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Influence of N and K Fertilization and Growth Temperature on $^{13}C/^{12}C$ Ratios of Timothy (*Phleum pratense* L.)

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Summary. Measurements have been reported of carbon isotope ratios of timothy grown at different temperatures and with varying nitrogen and potassium supplies. Both total plant tissue and extracted plant tissue have been analyzed. The $^{13}C/^{12}C$ ratios were found to vary both with temperature and with nutrient level; the highest values of $\delta^{13}C$ were found under the most optimum growth conditions.

Introduction

The effect of environmental parameters such as light intensity, oxygen concentration, and growth temperature on the $^{13}C/^{12}C$ ratios of the carbon fixed in plant tissues has been studied by several laboratories and varied results have been reported for the temperature effect. While in some studies (Troughton, 1972; Smith et al., 1973; Troughton and Card, 1975; Smith et al., 1976) a correlation was found between temperature and $^{13}C/^{12}C$ ratio expressed as $\delta^{13}C$ value; in another, there was no clear relationship between temperature and carbon isotope fractionation. In one study (Troughton and Card, 1975) a linear relationship was reported but other studies (Smith et al., 1973; Smith et al., 1976) which reported a change in $\delta^{13}C$ value did not find that the change was linear with temperature. These latter two studies showed that isotopic fractionation appeared to be least when growth conditions were most favorable for a specific plant and that a particular plant may show slightly different $\delta^{13}C$ values when grown in different localities. We have studied the change in $\delta^{13}C$ values of a plant not only with temperature but with changes in the nutrient level of the growth medium at three temperatures.

Material and Methods

Timothy (*Phleum pratense* L.) samples which had been grown under controlled conditions for the study of the effect of nitrogen and potassium on the growth and chemical composition of the plant were made available to us for carbon isotope study. The timothy, a C_3 plant of temperate origin, had been grown in three chambers with three day/night temperature regimes (13/7, 19/13, and 32/26° C); 18 h photoperiod; 18,000 lux (at pot height). The growth chambers were all equally ventilated and supplied by the same air source. The $\delta^{13}C$ value of the carbon dioxide of the air supply is not known. Four nitrogen and potassium fertility combinations were used for the plants grown in each chamber, −N and +N equivalent to additions of 10 and 400 kg/ha, −K and +K equivalent to 0 and 400 kg/ha. No potassium was added to the −K treatments because of the presence of 67 kg/ha in the sand in which the samples were planted.

The timothy samples were dried at 70° C. Isotope measurements were made both on whole stem base tissue and on extracted plant tissue. The latter were extracted with a 9:1 acetone-water solution at room temperature, treated with boiling water, and again dried at 70° C. In addition the high fertility samples from each temperature regime were reduced to cellulose by the standard chlorite method (Green, 1963). All samples were combusted in a Parr bomb and the isotope ratios of the CO_2 produced were measured on a Nuclide RMS 6–60 mass spectrometer. The $\delta^{13}C$ values are reported compared to the PDB carbonate standard. Replicate preparations and measurements of a third of the samples were performed to verify consistency of the methods.

Results and Discussion

The results show that there is clearly a relationship between $\delta^{13}C$ value and growth temperature at high fertilization levels. The temperature effect is, however, much more pronounced in the total plant tissue (Fig. 1) than in the extracted plant tissue presumably because of the larger quantity of extractable non-structural materials accumulated under the optimum growth conditions. The extraction procedure removed nonstructural sugars and organic acids whose $\delta^{13}C$ values have been shown to be higher than those of total plant tissue (Whelan et al., 1970). The values of the cellulose and extracted tissue are essentially the same at the two lower temperatures; the cellulose value is slightly higher for the 32/26° timothy. Thus it appears that a large fraction of the change of $\delta^{13}C$ of the carbon in the whole tissue of the timothy in response to changes in environmental conditions is the result of change in the non-structural chemical composition of the plant. There is, however, even in the cellulose a small effect of temperature on the $\delta^{13}C$ values of the plants grown with high fertilization.

The isotope values show a definite parallel with the biochemical study of the plant response to varying nutrient levels. With high N and K levels Balasko (1971) reported that timothy produced the highest total plant growth at the 13/7° temperature regime; the $\delta^{13}C$ value of the plant tissue is also the highest under these conditions. At the high fertility level the growth rate and total herbage of the timothy was reported as least at 32/26° and the $\delta^{13}C$ value as the most negative. The isotope values of both extracted and unextracted samples thus reinforce the hypothesis

