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Molecular Weight and ^{14}C Distribution of Fructosan in Timothy Stem Bases at Three Stages of Development[1]

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ABSTRACT

Variation in fructosan concentration in the stem base of timothy (*Phleum pratense* L.) at three stages of growth (initiation of stem elongation, ear emergence, and anthesis) from 1 to 168 hours after exposure to $^{14}CO_2$ was investigated. Molecular weight distribution of ^{14}C-labeled fructosan was determined. From initiation of stem elongation to anthesis, fructosan concentration increased from about 13 to almost 40%. In the first two growth stages, fructosan showed a broad molecular weight distribution, $\overline{M}_n$ = 18,000 to $\overline{M}_n$ = 2,000. There was a tendency by 168 hours after initiation of stem elongation for the plants to accumulate primarily higher molecular weight fructosan, while by ear emergence, there was a distinct accumulation of long-chain fructosan molecules. In all growth stages, ^{14}C-labeled fructosan appeared as early as 1 hour after exposure to $^{14}CO_2$ with label distributed throughout the molecular weight range. High ^{14}C activity was found in long-chain fructosans by 168 hours after exposure at ear emergence and anthesis. Condensation of short-chain fructosans and synthesis of new fructosans using monosaccharides with high ^{14}C activity may explain the above observation.

Additional index words: **Gel-filtration, Carbohydrates, Grass.**

FRUCTOSAN is the major storage carbohydrate in most temperate origin forage grasses (10) and is used for regrowth after cutting or grazing or to endure stress conditions (6, 13, 14). Timothy (*Phleum pratense* L.) plants are capable of storing large quantities of fructosan in their stem bases (9). Timothy fructosans are composed of fructose molecules linked β-2,6 with a degree of polymerization (DP) among the highest for fructosan-storing grasses making timothy of special interest in studies of carbohydrate metabolism (3, 5).

Knowledge of the cyclic trends of accumulation and use of fructosan in grasses may be of importance in forage production. Schlubach and Gassman (11) and Kühbauch and Züchner (7) found that long-chain fructosans were decomposed to a lesser extent than short-chain fructosans during the drying period in the process of hay making, potentially saving energy by knowing the trend of long-chain fructosan accumulation and use.

The purpose of this study was to determine the extent of incorporation of ^{14}C into timothy stem base fructosans at different growth stages and the molecular weight range of the fructosans into which the ^{14}C was incorporated.

MATERIALS AND METHODS

Stem base samples were obtained from an experiment described previously (1). In the preceding experiment, timothy plants were exposed to $^{14}CO_2$ for 45 min at three stages of growth (initiation of stem elongation, Stage I; ear emergence, Stage II; and anthesis, Stage III). Stem bases were harvested at 1, 3, 8, 32, and 168 hour intervals following commencement of exposure to $^{14}CO_2$. Twenty-four stem bases were harvested at each sampling time at Stage I, while 16 stem bases were harvested at each sampling time at Stages II and III. Bulked stem base samples from each sampling time were dried immediately at 70 C and ground to 40-mesh size. Samples were analyzed for concentrations of reducing and nonreducing sugars, starch, and fructosan according to the methods of Smith (12).

Fructosan extracts for molecular weight and ^{14}C activity estimations were prepared by shaking 500 mg of stem base tissue with 200 ml of 85% ethanol (V/V) at room temperature for 1 hour. The slurry was filtered through Whatman No. 1 filter paper and the filtrate containing the sugars was discarded. The residue was air dried and washed into a 250-ml Erlenmeyer flask with distilled water. Fructosans were extracted by shaking the residue with 200 ml of distilled water for 1 hour. Protein was precipitated with 10% lead acetate; excess lead acetate was precipitated with potassium oxalate. The solution was then filtered through Whatman No. 1 filter paper into a round bottom flask and condensed to a few milliliters under reduced pressure at 50 C. The condensate was filtered and rinsed through Whatman No. 42 filter paper into a calibrated test tube. The volume was adjusted with distilled water to obtain about a 1% fructosan solution. The condensed extract was tested for sugar impurities using thin layer chromatography (4). Each extract was found to be essentially free of sugars.

