Translocation and distribution of photosynthetically assimilated $^{13}$C to ‘Tsugaru’ apple fruits

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Abstract

Photosynthetically assimilated carbon ($^{13}$C) is transported from source leaves to fruits depending on their development stage. To examine the translocation of the assimilated $^{13}$C in a shoot with a fruit throughout the development process, we constructed an in-situ $^{13}$CO$_2$ exposure chamber system for a fruit-bearing apple shoot and measured the assimilation of $^{13}$C in leaves and its translocation to the fruit in an early maturing apple (Malus domestica) cultivar ‘Tsugaru’. Fruit-bearing shoots were exposed three times during the development period (early development, fruit development, and mature stages) to $^{13}$CO$_2$ for 1 h in the chamber and collected 72 h after exposure, followed by analysis of $^{13}$C inventory in each organ (leaves, branch, and fruit). We evaluated the translocation of $^{13}$C using two indices: $^{13}$C remaining ratio ($^{13}$C inventory in each plant organ)/net assimilated $^{13}$C during exposure), and $^{13}$C distribution ratio ($^{13}$C inventory in each plant organ)/total $^{13}$C inventory in the shoot). Although the $^{13}$C remaining ratio in the fruit during the early development stage was slightly lower than that at the fruit development and mature stages, the ratio at the mature stage was similar to that at the fruit development stage, indicating that the ability of source leaves to allocate photoassimilates to fruit was maintained until fruit maturation. The $^{13}$C distribution ratio of the fruit was similar regardless of its development stage, although biomass proportion of fruit increased with the development stages. This suggests that the sink strength of the fruit-bearing shoots of ‘Tsugaru’ may not be altered by fruit development.

Key words: Carbon translocation, Early maturing apple, Growth curve, Net assimilation, Sink strength

1. Introduction

Photoassimilate translocation to fruit has been relatively well investigated in fruit trees (Hansen, 1967a; Hansen, 1971; Finazzo et al., 1994; Darnell and Birkhold, 1996; Zhang et al., 2005; Carini et al., 2006; Volpe et al., 2008), including apple (Malus domestica Borkh.) trees. Photoassimilates can be translocated from source leaves to fruit at different rates depending on the fruit development stage. It is well known that fruit sink strength affects photosynthetic translocation from source leaves to fruit; for example, low apple fruit loads decreased both leaf assimilation and fruit dry matter production at the whole-tree level (Palmer et al., 1997). In avocado trees, the distribution of photoassimilates depended on the biomass of each plant organ (Finazzo et al., 1994), suggesting that the sink strength of the plant organ, represented by its biomass, controlled the distribution. In contrast, there are a few studies that provide evidence that source leaves can control the translocation of newly assimilated C to the fruit. Klages et al. (2001) observed that the larger biomass of individual apple fruits in low-cropping trees was primarily due to the supply of photoassimilates from source leaves rather than fruit sink metabolic activity. In a study of source-sink manipulation of peach trees at the shoot level, fruiting shoots with a higher ratio of leaf area to fruit mass tended to translocate more assimilated C to fruit at the peak stage of fruit sink strength (Volpe et al., 2008), suggesting that the supply of photoassimilates from source leaves is important for their translocation into fruit.

In addition, sink strength can vary during the fruit development period depending on sink demands and source-sink balance (Flore and Layne, 1999), and thus temporal variations in sink strength may influence photoassimilate translocation to fruit. However, the transport of photoassimilates to sink fruit throughout its development period, including the maturation stage, has not been adequately studied in fruit trees and, to our knowledge, this information is not available for apple trees. To this end, we studied the translocation of photoassimilates in apple shoots throughout fruit development, and investigated whether fruit sink size and source-sink mass balance affect the translocation and distribution of photoassimilates to the fruit.

