. Based on gas exchange measurements, Waring and Silvester (1994) confirmed that branch hydraulic conductance was much higher in short branches of *Pinus radiata* than in long branches. The increasing length of branches as trees age was accompanied by a decreasing foliar Δ value.

Our results do not provide conclusive evidence of the causes for the tree-age effect on ^{13}C discrimination. Further studies are needed on the vertical stratification of δ_a values, especially in temperate forests, on the separation between the effects of irradiance and vapor pressure deficit on Δ and on the hydraulic architecture and photosynthetic changes according to the age of the tree. Whatever the causes, our results show that it is important to take this aging effect into account when comparing the stable-carbon isotope content of rings formed at different tree ages.

Long-term changes of basal area increment, δ_p, and water-use efficiency

The basal area of tree rings formed at age 40, which were analyzed for δ_p, increased after ≈1860 (Fig. 2). The same growth trend was observed for the complete set of trees, including all tree age classes (Bert 1992). It is also similar to the growth trends revealed by other studies concerning several species in different areas of France (Becker et al. 1995) and Europe (Kenk et al. 1989, Briffa 1992). On average, silver fir in even-aged stands displayed a significantly higher BAI than in selection forests (Fig. 2) because they were growing on more fertile sites. However, after 1950, the even-aged stands were not thinned enough and the competition limited growth. The decreasing trend observed from 1970 onward was due to repeated drought periods during the 1970s and 1980s (Bert 1993).

δ_p values showed no significant difference between these two types of stands (t value 0.96[NS], Fig. 3). Cal-

FIG. 4. Isotopic discrimination ($\Delta‰$) of 40-yr-old tree rings sampled in even-aged and selection stands according to the date (left axis). The P_i/P_a ratio (left axis) is calculated from $\Delta = a - d + (b - a) P_i/P_a$.
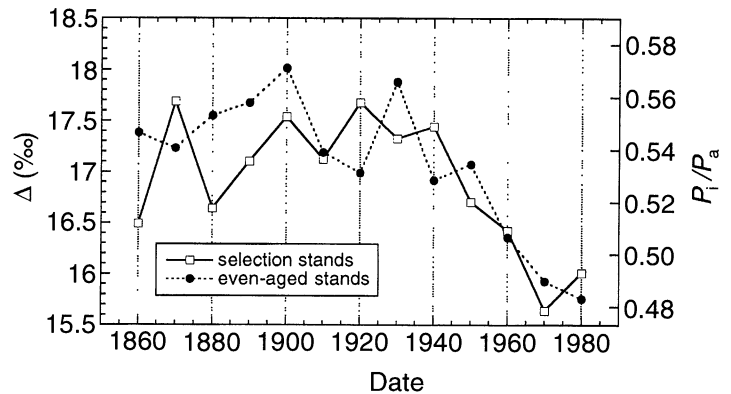
culated values of Δ, P_i, and A/g_w did not differ either. Consequently, values from both silvicultural systems were analyzed as a single data set.

If the plant fractionation was not affected by any long-term environmental changes, then δ_p should have paralleled δ_a. Our results show that variations of δ_p and δ_a were very different after $\approx$1930 (Fig. 3). Discrimination, Δ, calculated from Eq. 2, varied greatly around a mean of 17.3‰ from 1860 to 1930 (between 16.5‰ at the minimum and 18.0‰ at the maximum), with no obvious trend. From the 1930s to the 1980s, however, there was a significant decrease of Δ to a present value of 15.8‰ ($n = 12$, $P < 0.001$, $r^2 = 0.87$ for the linear regression of Δ values upon dates between 1930 and 1980; Fig. 4).

From 1860 to 1930, the P_i/P_a ratio calculated from Eq. 3 varied around a mean of 0.54, following Δ variations (Fig. 4). P_i significantly increased ($n = 16$, $P = 0.002$, $r^2 = 0.47$) at the same rate as P_a (Fig. 5) and thus the $P_a - P_i$ gradient kept constant. Following Eq. 5, A/g_w also remained fairly uniform (Fig. 6).

