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RESEARCH PAPER

^{13}C photoassimilate partitioning in sweet cherry (*Prunus avium*) during early spring

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Abstract

M. Ayala, and G.A. Lang. 2015. ^{13}C-Photoassimilate partitioning in sweet cherry (*Prunus avium*) during early spring. Cien Inv. Agr. 42(2):191-203. Stored reserves are critical for the early spring growth of reproductive and vegetative sinks in sweet cherry (*Prunus avium* L.). To study the distribution of carbon storage reserves on new, highly productive hybrid rootstocks in sweet cherry, an experiment was established using 5-yr-old cv. 'Regina' on the semi-vigorous rootstock 'Gisela®6' ('GI®6'). Using whole-canopy enclosure chambers, five trees were pulse-labeled three times with high levels of $^{13}CO_2$ during the fall. At leaf drop, leaves, buds, wood, bark and roots were sampled for gas chromatography-mass spectrometry (GC-MS) analysis. The storage organs with the highest percentages of excess ^{13}C atoms were the roots and the older wood in the trunks, branches and buds. During the spring, newly developing organs (flowers, immature fruits and leaves) were sampled weekly from bloom to stage III of fruit development for additional GC-MS analysis. ^{13}C-reserves were remobilized and partitioned to flowers, fruits and young leaves from before budbreak (side green stage) until 14 days after full bloom (DAFB). The isotopic composition differed significantly between phenological stages, and the highest ^{13}C levels in the growing sinks were detected between bloom and fruit set. The reproductive organs had the strongest sink activity until 14 DAFB, and competition with spur leaf sink activity was apparent. It is proposed that reserve source limitation may impact fruit set and spur leaf development, which may ultimately impact the availability of photoassimilate during the later stages of fruit growth.

Key words: Fruit set, 'Gisela®' rootstock, sink, spring remobilization stage I, storage reserves, transition.

Introduction

In most deciduous woody perennials, the primary sources of assimilates are newly synthesized photosynthates and accumulated reserves (Oliveira and Priestley, 1988). Storage reserves have been defined as nutritional substances (organic compounds and nutrients) accumulated in excess of current requirements, which may later be used to support metabolism and growth (Priestley, 1960). Carbohydrates (CH_2O) in the form of starch and soluble sugars are the major component of the storage reserves in trees (Loescher *et al*., 1990), which

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accumulate in various organs including the buds, leaves, branches, stems, roots, seeds and fruits.

Storage reserves are important to several life processes including winter survival, metabolism, respiration, defense and vegetative and reproductive growth (Kandiah, 1979 a,b; Oliveira and Priestley, 1988; Loescher *et al*., 1990). Several authors have indicated that the initial stages of spring growth in deciduous fruit trees depend upon the mobilization of reserves accumulated during the previous season until the new leaves become photosynthetically competent for the provision of new assimilates (Priestley, 1960; Hansen, 1967; Quinlan, 1969; Hansen, 1971; Oliveira and Priestley, 1988). Storage reserves are utilized in new growth and respiration to provide energy and materials for cellular structure before the uptake of root N and photosynthesis in the spring (Hansen, 1967; Cheng and Fuchigami, 2002).

In sweet cherry (*Prunus avium* L.), blooming usually occurs before the leaves are fully expanded, and the early stages of reproductive (flowers and fruits) and vegetative (spurs, extension shoots and roots) growth are dependent on the storage reserves accumulated during the previous fall (McCammant, 1988; Keller and Loescher, 1989). Lang (2001) indicates that, in sweet cherry, early fruit growth depends almost exclusively upon stored CH_2O reserves, but other deciduous trees, such as apple (*Malus domestica* Borkh.), are less dependent on stored reserves because the canopies have some photosynthetically competent leaves (Keller and Loescher, 1989; Hansen, 1971). An intermediate situation has been described for Japanese pear (*Pyrus pyrifolia* Nakai.), which usually has 30% of its final leaf area at full bloom (Teng *et al*., 1999).

The major accumulation of reserves in perennial structures begins after terminal bud set (Oliveira and Priestley, 1988), and in sweet cherry, storage reserves, mainly starch, accumulate in different organs after fruit ripening and the cessation of shoot extension and reach their maximum concentration at leaf abscission (McCammant, 1988; Keller and Loescher, 1989). In spring, activated meristems draw upon assimilates from the storage organs throughout the tree until new leaves become competent sources of photoassimilates. Premature leaf abscission might reduce CH_2O storage (Flore, 1994), so any type of biological stress (e.g., leaf damage due to pests and diseases) should be avoided because it might reduce the amount of stored CH_2O available for new growth in the next year (Flore, 1994).