In our study, leaves, a current-year branch, and a fruit of a fruit-bearing shoot were selected to represent source and sink, assuming that leaf assimilates are primarily translocated into nearby fruit (Hansen, 1971). We established an experimental system to expose a fruit-bearing shoot to $^{13}$CO$_2$ in-situ and to evaluate net assimilation under near-natural conditions but with relatively constant photosynthetic photon flux density (PPFD). Net amount of assimilated $^{13}$C during the exposure period and assimilated $^{13}$C accumulation in the shoot over a short period after the exposure were determined at three fruit developmental stages. Here the results are reported and discussed in relation to the sink strength of the fruit.
2. Materials and Methods

2.1 In-situ $^{13}$CO$_2$ exposure chamber for fruit-bearing shoot

We constructed a shoot chamber (0.20 m width, 0.41 m length, and 0.31 m height) for in-situ exposure of a fruit-bearing shoot to $^{13}$CO$_2$ (Fig. 1). The chambers were made of clear acryl boards (3 mm thickness for the main body) and consisted of upper and lower compartments, each of which had an edge with a silicone sponge rubber gasket (3 mm thickness) to fit each other. In the upper compartment, a slit (10 mm width and 15 mm height) was made at the center of a panel at the bottom of the compartment to set a shoot branch. The upper compartment had an air inlet hole and an axial fan fixed on side acryl boards at an angle of 45° near the inlet hole. A temperature/humidity (RTR-503, T&D Corporation, Nagano, Japan) sensor and a quantum sensor (Apogee Instruments, Inc., Logan, USA) were set in the upper compartment with cables passing through another hole. Fishing lines were stretched across the top of the upper compartment to keep leaves from direct contact with the top of the chamber. The lower compartment had an air outlet hole. The bottom board of the lower compartment was inclined at an angle of 20° in order to reduce chamber volume while maintaining enough space for a fruit. To circulate the air inside the chamber, an acryl board was set parallel to the bottom board.

A water jacket was attached to both the top and bottom compartments of the chamber to introduce water to the chamber by means of a water pump in order to remove infrared radiation and heat inside the chamber. The top board of the bottom water jacket was made of aluminum plate to ensure high thermal conductivity. The chamber was placed on a stand with a leveling tripod to adjust the height, allowing the shoot to be installed in as natural a position as possible.

After a fruit-bearing shoot was set between the upper and lower compartments with a shoot branch through the slit, the gap between the slit and the shoot branch was filled up with sealant putty (Cemedine Co., Ltd., Tokyo, Japan) and the edges of upper and lower parts were secured together with clips. The air was circulated inside the chamber using the axial fan just after enclosing the shoot. We used a potable LED light source (LG-600S, Suntech Co., Ltd., Tokyo, Japan) above the chamber for supplemental lightning, maintaining the average values of PPFD inside of chambers at a relatively constant value ranging from 735 to 745 μmol photon m$^{-2}$ s$^{-1}$ during the exposure experiment.

2.2 $^{13}$CO$_2$ supply system

We constructed three chamber systems to simultaneously expose three fruit-bearing shoots to $^{13}$CO$_2$ (Fig. 2). The air was supplied by an air compressor through two flow lines; one was fitted with a glass washing bottle (1 L) containing soda lime to remove CO$_2$ from the compressed air, while the other was without the bottle (Fig. 2a, air supplying unit). The two lines were connected to a three-way ball valve. Either compressed air or CO$_2$ free air was supplied from the valve to three parallel channels through mass flow controllers (CMQ-V, Azbil Corporation, Tokyo, Japan or SEC-E40MK3, Horiba, Ltd., Kyoto, Japan) at a flow rate of 7 L min$^{-1}$. $^{13}$CO$_2$ gas (99 atom%, SI Science Co., Ltd., Saitama, Japan) was supplied to each channel through a mass flow controller (ACE Inc., Kanagawa, Japan) at a flow rate of 3 mL min$^{-1}$ and mixed with the CO$_2$ free air in a series of three empty gas washing bottles (0.5 L). Total CO$_2$ concentration of the $^{13}$CO$_2$...
mixed air at the flow rates was calculated as 425 ppm, which was slightly higher than that of the atmosphere because of expected reduction in CO$_2$ concentration as a result of assimilation. The air was passed through two gas washing bottles (1 and 0.5 L) containing distilled water for humidification, and air flow to the humidifying bottles was adjusted using a valve. The pH of the distilled water was adjusted using L-ascorbic acid to a range of 4.0 to 5.0 to prevent CO$_2$ dissolution in the water.