During the period 1930–1980, Δ and P_i/P_a decreased (Fig. 4). Because P_i increased more slowly than P_a, the $P_a - P_i$ gradient gradually and significantly increased (Fig. 5, $n = 12$, $P < 0.001$, $r^2 = 0.96$). Consequently, the intrinsic water-use efficiency A/g_w also significantly increased 30% during this period ($n = 12$, $P < 0.001$, $r^2 = 0.96$, Fig. 6).

We do not have direct estimates of the errors for these calculated A/g_w values because we analyzed pooled samples only. However, the two sylvicultural systems we compared provided a replication of our measurements. The observed trends in these two types of forest stands were very close, especially after 1930, during the period of increasing water-use efficiency. Moreover, Leavitt and Long (1989*b*) measured a standard deviation of 0.7‰ for several pine species $\delta^{13}C$ at several sites, including instrumental errors and intertree variability. This estimate yields a 95% confidence interval for our pooled measurements mean of $\pm$0.5‰ (10 trees), which is lower than the observed trends with age or date. Variability of our measurements has probably also been reduced due to the selection process of rings from a very large initial set. Influences of atmospheric $\delta^{13}C$ errors have been previousy shown to be small (Dupouey et al. 1993).

The previous changes are in agreement with those recorded from analysis of historical collections of herbarium leaves: in the Mediterranean region, Peñuelas and Azcón-Bieto (1992) reported a 1‰ decrease of Δ during the last four decades, and in temperate ecosystems, Woodward (1993) observed a 15% increase of intrinsic leaf water-use efficiency starting between 1930 and 1950.

A combination of several phenomena may account for these trends. Silvicultural methods gradually im-

FIG. 5. Intraleaf CO_2 concentration calculated from 40-yr-old tree rings sampled in even-aged and selection stands (left axis), and atmospheric CO_2 content (right axis), according to the date.
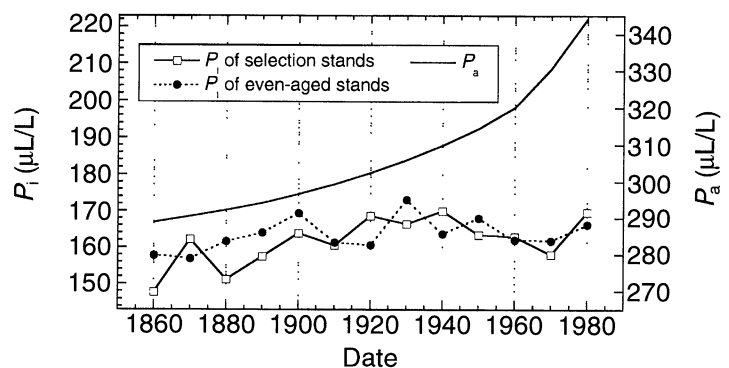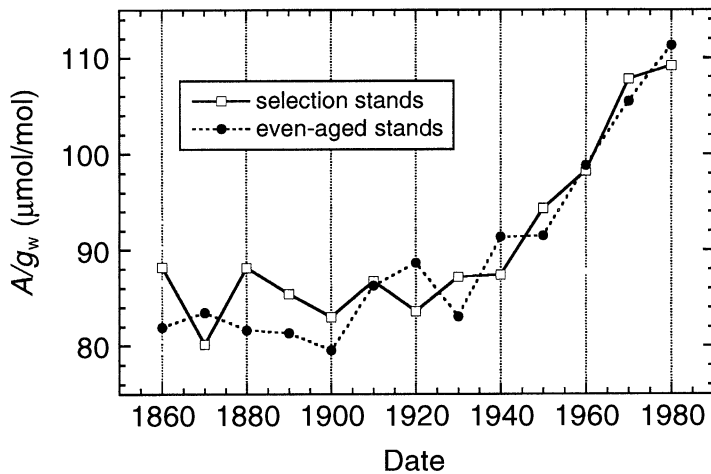
FIG. 6. Intrinsic water-use efficiency (A/g_w, the ratio of CO_2 assimilation rate to stomatal conductance for water vapor) calculated from 40-yr-old tree rings sampled in even-aged and selection stands according to the date.