Previous studies of the accumulation and remobilization of carbohydrate reserves in sweet cherry have been performed on non-precocious, vigorous, mature trees from *P. avium* seedling rootstocks. The Gisela® series of hybrid (usually *Prunus cerasus* x *P. canescens*) rootstocks advances the reproductive maturity of younger trees and reduces vegetative vigor to varying levels depending on the genotype, which may impact the distribution of carbohydrates. The hypothesis that storage reserves are a source of carbon (C) for initial fruit growth during stage I, before the current photoassimilates become the major C source, was therefore tested in sweet cherry using a highly productive, semi-vigorous hybrid rootstock. The distribution and remobilization of reserves in spring were analyzed in fruiting 'Regina' sweet cherry on the semi-dwarfing rootstock 'Gisela®6' ('GI®6': *Prunus cerasus* × *P. canescens)*, and ^{13}C was used as a tracer to distinguish between the two main sources of assimilates for early spring growth, those that were synthesized and those that were accumulated the previous fall (i.e., storage reserves), and the current photosynthates produced during the following spring by the newly expanded leaves. Tracing ^{13}C is a useful physiological technique to discriminate against $^{13}CO_2$ during photosynthesis, and it has been used to study the fate of C in other species (Farquhar *et al*., 1982; Boutton, 1991; Teng *et al*., 1999). High levels of $^{13}CO_2$ result in photoassimilates enriched in ^{13}C compared to the naturally lower levels of $^{13}CO_2$. In this experiment, increasing the content of ^{13}C in the stored CH_2O that was synthesized

during a distinct period of the growing season (fall) allowed us to follow its fate and use through the following growing season.

Materials and methods

Plant material

The experiment was conducted at Michigan State University's Clarksville Research Center (coordinates: N 42°42.6260; W 85°33.9580) in Clarksville, Michigan. Five-yr-old 'Regina' sweet cherry trees on the semi-dwarfing rootstock 'GI®6' ('Regina'/'GI®6') were selected for pulse-labeling with high levels of $^{13}CO_2$ following terminal bud set (Aug). All of the trees were trained to a central leader and had similar heights, trunk cross-sectional areas and leaf areas (LA: ~11.3±0.5 m^2), which were estimated by counting the total number of spurs (fruiting and non-fruiting) and current season shoots (CSS) prior to the ^{13}C pulses (Jul-Sep) and by measuring the LA of 5 detached fruiting spurs (FS), 5 non-fruiting spurs (NFS) and the CSS using a leaf area meter (LI-3100, LI-COR, Lincoln, Nebraska, USA). The study trees were not pruned during the experiment but were fertilized and irrigated by microsprinklers following standard commercial practices.

^{13}C *labeling*

After terminal bud set (Aug), five trees were enclosed in a transparent polyethylene (with an incident visible light transmission of 0.7) balloon (6.3 m^3) and pulsed with $^{13}CO_2$. A total of 5.1 mmol of $^{13}CO_2$ were injected into each balloon, which were sealed using a thin wire attached to the trunk, and $^{13}CO_2$ was generated by adding 80% lactic acid to each of the 1-L bottles containing barium carbonate (98 atom% ^{13}C). Labeling was carried out between 10:00 AM and 12:00 PM, and the three $^{13}CO_2$ pulses were conducted at 132, 145 and 158 days after full bloom (DAFB) on the same trees to ensure adequate labeling of C-reserves. Each $^{13}CO_2$ pulse lasted 30 min.

The canopy of the labeled trees was divided into 3 leaf layers (heights): low (1.2 m), medium (2.2 m) and high (3.2 m). Photosynthetic active radiation (PAR) and net assimilation rate (A) were measured on each labeling date with a CIRAS-2 infrared gas analyzer (PP-Systems Inc., Haverhill, Massachusetts, USA). The gas exchange measurements were carried out assuming a single leaf of 6 NFS, 6 FS and 6 CSS per tree at each pulse date, and FS, NFS and CSS were aligned to the 4 cardinal points. PAR and A ranged between 1234 and 1788 and from 8.6 and 14.1 $\mu molm^{-2} s^{-1}$, respectively, and the CO_2 concentration in the PLC6 (U) universal automatic leaf cuvette was 360 ppm.

Growth measurements

The total number of apical and lateral meristems (FS, NFS, and single buds) was recorded soon after budbreak (Apr) and at each subsequent terminal bud set (Aug). Additionally, flower and fruit number were recorded using a counter during bloom and fruit set (Apr), respectively (data not shown).

The growth of flowers, fruits, FS, NFS and CSS (i.e., CSG, the current season growth) were quantified weekly during the spring from a group of 30 representative trees without ^{13}C-labeling. From this population of trees, 5 individuals were chosen to measure 4 CSS per tree (one CSS per cardinal point at a height of 2.2 m) as well as extension growth and leaf number (folded and unfolded leaves). A sample of 5 fruits per tree (5 trees in total) was measured for fresh weight (FW), diameter (mm) and soluble solid concentration (SSC, %). Furthermore, a sample of 10 FS, 10 NFS, 10 CSS, 50 flowers during bloom and 50 developing fruits (at stage I, II and III of fruit development) were collected from 20 additional trees for fresh (FW) and dry (DW) weight measurements.

Destructive sampling of the fruits, FS, NFS and CSS allowed the definition of the beginning of

each developmental stage through the measurement of size (diameter and length), FW, DW and seed enlargement. Growth curves were developed to precisely identify changes in the developmental rates of fruit and LA.

Growing degree days (GDD; based on 4.4 °C), which are a measure of heat accumulation in plants, was used as an indicator of the phenological stages and development rates of fruits, leaves and shoots from budbreak to the end of stage III.

