Plant Physiol. (1986) 80, 242–245
0032-0889/86/80/0242/04/$01.00/0

Compensation Point and Isotopic Characteristics of C_3/C_4 Intermediates and Hybrids in *Panicum*[1]

Received for publication June 18, 1985

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ABSTRACT

Leaf CO_2 compensation points and stable hydrogen, oxygen and carbon isotope ratios were determined for *Panicum* species including C_3/C_4 intermediate photosynthesis plants, hybrids between C_3/C_4 intermediates and C_3 plants, C_3 and C_4 plants in the *Panicum* genus as well as several other C_3 and C_4 plants. C_3 plants had the highest compensation points, followed by hybrids, C_3/C_4 intermediates, and C_4 plants. $\delta^{13}C$ values of cellulose nitrate and saponifiable lipids from C_4 plants were about 10‰ higher than those observed for cellulose nitrate and saponifiable lipids of C_3/C_4 intermediates, hybrids, and C_3 plants. Oxygen isotope ratios of cellulose as well as those of leaf water were similar for all plants. There was substantial variability in the δD values of cellulose nitrate among the plants studied. In contrast, such variability was not observed in δD values of water distilled from the leaves, nor in the δD values of the saponifiable lipids. Variability in δD values of cellulose nitrate from C_3/C_4 intermediates, hybrids, C_3, and C_4 plants is due to fractionations occurring during biochemical reactions specific to leaf carbohydrate metabolism.

Most plants utilize the C_3, C_4, or CAM modes of fixing CO_2 from the atmosphere. Observations over the last decade, however, indicate that individual species in several genera cannot be classified into only one of these photosynthetic modes. These plants exhibit several physiological, anatomical, or biochemical characteristics of two different photosynthetic modes. For example, several species of *Peperomia* as well as species in *Codananthe*, *Pereskia*, *Cissus*, and *Sedum* have characteristics both of CAM and C_3 plants and are called CAM-cyclers (15, 16, 19, 24–27). Species in the *Portulaca* genus have physiological/biochemical characteristics of C_4 photosynthesis and Kranz anatomy, as well as succulence and acid flux typical of CAM plants (13, 14). Species in *Panicum*, *Flaveria*, *Mollugo*, *Moricandia*, and *Neurachne* also have physiological, biochemical, or anatomical characteristics of both C_3 and C_4 plants (17). *Panicum* and *Flaveria* are of particular interest because hybridization between species within each genus have been successful.

Our interest in C_3/C_4 intermediate species in this paper is limited to the *Panicum* genus. There are numerous C_3 and C_4 *Panicum* species as well as three species (*Panicum milioides*, *P. Spathellosum* [syn. *P. schenkii*], and *P. decipiens*) that exhibit characteristics intermediate to C_3 and C_4 plants (4–6). *Panicum* species with intermediary photosynthetic modes have Kranz anatomy and a lowered compensation point that are characteristics of C_4 plants (4–6). The *Panicum* intermediates, however, lack several of the enzyme activities typical of C_4 plants. In the *Panicum* genus, including hybrids between C_3/C_4 intermediates and C_3 plants, a range of photosynthetic compensation point values are known from C_3 through C_4 values (4, 6, 12). We have sought to understand the components regulating the compensation point in plants (6, 12). Therefore, in this work we will compare the compensation values of *Panicum's* with their isotopic values in a continuing effort to understand C_3/C_4 intermediate metabolism.

Isotope analysis of plant matter provides a powerful method of studying photosynthetic modes. In addition to the well known separation of C_3 plants from C_4 and CAM plants based on stable carbon isotope ratios (17), oxygen and hydrogen isotope ratios of cellulose and cellulose nitrate, respectively, also are influenced by photosynthetic mode (20, 22–25). Analysis of C_3, C_4, and CAM plants growing in the vicinity of each other show that cellulose nitrate from CAM plants is enriched in deuterium relative to C_3 and C_4 plants (20, 22, 25). Sternberg *et al.* (20, 22–25) concluded that the difference in δD values between CAM plants and C_3 and C_4 plants are due to fractionations occurring during biochemical reactions particular to CAM plants. C_4 plants also tend to have higher abundances of deuterium and ^{18}O than C_3 plants (20, 22, 27). Sternberg *et al.* (22) suggested that the difference in δD and $\delta^{18}O$ values observed between C_3 and C_4 plants are due to differential responses of photosynthesis to low RH.

