Changes of tree-ring $\delta^{13}$C and water-use efficiency of beech (*Fagus sylvatica* L.) in north-eastern France during the past century

A. DUQUESNAY, 1 N. BRÉDA, 1 M. STIEVENARD 2 & J. L. DUPOUHEY 1

1 Ecophysiology Unit, INRA-Nancy, 54280 Champenoux, France, and 2 LMCE, CEA Saclay, 91191 Gif-sur-Yvette, France

ABSTRACT

We investigated variation in intrinsic water-use efficiency during the past century by analysing $\delta^{13}$C in tree rings of beech growing in north-eastern France. Two different silvicultural systems were studied: high forest and coppice-with-standards. We studied separately effects related to the age of the tree at the time the ring was formed and effects attributable to environmental changes. At young ages, $\delta^{13}$C shows an increase of more than 1‰. However, age-related trends differ in high forest and coppice-with-standards. Changes in microenvironmental variables during stand maturation, and physiological changes related to structural development of the trees with ageing, could explain these results. During the past century, $\delta^{13}$C in tree rings shows a pattern of decline that is not paralleled by air $\delta^{13}$C changes. Isotopic discrimination has significantly decreased from 18.1 to 16.4‰ in high forest and varied insignificantly between 17.4 and 16.9‰ in coppice-with-standards. As a consequence, intrinsic water-use efficiency has increased by 44% in high forest and 23% in coppice-with-standards during the past century. These results accord with the increased water-use efficiency observed in controlled experiments under a CO2-enriched atmosphere. During the past century, $\delta^{13}$C in tree rings shows a pattern of decline that is not paralleled by air $\delta^{13}$C changes. Isotopic discrimination has significantly decreased from 18.1 to 16.4‰ in high forest and varied insignificantly between 17.4 and 16.9‰ in coppice-with-standards. As a consequence, intrinsic water-use efficiency has increased by 44% in high forest and 23% in coppice-with-standards during the past century. These results accord with the increased water-use efficiency observed in controlled experiments under a CO2-enriched atmosphere. However, other environmental changes, such as nitrogen deposition, may be responsible for such trends.

Key-words: *Fagus sylvatica* L., age; $^{13}$C/$^{12}$C ratio; isotopic discrimination; long-term trends; north-eastern France; silviculture; water-use efficiency.

INTRODUCTION

Elevated atmospheric CO2 concentration affects the gas-exchange metabolism of trees in several ways. In controlled experiments, CO2 assimilation is generally stimulated and stomatal conductance reduced. As a consequence, water-use efficiency (WUE), the ratio of carbon assimilated to water transpired, increases markedly (Eamus & Jarvis 1989; Ceulemans & Mousseau 1994). If these results, usually obtained from young trees in short-term experiments, can be extrapolated to the long term and to adult trees in forest ecosystems, they could have important implications for the global carbon cycle and the geographical range of trees (Amthor 1995) and for the hydrological cycle (Farquhar 1997).

This extrapolation is still a matter of speculation, partly because of the lack of long-term field experiments to examine tree responses to elevated atmospheric CO2. However, the response to atmospheric CO2 increase during the past century can be indirectly assessed using tree-rings and herbarium samples.

Leaf stomatal density controls maximal values of stomatal conductance and therefore influences WUE. Herbarium leaves of many tree species show an average reduction in stomatal density over the past century (see Woodward & Kelly 1995). For beech (*Fagus sylvatica* L.), different trends have been observed, ranging from an increase of 6.3% to a decrease of 43% during the past 200 years (Paolletti & Gellini 1993; Woodward & Kelly 1995).

The $^{13}$C/$^{12}$C ratio also gives information about past WUE variations. This ratio is expressed as $\delta^{13}$C, the proportional deviation from the international Pee dee belemnite (PDB) carbonate standard (Craig 1957):

$$\delta^{13}C (\%) = \left(\frac{^{13}C}{^{12}C}_{\text{sample}} \right)_{\text{PDB}} - 1 \times 1000. \quad (1)$$

During carbon fixation, fractionation associated with physical and enzymatic processes causes plant matter to be $^{13}$C-depleted in comparison with the air. This carbon isotopic discrimination is expressed as

$$\Delta (\%) = \frac{\left(\delta_{\text{air}} - \delta_{\text{plant}}\right)}{\left(\delta_{\text{air}} + \delta_{\text{plant}}\right)} \times 1000. \quad (2)$$

The relative rates of carbon fixation and stomatal conductance are the primary factors determining $\Delta$. According to the model proposed by Farquhar, O’Leary & Berry (1982),

$$\Delta (\%) = a + (b - a)(C_j/C_a) \quad (3)$$

where $a$ is the discrimination against $^{13}$CO2 during CO2 diffusion through the stomata ($a = 4.4\%e$, O’Leary 1981), $b$ is the discrimination associated with carboxylation ($b = 27\%e$, 1981).
Farquhar & Richards (1984) and \( C_i \) and \( C_a \) are intercellular and ambient \( \text{CO}_2 \) concentrations.