To estimate the molecular weight distribution of the fructosan extracts, 1 ml of each extract was eluted through a G-75 superfine Sephadex column with a 0.13% solution of NaN_3. The NaN_3 was used as a bacteriostat. One ml of a 1% solution of Dextran 10 and Dextran 20 (T-10 and T-20, Pharmacia) was also eluted through the above column. The peak for Dextran 10 occurred in fraction number 35 (molecular weight ($\overline{M}_n$) = 6,200) and the peak for Dextran 20 occurred in fraction number 25 ($\overline{M}_n$ = 15,000). These peaks were used in estimating fructosan molecular weights according to the regression equation, $Y = a + bX$, where Y is the fructosan molecular weight, a is the Y intercept ($\overline{M}_n$ = 37,000), b is the regression coefficient (−880 $\overline{M}_n$/fraction number), and X is the fraction number.

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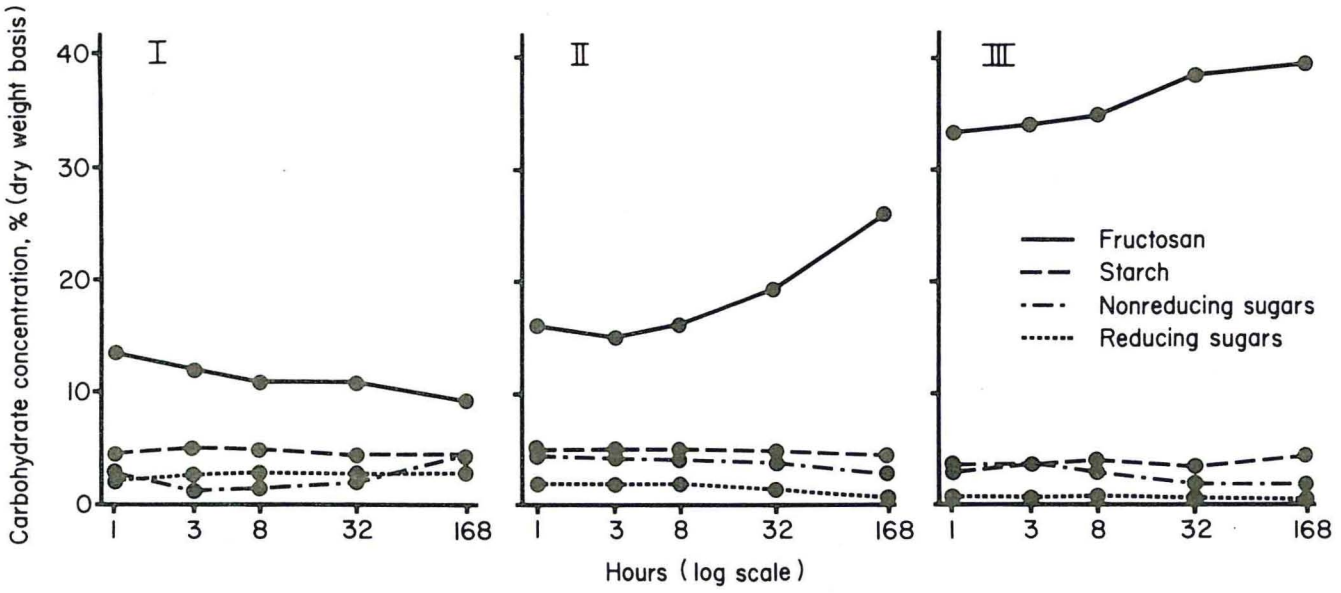

Fig. 1. Carbohydrate concentration in timothy stem bases at 1, 3, 8, 32, and 168 hours following commencement of exposure to $^{14}CO_2$ at initiation of stem elongation (Stage I), ear emergence (Stage II), and anthesis (Stage III).

The column bed was 81 × 1.5 cm, had a void volume of 42.5 ml, and a flow rate of 6 ml/hour. The eluant was collected in 2 ml fractions until essentially all dextran and fructosan had been eluted through the column as determined on a 0.5 ml aliquot of each fraction using the anthrone procedure of McCready, et al. (8).

To estimate ^{14}C activity, one ml of each fraction was pipetted into 20-ml scintillation vials. To prevent precipitation of fructosan by the scintillation solution each sample was hydrolyzed with 0.1 ml of 1 *N* HCl (15 min at 100 C), cooled, and neutralized with 0.1 ml of 1 *N* NaOH before addition of the scintillation solution. Quenching was not a problem when using the above hydrolysis procedure. To each vial, 10 ml of scintillation solution (13.3 g PPO; 190g naphthalene; 1900 ml dioxane) were added. Radioactivity was counted at 94.3% efficiency in a Beckman Liquid Scintillation System LS-11. Radioactivity was calculated for the entire 2 ml fraction.