^{13}C Sampling and analysis

Trunks, branches and roots were sampled after leaf abscission (Nov) and at the side green stage (Apr). Small (5×5 cm) patches of wood and bark were removed from each tree at different locations along the trunk and branches. Wood samples consisted of the xylem tissue, and the bark samples included the periderm, phloem and cambium. FS and single buds were randomly collected throughout the canopy, and roots were collected from the first 60-80 cm of the root zone below the soil surface by excavating at four points between and within rows around the trunk. Roots were then separated according to their size as fine (< 1 mm), medium (1-5 mm) or coarse (> 5 mm). Additionally, the actively growing vegetative and aerial reproductive organs were sampled during the spring to represent the entire period of fruit development including FS, NFS, single buds, single flowers, spur flowers, young leaves at the tip of the CSG (shoots) and fruits. Additional samples of the same organs were collected from three unlabeled trees to calculate natural abundance. Samples were immediately frozen in liquid N for the subsequent determination of ^{13}C enrichment by gas chromatography-mass spectrometry (GC-MS). Three unlabeled trees were destructively removed to calculate DW partitioning among the different organs at stage I of fruit development (35 DAFB).

The plant material was oven-dried at 70 °C for 72 h and subsequently ground using a Wiley mill (20 mesh). ^{13}C enrichment was calculated following Boutton (1991) and Vivin *et al*. (1996) as follows:

$$\delta^{13}C\ (‰) = [R\ sample\text{-}R\ standard/R\ standard] \times 1000 \quad \text{Eq. (1)}$$

$$R\ sample = {}^{13}C/{}^{12}C = [\delta^{13}C/(1000 + 1)] \times R_{PDB} \quad \text{Eq. (2)}$$

$$F = {}^{13}C/({}^{13}C+{}^{12}C) = R/(R+1) \quad \text{Eq. (3)}$$

$$Atom\%\ excess = (F_{postdose}\text{-}F_{baseline}) \times 100 \quad \text{Eq. (4)}$$

$$New\ {}^{13}C\ content = (Atom\%\ excess/100) \times dry\ matter \times [C] \quad \text{Eq. (5)}$$

where the value of $\delta^{13}C$ (‰) is calculated from the carbon isotope ratios measured from the sample and standard gases (Eq. 1). The absolute ratio (R) of a sample is defined by Eq. 2, where RPDB = 0.0112372. Atom% excess is used as an index to determine the enrichment level of a sample following the administration of the ^{13}C tracer in excess of the ^{13}C baseline prior to the $^{13}CO_2$ pulse (Eq. 3 and 4). The new ^{13}C pool is calculated for the different branch components based on the dry mass and C concentration (Eq. 5).

Two types of information are reported: the ^{13}C abundance (expressed as ^{13}C atom % excess) and the absolute amount of ^{13}C recovered for each organ (expressed as $\mu g^{13}C$). The first value is indicative of the gain in ^{13}C per individual organ with respect to its natural ^{13}C abundance level, which constitutes an index of sink activity, and the second value represents the amount of ^{13}C partitioned to a specific organ in terms of dry matter (i.e., total number of units) and provides an index of the sink strength of each organ.

Statistical analysis

An analysis of variance was conducted using the PROC MIXED procedure of the SAS statistical analysis program (SAS Institute Inc., Cary, NC). Repeated measurements covariance and ANOVA analyses were conducted during the spring to validate the proposed hypothesis. The

covariance analysis indicated that there was not a significant covariate effect of total ^{13}C fixation on the distribution of ^{13}C among organs.

Results

Phenological characterization

The side green stage was observed 15 days before full bloom (DBFB) after an accumulation of 148 GDD. At first bloom (4 to 6 DBFB; 239-263 GDD), only single and spur flowers and NFS were actively growing. The single flower buds at the base of the 1-yr-old wood bloomed earlier than the spur flower buds, and at full bloom (264-287 GDD), growth was evident for the organs described above as well as FS and young CSS leaves. Fruit set (ovary with 6.2±0.2 mm) was observed 4 to 7 days after full bloom (DAFB) at 288-342 GDD, and stages I, II and III of fruit development occurred from 8 to 28 DAFB, 29 to 48 DAFB and 49 to 63 DAFB, respectively.

The sequential order in which the organs began exhibiting visual signs of growth was as follows: single flower, spur flower and NFS meristems, terminal CSS meristems and FS meristems. Dry matter increased in all organs until 35 DAFB, and at that point, the foliar expansion of FS and NFS ceased, but the shoots and fruits continued accumulating DW.

Shoot length and leaf number rapidly increased from 14 DAFB until 49 DAFB (Table 1), and the fruits showed a rapid increase in growth beginning at 42 DAFB with 40% of the final size achieved during stage III (Table 2).

^{13}C labeled storage reserves at leaf abscission

The ^{13}C in all of the organs sampled at leaf abscission (Nov) were above the natural levels of abundance, but it varied significantly among organs (Figure 1). The highest ^{13}C levels, expressed as higher ^{13}C atom % excess, were detected in the 2- and 3-yr-old wood of the trunk, roots (coarse and medium) and vegetative buds. Significant ^{13}C

Table 1. Current season growth (shoot) measurements of 'Regina'/'Gisela ®6' sweet cherry trees between bloom and terminal bud set (2003). Mean ± SE; n=20.