In this study we grew plants under greenhouse conditions and measured their CO_2 compensation points plus their carbon, hydrogen and oxygen isotope ratios. We studied C_3 and C_4 grasses, C_3/C_4 intermediates and C_3 *Panicum* species (4). We thus were able to determine whether the stable isotope ratios of species having intermediate photosynthetic modes and compensation points are intermediate to those observed in C_3 and C_4 plants.

MATERIALS AND METHODS

Panicum species and hybrids were obtained from R. Harold Brown and Joe H. Bouton in the Agronomy Department of the University of Georgia. All plants were grown under greenhouse conditions, watered daily, and fertilized weekly with Peter's special (15-15-15). Compensation points (Γ) were measured as in Brown and Brown (6) at 30°C illuminated at 1000 $\mu E \cdot m^{-2} \cdot s^{-1}$ and are expressed as μl of CO_2 L^{-1}. Two sets of plants were grown for isotope analysis: one set was grown and harvested by March 1984 and the second set was grown and harvested by

[1] Supported by the National Foundation grants DMB 84-05003 (M. J. D.) and DMB 84-06331 (C. C. B.).

August 1984.

Hydrogen and carbon isotope ratios of cellulose nitrate and oxygen isotope ratios of cellulose were determined as described previously (22). Extraction and saponification of lipids and isotope analysis were done as described previously (21). Plant water was extracted by distillation under vacuum at 100°C. Hydrogen isotope ratios were determined on small water samples in capillary tubes as in Bigeleisen *et al.* (2). Oxygen isotope ratios of water were determined as described by Epstein and Mayeda (9).

Isotope ratios are expressed as δ values, where

$$\delta = \left[\frac{R \text{ sample}}{R \text{ standard}} - 1\right] \times 1{,}000\ (‰).$$

and R represents D/H for hydrogen, $^{13}C/^{12}C$ for carbon and $^{18}O/^{16}O$ for oxygen. The standards were standard mean ocean water for hydrogen and oxygen and Peedee belemnite carbonate for carbon. The precisions of istopic analysis were ±2‰ for δD values, ±0.2‰ for $\delta^{13}C$ values, and ±0.5‰ for $\delta^{18}O$ values.

RESULTS AND DISCUSSION

Compensation points for all species studied here are given in Brown *et al.* (4), though we repeated the determinations for verification. Compensation points are plotted against $\delta^{13}C$ values of cellulose nitrate and saponifiable lipids in Figure 1, A and B, against $\delta^{18}O$ values of cellulose and leaf water in Figure 2, A and B, and against δD values of cellulose nitrate, leaf water, and saponifiable lipids in Figure 3, A, B, and C, respectively. No relationship was observed between isotope ratios of the plant components analyzed here and their compensation points. Neither carbon or oxygen isotope ratios fluctuate with compensation points. The relationship between hydrogen isotope ratios of cellulose nitrate and compensation points is not clear.

As has been observed previously, C_4 plants had the lowest CO_2 compensation point followed in order by the C_3/C_4 intermediates, the hybrids and the C_3 plants (4–7). It has been suggested that the close proximity of chloroplasts, mitochondria, and peroxisomes in the *Panicum* intermediates might be responsible for their ability to refix photorespired CO_2 (4, 7), thus lowering their