RESULTS AND DISCUSSION

Carbohydrate Concentrations

Reducing and nonreducing sugars, starch, and fructosan concentrations in the stem bases of timothy plants from 1 to 168 hours after exposure to $^{14}CO_2$ at three stages of growth are shown in Fig. 1. Reducing and nonreducing sugar and starch levels showed little variation during the week following exposure to $^{14}CO_2$ at the three growth stages, while fructosan levels increased dramatically from Stage I through Stage III. Following exposure at Stage I, a 4.5% decrease in fructosan concentration was noted after 168 hours. In contrast, 168 hours following exposure at Stage II, fructosan concentration increased considerably before beginning to level off at Stage III. Leaves and stems are almost fully developed by Stage II and stem bases had accumulated nearly 26% fructosan (dry weight basis) by 168 hours after $^{14}CO_2$ exposure, and almost 40% fructosan by 168 hours after exposure at Stage III.

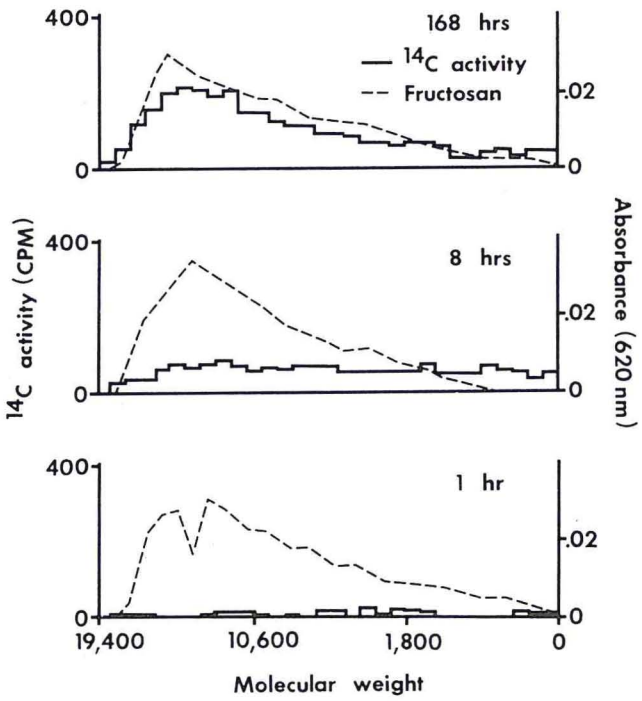

Fig. 2. Distribution of ^{14}C activity and fructosan in timothy stem bases after elution through Sephadex G-75 superfine. Plants were exposed to $^{14}CO_2$ for 45 min at the initiation of stem elongation (Stage I) and harvested 1, 8, and 168 hours following commencement of exposure. Fructosan and ^{14}C activity at molecular weight 0 may have been caused by tailing in the column or breakdown of minute amounts of low molecular weight fructosans during handling. All ^{14}C activities minus background. Standard error at 1 hour = 1.39.