Developmental stage	Days relative to full bloom	Shoot Length (cm)	Leaf Number		
			Total	Folded	Unfolded
First white/first bloom[1]	-6	0.3 ± 0.01[2]	3.1 ± 0.2	2.5 ± 0.1	0.6 ± 0.1
Full bloom	0	0.5 ± 0.0[1]	8.0 ± 0.2	4.3 ± 0.2	3.7 ± 0.2
Fruit set	7	1.9 ± 0.1	9.1 ± 0.2	2.1 ± 0.1	7.0 ± 0.2
Stage I	14	4.8 ± 0.2	9.6 ± 0.2	1.6 ± 0.1	8.0 ± 0.2
Stage I	21	9.1 ± 0.4	10.5 ± 0.2	1.8 ± 0.1	8.7 ± 0.2
Stage I	28	14.6 ± 0.5	12.0 ± 0.2	2.0 ± 0.1	10.0 ± 0.2
Stage II	35	24.2 ± 0.9	14.8 ± 0.2	2.5 ± 0.1	12.3 ± 0.2
Stage II	42	30.6 ± 1.1	16.9 ± 0.3	2.7 ± 0.2	14.2 ± 0.2
Stage III	49	38.5 ± 1.3	18.9 ± 0.4	1.9 ± 0.2	17.0 ± 0.3
Stage III	56	42.9 ± 1.6	19.6 ± 0.4	1.3 ± 0.2	18.3 ± 0.4
Stage III	63[y]	45.6 ± 1.9	20.2 ± 0.5	1.0 ± 0.1	19.2 ± 0.5
Stage III	70	46.6 ± 2.0	20.7 ± 0.5	0.5 ± 0.2	20.2 ± 0.5
Terminal bud set	77	47.0 ± 2.0	20.8 ± 0.6	0.3 ± 0.2	20.5 ± 0.5

[1]Developmental stages overlapped during this week.
[2]At this date, a few rudimentary shoots emerged from terminal buds.

Table 2. Measurements of 'Regina'/ 'Gisela®6' sweet cherry fruit growth from fruit set through stage III. Mean ± SE; n= 25.

Developmental stage	DAFB[1]	GDD[2]	Fresh Weight (g)	Diameter (mm)
Fruit set	7	342	0.3 ±0.02	6.2 ± 0.2
Stage I	14	405	1.2 ±0.1	11.5 ± 0.5
	21	469	1.5 ±0.1	13.0 ± 0.2
	28	533	1.7 ±0.1	13.6 ± 0.2
Stage II	35	618	1.9 ±0.1	14.6 ± 0.2
	42	722	2.5 ±0.1	16.2 ± 0.2
Stage III	49	843	4.8 ±0.3	19.8 ± 0.4
	56	968	8.8 ±0.5	25.4 ± 0.6
	63	1087	11.2 ±0.7	26.0 ± 4.9

[1]DAFB: days after full bloom.
[2] GDD: growing degree days.

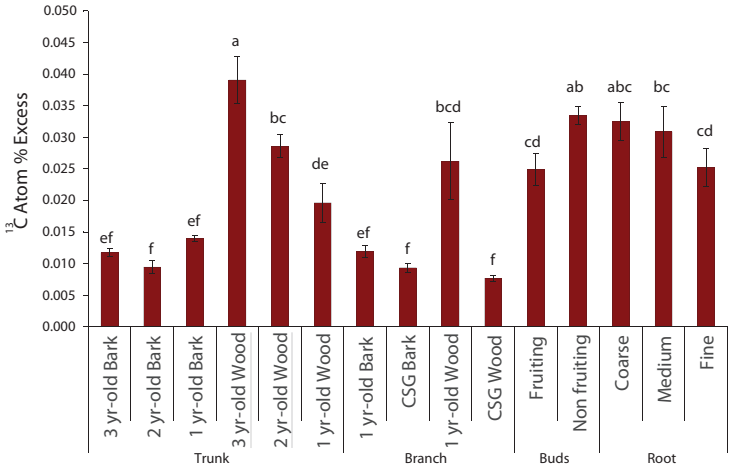

Figure 1. ^{13}C atom % excess in different organs of 'Regina'/'Gisela ®6' sweet cherry at leaf abscission. Colored bars indicate the mean for each organ, and vertical lines indicate SE; n=5. Means followed by the same lowercase letter are not significantly different at $\alpha = 0.01$. The p-value is ≤ 0.0001.

levels were also found in the younger wood of the trunk as well as the branches, fruiting buds and fine roots. CSG and bark from sections of various ages had significantly lower ^{13}C atom % excess, and at leaf abscission, the ^{13}C content in the leaves was 74% lower than that measured immediately after labeling.

^{13}C reserves partitioning at budbreak

The ^{13}C in all of the organs collected at the side green stage (Apr) remained above the natural levels of abundance (Figure 2), and the highest ^{13}C atom % excess values at budbreak were detected in the fruiting buds, non-fruiting buds and coarse roots (p-value ≤0.0001). Significantly higher ^{13}C levels were also detected in the medium roots, fine roots and wood (trunk and branches). The rest of the organs had lower ^{13}C atom % excess values, which were not significantly different from each other.