Given Fick’s law:

\[
A = \frac{g_{\text{CO}_2} (C_a - C_i)}{\text{CO}_2}
\]

where \( A \) is the net photosynthesis, measured as \( \text{CO}_2 \) uptake, and \( g_{\text{CO}_2} \) is the leaf conductance to \( \text{CO}_2 \), and given that \( g_{\text{H}_2\text{O}} \) the leaf conductance to water vapour is \( 1.6 \frac{g}{A} \)

\( \Delta \) can be related to the ratio \( A/g_{\text{H}_2\text{O}} \) by

\[
\Delta (\%) = a + (b - a) \left( 1 - \frac{1.6}{C_a} \frac{A}{g_{\text{H}_2\text{O}}} \right). \tag{5}
\]

\( A/g_{\text{H}_2\text{O}} \) is called intrinsic WUE (Osmond, Bjorkman & Anderson 1980). It is a component of plant transpiration efficiency, the long-term expression of biomass gain with respect to water loss at the level of the whole plant.

Peñuelas & Azcón-Bieto (1992) observed that isotopic discrimination \( \Delta \) of herbarium leaves of Mediterranean trees has decreased by 1‰ and thus intrinsic WUE has increased during the past four decades. Woodward (1993), also using herbarium leaves, showed that the intrinsic WUE of eight temperate tree species has increased by 30% since 1930.

A few results have been obtained by investigating \( \delta^{13} \text{C} \) in tree rings. Feng & Epstein (1995) showed a significant intrinsic WUE increase for four species over the past century. Likewise, Bert, Leavitt & Dupouey (1997) observed a 30% increase in the intrinsic WUE of Abies alba Mill. between 1930 and 1980. Conversely Marshall & Monsrud (1996) showed homoeostasis for WUE in several species of conifer between the beginning and the end of the century. Almost all such observations have been made in coniferous species.

In the present study, we investigated intrinsic WUE changes during the past century in a deciduous species, beech, growing in the Lorrain plain. A previous dendrochronological study (Badeau et al. 1995) had shown an increasing long-term growth trend during this period. Furthermore, growth trends depend on silvicultural system and stand density. Therefore in our study we took separate samples from high forest and coppice-with-standards.

Finally, key factors in such long-term studies are the confounding effects of environmental changes and tree or stand ageing. Wood formed during the first years of tree life is generally depleted in \( ^{13} \text{C} \) in comparison with wood formed later (Francey & Farquhar 1982; Bert, Leavitt & Dupouey 1997), a phenomenon called ‘age effect’ or ‘juvenile effect’. The corresponding \( \delta^{13} \text{C} \) increase varies around 1.5‰. However, the reported time span of this increase is highly variable: from the first 20 years of tree life (Freyer 1979) to 215 years (Mazany, Lerman & Long 1980). In our study, we devised a sampling scheme to study separately this age effect and long-term trends attributable to external environmental factors.

**MATERIALS AND METHODS**

**Sampling strategy**

The study was carried out in the calcareous plateaux of Lorrain (north-eastern France), where the climate is intermediate between continental and atlantic. The average annual rainfall is 730 mm and the average annual temperature 9.7 °C.

We sampled separately two types of silvicultural system: high stands and coppice-with-standards (Fig. 1). In the first system, stands are of even age and trees experience a high level of competition during their life. The second system is characterized by two different strata that develop differently: the coppice, growing from stumps, which is clear-cut every 25–30 years, and a few high trees, the standards, which grow from seeds, initially within the coppice, then above the coppice, at low density. The standards are harvested after several rotations of the coppice, roughly every 150 years.

Cores used in the present study were selected from a larger set of cores previously collected for a dendroecological study (Badeau et al. 1995). In this previous study, 102 sites with similar ecological characteristics (topography, soil, vegetation, microclimate) and free from any fertilization had been sampled. Ten trees, selected among the dominants in high stands and the standards in coppice-with-standards, had been cored to the pith at breast height in each site (one core per tree). A total of 1025 trees had been sampled.