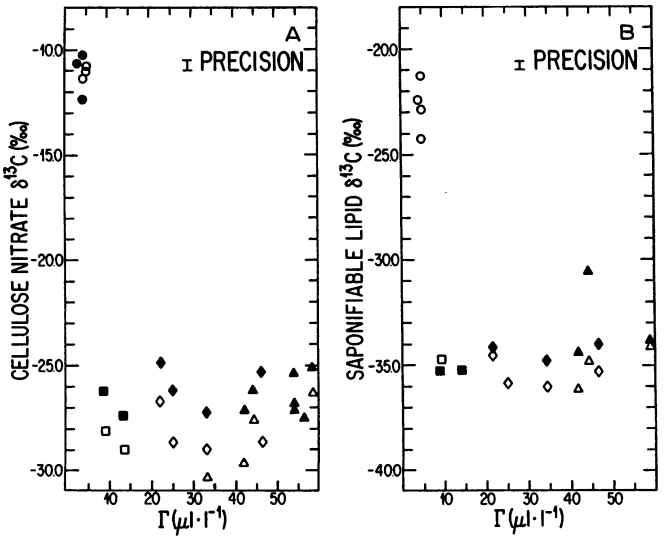

FIG. 1. Photosynthetic CO_2 compensation points *versus* (A) $\delta^{13}C$ values of cellulose nitrate and (B) $\delta^{13}C$ values of saponifiable lipids for plants having the C_4, C_3, and C_3/C_4 intermediate photosynthetic modes as well as values for hybrids between C_3/C_4 intermediates and C_3 plants. Closed symbols are for the sample set harvested at March 1984 and open symbols are for the sample set harvested in August 1984. (△, ▲), C_3 plants; (◇, ◆), hybrids; (□, ■), C_3/C_4 intermediates; (○, ●), C_4 plants.

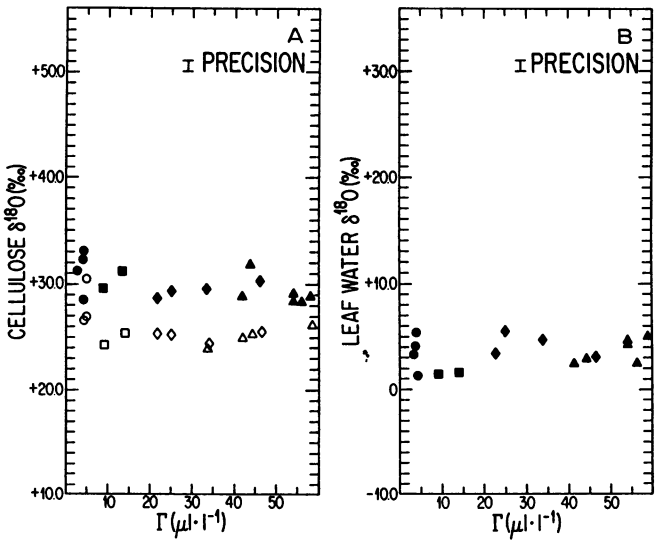

FIG. 2. Photosynthetic CO_2 compensation points *versus* (A) $\delta^{18}O$ values of cellulose and (B) $\delta^{18}O$ values of plant water for plants described in legend of Figure 1.

compensation points. Whatever the causes for the relatively low CO_2 compensation points in the *Panicum* intermediates, compensation point is a heritable trait since the hybrids had compensation points intermediate to those of their parent plants.

The $\delta^{13}C$ values of cellulose nitrate for the C_3, C_3/C_4 intermediates and hybrids were all C_3-like (1), being between −25 and −30‰ (Fig 1A). Cellulose nitrate from C_4 plants had $\delta^{13}C$ values typical of C_4 plants (1), being in the range of −10 to −12‰. The same relationship among C_4, C_3, C_3/C_4 intermediates and hybrids is observed in the carbon isotope ratios of saponifiable lipids (Fig. 1B). The lipids, however, are about 10‰ depleted in $\delta^{13}C$ relative to the carbon isotope ratio of the cellulose nitrate, as has been previously reported (21). Most workers agree that the similarity of carbon isotope ratios of cellulose nitrate from C_4 plants to $\delta^{13}C$ values of atmospheric CO_2 (−7‰) is caused by two factors. The first is the fact that PEP[2] carboxylase (the carboxylating enzyme of C_4 plants) does not discriminate against ^{13}C as much as does RuBP carboxylase (the carboxylating enzyme of C_3 plants) (18). The second factor is that a typical C_4 plant is able to concentrate CO_2 in its bundle sheaths, thus forming a semiclosed system since most of the CO_2 is refixed. With this argument in mind, it is not surprising that the C_3/C_4 intermediates in the *Panicum* genus do not have $\delta^{13}C$ values intermediate to those of C_3 and C_4 plants. The *Panicum* intermediates have very little PEP carboxylase and lack several of the enzymes responsible for the CO_2 concentrating mechanism found in C_4 plants (12, 17). In addition, physiological measurements show that *Panicum* intermediate species cannot concentrate CO_2 into their bundle sheaths (17). We expect, however, that plant species in the *Flaveria* genus will have $\delta^{13}C$ values intermediate to C_3 and C_4 plants, since they seem to have the enzymes necessary for a CO_2 concentrating mechanism (17).