In most of the organs, the ^{13}C atom % excess values detected at budbreak were lower or similar to those measured at leaf abscission; the only exceptions were fruiting and non-fruiting buds, which had higher ^{13}C atom % excess at budbreak than at leaf abscission. The greatest increase (45%) in ^{13}C atom % excess values was detected in fruiting

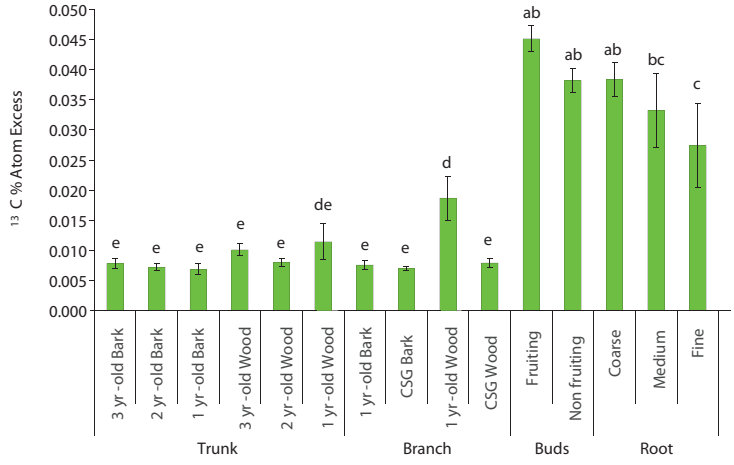

Figure 2. ^{13}C atom % excess in different organs of 'Regina'/'Gisela®6' sweet cherry at budbreak. Colored bars indicate the mean for each organ, and vertical lines indicate SE; n=5. Means followed by the same lowercase letter are not significantly different at $\alpha = 0.05$ and $\alpha = 0.01$. The p-value is ≤ 0.0001.

buds, and the greatest reductions in ^{13}C atom % excess were detected in the 2- and 3-yr-old wood of the trunk followed by that of most of the bark sections. The ^{13}C levels in the roots of all sizes and 1-yr-old wood did not change significantly between leaf abscission and budbreak.

^{13}C reserves partitioning from bloom

Significantly higher ^{13}C levels than natural were detected from the first bloom (6 DBFB) until the beginning of stage I (14 DAFB). Significant differences in ^{13}C atom % excess values were detected among the aerial organs within specific developmental stages, indicating differences in the levels of dependency on storage reserves (Figure 3). Moreover, ^{13}C levels decreased in all organs with time, and there were significant differences between stages. From 21 to 35 DAFB, ^{13}C contents were relatively constant for all organs.

^{13}C atom % excess values were highest during first and full bloom; during first bloom, the spur flowers had the highest ^{13}C levels followed by single flowers and the NFS leaves. At this stage, FS leaves and CSS leaves had not yet begun to grow, but at full bloom, similar ^{13}C levels were detected in flowers (single and spur clusters) and the leaves of young shoots followed by NFS leaves. FS had the lowest ^{13}C content. Furthermore, at this stage, shoots were 0.3 cm in length and had 3 very small developing leaves (Table 1).

Between full bloom and fruit set, a lower % excess ^{13}C atom value (i.e., less ^{13}C enrichment) was observed in all organs, but the ^{13}C levels were still higher than the natural abundance values. At fruit set (7 DAFB), FS leaves and very small immature fruits (0.3 g FW) had the highest ^{13}C contents followed by NFS leaves. In contrast to full bloom, FS leaves had the highest, and CSG the lowest, gains in ^{13}C. At this stage, shoots were 1.9 cm in length with 9 developing leaves (Table 1).

At the beginning of stage I (14 DAFB), fruits had the highest ^{13}C levels followed by FS leaves. NFS leaves and CSG had the lowest ^{13}C levels, but later in stage I (21 DAFB), ^{13}C levels reached their lowest point. For the first time, NFS spurs had the highest ^{13}C atom % excess values compared to the rest of the organs while the ^{13}C levels in the shoots (4.8 cm in length and 10 leaves) were closest to the natural abundance values. After this stage, relatively constant ^{13}C levels for all organs were observed, especially for CSG, which

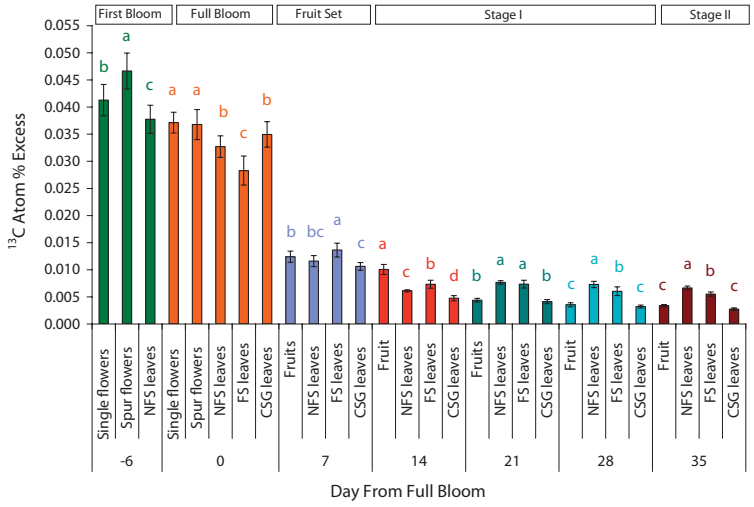

Figure 3. ^{13}C atom % excess for different aerial organs of 'Regina'/'Gisela®6' sweet cherry during the spring. Colored bars indicate the mean for each organ, and vertical lines indicate SE; n=5. Means within a given sampling period followed by the same lowercase letter are not significantly different at $\alpha = 0.05$. FS: fruiting spur; NFS: non-fruiting spur; CSG: current season growth.

indicated minimal additional contributions from the ^{13}C reserves.