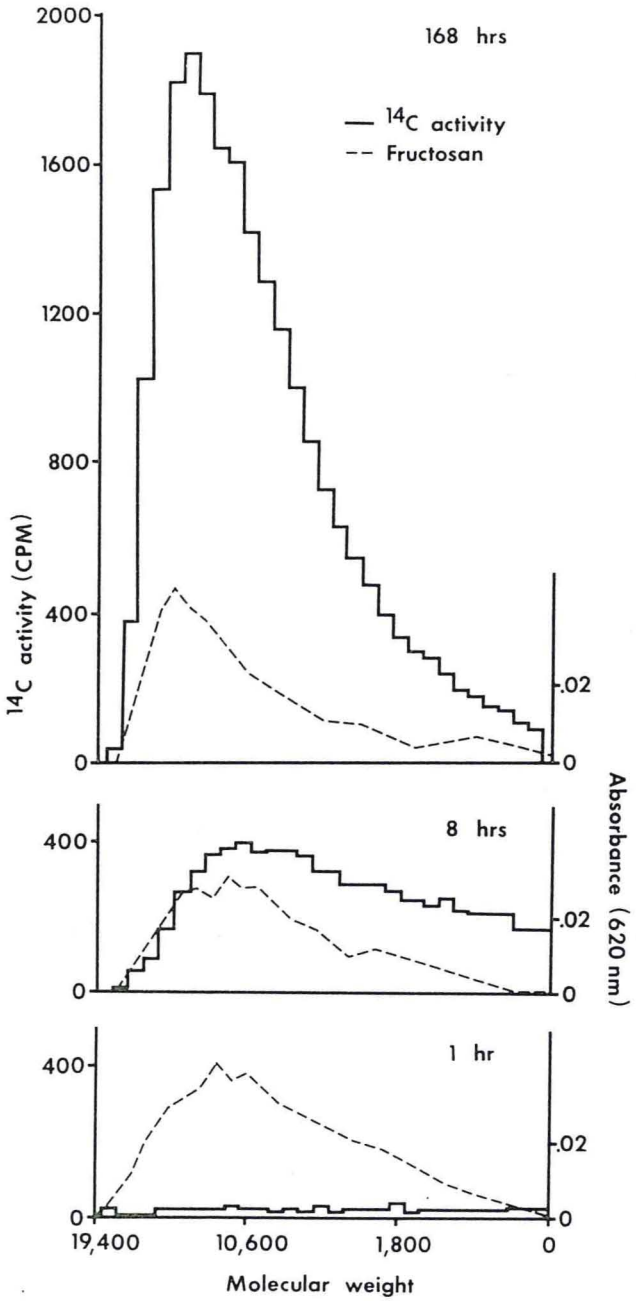

Fig. 3. Distribution of ^{14}C activity and fructosan in timothy stem bases after elution through Sephadex G-75 superfine. Plants were exposed to $^{14}CO_2$ for 45 min at ear emergence (Stage II) and harvested 1, 8, and 168 hours following commencement of exposure. Fructosan and ^{14}C activity at molecular weight 0 may have been caused by tailing in the column or breakdown of minute amounts of low molecular weight fructosans during handling. All ^{14}C activities minus background. Standard error at 1 hour = 2.00.

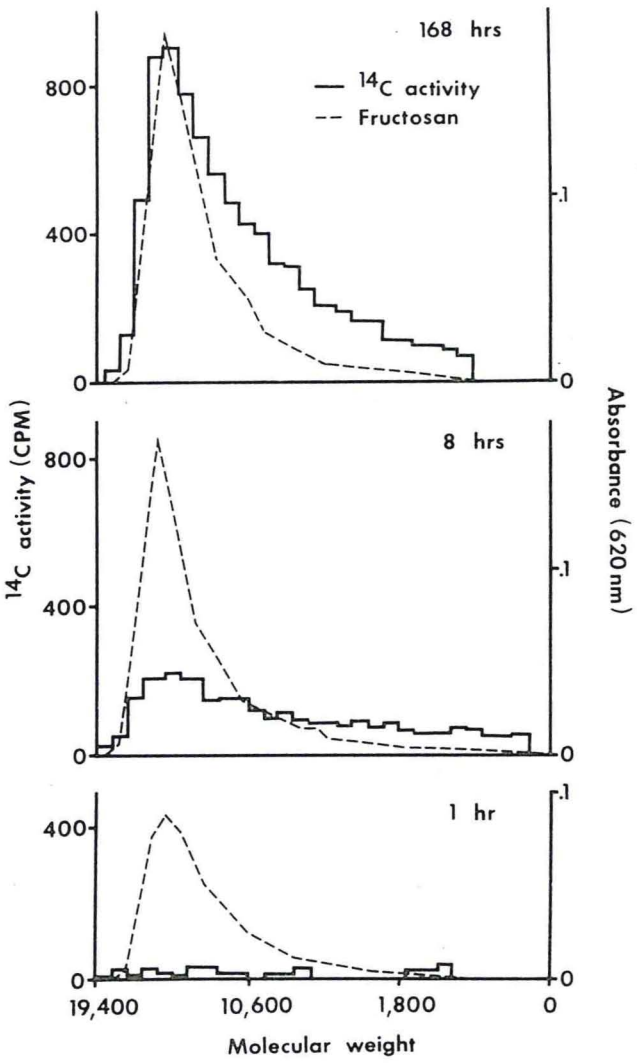

Fig. 4. Distribution of ^{14}C activity and fructosan in timothy stem bases after elution through Sephadex G-75 superfine. Plants were exposed to $^{14}CO_2$ for 45 min at anthesis (Stage III) and harvested 1, 8, and 168 hours following commencement of exposure. Fructosan and ^{14}C activity at molecular weight 0 may have been caused by tailing in the column or breakdown of minute amounts of low molecular weight fructosans during handling. All ^{14}C activities minus background. Standard error at 1 hour = 2.36.

