NOTE

Variations in the stable carbon isotope ratios of *Zelkova serrata* leaves from roadside trees in Toyama City, Japan

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Nitrogen contents and stable carbon isotope ratios of *Zelkova serrata* leaves collected from roadside trees in Toyama City, Japan were measured in order to judge the stress due to water availability and nutrient efficiency. Furthermore, the source of urban atmospheric CO₂ was examined from the isotopic discrimination between *Zelkova serrata* leaves and CO₂ in the ambient air based on the correlation between carbon isotope ratio and content of CO₂, since there was no stress associated with the roadside trees. The carbon isotope ratio of roadside tree leaves decreases with increasing traffic. This suggests that the atmospheric CO₂ in urban street is contaminated by exhaust gases of car.

INTRODUCTION

When plants fix atmospheric CO₂ by photosynthesis, carbon isotope fractionation associated with carboxylation and diffusion occurs. Therefore, plants are enriched in ¹²C relative to the atmosphere. The isotopic discrimination between C₃ plants and atmospheric CO₂ is shown in the following equation (Farquhar *et al.*, 1989):

$$\delta^{13}C_p = \delta^{13}C_a - (4.4 + 22.6 \times C_i/C_a) \quad (1)$$

where $\delta^{13}C_p$ is the carbon isotope ratio of plants, $\delta^{13}C_a$ the carbon isotope ratio of atmospheric CO₂, C_i the internal gas-phase content of CO₂ and C_a the external CO₂ content. When isotope discrimination between plants and atmospheric CO₂ is established, the leaf carbon isotope ratio depends on that of the CO₂ in the ambient air.

The C_i/C_a ratios of plants, however, change according to the long-term water stress and/or nutrient conditions (e.g., Toft *et al.*, 1989; Read *et al.*, 1992). For example, the ratios vary from 0.64 to 0.40 for *Zelkova serrata* due to soil water content (Takahashi, 1995). Consequently, leaves

exhibit more positive $\delta^{13}C$ values under water stressed conditions, values changing by as much as 5.4‰. On the other hand, if there is no stress on urban roadside trees, the $\delta^{13}C$ values of leaves are mainly controlled by those of atmospheric CO₂.

The aim of present study is to judge the stress due to water availability and nutrient efficiency from investigation of *Zelkova serrata* carbon isotope and nitrogen content at Sakuradori Street in Toyama City. We also would like to clarify the source of atmospheric CO₂ from the isotopic discrimination between this plant and CO₂ when there is no stress associated with *Zelkova serrata*.

EXPERIMENTAL

Sampling

Zelkova serrata leaves at height of 4 m from Sakuradori roadside trees were collected in 6 September 1996 and the ambient air from street crossings in Toyama City, monthly during May–November in 1996–1997. The air was sampled in a Aluminized Polyethylene bag by a two way gas-tight syringe. The traffic at street crossings was

averaged from the data of May–September in 1996.

Analytical method

Leaf samples were dried thoroughly for 6 hr at 80°C prior to combustion and ground to a powder. After 4 mg of leaf samples has been weighed into a cut quartz tube, the sample was mixed with 0.5 g of CuO by gentle shaking. Approximately 0.25 g of reduced copper was added to the combustion tube on top of the sample/CuO mixture. When a vacuum was achieved, the combustion tubes were sealed with a gas/oxygen torch. The muffle furnace in which each sealed tube was placed was heated to 900°C and the temperature maintained for 2 hr. The gases produced by combustion of leaf sample revealed the presence of only CO₂, H₂O and N₂. After the CO₂ and N₂ in leaf sample were separated with Porapak Q by helium carrier and their contents were determined by a TCD gas chromatograph, then the remaining CO₂ was separated from the other gases by cryogenic distillation (Kidoguchi, 1996). The CO₂ produced was manometrically measured.

The concentrations of atmospheric CO₂ were measured by a FID gas chromatograph equipped with methanizer. For the measurement of carbon isotope ratios, atmospheric CO₂ is condensed out in a trap cooled by liquid nitrogen (Craig, 1953). However, minor amount of atmospheric N₂O, which has the same molecular masses as CO₂ but has quite different abundances of isotope species, is also trapped with CO₂ because both molecules have almost the same physical properties. In the present study, we reduced N₂O to N₂ through CO₂-N₂O mixture over copper heated at 500°C and pumped away the N₂ (Kidoguchi, 1998).

The CO₂ thus purified was analyzed for its $\delta^{13}\text{C}$ with the Micromass PRISM mass spectrometer. The overall reproducibility for both the leaf and air samples was $\pm 0.1\text{‰}$.