As indicated above, the ^{13}C content not only varied among organs but also among developmental stages. The reproductive and vegetative organs collected at leaf abscission and at and after budbreak exhibited distinct ^{13}C seasonal fluctuation patterns. At leaf abscission, the vegetative meristems were more highly enriched with ^{13}C than reproductive buds, but this was reversed from budbreak through bloom. The ^{13}C contents of the reproductive organs (flowers and fruits) were also higher than those of the vegetative buds at 14 DAFB.

To calculate the gain in ^{13}C, the total DW for a particular organ at each developmental stage and the total distribution of ^{13}C (expressed as µg ^{13}C) were calculated for each of the aerial organs sampled through 28 DAFB. After that time, the levels of ^{13}C in all of the organs remained close to the natural levels of abundance and did not vary considerably. However, the greatest relative partitioning was to NFS (Figure 4); the highest recovery for NFS occurred at full bloom, when the spur leaves were actively growing, and the single and spur flowers used low amounts of ^{13}C reserves relative to those partitioned to NFS. In terms of flower types, the spur flowers had a greater relative ^{13}C demand than the single flowers.

Between fruit set and 28 DAFB, the pattern of ^{13}C distribution among the organs was consistent. NFS leaves attracted most of the labeled reserves while partitioning to the fruit, FS leaves and CSG leaves resulted in similarly low ^{13}C contents. Fruit did not attract a significant amount of ^{13}C, and the highest gains were detected at 7 and 14 DAFB when the DWs of the fruit were only 33 and 150 mg, respectively.

Discussion

Carbon partitioning in the fall and the remobilization of reserves in the spring were studied in 5-yr-old sweet cherry trees on a semi-vigorous rootstock. ^{13}C was used as a tracer to distinguish between the two main sources of assimilates for early spring growth: those synthesized and accumulated the previous fall (i.e., storage reserves) and the current

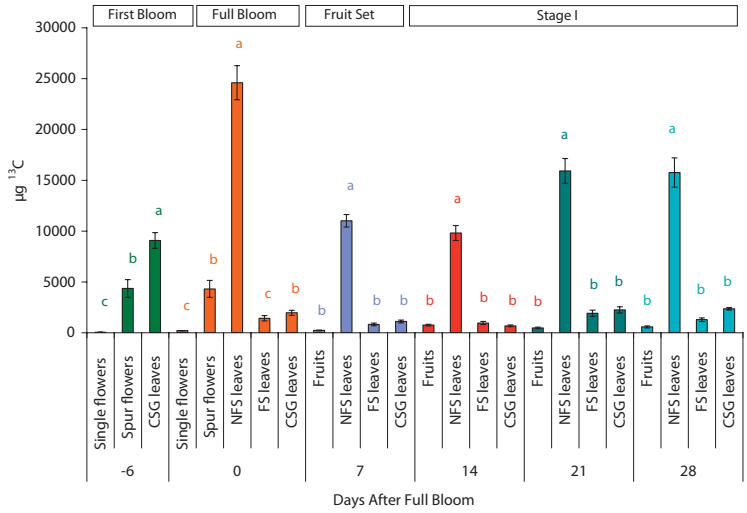

Figure 4. Total ^{13}C content (µg ^{13}C) for the different organs of 'Regina'/'Gisela®6' sweet cherry trees during the spring (May to Jun). Colored bars indicate the mean for each organ, and vertical lines indicate SE; n=5. Means within a given stage followed by the same letter are not significantly different at $\alpha = 0.05$. FS: fruiting spur; NFS: non-fruiting spur; CSG: current season growth.

photosynthates produced during the following spring by newly expanded leaves. At leaf abscission, the ^{13}C content in leaves was 74% lower than that measured immediately after labeling, either indicating the translocation of ^{13}C to other organs, respiratory loss or both. ^{13}C loss due to leaf abscission was 14% of the ^{13}C fixed in the fall.

The higher ^{13}C reserve accumulations were detected in the older wood of the trunk, the coarse roots and the vegetative buds while the younger wood of the trunk and branches, fruiting buds and fine roots registered lower ^{13}C accumulations. Bark from different sections did not store much ^{13}C compared with the other organs, and these results were consistent with high accumulation of CH_2O in the wood of the trunk and older branches of sweet cherry (Keller and Loescher, 1989). Basipetal translocation of CH_2O from leaves to perennial storage organs, following terminal bud set but prior to leaf drop, which allow it to become part of structural growth or storage reserves (Oliveira and Priestley, 1988), have been reported in apple (Quinlan, 1969; Kandiah, 1979 a,b), Japanese pear (Teng *et al*., 1999), grape (Hale and Weaver, 1962) and sweet cherry (Loescher *et al*., 1990).

^{13}C reserves were either remobilized or utilized during the period between leaf abscission and budbreak. At budbreak, the pattern of ^{13}C distribution throughout the tree was different from that at leaf drop, and the ^{13}C content of wood and bark from older sections had significantly decreased. However, the fruiting buds had dramatically higher ^{13}C contents than those at leaf drop, indicating that ^{13}C reserves were remobilized from other storage organs during the period between dormancy and budbreak. Based on the changes in ^{13}C levels between leaf drop and budbreak, it is likely that ^{13}C reserves were translocated, prior to bloom, from the wood and bark of the trunk to the reproductive meristems.

