Current Season Photoassimilate Distribution in Sweet Cherry

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Additional Index Words. fruit development, leaf area, partitioning, Prunus avium, sink-source, stage III, 13C

Abstract. Sweet cherry (Prunus avium) tree canopies comprise three types of leaf populations: fruiting spur (FS), nonfrooting spur (NFS), and extension shoot (ES) leaves. The contribution of each leaf population as sources of photoassimilate synthesis and distribution for sweet cherry fruit development has not been described previously. To determine how carbon fixed by different leaf populations is distributed to reproductive and vegetative sinks during fruit development, fruiting branches of 7-year-old 'Ulster' sweet cherry trees grown on 'Gisela'6 (G6) (Prunus cerasus × Prunus canescens) rootstock at Michigan State University’s Clarksville Research Center (Clarksville, MI) were exposed to 13CO2 labeling on five dates in 2003 [25, 40, 44, 56, and 75 days after full bloom (DAFB)] (which occurred on 30 Apr.), comprising the period from late Stage I (SI) to late Stage III (SIII) of fruit development. Forty-eight hours after labeling, whole branches were removed and separated into different organs for 13C analysis by gas chromatography–mass spectrometry (GC-MS). The organs analyzed included: FS leaves, NFS leaves, ES leaves, fruit, and wood + bark from the segment of the branch corresponding to each leaf population. Relative distribution of C from each leaf population source to each sink varied during fruit development. Overall, the proportion of 13C recovered in the fruit was highest for the FS leaf population (which included fruit exposure to 13CO2), followed by the NFS leaves, then ES leaves. From SI to SIII, ~60% of the 13C recovered in the FS portion of the branch was found in the fruit, except during the exponential growth of fruit in mid-SIII (56 DAFB) when this proportion was nearly 80%. About 30% of the 13C fixed by NFS leaves was found in the fruit during Stage II (SII) (40 DAFB) and early (44 DAFB) and late (75 DAFB) SIII, with higher proportions at SI (45% at 25 DAFB) and mid-SIII (70%). About 25% of the 13C fixed by ES leaves was found in the fruit during SI, SII, and late SIII, with a lower proportion (17%) at early SIII when shoot growth was exponential, and a higher proportion (nearly 60%) at mid-SIII. The proportion of 13C fixed and translocated to ES growth was minimal from FS and NFS leaves throughout the sampling dates, but that by the ES leaves was significant, peaking at early SIII. The results illustrate the dynamics of C contribution from each leaf population between vegetative and reproductive sinks during growth in sweet cherry orchards, which provides useful physiological information for canopy pruning and crop load regulation.

In most tree fruit, carbon for fruit and canopy structural growth is provided initially by storage pools, transitioning to current assimilates as leaves become photosynthetically competent (Corelli-Grappadelli et al., 1994; Roper et al., 1988; Teng et al., 2001, 2002; Wünsche et al., 2005). In sweet cherry, reproductive and vegetative growth occurs simultaneously during spring and early summer (Roper et al., 1987). FS and NFS leaf area (LA) is derived from preformed vegetative meristems and reaches a maximum within 3–4 weeks of budbreak (Lang, 2005). However, on well-managed trees, ES LA continues developing from budbreak through harvest, transitioning from preformed to neoformed vegetative meristem activity and leaf expansion. Actively growing aerial sinks (i.e., flowers, fruit, spurs, and ES) compete for the C provided by these different leaf populations (Ayala and Lang, 2008; Roper et al., 1988). Roper et al. (1987) suggested that import of photoassimilates synthesized by leaves distal to FSs may be required for optimal fruit development, and branch girdling and defoliation studies demonstrated negative effects on quality traits when fruit were isolated from the major sources of photoassimilates (Ayala and Lang, 2004). Fruit trees can be considered as a collection of individual sinks (reproductive and vegetative) that compete with each other (DeJong, 1999; Wright, 1989). The sink demand of an organ and its competitive ability to attract assimilates varies by developmental stage during the season (Fischer et al., 2012; Frest and Layne, 1999; Wright, 1989). The C available to individual organs depends on the supply of photoassimilates from sources (i.e., leaves and storage reserves) and the organ’s sink demand (Ayala and Lang, 2015; Basile et al., 2002). Farrants (1996) and Minchin et al. (1997) suggested that the distribution of assimilates is controlled by the entire source–sink pathway in the plant system and is not a property of sinks alone. By contrast, dry weight (DW) partitioning among sinks is regulated by sink development (Li et al., 2015; Marcelis, 1996).