**Tree-age effect on isotopic discrimination**

To avoid any effect of long-term environmental changes, we chose tree rings formed between 1965 and 1974 but by trees of different cambial ages (the age of the tree when the ring was formed). Eleven 10-year age classes were studied:

---

**Figure 1.** Stand-structure changes along a silvicultural rotation in (a) high stands and (b) coppice-with-standards. In high stands, trees pass through a juvenile stage (1) and an adult stage (2). In coppice-with-standards two strata initially coexist: the coppice (cross-hatching) and the standards (1); then, every 25–30 years, the coppice is clear cut (2).
5–14 years, 15–24 years, etc. Ten-ring sequences were pooled and analysed together to limit the influence of particular climatic events. The 1965–1974 decade was chosen for two reasons: a large number of rings were available for this decade, and it was characterized by a low water stress level (Fig. 2). Drought reduces beech isotopic discrimination (Dupouey et al. 1993; Saurer, Siegenthaler & Schweingruber 1995) and we wanted to limit possible interactions between age and drought effects.

Long-term changes of isotopic discrimination

In this part of the work, we analysed tree rings formed between 1850 and 1990, pooled by 10-year sequences. Only tree-rings formed at 38–47 years of age in high stands and 43–52 years of age in coppice-with-standards were studied to avoid the age effect. We selected these age classes because a large number of rings were available and because a juvenile effect has been observed on radial growth curves below 40 years of age (Picard 1995).

For each of the previously chosen age and date classes, 10 trees were selected according to their history: by looking at individual radial growth curves, we discarded trees in which growth had been suppressed at any time. Site effects were limited by choosing the 10 trees from different locations. For each selected tree, the chosen tree-ring sequence was set by counting from the pith, then excised from the core with a razor blade.

Stable carbon isotope analysis

The holocellulose fraction was extracted from the tree-ring samples before carbon isotope analysis to avoid the effects of radial translocation of carbon (Tans, de Jong & Mook 1978) or varying proportions of wood compounds (Park & Epstein 1961; Wilson & Grinsted 1977; Benner et al. 1987). Ten-ring sequences were ground in a mill, and subsequently boiled for 10 h in a soxhlet with 2:1 toluene:ethanol, then with 100% ethanol for the same time. The treated wood was then boiled in deionized water and bleached in acetic acid solution at 70 °C to which sodium hypochlorite (NaClO2) was added to decompose the lignin (Leavitt & Danzer 1993). Holocellulose was obtained by soaking samples in sodium hydroxide (17%, 1 h) then in acetic acid (10%, 10 min) following Da Silveira & Sternberg (1989).

Holocellulose was combusted to CO2 at 500 °C in sealed evacuated Pyrex tubes containing precombusted copper oxide as the oxygen source (Sofer 1980). The CO2 was separated from other combustion products by cryogenic distillation. The 13C/12C ratio was measured with a Finnigan Mat 252 mass spectrometer.

Calculation of Δ, C, and WUE — error estimation

Δ, C, and Al/gH2O were calculated using Eqsns 2, 3 and 5, respectively.

For Eqn 2, we collected published values for air δ13C from ice-core measurements (Friedli et al. 1986), direct atmospheric measurements (Keeling, Mook & Tans 1979; Keeling, Carter & Mook 1984; Keeling et al. 1989; Leavitt & Long 1989) and inferred from C4 plants (Marino & McElroy 1991).

For Eqsns (3) and (5), we used atmospheric CO2 concentrations collected from ice-core data (Neftel et al. 1985; Raynaud & Barnola 1985; Friedli et al. 1986; Pearman et al. 1986; Staffelbach, Stauffer & Sigg 1991) and direct atmospheric measurements (Keeling et al. 1979; Mook et al. 1983; Keeling et al. 1984; Fraser, Elliot & Waterman 1986; Friedli et al. 1987; Keeling et al. 1989; Leavitt & Long 1989; Keeling & Whorf 1991).

For the study of tree-age effects, we used air δ13C and C values of the 1970 decade (average of values between 1964 and 1975) for the calculation of Δ, C, and Al/g.

Meteorological data from the Nancy station were used for the calculation of potential evapotranspiration (PET), according to the Penman equation.

Two types of error were estimated: (1) the error associated with holocellulose combustion and mass-spectrometer measurements, which was assessed by repeated analysis of a laboratory standard extracted from a beech-wood sample from Lorraine; (2) the error associated with our sampling design. For the latter purpose, in one case (1970 decade), the 10 tree-ring sequences of one batch were separately analysed. This error term, which also includes the type (1) error, was used to assess the significance of long-term δ13C variations.