Even before budbreak, the reproductive meristems were the strongest sinks for the remobilized ^{13}C-assimilates, and these continued to be a priority for ^{13}C partitioning until 14 DAFB. The remobilization and utilization of storage reserves for metabolism during dormancy has been previously reported (Priestley, 1981; Oliveira and Priestley, 1988), but it was not the aim of this research to develop a comprehensive accounting of ^{13}C-reserve use by non-cropping-related sink activities (such as

those of the roots, phloem and cambial growth). However, it is possible that before budbreak, some of the ^{13}C-reserves had already been used for these additional sink activities (Oliveira and Priestley, 1988). Keller and Loescher (1989) conclude that these aboveground sweet cherry tissues begin to utilize CH_2O in the late winter, and the interconversions of starch to soluble sugars in wood and bark occur during dormancy. Similar reductions in the amount of reserves and the remobilization from roots and stems to meristematic regions over winter have been reported for apple (Hansen, 1967; Quinlan, 1969; Priestley, 1981), and CH_2O reserve depletion and concentration gradients during dormancy have been attributed to maintenance respiration and bud development, which is related to temperature (Oliveira and Priestley, 1988).

The ^{13}C content of the roots, which are considered to be the major storage organs in sweet cherry (Loescher *et al*., 1990), did not vary until budbreak, at least. The contribution of root reserves to new spring growth is unclear and might depend on species, tree age (i.e., root to shoot ratio), cultivar and rootstock (Priestley, 1981; Loescher *et al*., 1990). In peaches, rootstock vigor, crop load and ripening time affect the extent of the utilization of CH_2O reserve among cultivars (Inglese *et al*., 2002), which is soil temperature dependent with little depletion at temperatures <10 °C (McCammant, 1988). Additional information is required to elucidate the role of fine and coarse roots as storage organs in sweet cherry on dwarfing and semi-vigorous, highly productive rootstocks.

Various studies have reported that storage reserves are important to early spring growth (flowers, leaves, shoots and fruits) in deciduous species (Quinlan, 1969; Hansen and Grauslund, 1973; Oliveira and Priestley, 1988; Loescher *et al*., 1990). In this study, the early spring growth of sweet cherry flowers, fruits, spur leaves and shoots was supported by ^{13}C reserves accumulated the previous fall, and the mobilization of stored ^{13}C relative to new aerial growth was detected before budbreak and continued until 14 DAFB when the spur and shoot leaves were not yet fully developed. Therefore, reproductive and vegetative growth competed strongly for remobilized storage reserves during bloom and initial fruit growth. The use and competition for storage reserves in early stages have been studied in apple (Hansen, 1967; Quinlan, 1969; Hansen, 1971; Kandiah, 1979 a,b), grape (Scholefield *et al*., 1978), and Japanese pear (Teng *et al*., 1999). The level of dependence on, and competition for, reserves may be influenced by the order in which organs begin growing in the spring. The importance of budbreak phenologies to potential partitioning effects among cultivars has been previously reported for a southern highbush blueberry (*Vaccinium corymbosum* L.; Maust *et al*., 2000). In the Japanese pear, the initial growth of leaves and shoots is more dependent on storage reserves than the organs that develop later (Teng *et al*., 1999). Similarly, in apple extension shoots, the leaves that developed earlier in the spring were more dependent on storage reserves than the upper (later developing) leaves (Quinlan, 1969).

The greatest dependence of the sweet cherry cv. 'Regina' on storage reserves was at bloom, after which the utilization of ^{13}C-reserves declined. At fruit set, the ^{13}C levels of the different growing organs were lower, but the competition continued. The demand of the individual organs varied during this period, which was reflected in their ^{13}C concentrations, and the highest sink activity was detected in the reproductive organs (flowers and fruit). However, considering the total mass of particular organs, the vegetative structures had the greatest sink strength for the ^{13}C reserves.

At 14 DAFB, the concentration of ^{13}C in all of the organs declined, indicating decreasing dependence on or depletion of the ^{13}C reserves. From 21 to 35 DAFB, ^{13}C contents were relatively constant for all organs, suggesting either a decline in dependence or a steady depletion of ^{13}C storage reserves. Keller and Loescher (1989) demonstrated that the CH_2O in sweet cherry roots, wood and bark decline rapidly during full bloom, and similar reductions in storage reserves have been reported for apple

during spring, especially in the roots (Priestley, 1960; Hansen, 1967; Quinlan, 1969; Kandiah, 1979a,b), and Japanese pear (Teng *et al*., 1999). The depletion of storage reserves after bloom has been associated with the abscission of floral tissues and unfertilized flowers (Hansen, 1971; Teng *et al*., 1999), and other reports indicate that reserves decline after budbreak primarily due to respiratory loss with a small portion devoted to new reproductive and vegetative growth (Hansen, 1967; Hansen and Grauslund, 1973; Kandiah, 1979a,b). In apple, most of the fruit growth depends on photosynthates produced by newly formed leaves, and only a small portion (<20 to 25%) of the reserves is used for new growth (Hansen, 1967; Hansen and Grauslund, 1973; Kandiah, 1979b; Johnson and Lakso, 1986). Hansen (1971) suggested that 50 to 75% of the structural materials in the flowers and shoots come from storage reserves until the flowers show color and the shoots have developed 5 to 6 leaves (i.e., 200 mg DW/spur and 500-1000 mg DW/extension shoot). This seems to be the case in sweet cherry as well as the results indicate that of the total ^{13}C fixed, between 3 to 11% was partitioned to new aerial organs until 14 DAFB when the shoots had 5 leaves and the fruits were 12 mm in diameter.