The air was supplied to the inlets of exposure chambers through mass flow controllers (SEC-E40MK3) at a flow rate of 6 L min$^{-1}$ (Fig. 2h, chamber unit), which was predetermined based on preliminary experiments. The residual air from the inlets and outlets of the chambers were removed passively through vents with flow adjusting valves.

Inlet and outlet air samples were collected with each air-sampling bag (Aluminum bag, AAK-1, GL Science Inc., Tokyo, Japan) (Fig. 2c, air sampling unit). Six three-way cocks were used for continuous sampling during exposure period. Part of the air was pumped from inlets and outlets by six diaphragm pumps (0.6–1.0 L min$^{-1}$, APN-60G1D3-W, Iwaki Co., Ltd., Tokyo, Japan) and sampled through six flow meters with precision needle valves adjusted to the flow rate of approximately 0.1 L min$^{-1}$ (RK1250, Kojima Instruments Inc., Kyoto, Japan). The remaining air was removed passively through vents.

### 2.3 Study sites and trees

The experiment was conducted in the experimental apple orchard at the Apple Research Division, Institute of Fruit Tree and Tea Science, National Agriculture and Food Research Organization (NARO), Morioka City, Iwate, Japan (39°45′N; 141°7′E, 190 m elevation) during the fruit-growing season in 2016. Monthly mean air temperature, total precipitation, and solar radiation near the study site between May and September ranged 15.4–24.1°C, 99–259 mm and 375–592 MJ m$^{-2}$, respectively (meteorological observation data, NARO Tohoku Agricultural Center).

Three 29-year-old *M. domestica* ‘Tsugaru’ apple trees on M.9 stocks were used in the experiment. ‘Tsugaru’ is one of the commercially important and early maturing cultivars in Japan. The height and canopy width of the trees were 3.6 ± 0.4 and 4.1 ± 0.3 m (mean ± standard deviation, $n = 3$), respectively. The dates of bud break and flowering were not observed in the experimental orchard. The bud break, leaf expansion, flowering, full bloom, and petal fall of ‘Tsugaru’ in 2016 in Kuroishi City, approximately 100 km northwest from the study site, were reported to be 1, 15 April, 4, 8, and 13 May, respectively (Apple Research Institute, 2016). These dates were 4–7 days earlier than those averaged from 1996 to 2015. Leaves were manually thinned (particularly fruit leaves) to improve fruit coloration as a routine procedure for the orchard on 7 September. Fruits were harvested in mid-September.

### 2.4 CO$_2$ exposure experiment

We defined ‘Tsugaru’ fruit growth stages based on our estimated growth curve in days after bud break (DAB) discussed later (Fig. 3) as follows: early fruit development stage (EFD) from fruit set to 100 DAB; fruit development stage (FD) from 100 DAB to 150 DAB; fruit maturation stage (FM) from 150 DAB to 170 DAB at harvest. As shown in Fig. 3, the fruit grew slowly at the EFD stage, prominently at FD, and a little slower at FM than at FD. Exposure of selected shoots to $^{13}$CO$_2$ was conducted twice at EFD (63 and 88 DAB) and once at FD (111 DAB) or FM stages (161 or 167 DAB) as shown in Table 1. Unfortunately, all fruits on the shoots exposed at 63 DAB had been lost after 72 h of exposure, probably because of natural fruit drops, which are common in spring in this cultivar. Three shoots were simultaneously exposed to $^{13}$CO$_2$ during each growth stage excluding the exposure at FM. At FM, one shoot was exposed to $^{13}$CO$_2$ at 161 DAB, and two at 167 DAB. The data for both days at the FM stage were combined and treated as data at 165 DAB in this report.