RESULTS

Error estimation

Repeated combustion and analysis of the reference cellulose gave a confidence interval of ± 0.09‰ (P < 0.05, n = 24). Within the 1970 decade, the standard deviation of the 10 samples was 0.48‰ for high stands (SEM ± 0.15‰,
n = 10) and 1.11‰ for coppice-with-standards (SEM ± 0.35‰, n = 10). The corresponding SEMs of $\Delta$, $C_i$ and $A/g$ for high stands and coppice-with-standards were, respectively: ± 0.16‰ and ± 0.36‰; ± 2.3 cm$^3$ m$^{-2}$ and ± 5.2 cm$^3$ m$^{-2}$; and ± 1.4 µmol mol$^{-1}$ and ± 3.3 µmol mol$^{-1}$.

**Age effect**

$\delta^{13}C$ variations according to age for high stands and coppice-with-standards are shown in Fig. 3. There is a clear difference between the two silvicultural systems: for high stands, $\delta^{13}C$ increases from –24.5‰ to –23.25‰ from 10 to 50 years of age and then up to 140 years gradually decreases to its initial value. For coppice-with-standards, $\delta^{13}C$ values increase from –24.75‰ to –23.5‰ from 20 to 80 years of age and then remain relatively constant up to 140 years. Thus, the increase of $\delta^{13}C$ in the first stage of life shows a lag of 30 years between high forest and coppice-with-standards. Furthermore, $\delta^{13}C$ variations in high stands are larger than the confidence interval, whereas $\delta^{13}C$ variations in coppice-with-standards are smaller than the confidence interval.

**Variations during the past century**

Variations of air $\delta^{13}C$ and tree $\delta^{13}C$ values during the past century are shown in Fig. 4(a). $\delta^{13}C$ values vary between –23.2 and –24.3‰. Both long-term, low-frequency variations and short-term, interdecade variations are observed.

Simple or multiple regression of these latter high-frequency variations upon climatic variables (temperature, precipitation and potential evapotranspiration) gave only loose relationships ($r = 0.38$, $n = 10$, not significant, for the correlation between $\Delta$ and precipitation minus potential evapotranspiration averaged by decades). The water-stress effect on $\delta^{13}C$ variations is therefore probably smoothed at the decade level and was not factored out in subsequent analyses.

**Discussion**

Our results confirm the existence of an age effect on $\delta^{13}C$, often also called the juvenile effect. Values increase by 1‰ during the first stage of life in both high forest and coppice-with-standards, but the timing of this age effect differs between the two systems. This age effect may be attributed to two different causes: changes in microenvironmental variables during stand maturation, and physiological changes linked to tree structural development.

During stand maturation, canopy height, leaf area and tree density change. At the young stage, small trees may assimilate more soil-respired, and therefore isotopically lighter, CO$_2$ (Vogel 1978; Sternberg et al. 1989). It has also been shown that $\delta^{13}C$ increases with an increase in vapour pressure difference (Farquhar, Ehleringer & Hubick 1989) or irradiance (Francéy & Farquhar 1982; Collet et al. 1993; Zimmerman & Ehleringer 1990), two factors that change as trees grow.

The above mechanisms could explain the observed increase of $\delta^{13}C$ with age in our stands. The 30-year lag between the increase in high-stands $\delta^{13}C$ values and the increase in coppice-with-standards values could be explained by the fact that, in coppice-with-standards, trees destined to become standards are sheltered by the existing standards and compete with the dense coppice for 25–30 years. Thus, during the first stage of their life, coppice-with-standards trees grow in a more humid and probably CO$_2$-enriched environment, and at a lower irradiance level. For older trees, a reverse situation is observed: in coppice-with-standards, old trees grow isolated in an open stand, whereas the tree density in high stands is high and the canopy closed. Indeed Buchmann, Kao & Ehleringer (1997) observed a stronger vertical gradient of air CO$_2$ and $\delta^{13}C$ in more closed broadleaf canopy compared with more open stands, leading to more negative leaf $\delta^{13}C$.
Figure 4. Changes during the past century in (a) $\delta^{13}C$, (b) isotopic discrimination $\Delta$, (c) $C_i$ and (d) $A/g$ for high forest (●) and coppice-with-standards (○). Air $\delta^{13}C$ values and atmospheric CO$_2$ concentrations are shown, respectively, on the right y-axis of graphs (a) and (c). Standard errors of the means associated with the 1970 decade are shown for high forest and coppice-with-standards by the solid and dotted lines, respectively.

values. Leavitt (1993) observed that wood δ13C was higher in open-canopy stands than in closed-canopy stands. However, the decrease of δ13C in old high stands remains to be explained.