Fruit are major sinks for assimilates (DeJong and Walton, 1989). In Prunus species, fruit development follows a double sigmoidal curve, which is divided into three stages (Berman and DeJong, 1996; Frest, 1994). Following pollination and fruit set, SI is characterized by active cell division and rapid initial fruit growth. SII or “pit hardening” is associated with endocarp...
lignification and slower growth of the pericarp. SIII or “final swell” is a period of rapid fruit growth characterized by mesocarp cell enlargement and DW accumulation. The length of each phenological stage is influenced by the ripening characteristics of the variety, which may vary by up to 8–9 weeks in sweet cherry. The shortest stage of cherry fruit development is SII, and 50% to 80% of fruit growth occurs during SIII (Flores, 1994). Roper et al. (1988) suggested that because sweet cherry fruit development occurs during a relatively short timeframe (60–70 d), fruit might be high priority during SIII (Flore, 1994). Roper et al. (1988) suggested that development is SII, and 50% to 80% of fruit growth occurs weeks in sweet cherry. The shortest stage of cherry fruit

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respectively short timeframe (60–70 d), fruit might be high priority sinks. In peach (Prunus persica) and apple (Malus x domestica), periods of resource limitation lead to a competition for photoassimilates between reproductive and vegetative organs (Streetman and De Jong, 1995; Pavel and De Jong, 1993; Reyes et al., 2016).

The objective of this study was to determine how C assimilated by FS, NFS, and ES leaf populations on fruiting sweet cherry branches is distributed to competing sinks. It was hypothesized that the distribution of C fixed by each leaf population would be differentially influenced by dynamic changes in competing reproductive and vegetative sink demands from fruit set to ripening. The contribution of each leaf population as sources of photoassimilate synthesis and distribution for sweet cherry fruit development has not been described previously.

Materials and Methods

PLANT MATERIAL AND ENVIRONMENT. The experiment was conducted at Michigan State University’s Clarksville Research Center, Clarksville, MI (lat. 42.8°N, long. 85.2°W). In 2003, mature (7 years old) ‘Ulster’ sweet cherry trees on ‘Gisela 5’ (Gi5) as the cross-pollen source. The trees were

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PHENOCLOGICAL CHARACTERIZATION BEFORE 13CO2 PULSING. Six hundred 2-year-old fruiting branches having similar vigor, crop load, length, and diameter were selected for 13CO2 pulse-labeling and growth measurements. Morphological measurement range means for the selected branches were: length (97.4–100.8 cm), diameter (18.1–22.1 mm), number of FS and NFS (12–14), FS and NFS leaf number (4.7–6.0), and fruit number (two to three fruit per spur). Most of the branches were located in the middle to upper sections of the canopy, 1.5–2.5 m above the ground. Terminal ES growth (i.e., length and leaf number) was measured weekly from budbreak until terminal budset for all branches. LA for each experimental branch was estimated by counting the total spur number (FS and NFS) and number of ES nodes before each 13C-pulse, and measuring the LA of 15 FS, NFS, and ES using detached leaves and a LA meter (LI-3100; LI-COR, Lincoln, NE). Leaf area per spur (FS and NFS) ranged from 68.1 ± 5.3 to 134.4 ± 5.2 cm². Leaf area of ES at terminal budset ranged between 785 ± 7.3 and 876 ± 9.3 cm².