Molecular Weight Distribution of Fructosan

Fructosan in the stem base showed a very broad molecular weight distribution at Stage I (Fig. 2). Most fructosan was between $\overline{M}_n$ = 18,000 and $\overline{M}_n$ = 10,000 with lesser amounts down to $\overline{M}_n$ = 2,000.

At Stage II, fructosan molecular weight distribution showed a broad distribution pattern with the largest amount of fructosan at about $\overline{M}_n$ = 11,000 (Fig. 3). One week later, however, fructosan tended to consist of long-chain molecules between $\overline{M}_n$ = 18,000 and $\overline{M}_n$ = 11,000. Stage II seems to have been an intermediate step in the development of long-chain fructosan molecules with a relatively narrow molecular weight range. As was found in previous studies (5, 15), the molecular weight range of fructosan in the stem base at Stage III showed a relatively narrow distribution around $\overline{M}_n$ = 16,000 (Fig. 4).

Balasko and Smith (1) found that whole plants (identical to those used in our experiment) transported large amounts of mono- and dissaccharides from

leaf blades and sheaths to stems and stem bases. At Stage III, increasing fructosan concentration (Fig. 1) and fructosan molecules with a high molecular weight (Fig. 4) together with the findings of Balasko and Smith (1) are consistent with the theory that grasses of temperate origin prevent increases in osmotic pressure by polymerizing fructosan molecules (6).

^{14}C Distribution of Fructosan

Activity of ^{14}C in stem base fructosans 1 hour after exposure at all growth stages showed that assimilates were rapidly incorporated into fructosan molecules, ^{14}C activity in the stem base ranging from 7 to 2%, Stages I through III, respectively, of total plant ^{14}C activity (1). This agrees with the rapid distribution of ^{14}C following exposure to $^{14}CO_2$ previously observed in plants (2). In our study, ^{14}C activity was distributed uniformly throughout the entire molecular weight range as early as 1 hour after exposure (Fig. 2, 3, 4). Eight hours after exposure to $^{14}CO_2$, high levels of activity were also found over the entire molecular weight range with slightly higher levels noted for larger molecular weight fructosans. Accumulation of ^{14}C took place independently of fructosan accumulation in the stem base, i.e., at Stage I, fructosan concentration decreased 4.5% from 1 to 168 hours following exposure to $^{14}CO_2$ (Fig. 1) while ^{14}C activity in the fructosan fraction increased distinctly (Fig. 2) and at Stage II, fructosan concentration remained fairly stable from 1 to 8 hours (Fig. 1) while ^{14}C activity in the fructosan fraction increased dramatically (Fig. 3).

Several factors might influence the large increase in ^{14}C activity in the stem base fructosans 168 hours after exposure at Stages II and III. In relation to the distribution of fructosans of varying molecular weight, high ^{14}C activity was found in the low molecular weight range 8 hours after $^{14}CO_2$ exposure at Stage II, while by 168 hours, ^{14}C activity was lower in the same molecular weight range (Fig. 3). While this data may support the theory that long-chain molecules are in part built up from short- and medium-chain molecules, the amount of the latter is not sufficient to account for the tremendous increase in ^{14}C activity of long-chain fructosans as seen in Fig. 3.

Balasko and Smith (1) reported that ^{14}C activity of the mono- and disaccharide fractions in the stem base decreased at about the same rate as ^{14}C activity in the fructosan fraction increased. Consequently, highly labeled long chain fructosans in the stem base 168 hours after exposure a Stages II and III may also be due to the incorporation of ^{14}C monosaccharides. During Stage I an increase in ^{14}C activity of the fructosan fraction was not seen (Fig. 2); as reported earlier (1, 6), this may be due to the high demand for assimilates needed for stem construction.

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