Variations of hydraulic conductance linked with tree ageing and branch elongation have also been shown to modify leaf gas exchanges and thus plant δ13C. Younger trees have shorter branches and therefore more negative tree-ring or leaf δ13C values (Waring & Silvestre 1994; Yoder et al. 1994; Panek & Waring 1995; Panek 1996). However, these relationships were obtained only with coniferous species. Fagus sylvatica has a more complex structure, characterized by a balance between short and long internodes, and this balance is the key factor in hydraulic conductance variability in ring-porous species such as Fraxinus excelsior L. or Fagus sylvatica (Cochard et al. 1997). The relationships in these species between the variations of internode length and δ13C changes with age warrant further investigation.

**Long-term changes**

Our results are in agreement with those of Peñuelas & Azcón-Bieto (1992), Woodward (1993), Ehleringer & Cerling (1995), Feng & Epstein (1995) and Bert et al. (1997), who reported similar decreases of isotopic discrimination Δ or increases of WUE during the same period, using historical records in leaves or wood. Our results also accord with predicted values of WUE increase obtained from a gas-exchange model of the effect of current environmental changes (Beerling 1994). In all previous studies, the C3/C4 ratio remained constant or decreased during the past century. Our results do not support the hypothesis of a regulation of either C3 or C3/C4 at least in the long term. However, Marshall & Monserud (1996) observed an increase of C3/C4 since the beginning of the century for three coniferous species in the western USA, and hence homoeostasis in WUE. Marshall & Monserud compared two groups of 10-ring sequences, one at the beginning of the century, and one at the end of the century. Our study has shown a large interdecade variability of δ13C, which could hide long-term trends.

Results obtained in controlled experiments suggest that the trend of increasing WUE that we observed could be attributable to the increase in atmospheric CO2 concentration. This effect has been well documented for many woody plant species (see Ceulemans & Mousseau 1994) and is one of the most pronounced responses to elevated CO2. For Fagus sylvatica, Overdieck & Forstreuter (1994) demonstrated improved WUE in saplings growing at elevated CO2 concentration. Using branch-bags, Dufêne, Pontailler & Saugier (1993) also observed an increase in WUE in branches of 20-year-old beech trees. This effect is generally the result of an increase in assimilation rate and a decrease in stomatal conductance (but see Heath & Kerstiens 1997). These experimental results were obtained at ambient and twice-ambient CO2 concentration, whereas the trees we studied grew at CO2 concentrations of 290–360 cm3 cm−3.

Polley et al. (1993, 1996) gave experimental evidence of the existence of a large positive effect on WUE of CO2 increase from subambient level. In addition to these short-term gas-exchange adjustments to elevated CO2, slow adaptive processes could also have enhanced WUE. The decrease during the past century in leaf stomatal density observed on herbari- um leaves could by itself explain the concomitant WUE increase (Woodward 1993; Kürschner 1996).

Other environmental factors, such as nitrogen supply, could also play a part in the observed trends. Nitrogen deposition has increased markedly in the studied area during the past decades (Ulrich & Williot 1993). Guehl, Fort & Ferhi (1995) observed that WUE assessed by gas-exchange measurements increases when nitrogen supply is optimum for maritime pine. Högborg, Johannisson & Hällgren (1993) observed a 1‰ decrease in Δ in pine needles after nitrogen fertilization. However, Mitchell & Hinckley (1993), in Douglas fir, found no difference in instantaneous WUE with high and low levels of nitrogen supply.

**CONCLUSION**

The aim of the present study was to examine variations of δ13C and intrinsic WUE during the past century. Our results show that isotopic discrimination Δ has decreased significantly. Intrinsic WUE has increased by 44% in high stands over the past century. These results, obtained from a sample of adult forest trees, are consistent with observations on the consequences for young plants in controlled conditions of an increase in atmospheric CO2 concentration. However, the observed changes cannot be attributed to this single cause, because of the many other factors involved. Moreover, complex patterns in the variation of δ13C with tree age show that isotopic values cannot be simply compared at different tree ages.

Few in situ studies of δ13C long-term trends have been carried out in forest ecosystems, and trends in isotopic discrimination (Δ) and WUE over the past century observed by different authors do not all correspond. Further studies are needed, with other species and in other biomes.

**ACKNOWLEDGMENTS**

We thank P. Behr for technical assistance during the field work, and V. Lenoble for valuable suggestions during the preparation of isotope samples. We are also grateful to V. Badeau and J. M. Guehl for helpful discussions and comments, and to C. Powell for correcting the English of the manuscript.

**REFERENCES**


Received 26 September 1997; received in revised form 18 February 1998; accepted for publication 18 February 1998.