Twenty fruit (F) per branch were measured for fresh weight [FW (grams)] and DW (grams), diameter (millimeters), and soluble solids content [SSC (percent)]. Thirty FS, NFS, and ES were measured for FW, DW, leaf number, and LA.

TREATMENTS (T) AND 13C LABELING. From the 600-branch population previously characterized, a group of 200 branches were selected randomly for growth measurements, and a group of 400 branches were selected randomly for labeling (or as nonlabeled controls). For each leaf population (T = 3) and pulse date (Date = 5), 10 single branch replications (n = 10) were used for each sampling date (immediately and 48 h after pulsing). Branch sections corresponding to FS (leaves plus fruit), NFS (leaves only), and ES (leaves plus new shoot) leaf populations were labeled with short pulses of 13CO2 at 25 d (SI), 40 d (SII), 44 d (early SIII), 56 d (mid-SIII), or 75 d (late SIII (terminal budset)] DAFB. Each labeling date corresponded to the noted phenological stage during fruit development and was a sunny day with the following mean daily temperatures: SI at 11 °C, SII at 16 °C, early SIII at 18 °C, mid-SIII at 27 °C, and late SIII at 21 °C. There was no rain between pulse date and sampling 48 h later except following the mid-SIII pulse, which had 2.8 mm of rain in between pulsing and sampling. Three additional nonlabeled branches were removed at each date to quantify natural 13C abundance in leaves.

For each labeled branch, one complete section (FS leaves plus fruit, NFS leaves, or ES leaves plus new shoot) was enclosed in a transparent polyester film (Mylar®; DuPont, Wilmington, DE) balloon chamber of appropriate volume and pumped for 15–20 min with 13CO2 when assimilation rates were positive, between 1000 and 1200 hr. 13CO2 was generated by injecting 80% lactic acid into a 1-L wash bottle containing barium carbonate (98 atom percent 13C), and swirling and pumping the bottle to deliver a total of 3.9 mmol of 13CO2 into each chamber. The average rate of CO2 uptake for each date was calculated. Photosynthetically active radiation ranged between 1456 and 1835 μmol•m²•s⁻¹ during 13C labeling. Single leaf gas exchange was measured with an IR gas analyzer (CIRAS-2; PP-Systems, Haverhill, MA) for FS, NFS, and ES leaves on selected branches before and during the pulse-labeling. Net assimilation rate (A) varied between 15.7 and 20.4 μmol•m⁻²•s⁻¹ CO2 among the three leaf populations and dates.

SAMPLING AND 13C ANALYSIS. Immediately after labeling, three fully expanded leaves were sampled from each leaf type to estimate the initial total 13C fixed by each leaf population. When FS were the labeled population, individual fruit were also sampled to estimate the 13C fixed because of fruit photosynthesis.

At 48 h after each pulse-labeling, each branch was removed at its base to measure FW and DW of different organs and prepare them for 13C enrichment analysis by GC-MS (20–20 mass spectrometer and ANCA-GSL sample combustion unit; PDZ Europa, Sandbach, UK). The organs analyzed included: FS leaves, NFS leaves, ES leaves, fruit, and wood + bark from the segment of the branch corresponding to each leaf population. Extension shoots were divided further into mature fully expanded leaves, developing leaves, young leaves, and wood. In addition, 10 single fruit from the FS section that had been labeled directly with 13C were divided into pericarp (epicarp + mesocarp) and endocarp. All plant materials were oven-dried at 70 °C for 72–96 h and ground using a Wiley mill (20 and 40
Growth and fruiting of 2-year-old branches

Extension shoot growth on ‘Ulster’/Gisela®6 sweet cherry fruit fresh weight (FW) and growth data for 2-year-old branches (n = 10) at each 13CO2 pulse-labeling date [25, 40, 44, 56, and 75 d after full bloom (DAFB)]. Full bloom was on 30 Apr.