proved in western Europe during the last century. Thus, stand structures and competition levels could have slowly evolved, changing the microclimatic environment of trees. Macroclimatic changes also occurred, as indicated by increasing winter temperatures and precipitation since 1860 in the eastern part of France (D. Bert, unpublished report). Atmospheric CO_2 concentration has increased from 280 to 350 μL/L over the past 150 yr (Friedli et al. 1986, Keeling and Whorf 1991). Finally, atmospheric deposition of pollutants, especially nitrogen and other acidifying compounds, strongly increased in Europe during the last 40 yr (Asman et al. 1987, Skeffington and Wilson 1988). Total annual nitrogen bulk deposition has been measured at 10 kg·ha^{-1}·yr^{-1} in the area (Ulrich et al. 1995).

Among these possible causes, the effects of increasing CO_2 on water-use efficiency has been extensively studied in controlled experiments. Woody plant species commonly react by increasing assimilation and decreasing stomatal conductance, which leads to a sharp rise of intrinsic water-use efficiency (see, e.g., Morison 1993). Beerling (1994), modeling gas exchange responses to the recent increase in the atmospheric CO_2 concentration, calculated a 20% increase in water-use efficiency of temperate trees, close to the value we observed in the present study. However, despite the scarcity of direct experimental evidence, the other possible causes of increasing water-use efficiency cannot be ruled out. Air pollutants such as ozone or sulphur dioxide have been shown to promote a decrease in Δ of 1‰ or greater, even under modest exposure in controlled experiments (Farquhar et al. 1989), or in the wood of trees under chronic exposure (Martin and Sutherland 1990). Differing effects of nitrogen fertilization on plant isotopic composition and water-use efficiency have been reported in controlled experiments (see, e.g., Mitchell and Hinckley 1993). They varied between having no effects to a 1.4‰ decrease in Δ found in needles formed the year after 2000 kg N/ha of nitrogen were added (Högberg et al. 1993).

The simplified model (Eq. 3) used for the calculation of P_i/P_a neglects some potential sources of variation in Δ, namely variations of the boundary layer and mesophyll conductances (see Farquhar and Lloyd 1993). Due to the coarse wave of the canopy of the silver fir stands, changes in aerodynamic resistance could only play a minor role on long-term variations of Δ if at all. The d coefficient in Eqs. 3 and 5 expresses the fractionation due to resistance during transport of CO_2 from the stomatal cavity to the sites of carboxylation, including diffusion through intercellular air spaces and liquid phase diffusion. For hypostomatous species such as silver fir, this resistance can be substantial (Farquhar and Lloyd 1993, Parkhurst 1994) and its long-term variations are unknown.

CONCLUSION

Results obtained in controlled experiments on tree reaction to environmental changes are often difficult to extrapolate to natural ecosystems because observations are made on young trees over a short period of time and without the complex interaction of the numerous ecological factors existing in the forest. Our observation of an in situ increase of intrinsic water-use efficiency of a forest tree species is a first upscaled confirmation of similar observations made in controlled experiments, under the influence of CO_2 increase and other simulated environmental changes. However, we are faced with the converse problem, namely the difficulty of ascribing this observed ecophysiological change in the forest to the effect of one particular environmental factor. Repeating such field studies in different regions of the world, with or without high levels of nitrogen input or human management, should provide a means of differentiating between the different possible causes.

Whatever its causes, these long-term variations of intrinsic water-use efficiency have important implications for the use of carbon isotope ratios in tree rings as a palaeorecorder of past environments. First, this

bio-indicator is strongly dependent on the age of the tree. Thus, the low-frequency component of isotopic variability in tree rings cannot be unambiguously used for the reconstruction of absolute environmental values (Dupouey et al. 1992, 1993). Like tree-ring widths, these measurements are better suited for the reconstruction of year-to-year deviations of climatic parameters from their long-term average. Moreover, additional low-frequency variance in the isotopic signal is due to the ecophysiological changes in the tree in relation with long-term environmental changes. Thus, the relationship between the isotopic composition of tree rings and climatic parameters, often calibrated during the recent period of rapid environmental changes, may perhaps not be directly applicable to previous time periods, at least for the reconstruction of absolute climatic values.

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