Fruit-bearing shoots, which consisted of leaves, a current-year branch, and a fruit, positioned on the top of twigs were used for the exposure experiment. We selected a fruit-bearing shoot from each tree at the height between 50 and 150 cm for exposing to $^{13}$CO$_2$ for each date.

After we supplied the compressed air for 10 min to check the exposure system, the shoots were exposed to $^{13}$CO$_2$ for 1 h, followed by supplying the compressed air for 30 min to flush out the remaining $^{13}$CO$_2$. The time of the exposure experiments was from 9:50 to 14:10 (Table 1). During the exposure experimental period of 100 min in total, we collected inlet and outlet air samples in the air-sampling bags through the three-way cocks operated manually at 10 min intervals. Five leaf disk samples (1-cm diameter) from five different leaves on each shoot were sampled just after the exposure experiment to confirm $^{13}$CO$_2$ assimilation. The exposed shoots were collected at 72 h after finishing the $^{13}$CO$_2$ exposure. The sampling timing at 72 h after exposure was determined based on previous studies (Hansen, 1967b; Yamamoto, 2001; Okawa et al., 2002), which indicated that the translocation of assimilated $^{13}$C from source leaves to other parts mostly occurred for a few days.

A fruit-bearing shoot from each tree was also selected as a control sample before exposure. Since our exposure was conducted on the same trees repeatedly, experimental shoots were selected at different positions each time to minimize the effects of previous exposure. The concentrations of $^{13}$C in the control leaves, branch, and fruit before exposure were approximately 1.08 atom%, almost natural abundance, and did not vary among different stages (Fig. S1), indicating that the $^{13}$C fixed at the previous exposures did not affect the $^{13}$C abundance level in the experimental shoots.

### 2.5 Sample treatment and analyses

The shoot samples collected before and after exposure were divided into leaves, branch, and fruit. Area of the leaves of the exposed shoots was measured using a leaf area meter (LI-3100C Area Meter, LI-COR, Inc., Lincoln, NE, USA). After the samples of leaf disks, leaves, branches, and fruits were oven-dried at 60°C for >72 h, their dry weights were measured, after which they were ground into powders using a vibration sample mill or a grinder mill. Total C and N concentration of the ground samples was analyzed using a NCH analyzer (NCH-22F, Sumika Chemical Analysis Service, Ltd., Tokyo, Japan). The specific leaf area (SLA, leaf area per leaf biomass), leaf N content per
Fig. 3. The biomass of leaves (LB, a), branch (BB, b), and fruit (FB, c) at the shoot level of 29-year-old Tsugaru/M.9 trees from mid-May to mid-September, except for the leaves, where data were shown until early-September (156 DAB). Each growth curve was fitted with the Logistic (LB or BB = $a / (1 + b \times e^{-c \times DAB})$) or FB = $a / (1 + b \times e^{-c \times DAB - d})$) using the biomass data of each plant organ; LB: $a = 5.6734$, $b = 43.742$, $c = 0.0537$ (a), BB: $a = 1.816$, $b = 481.40$, $c = 0.0782$ (b), FB: $a = 56.474$, $b = 160.18$, $c = 0.0536$, $d = 33$ (c). The ratios of biomass allocation (d), absolute growth rate (AGR), and relative growth rate (RGR) (e) of leaves, branch, and fruit were calculated based on the growth curves. Solid arrows indicate exposure dates (DAB) and dotted arrows indicate sampling timing at 72 after finishing the $^{13}$CO$_2$ exposure (c).

Table 1. Experimental conditions of in-situ exposure of 29 years old ‘Tsugaru’ apple trees to $^{13}$CO$_2$ and the resulting net assimilated $^{13}$C.