| DAFB | Fruit FW (g/fruit) | ES length (cm) (mean ± se) | LA (cm²/branch) (mean ± se) | Fruit (no./branch) (mean) | LA (cm²/fruit) (mean) |
|------|------------------|-----------------------------|-----------------------------|---------------------------|-----------------------|
| 25   | 1.2 ± 0.0        | 9.4 ± 0.2                   | 10.1 ± 0.1                  | 2,984 ± 67                | 94.5 ± 3.3             | 31.6                  |
| 40   | 1.5 ± 0.1        | 17.9 ± 0.3                  | 13.3 ± 0.1                  | 3,292 ± 83                | 79.8 ± 3.4             | 41.2                  |
| 44   | 1.7 ± 0.1        | 23.7 ± 0.4                  | 15.4 ± 0.1                  | 3,446 ± 62                | 67.1 ± 3.6             | 51.4                  |
| 56   | 4.1 ± 0.2        | 28.2 ± 0.5                  | 17.6 ± 0.2                  | 3,900 ± 80                | 68.7 ± 3.0             | 56.8                  |
| 75   | 6.5 ± 0.3        | 33.9 ± 0.9                  | 19.1 ± 0.1                  | 3,994 ± 126               | 66.1 ± 3.2             | 60.4                  |

ES = extension shoot; LA = leaf area.

Results and Discussion

Table 1. ‘Ulster’/Gisela®6 sweet cherry fruit fresh weight (FW) and growth data for 2-year-old branches (n = 10) at each 13CO2 pulse-labeling date [25, 40, 44, 56, and 75 d after full bloom (DAFB)]. Full bloom was on 30 Apr.
Table 2. Total $^{13}$C content recovered in 2-year-old branches of ‘Ulster’/‘Gisela’6’ sweet cherry immediately (after chamber removal) following $^{13}$C pulse-labeling to fruiting spur (FS), nonfruiting spur (NFS), and extension shoot (ES) branch sections ($n = 10$) at 25, 40, 44, 56, and 75 d after full bloom (DAFB). Calculations were made based on total dry weight (DW) of branches. Full bloom was on 30 Apr.

| $^{13}$C-pulsed leaf population | 13C-pulse date (DAFB) | Total $^{13}$C content (mg/branch) |
|-------------------------------|----------------------|-----------------------------------|
|                               | 25 | 40 | 44 | 56 | 75 |                               |
| FS leaves                     | 42.9 a | 34.9 a | 36.8 a | 47.1 a | 46.0 a |                               |
| NFS leaves                    | 34.5 ab | 31.8 a | 38.1 a | 38.9 a | 42.7 a |                               |
| ES leaves                     | 28.5 b | 32.7 a | 35.2 a | 30.8 a | 44.7 a |                               |

Means within a column followed by the same small letter are not significantly at different $\alpha = 0.05$. Mean separation using Tukey’s test at $P = 0.05$.

DAFB, when the $^{13}$C remaining in all of the pulsed leaf populations was at the lowest relative levels compared with all other pulse dates. High sink activity related to rapid fruit growth and reduced leaf CH$_2$O levels has been reported previously in sweet cherry (Roper et al., 1987, 1988) and Japanese pear [Pyrus pyrifolia (Teng et al., 2001)].

At 48 h after pulsing, there were significant differences in $^{13}$C content among different organs of the branch, depending on the pulsed leaf population (Tables 4–6). For the three source leaf populations, the greatest proportion of translocated $^{13}$C was detected in fruit throughout SI, SII, and SIII. However, there were significant differences among leaf populations regarding the relative amount of $^{13}$C accumulated in fruit for each pulse-labeling date. The highest relative $^{13}$C levels in fruit were detected when FS leaves were the labeled source; these ranged from 57% to 63% across all labeling dates except at 56 DAFB when levels peaked at 79% (Table 4). The second most important C source for fruit was NFS leaves, with relative $^{13}$C levels ranging from around 32% at 40, 44, and 75 DAFB to 71% at 56 DAFB (Table 5). The lowest relative $^{13}$C levels recovered in fruit, around 22% to 28%, were from ES leaves as the source of photoassimilates, although again at 56 DAFB, relative $^{13}$C levels peaked at 59% (Table 6). These consistently high relative $^{13}$C levels recovered in fruit from all source leaf populations during mid-SIII suggest that this is the period of strongest fruit sink strength and the period when additional source LA is most needed to optimize fruit growth.