RESULTS AND DISCUSSION

Carbon isotope ratios and nitrogen contents of *Zelkova serrata* leaves range from -32.4 to

-29.7‰ and from 1.6 to 2.1 wt. %, respectively (Table 1). On the contrary, $\delta^{13}\text{C}$ values and contents of atmospheric CO₂ during May–November, 1996–1997 at street crossings in Sakuradori Street are -11.2 to -9.2‰ and 402 to 440 ppm, respectively (Fig. 1). The carbon isotope ratio of CO₂ decreases with increasing CO₂ content of air as shown in Fig. 1. No seasonal variations in $\delta^{13}\text{C}$ values of tree leaves and atmospheric CO₂ at street crossings along Sakuradori Street were clearly observed (Kidoguchi, 1998).

Isotope discrimination

In order to judge the stress, substituting the averaged $\delta^{13}\text{C}$ value (-10.1‰) of atmospheric CO₂ along Sakuradori Street and the C_i/C_a ratios from 0.64 and 0.40 into Eq. (1) the carbon isotope ratios of *Zelkova serrata* ranging from -29.0 to -23.5‰ are obtained. Since the tree leaves are depleted in $\delta^{13}\text{C}$ relative to the calculated values by amounts up to about 5‰, there are no stress associated with the roadside trees. In this case, the $\delta^{13}\text{C}$ values of *Zelkova serrata* leaf should have been constant, but the values are scattered actually. At

Table 1. Carbon isotope ratio, and the carbon and nitrogen contents of *Zelkova serrata* leaves (sampled on September 6, 1996) from sixteen trees along Sakuradori Street in Toyama City

Sample No.	Carbon (wt. %)	Nitrogen (wt. %)	$\delta^{13}\text{C}$ (‰)
ZS-01	46	2.1	-31.6
ZS-02	47	1.8	-32.4
ZS-03	45	1.7	-31.4
ZS-04	44	2.1	-31.7
ZS-05	47	1.8	-32.0
ZS-06	47	1.9	-32.0
ZS-07	47	2.1	-30.1
ZS-08	47	2.1	-32.2
ZS-09	42	1.6	-29.7
ZS-10	47	1.9	-31.2
ZS-11	49	1.8	-30.0
ZS-12	41	2.1	-30.5
ZS-14	47	2.1	-30.8
ZS-15	43	1.8	-32.4
ZS-16	46	1.8	-30.8

ZS-01, ZS-08, ZS-12, ZS-15, ZS-16: Street crossings.

the sampling point of present study, there are no differences in meteorological conditions, such as relative humidity and light intensity that would have caused the change in the isotope ratio (e.g., Madhavan *et al.*, 1991). Therefore, variations in $\delta^{13}\text{C}$ values of *Zelkova serrata* depend on those of the CO_2 in the air and reflect the fractionation in the atmospheric CO_2 .

From the *Zelkova serrata* leaf nitrogen content, it was expected that all of these trees were nutritionally deficient, judging from the values reported by Takahashi (1995). Since the stress due to nutrition deficiency, however, did not affect the leaf $\delta^{13}\text{C}$ values, it was not responsible for the variation in isotope ratios (Kidoguchi, 1996).

Therefore, the variations depend on source of atmospheric CO_2 .

Source of CO_2 in urban street

As shown in Fig. 2, the CO_2 content at some street crossings increases with increasing the traffic in Toyama City. Furthermore, the $\delta^{13}\text{C}$ value of atmospheric CO_2 become more negative with increasing CO_2 content in the ambient air. On the contrary, the roadside tree leaves exhibit more negative $\delta^{13}\text{C}$ values with increasing the traffic (Fig. 3). This suggests that most of the atmospheric CO_2 in urban street is mainly derived from exhaust gases of cars. Consequently, the atmospheric CO_2 content can be estimated by measuring leaf

Fig. 1. Plot of $\delta^{13}\text{C}$ values versus content of CO_2 from air samples during May–November, 1996–1997, at Sakuradori Street in Toyama City. The line is drawn by the least-squares method.

Fig. 2. Relationship between atmospheric CO_2 contents vs. traffic at street crossings in Toyama City. The air was sampled on September 6, 1996, and the traffic (car numbers per day) is the average value for the data of May–September in 1996. Closed circles are from Sakuradori Street (at the crossings corresponding to the tree positions of ZS-01, ZS-08, ZS-15, ZS-16 in Table 1) and open circles from other streets.

Fig. 3. Carbon isotope ratios in *Zelkova serrata* leaves (sampled on September 6, 1996, ZS-01, ZS-08, ZS-12, ZS-15, ZS-16 in Table 1) vs. traffic (May–September, 1996) at street crossings in Sakuradori Street. The traffic indicates car numbers ($\times 1000$) per day.

$\delta^{13}\text{C}$ values, if the traffic along each street is presumed. Assuming that in the roadside tree there is no stress such as water deficiency, the behavior of urban atmospheric CO_2 can be clarified from the examination of the leaf $\delta^{13}\text{C}$ values for all city roadside trees.

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