In sweet cherry, final fruit size depends on cell division (stage I) and subsequent cell elongation during the final swell (stage III). Histological studies of sour cherry (*Prunus cerasus* L.) fruit have indicated that mesocarp cells increase in number during the pre-bloom stage and stage I, which is the period of maximum division (Tukey and Young, 1939). Olmstead *et al*. (2007) reported that the number of sweet cherry cells in the mesocarp is genetically stable, differing little between small and large fruits of the same genotype, so cell size is the main determinant of fruit size at harvest. Lang (2001) proposed that N and CH_2O reserves are critical for final flower development, bloom and fruit set in sweet cherry; at this time, cells are dividing rapidly in young shoots and fruits, defining the final potential fruit size and perhaps, just as importantly, spur leaf area. This study confirms that storage reserves are an important source of C for reproductive organs during bloom and initial fruit cell division as well as for initial spur leaf development, which will then become the major source of C during fruit cell expansion (Ayala and Lang, 2008). At the stages of budbreak and fruit set, competing sinks are source-limited because the canopy is not fully expanded; source limitation results in insufficient C to support potential organ growth (DeJong and Grossman, 1995). The timing of reserve utilization by reproductive meristems and developing spur leaves, in competition with other sinks through 7 to 14 DAFB, suggests that reserve levels may be a potential determinant of the variation in fruit set and final fruit size in highly productive and less vigorous sweet cherry trees. In such situations, the regulation of crop load is required to avoid a period of extreme resource limitation during bloom and fruit set, until about the 4^{th} or 5^{th} leaf, that can diminish leaf area and reduce final fruit size.

In summary, this study documents the dynamic hierarchy of the distribution of stored C among the aerial organs of a 5-yr-old sweet cherry trees from budbreak through stage I of fruit growth. Reproductive organs are the highest sinks of storage reserves until 14 DAFB, but strong competition between flowers, fruits and different leaf populations also occur. Late in stage I, when shoots are ~5 cm in length and there are 10 leaves and fruits are 13.0±0.2 mm, the storage reserves no longer constitute the main source of assimilate, and the newly expanded leaves become the major C source for fruit and shoot growth.

These results advance the understanding of the importance of storage reserves for early spring growth in sweet cherry using vigor-reducing rootstocks that increase precocity and yields. The practical recommendations derived from this research include the maintenance of healthy photosynthetic sources during the previous fall to promote optimal reserve accumulation in storage sites, a precise manipulation of canopy structure to achieve a more balanced

partitioning of reserves between reproductive and vegetative organs, especially the spur leaves, during the early spring, and the avoidance of late summer stresses, such as drought or defoliation due to diseases or insects. Good and coordinated horticultural practices after harvest (i.e., timing of N fertilization, pest and disease control, irrigation and appropriate summer pruning) will promote an optimum CH_2O supply for storage and subsequent use in new growth during the early spring.

Resumen

M. Ayala y G.A. Lang. 2015. Distribución de 13C-fotasimilados en cerezo dulce a inicio de primavera. Cien. Inv. Agr. 42(2): 191-203. En cerezo dulce (*Prunus avium* L.), las reservas de almacenaje son importantes para el crecimiento de sumideros reproductivos y vegetativos en temprano en primavera. Para estudiar la distribución de reservas de almacenaje carbonadas en portainjertos híbridos altamente productivos, se realizó un experimento utilizando árboles del cv 'Regina' injertados en el portainjerto semivigoroso 'Gisela®6' ('GI®6'). La copa completa de cinco árboles fue encerrada con cámaras transparentes de Mylar® para luego ser enriquecidos con altos niveles de $^{13}CO_2$ en tres ocasiones durante el otoño. Al estado fenológico de caída de follaje, yemas, hojas, madera, corteza y raíces fueron muestreadas para ser analizadas mediante cromatografía de gases y espectrometría de masa. Los órganos de almacenaje con los mayores niveles del parámetro Exceso Atom ^{13}C (%) fueron raíces, madera más vieja del tronco y ramas y yemas. Durante primavera órganos en desarrollo (flores, frutos inmaduros y hojas) fueron muestreados semanalmente desde floración hasta Fase III de desarrollo de fruto para un análisis usando cromatografía de gases y espectrometría de masa. La composición isotópica fue significativamente diferente entre órganos y estados fenológicos. Los mayores niveles de ^{13}C en sumideros en desarrollo fueron detectados entre floración y cuaja frutal. Los órganos reproductivos tuvieron las mayor actividad sumidero hasta 14 (días después de plena flor, DDPF) compitiendo con las hojas de dardos. Según lo observado, se propone que una limitación en las reservas de almacenaje impactaría la cuaja de fruta y el desarrollo de área foliar, lo cual en definittiva afectaría la disponibilidad de fotoasimilados durante estados fenológicos más tardíos en el periodo de desarrollo de frutos en cerezo dulce.

Palabras clave: Cuaja de frutos, portainjertos 'Gisela®', sumidero, remobilización en primavera, fase I, reservas de almacenaje, transición.

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