Fruiting spur, NFS, and ES leaves all supplied current photoassimilates to fruit and vegetative growth during SI, SII, and SIII of fruit development. Across all pulse-labeling dates, $^{13}$C fixed by FS leaves was translocated predominantly to fruit and wood subtending those leaves (Table 4). In 87% of $^{13}$C-enriched branches, very minor acropetal translocation of $^{13}$C was detected in NFS leaves, NFS wood, ES leaves, and wood. $^{13}$C fixed by NFS leaves was translocated predominantly basipetally to fruit and fruiting wood (Table 5). Significant amounts of $^{13}$C were detected in the wood subtending the NFS leaves. However, acropetal $^{13}$C translocation to ES wood and leaves also was observed. Only 12% of enriched branches did not exhibit translocation to either FS or ES. Bidirectional translocation from the NFS leaves has been shown previously for sweet cherry on the dwarfing rootstock Gi5, in which NFS leaves translocated $^{13}$C primarily to fruit during final swell, but also to ES growth (Ayala, 2004). Unidirectional and bidirectional transport from different leaf populations also have been reported for apple (Corelli Grappadelli et al., 1994; Hansen, 1969; Toselli et al., 2014; Wang et al., 2003; Zhou et al., 2015), sour cherry (Kappes and Flore, 1986; Toldt-Andersen, 1998), Japanese pear (Zhang et al., 2005), pecan [Carya illinoinensis (Davis and Sparks, 1974)], persimmon [Diospyrus kaki (Nakano et al., 1998; Simkhada et al., 2007)], and grape [Vitis vinifera (Hale and Weaver, 1962)]. The highest total recoveries of $^{13}$C for both spur leaf populations occurred 56 DAFB, coincident with peak fruit growth as shown in Fig. 1.

ES growth was not a strong sink for assimilates during sweet cherry fruit development in these relatively heavily-cropped ‘Ulster’ trees on the semivigorous rootstock Gi6. Minimal amounts of $^{13}$C (<1%) were found in ES when FS and NFS leaves were labeled (Tables 4 and 5). Conversely, Kappel (1991) reported that, with ‘Lambert’ sweet cherry on vigorous P. avium seedling rootstocks, ES growth had a greater sink strength for photosynthates than fruit. Source–sink relationships and relative C distribution can be influenced by rootstock genotype (Caruso et al., 1997; Moing and Gaudillere, 1992). Fruit growth can affect ES development negatively in sweet cherry trees on dwarfing rootstock (Whiting, 2005; Whiting and Lang, 2004). In ‘Bing’ on semidwarfing Gi5, heavy cropping reduced ES elongation and DW accumulation (Corea, 2008). Similarly in peach, the presence of fruit influenced primary and secondary growth (Costes et al., 2000), and stem length and DW accumulation, suggesting competition for C between vegetative growth and fruit (Grossman and DeJong,
Table 3. Total $^{13}$C content recovered in ‘Ulster’/’Gisela’6’ sweet cherry fruit sampled from 2-year-old branches ($n = 10$) immediately (after chamber removal) following $^{13}$C pulse-labeling of the fruiting spur (FS) section at 25, 40, 44, 56, and 75 d after full bloom (DAFB). Full bloom was on 30 Apr.

| $^{13}$C-pulse date (DAFB) | 25 | 40 | 44 | 56 | 75 |
|--------------------------|----|----|----|----|----|
| Fruit $^{13}$C content (μg g$^{-1}$ DW) | 188.3 a | 69.3 b | 98.0 b | 8.4 c | 11.6 c |

$^{a}$Means within a row followed by the same small letter are not significantly different at $\alpha = 0.05$. Mean separation using Tukey’s test at $P = 0.05$.