Theoretically, oxygen isotope ratios of plant cellulose reflect the oxygen isotope ratios of the water at the site of cellulose synthesis (8, 10). The $\delta^{18}O$ values of cellulose from submerged aquatic plants, which do not transpire, are about 27‰ higher than that of the water in which they grew (8, 10, 23). The biochemical pathway of CO_2 fixation does not seem to affect the relationship between $\delta^{18}O$ values of cellulose and $\delta^{18}O$ values of the water at the site of synthesis, since Sternberg *et al.* (23)

[2] Abbreviations: PEP, phosphoenolpyruvate; RuBP, ribulose 1,5-bisphosphate.

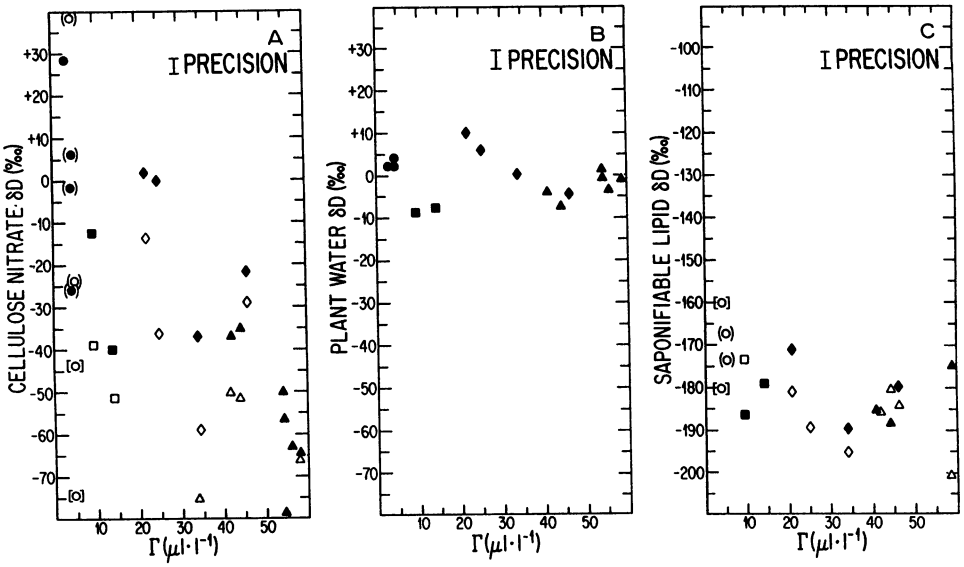

FIG. 3. Photosynthetic CO_2 compensation points *versus* (A) δD values of cellulose nitrate, (B) δD values of plant water, and (C) δD values of saponifiable lipids for plants described in legend of Figure 1. In addition, for the C_4 plants, symbols in parentheses represent plants which are known to have NADP+ or NAD+ malic enzyme as the decarboxylating enzyme, while symbols in brackets indicate plants using PEP-carboxykinase as the decarboxylating enzyme.

observed that aquatic CAM plants and non-CAM plants all have the same enrichment in ^{18}O relative to the water in which they grew. The same isotopic relationship between cellulose and water at the site of cellulose synthesis has even been observed for tunicates, which are aquatic cellulose-producing heterotrophic organisms (8).

For terrestrial plants, however, the interpretation of the oxygen isotope ratios of cellulose is complicated by transpiration. Transpiration will enrich leaf water in ^{18}O relative to groundwater. This enrichment then influences the oxygen isotope ratio of the leaf cellulose (11). Thus, differences in oxygen isotope ratios of cellulose for plants which are exposed to the same irrigation water will be determined by two factors: the amount of transpiration a plant undergoes and the sensitivity of photosynthesis to desiccating conditions. A plant which is sensitive to desiccating conditions will cease photosynthesis and not record high $\delta^{18}O$ values of leaf water in the plant cellulose during dry periods. A plant that continues photosynthesis during such periods will record high leaf water $\delta^{18}O$ values in its cellulose.

A consistent oxygen isotopic difference was observed between the sample sets grown during different periods (Fig. 2A). This difference probably reflects a slight difference in the $\delta^{18}O$ values of irrigation water, which fluctuate throughout the year. No substantial differences were observed in the $\delta^{18}O$ values among the various photosynthetic modes within each sample set. The $\delta^{18}O$ values of leaf cellulose (Fig. 2A) from samples for which we extracted leaf water (Fig. 2B) were all about 27‰ higher than the $\delta^{18}O$ values of the extracted water. This result confirms our previous conclusion that the biochemical pathway of photosynthetic CO_2 fixation does not seem to affect the relationship between $\delta^{18}O$ value of cellulose and $\delta^{18}O$ value of the water at the site of cellulose synthesis (23).