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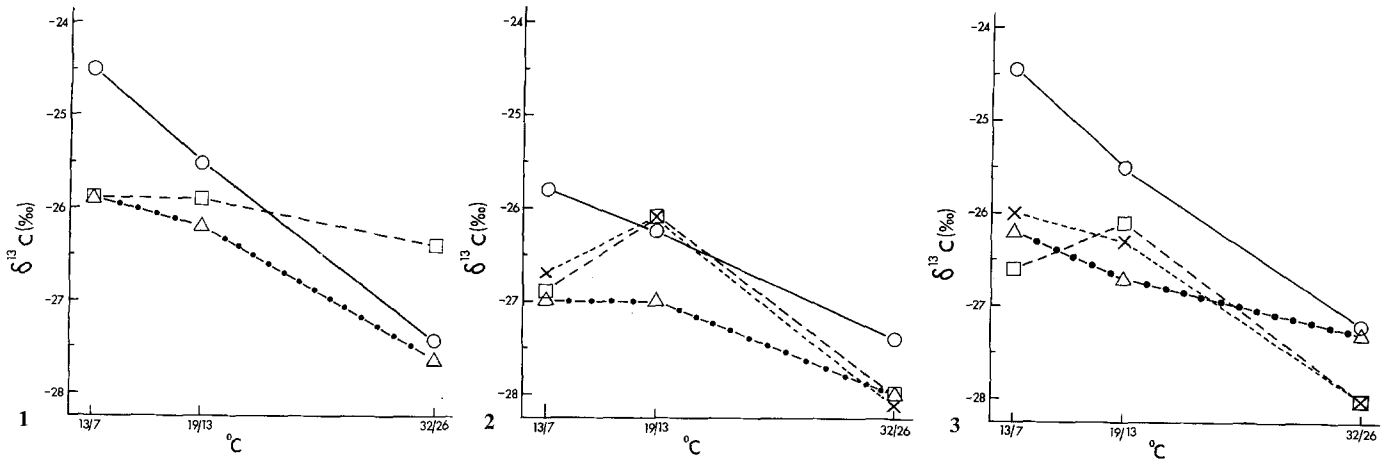

Fig. 1. $\delta^{13}C$ values of whole plant tissue (○), extracted tissue (△), and cellulose (□) of timothy grown with high N and K at three day/night temperatures

Fig. 2. $\delta^{13}C$ values of acetone-water extracted tissue of timothy grown with varying nutrient and day/night temperature regimes. ○ = +N+K; △ = +N−K; □ = −N+K; X = −N−K

Fig. 3. $\delta^{13}C$ values of whole plant tissue of timothy grown with varying nutrient and day/night temperature regimes. ○ = +N+K; △ = +N−K; □ = −N+K; X = −N−K

that under optimum growth conditions the isotopic fractionation of a plant is at a minimum.

Balaska also reported that, while herbage and total plant weights of timothy were highest in the low temperature regime at all levels of fertility and lowest in the high temperature regime, the total plant growth rates were greatest at 19/13° C except for the +N+K treatment. At this high fertility level the total plant growth rates were greatest at 13/7°. The isotope values of the extracted samples (Fig. 2) parallel the data on growth rate; the $\delta^{13}C$ values are highest for the samples grown at 19/13° except that at high fertility levels the highest $\delta^{13}C$ value is that of the timothy grown at 13/7°. The $\delta^{13}C$ values of the unextracted whole plant tissue (Fig. 3), on the other hand, parallel the herbage and total plant weights. The growth at 13/7° was most affected by decreases in N and K; that at 32/26° the least affected. The isotope fractionation at 13/7° is also markedly affected by the change in nutrient levels. While there is a temperature effect on the isotope ratios in that the $\delta^{13}C$ values are always lowest for the plants grown at 32/26°, the relationship is obviously not linear at lower nutrient levels.

The results suggest that one possible explanation for the variation in $\delta^{13}C$ values found for a specific plant in different localities might be varying environmental parameters such as differing nutrient levels of the soil in addition to differences in growth temperature. If other C_3 plants such as trees are susceptible to the same influences, varying nutrient levels could complicate the study of isotope ratios in tree rings.

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References

Balasko, J.: Influence of nitrogen and potassium fertilization and temperature on growth and chemical composition of switchgrass (*Panicum virgatum* L.) and timothy (*Phleum pratense* L.). Ph.D. dissertation, University of Wisconsin-Madison (1971)

Berry, J., Troughton, J.H., Björkman, O.: Effect of oxygen concentration during growth on carbon isotope discrimination in C_3 and C_4 species of *Atriplex*. Carnegie Inst. Wash. Yr. Bk, **71**, 158–161 (1972)

Green, J.W.: Wood cellulose. In: Methods in Carbohydrate Chemistry, Vol. III (R.L. Whistler, ed.), pp. 9–21. New York: Academic Press 1964

Smith, B.N., Herath, H.M.W., Chase, J.B.: Effect of growth temperature on carbon isotopic ratios in barley, pea and rape. Plant and Cell Physiol. **14**, 177–182 (1973)

Smith, B.N., Oliver, J., McMillan, C.: Influence of carbon source, oxygen concentration, light intensity, and temperature on $^{13}C/^{12}C$ ratios in plant tissues. Bot. Gaz. **137**, 99–104 (1976)

Troughton, J.H.: Carbon isotope fractionation by plants. In: Proc. 8th International Conf. on Radiocarbon Dating, Vol. 2 (T.A. Rafter, T. Grant-Taylor, eds.) pp. 420–438. Roy. Soc. New Zealand, Wellington 1972

Troughton, J.H., Card, K.A.: Temperature effects on the carbon-isotope ratio of C_3, C_4 and crassulacean-acid-metabolism (CAM) plants. Planta (Berl.) **123**, 185–190 (1975)

Whelan, T., Sackett, W.M., Benedict, C.R.: Carbon isotope discrimination in a plant possessing the C_4 dicarboxylic acid pathway. Biochem. Biophys. Res. Commun. **41**, 1205–1210

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