$^{b}$DW = dry weight.

Table 4. Total $^{13}$C recovered per branch and relative $^{13}$C distribution among organs on 2-year-old branches of ‘Ulster’/’Gisela’6’ sweet cherry trees 48 h after $^{13}$CO$_2$ pulsing of the fruiting spur (FS) branch section ($n = 10$). Percentages are based on absolute amounts of $^{13}$C recovered for each organ at each $^{13}$CO$_2$ pulse-labeling date [25, 40, 44, 56, and 75 d after full bloom (DAFB)]. Full bloom was on 30 Apr.

| Organ sampled | Relative $^{13}$C distribution (%) |
|---------------|---------------------------------|
| Fruit         | 63.2$^a$ 59.9$^e$ 58.9$^f$ 79.1$^e$ 57.3$^e$ |
| FS leaves     | 32.5$^e$ 30.5$^e$ 34.1$^e$ 17.9$^e$ 36.4$^e$ |
| NFS leaves    | 0.7$^a$ 0.2$^c$ 0.0 b 0.3 a 0.2 a |
| ES leaves     | 0.1 a 0.0 c 0.0 b 0.0 a 0.2 a |
| FS wood       | 3.0 a 8.8 a 7.0 a 2.6 a 5.4 a |
| NFS wood      | 0.5 a 0.6 b 0.0 b 0.1 a 0.4 a |
| ES wood       | 0.0 a 0.0 c 0.0 b 0.0 a 0.1 a |

$^{a}$Means within a column followed by the same letter are not significantly different at $\alpha = 0.05$. Mean separation using Tukey’s test at $P = 0.05$.

$^{b}$Organ sampled with $^{13}$C directly were not considered in the statistical analysis.

Table 5. Total $^{13}$C recovered and relative $^{13}$C distribution among organs on 2-year-old branches ($n = 10$) of ‘Ulster’/’Gisela’6’ sweet cherry trees 48 h after $^{13}$CO$_2$ pulsing of the nonfruiting spur (NFS) branch section. Percentages are based on absolute amounts of $^{13}$C recovered for each organ at each pulse-labeling date [25, 40, 44, 56, and 75 d after full bloom (DAFB)]. Full bloom was on 30 Apr.

| Organ sampled | Relative $^{13}$C distribution (%) |
|---------------|---------------------------------|
| Fruit         | 45.8$^a$ 31.7 a 31.3 a 70.9 a 32.7 a |
| FS leaves     | 0.2 a 0.7 d 0.0 c 0.5 c 0.2 c |
| NFS leaves    | 41.2$^a$ 42.7$^e$ 46.1$^f$ 19.9$^e$ 49.3$^e$ |
| ES leaves     | 0.2 c 0.1 d 0.3 c 0.2 c 0.5 c |
| FS wood       | 8.4 b 16.9 b 14.5 b 5.1 b 12.1 b |
| NFS wood      | 4.2 bc 7.8 c 7.6 bc 3.3 bc 5.0 bc |
| ES wood       | 0.0 c 0.1 d 0.2 c 0.1 c 0.2 c |

$^{a}$Percentages within a column followed by the same letter are not significantly different at $\alpha = 0.05$. Mean separation using Tukey’s test at $P = 0.05$.

$^{b}$Organs labeled with $^{13}$C directly were not considered in the statistical analysis.

Table 6. Total $^{13}$C recovered and relative $^{13}$C distribution among organs on 2-year-old branches ($n = 10$) of ‘Ulster’/’Gisela’6’ sweet cherry trees 48 h after $^{13}$CO$_2$ pulsing of the extension shoot branch section. Percentages are based on absolute amounts of $^{13}$C recovered for each organ at each pulse-labeling date [25, 40, 44, 56, and 75 d after full bloom (DAFB)]. Full bloom was on 30 Apr.