<table>
<thead>
<tr>
<th>Fruit development stage</th>
<th>DAB</th>
<th>Exposure start time</th>
<th>Air temperature ($^\circ$C)</th>
<th>Relative humidity (%)</th>
<th>PPFD ($\mu$mol photon m$^{-2}$ s$^{-1}$)</th>
<th>Number of leaves</th>
<th>Leaf area (cm$^{-2}$)</th>
<th>Net assimilated $^{13}$C per leaf area (mg $^{13}$C m$^{-2}$ h$^{-1}$)</th>
<th>Leaf disk $^{13}$C concentration (atom% excess)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early fruit development (EFD)</td>
<td>63**</td>
<td>10:50</td>
<td>21.4 ± 0.6</td>
<td>72.0 ± 8.2</td>
<td>745 ± 46</td>
<td>17.0 ± 2.6</td>
<td>292 ± 39</td>
<td>190 ± 28$^a$</td>
<td>0.71 ± 0.15$^a$</td>
</tr>
<tr>
<td>Early fruit development (EFD)</td>
<td>88</td>
<td>14:10</td>
<td>25.9 ± 0.5</td>
<td>80.7 ± 8.9</td>
<td>736 ± 23</td>
<td>18.7 ± 5.5</td>
<td>447 ± 133</td>
<td>446 ± 44$^a$</td>
<td>0.95 ± 0.43$^a$</td>
</tr>
<tr>
<td>Fruit development (FD)</td>
<td>111</td>
<td>13:30</td>
<td>33.5 ± 2.1</td>
<td>72.6 ± 7.9</td>
<td>745 ± 12</td>
<td>25.3 ± 4.0</td>
<td>479 ± 36</td>
<td>321 ± 11$^b$</td>
<td>0.60 ± 0.21$^b$</td>
</tr>
<tr>
<td>Fruit maturation (FM)</td>
<td>165</td>
<td>9:50 or 11:00</td>
<td>28.5 ± 1.5</td>
<td>71.6 ± 5.8</td>
<td>735 ± 14</td>
<td>9.7 ± 3.1</td>
<td>244 ± 43</td>
<td>379 ± 62$^{ab}$</td>
<td>0.65 ± 0.17$^a$</td>
</tr>
</tbody>
</table>

Different lower case letters show significant differences ($P < 0.05$, ANOVA followed by Tukey HSD test).
DAB: days after bud break; PPFD: photosynthetic photon flux density.
* Mean and standard deviation of three duplicate samples.
** All fruits on the exposed shoots were naturally dropped between exposure and sampling.
leaf area ($N_{\text{leaf}}$), the ratio of leaf biomass to fruit biomass (leaf/fruit mass ratio), and the ratio of leaf area to fruit mass (leaf area/fruit mass ratio) were calculated at the shoot level.

The CO$_2$ concentration in the inlet and outlet air samples was analyzed using a gas chromatograph mass spectrometer (GCMS-QP 2010, Shimadzu, Kyoto, Japan). The $^{13}$C/$^{12}$C atom ratio in the air and plant samples was analyzed by mass spectrometry (DELTA V Plus, Thermo Fisher Scientific K.K., Kanagawa, Japan and IsoPrime 100, IsoPrime Ltd., Cheadle Hulme, UK).

### 2.6 Measurement of biomass growth

In addition to shoot samples discussed above, we collected fruit-bearing shoots at 42, 45, 70, 73, 153, 156, 173, and 176 DAB for measuring biomass. The shoots were divided into leaves, branch, and fruit, followed by measuring their dry weights after oven-drying at 60°C for >72 h.

### 2.7 Data analyses

We determined the amount of $^{13}$C assimilated by leaves (net assimilated $^{13}$C) from the differences in the $^{13}$C amounts between the inlet and outlet air samples at a 10-min interval. The data were integrated for 60 min during the $^{13}$CO$_2$ exposure period and 10 min after the exposure because of the time lags of sampling between the inlet and outlet. Excess $^{13}$C concentration in the leaves, branch, and fruit at 72 h after $^{13}$CO$_2$ exposure was obtained by subtracting $^{13}$C concentration of the control samples. We evaluated the translocation of $^{13}$C using two indices: $^{13}$C remaining ratio ($^{13}$C inventory in each plant organ)/(net assimilated $^{13}$C during exposure), and $^{13}$C distribution ratio ($^{13}$C inventory in each plant organ)/(total $^{13}$C inventory in the shoot).