Field samples studied previously showed differences between $\delta^{18}O$ values of cellulose from C_4 and C_3 plants in the range of 5 to 8‰ (20, 22, 25). For these samples, we hypothesized that the differences are due to the fact that photosynthesis in C_4 plants is less sensitive than that in C_3 plants to dessicating conditions where leaf water is enriched in ^{18}O. If this hypothesis is correct, one would expect that under humid and nonwater-stressed conditions the differences in oxygen isotope ratios between C_3 and C_4 would be eliminated or minimized since C_3 photosynthesis would not be inhibited. The lack of significant differences in cellulose $\delta^{18}O$ values between C_3 and C_4 plants in this greenhouse grown sample set is consistent with our expectations. The humid greenhouse conditions under which the plants studied here were grown eliminated the differences in oxygen isotope ratio between C_3 and C_4 plants (Fig. 2A).

Like oxygen, D/H ratios of cellulose nitrate from the second sample set are slightly lower than those from the first sample set (Fig. 3A). Again, the difference in hydrogen isotope ratio between two sample sets might be due to slight variations in the hydrogen isotope ratios of the irrigation water, which also show annual variations.

There is a large variability in δD values of cellulose nitrate within each photosynthetic mode. We investigated the possibility that this variability might be due to differences in δD values of plant water between different plant species by extracting their waters and measuring their hydrogen isotope ratios. No substantial differences were observed for hydrogen isotope ratios of water among the various plant studies (Fig. 3B). We measured hydrogen isotope ratios of saponifiable lipids for several plants, and did not find a substantial difference among δD values of saponifiable lipids from C_4, C_3/C_4 intermediates, C_3, and hybrid plants (Fig. 3C). Taken together, our hydrogen isotopic measurements indicate that the type of reactions responsible for variability in δD values of cellulose nitrate in the C_4, C_3/C_4 intermediates, hybrids, and C_3 plants are specifically involved in carbohydrate metabolism that leads to cellulose synthesis.

Among all the photosynthetic modes studied here, the C_4 plants had the highest variability in their δD values of cellulose nitrate (Fig. 3A). Some C_4 plants had cellulose nitrate with unusually high δD values. As we have previously observed, this variability is probably due to isotopic fractionations occurring during biochemical reactions involved in carbohydrate metabolism. The deuterium enrichment or depletion in C_4 plants also may be related to their specific decarboxylation reactions. The C_4 plants with higher δD values have NADP+ or NAD+ malic enzyme as their decarboxylating enzyme, while those with the lower hydrogen isotope ratios have PEP carboxykinase as their decarboxylating enzyme (Fig. 3A). Malic enzyme and PEP carboxykinase have different products from the malic acid decarboxylation. Specifically, malic enzyme catalyzes an oxidative decarboxylation involving pyridine nucleotide reduction where deuterium fractionation may occur; whereas PEP carboxykinase involves no pyridine nucleotide metabolism. Hence, these products may have different degrees of deuterium enrichment that would subsequently be incorporated into the carbohydrate pool.

In conclusion, our measurements of leaf CO_2 compensation points show that hybrids between C_3/C_4 intermediates and C_3 plants have compensation points intermediate to the C_3/C_4 and

C_3 plants, in agreement with other workers (4–7). Thus, the processes which are responsible for low CO_2 compensation points in C_3/C_4 plants are heritable in a coordinated fashion so as to lower the compensation point of the F1 hybrids. Our carbon and oxygen isotope measurements of cellulose nitrate and cellulose respectively indicate that C_3/C_4 plants as well as C_3 plants do not differ in $\delta^{13}C$ and $\delta^{18}O$ values. Thus, the heritability of biochemical and physiological processes responsible for fractionation of these isotopes could not be checked. We note that a previous study on hybrids between C_4 and C_3 *Atriplex* species showed that the F1 generation had $\delta^{13}C$ values similar to those of their C_3 parent plants (3). Hydrogen isotope ratios of cellulose nitrate were highly variable, especially for the C_4 plants. We propose that the variability observed in hydrogen isotope ratios of cellulose nitrate from these plants are related to fractionations occurring during carbohydrate metabolism.

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