| Organ sampled | Relative $^{13}$C distribution (%) |
|---------------|---------------------------------|
| Fruit         | 27.2$^a$ 22.3 a 17.5 a 59.2 a 28.3 a |
| FS leaves     | 0.2 a 0.0 c 0.1 d 0.5 b 0.0 c |
| NFS leaves    | 0.4 c 0.1 c 0.0 d 0.4 c 0.2 c |
| ES leaves     | 50.4$^e$ 46.3$^e$ 59.8$^h$ 28.1$^e$ 45.0$^e$ |
| FS wood       | 8.8 b 10.1 b 4.8 c 5.0 b 10.7 b |
| NFS wood      | 8.1 b 15.7 ab 9.1 b 3.7 b 10.0 b |
| ES wood       | 4.9 bc 5.5 b 8.7 b 3.1 b 5.8 b |

$^{a}$Percentages within a column followed by the same letter are not significantly different at $\alpha = 0.05$. Mean separation using Tukey’s test at $P = 0.05$.

$^{b}$Organs labeled with $^{13}$C directly were not considered in the statistical analysis.

(i.e., 22 leaves) in length. The lowest total recovery of $^{13}$C was at 25 DAFB, when ES were 10 cm in length and had only 10 leaves. The lowest $^{13}$C export from ES was at the beginning of SIII (44 DAFB, 570 GDD), when shoots were elongating rapidly.

Translocation of $^{13}$C to, and within, fruit

When the total $^{13}$C translocation to the fruit, from all sources, is combined for each pulsing date, the extremely strong fruit sink activity at 56 DAFB is apparent with more than twice the total $^{13}$C content (65,055 μg/fruit) than for that found at any other date (Table 7). On a relative $^{13}$C distribution basis, the FS leaf population always contributed the most, around half of that...
Table 7. Total $^{13}$C content in sweet cherry fruit derived from all pulsed sources on 2-year-old branches ($n = 10$) of ‘Ulster’/‘Gisela®6’ trees 48 h after $^{13}$CO$_2$ pulsing of fruiting spur (FS) leaves and fruit, nonfruiting spur (NFS) leaves, and extension shoot (ES) leaves. Percentages are based on absolute amounts of $^{13}$C recovered in fruit at each pulse-labeling date [25, 40, 44, 56, and 75 d after full bloom (DAFB)]. Full bloom was on 30 Apr.

| DAFB | Relative $^{13}$C contribution from each pulsed source (%) | Total $^{13}$C content in fruit (μg) |
|------|----------------------------------------------------------|-------------------------------------|
|      | FS leaves | NFS leaves | ES leaves |                              |
| 25   | 49.4 a     | 40.9 b     | 9.8 c     | 26,851.7                        |
| 40   | 50.5 a     | 30.4 b     | 19.1 c    | 20,648.2                        |
| 44   | 53.9 a     | 31.1 b     | 15.0 c    | 23,223.4                        |
| 56   | 43.9 a     | 37.6 a     | 18.5 c    | 65,055.0                        |
| 75   | 46.5 a     | 28.4 b     | 25.1 b    | 31,550.6                        |

*Means within a row followed by the same small case are not significantly different $α = 0.05$. Mean separation using Tukey’s test at $P = 0.05$.

Table 8. Relative $^{13}$C partitioning between ‘Ulster’/‘Gisela®6’ sweet cherry fruit pericarp and endocarp ($n = 5$) 48 h after $^{13}$CO$_2$ pulsing of fruiting spur (FS) leaves and fruit at 25, 40, 44, 56, and 75 d after full bloom (DAFB). Calculations and statistical analyses are based on absolute amounts of $^{13}$C recovered at each pulse-labeling date. Full bloom was on 30 Apr.

| Tissue | Relative $^{13}$C distribution (%) | DAFB |
|--------|-----------------------------------|------|
|        |                                   | 25   |
|        |                                   | 40   |
|        |                                   | 44   |
|        |                                   | 56   |
|        |                                   | 75   |
| Pericarp | 25.8 a | 21.4 a | 32.3 a | 77.4 b | 83.4 b |
| Endocarp | 74.2 a | 78.6 a | 67.7 ab | 22.6 a | 16.6 a |