The net photosynthetic rate ($P_n$) during $^{13}$CO$_2$ exposure was determined at a leaf area basis. The $P_n$ was determined from the differences in CO$_2$ concentrations between the inlet and outlet air samples at a 10-min interval for each chamber. The inlet and outlet CO$_2$ concentrations decreased at the time of transition from the compressor air to $^{13}$CO$_2$ and increased with time, then became relatively stable at the period from 30 to 60 min after starting $^{13}$CO$_2$ exposure. We used the stable value of $P_n$ at a 10-min interval in order to evaluate the ability of carbon assimilation at each exposure date.

The growth of biomass of each plant organ was analyzed by regression following a logistic function using the least square method. The biomass data used for the analysis were from mid-May to mid-September, except for the leaves, where data were until early-September (156 DAB) because of leaf thinning after the date.

\[
\text{Biomass of each plant organ} = a / (1 + b \times e^{-c \times \text{DAB}})
\]  

The absolute growth rate (AGR) and relative growth rate (RGR) of the biomass of leaves, branch, or fruit at the shoot level were calculated based on each regression curve.

One-way analysis of variance (ANOVA) followed by Tukey HSD multiple comparison test were used to determine the effects of various factors on target variables. Data were transformed (logarithmic or arcsine-square-root) to confirm the assumptions of normality and homogeneity when necessary. The data of $^{13}$C inventory and remaining and distribution ratios in each plant organ obtained from the experiment at 63 DAB were removed from the analysis because natural fruit drop occurred between exposure and sampling. All statistical analyses were performed using R (version 3.2.3).

### 3. Results

#### 3.1 Biomass growth of leaves, branch, and fruit

Results of biomass growth of leaves, branch, and fruit are shown in Fig. 3. The biomass of leaves increased rapidly from the beginning of sampling to 60 DAB (Fig. 3a), while that of the branch increased rapidly from 60 to 90 DAB (Fig. 3b). The fruit biomass rapidly increased between 100 and 150 DAB as presented in Fig. 3c. The regression equations of biomass growth of the plant organs are also shown in the caption of the figures. Fig. 3d shows the biomass ratio in each plant organ to the whole shoot estimated by the regression curves, indicating that the proportion of fruit biomass exceeded 0.5 at approximately 80 DAB and reached 0.9 near the harvest.

The AGR and RGR of each plant organ by the regression curves are presented in Fig. 3e and Fig. 3f, respectively. As shown in the figure, the AGR of leaves and branch had peaks at ~60 DAB and ~80 DAB. The AGR of fruit increased rapidly and reached its peak at ~130 DAB and gradually decreased until harvest. The RGR of leaves and branch tended to be higher during the early stages and gradually decreased until harvest, while the fruit RGR was higher during the early stages and exponentially decreased (Fig. 3f).

#### 3.2 Net assimilation and leaf traits

The supplemental lightning ensured similar light conditions in the chambers among the different exposure dates irrespective of outside solar radiation (Table 1). As a result, the $P_n$ during exposure was not significantly different from the EFD to FM stages (11-14 μmol m$^{-2}$ s$^{-1}$; Fig. 4a, the inlet and outlet CO$_2$ concentrations are presented in Table S1), although the exposure was conducted at various start times (9:50-14:10, Table 1). Thus, the exposed shoots exhibited similar photosynthetic rates irrespective of different developmental stages and exposure times.

The $^{13}$C concentrations of leaf disk samples after the exposure period are presented in Table 1, together with the net assimilated $^{13}$C per leaf area during exposure. The net assimilated $^{13}$C per leaf area and $^{13}$C in leaf disk just after the experiment were highest at 88 DAB (the inlet and outlet $^{13}$C concentrations are shown in Table S2).

The SLA at the shoot level was significantly higher at the EFD than FD and FM stages (Fig. 4b), indicating that an increase in leaf thickness after leaf expansion occurred between EFD and FD. The $N_{\text{leaf}}$ did not change among the different stages (Fig. 4c). This, together with the non-significant difference in the $P_n$ among developmental stages (Fig. 4a), shows that we selected shoot leaves with similar ability of assimilation at different stages.