*Means within a column followed by the same letter are not significantly different $α = 0.05$. Mean separation using Tukey’s test at $P = 0.05$.

recovered. At 25 DAFB, the NFS leaves were an important source for $^{13}$C, and during the period of greatest fruit sink demand, the NFS leaves were statistically similar to the FS leaves. As the ES leaf population reached its maximum LA (75 DAFB), the $^{13}$C provided by those leaves became statistically similar to that of the NFS leaf population, with each providing $\approx 25\%$ of the $^{13}$C found in the mature fruit.

In this study, the sink demand of sweet cherry fruit varied during development and fruit were stronger sinks for photoassimilates than shoots, as has been reported for peach (Grossman and De Jong, 1995). In sour cherry the highest fruit sink strength was during SI (Flores and Layne, 1999). During SI, sink activity of a small sweet cherry fruit requires $^{13}$C assimilates for cell division. At this stage in our study, the highest $^{13}$C atom percent excess per unit basis was detected in fruit. Hansen (1987) indicated that increased sink activity of fruit promotes the uptake of assimilates, which in turn accelerates its growth rate. Sour cherry small fruit exhibit strong sink activity by removing C from the translocation system, which supports their high specific growth rate during early fruit development (Toldam-Andersen, 1998). Similarly, the sink activity of immature small grape berries is important for DW accumulation during the first week of growth when cell expansion is slow (Coombe, 1989).

The distribution of translocated $^{13}$C between the fruit pericarp (epicarp + mesocarp) and pit (endocarp + embryo) changed significantly during development (Table 8). From late SI through most of SII (25–40 DAFB), 74% to 79% of the total $^{13}$C in the fruit was recovered from the pit. The transition from SII to SIII (44 DAFB) marked the beginning of a shift in relative $^{13}$C distribution, from 2:1 pit:pericarp at 44 DAFB to 1:3 at 56

DAFB and 1:4 at 75 DAFB. Teng et al. (2001) reported that Japanese pear fruit accumulated most of the $^{13}$C in the flesh during the period of active growth. Similar results have been reported for peach (Corelli-Grappadelli et al., 1996).

**Conclusions**

This study examined how source–sink relationships at specific phenological stages influence the uptake and distribution of current season photosynthates in sweet cherry trees on highly productive rootstocks such as Gi6. On average, during fruit development, FS leaves contributed more $^{13}$C (60% to 80%) to fruit than did NFS (30% to 70%) and ES (18% to 60%) leaves. The exception was at 56 DAFB (mid-SIII, 812 GDD), a period of rapid cell enlargement and DW accumulation, when the amounts of $^{13}$C translocated to fruit were significantly higher from all $^{13}$C sources compared with the other pulse-labeling dates. In ‘Ulster’/Gi6 trees, SI (319–483 GDD) and mid-SIII (753–874 GDD) were the critical periods of potentially restricted C availability, because of limited ES LA during the former period and high fruit sink strength during the latter period.

Therefore, these results confirm the need to potentially reduce crop load during the early stages of fruit development to avoid negatively affecting fruit quality, particularly, size, SSC, firmness, and postharvest life. This has important implications for orchard management strategies such as dormant pruning, fruit thinning (i.e., intensity and type), and tree nutrition to maintain adequate LA to support fruit growth. To reduce the potential for resource limitations during fruit development, particularly in highly precocious dwarfing rootstock/scion combinations, more intensive dormant pruning could be imposed, with complementary flower or fruit thinning in orchards prone to excessively high yields. Heading cuts may be favored over thinning cuts to stimulate new ES LA and reduce the density of future spur flower bud formation. Fertilization strategies to build storage reserves for the promotion of larger FS and NFS leaf development in spring would also shift the LA:F ratio favorably. Additional studies of sweet cherry source–sink relationships at specific phenological stages would be valuable to further examine partitioning variations in relation to growing conditions and other rootstocks and varieties.

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