The leaf/fruit mass ratio was the highest at the EFD stage (Fig. 4d), then significantly decreased with fruit development due to both an increase in fruit biomass and a reduction in leaf biomass by leaf thinning at the FM stage. The leaf area/fruit
mass ratio was also the highest at the EFD stage, significantly higher than that at the FM stage (Fig. 4e).

3.3 $^{13}$C inventory and remaining and distribution ratios

All fruits on the exposed shoots naturally dropped between the exposure period and sampling period in early-June at the EFD stage. The $^{13}$C inventory, remaining ratio, and distribution ratio in early-June were not included in the analyses. The $^{13}$C inventory in the shoot tended to be higher at the EFD (late-June) and FD (late-July) stages than at the FM stage (early- to mid-September) (Fig. 5a). The averaged values of $^{13}$C inventory in the whole shoot at the EFD, FD, and FM stages were $16.4 \pm 3.9$, $16.2 \pm 0.9$, and $9.7 \pm 0.9$ mg $^{13}$C shoot$^{-1}$, respectively, while the averaged values of net assimilated $^{13}$C per shoot during exposure from mass balance of $^{13}$CO$_2$ between inlet and outlet air during the exposure period were $19.7 \pm 4.9$, $15.3 \pm 0.6$, and $9.1 \pm 1.0$ mg $^{13}$C shoot$^{-1}$ h$^{-1}$, respectively. These values are within a standard deviation of each value, and we used the data for calculating the $^{13}$C remaining ratio.

The $^{13}$C remaining ratio tended to be higher in the fruit than in the leaves and branch (Fig. 5b). The $^{13}$C remaining ratio in the leaves slightly increased with developmental stages, with a significant difference between the EFD and FM stages. In the branch, the $^{13}$C remaining ratio tended to decrease from EFD to FD, but no significant difference was found among the developmental stages. The $^{13}$C remaining ratio in the fruit significantly increased from EFD to FD and remained high until FM. The whole shoot (i.e., leaves, branch, and fruit) had significantly lower $^{13}$C remaining ratio at EFD than FM ($P < 0.05$, ANOVA followed by Tukey HSD test). Although the $^{13}$C remaining ratio in the whole shoot exceeded 1.0 at FD and FM, the lower $^{13}$C remaining ratio at EFD indicates higher $^{13}$C loss from the shoot during the EFD stage than at the other stages.

The $^{13}$C inventory ratio in each plant organ to that in the shoot ($^{13}$C distribution ratio) is shown in Fig. 5c. The temporal patterns of $^{13}$C distribution ratio in the leaves and branch were similar to

![Fig. 4](image-url) The net photosynthetic rate ($P_n$) during exposure (a), specific leaf area (SLA) (b), leaf N content per leaf area (c), ratio of leaf mass to fruit mass (leaf/fruit mass ratio) (d), and ratio of leaf area to fruit mass (leaf area/fruit mass ratio) (e) of the selected shoots of 29-year-old Tsugaru/M.9 trees exposed at 63, 88 (EFD), 111 (FD), and 165 DAB (FM). Error bars represent the SD of single measurements in three replicates (One-way ANOVA followed by Tukey HSD test, $P < 0.05$, n.s., non-significant; n.d., not determined).
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$^{13}$C export from the exposed shoot to other plant organs and/or more respiration loss from the shoot by 72 h after the exposure at the EFD stage. A linear relationship exists between biomass increment (i.e., relative growth rate) and growth respiration (Marcelis, 1996). In contrast, $^{13}$C-photoassimilate translocation from labeled fruiting shoots to adjacent non-labeled shoots is hardly observed on peach trees (Volpe et al., 2008). Since the relative growth rates of leaf, branch, and fruit biomass were higher at the earlier fruit development stages in our study, the greater loss of photoassimilates at the EFD stage may be attributable to higher growth respiration rate in the exposed shoots. However, we did not determine either photoassimilate export to other organs or respiration loss from each plant organ of the exposed shoot. More detailed studies are necessary to clarify this implication.

At the FD and FM stages, the $^{13}$C remaining ratio in the fruit was similar between the stages of fruit development, indicating that photoassimilate translocation was maintained until maturation. The fruit biomass, leaf/fruit mass ratio, and leaf area/fruit mass ratio tended to be, but not significantly, higher at the FD stage than at the FM stage. This suggests that photoassimilates translocated to fruit may be used for more growth at the FD stage and for more storage of soluble carbohydrates at the FM stage. Apple Research Institute (2016) reported that the sugar content of ‘Tsugaru’ apple fruits in northern Japan increased constantly from early August to mid-September. Such trend has been reported previously in midseason ‘Cox’s Orange Pippin’ and late maturing ‘Golden Delicious’ apples (Pavel and DeJong, 1995), in which rapid increase of sugars was observed during the later phase of the growth season.

Sink strength varies with time of development through changes in sink demands, the development (Flore and Layne, 1999), and starch and sugar contents of each plant organ (Beckles, 2012). In our study, similar values of $^{13}$C distribution ratio in the fruit were found regardless of the developmental stages although the proportion of fruit biomass varied with stages; the leaf/fruit mass ratio and leaf area/fruit mass ratio tended to be higher at the FD stage than at the FM stage. This suggests that apple fruit sink strength at the shoot level in trees would largely not vary with different fruit developmental stages, and photoassimilate translocation from source leaves to fruit would be influenced by the net assimilation of source leaves, which largely depend on environmental conditions, such as temperature and PPFD, and leaf traits, such as leaf area, SLA, and N content (Kumashiro et al., 1990; Lambers et al., 1998; Grappadelli, 2003). Similarly, in a previous study, it was observed that greater biomass of individual fruit in low-cropping ‘Braeburn’ apple trees than in high-cropping trees was primarily due to greater supply of assimilates from source leaves rather than fruit sink metabolic activity (Klages et al., 2001).

5. Conclusion

The $^{13}$C remaining ratio in the ‘Tsugaru’ apple fruit was significantly lower at the EFD than at the FD and did not change between the FD and FM stages, indicating that $^{13}$C-photoassimilate translocation from source leaves to fruit was maintained throughout the fruit development period, including maturation. We also found that the $^{13}$C distribution ratio in the fruit was

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Fig. 5. The $^{13}$C inventory (a), $^{13}$C remaining ratio (b), and $^{13}$C distribution ratio in the leaves, branch and fruit of the selected shoots of 29-year-old ‘Tsugaru’/M.9 trees collected at 72 h after finishing the $^{13}$CO$_2$ exposure at 63, 88 (EFD), 111 (FD), and 165 DAB (FM) (c). Error bars represent the SD of single measurements in three replicates. One-way ANOVA followed by Tukey HSD test was performed for each plant organ ($P < 0.05$). The results in early-June were not included in the analyses because of natural fruit drops.

those of the $^{13}$C remaining ratio. The $^{13}$C distribution ratio in the fruit did not differ significantly among the stages, irrespective of the changes in biomass proportion (Figs. 3d, 4d).

4. Discussion

We found that the $^{13}$C remaining ratio in the fruit was lower at the EFD stage than at the FD and FM stages, suggesting higher
similar among the fruit developmental stages, suggesting that the fruit sink strength may not vary with fruit development at the shoot level. When the fruit is still green, it can assimilate C on its skin (e.g., Pavel and DeJong, 1993). In our study, the exposed shoots included fruit, which might have increased the translocation of \(^{13}\)C to the fruit. Our results were obtained using an early maturing ‘Tsugaru’ apple, which matures in early- to mid-September in northern Japan. Further study is needed to determine whether these patterns will hold for midseason and late-maturing apple cultivars, particularly in the later season when environmental factors (e.g., low air temperature) and leaf traits (e.g., low N content) influence leaf assimilation.

**Supplemental information**

Supplemental information for this paper is available at http://doi.org/10.2480/agrmet.D-17-00014

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**References**


