Effect of Prebloom Pruning on ^{13}C and ^{15}N Distribution during Early Spring in Sweet Cherry

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Abstract. In sweet cherry, highly advanced dwarf combinations using ‘Gisela’ rootstocks promote higher productivity than do more vigorous combinations but require maintaining the leaf area to fruit area (LA:F) ratio. An experiment using double isotopic enrichment with ^{13}C and ^{15}N was carried out in 5-year-old ‘Bing’/‘GI 6’ trees in a commercial orchard located in Santa Cruz, Chile (34°39’ S; 71°19’ W), and characterized by a Mediterranean climate. Forty whole sweet cherry trees (TR₁) trained as central axes were pruned in winter [July 20, 60 days before full bloom (DBFB)] and another 40 trees were left without pruning (TR₂). A single $^{13}\text{CO}_2$ pulse and ^{15}N -urea application to the mature leaves of individual 4-year-old branches on trees of both TRs was carried out during stage III (SIII). Winter pruning reduced yield by 44%, improved fruit quality [i.e., size, weight, and soluble solids content (SSC)] and induced the growth of extension shoots (ES) (i.e., number, length, and LA). For both TRs, fruits were stronger sinks for ^{13}C -photoassimilates and ^{15}N than were ES. ES of pruned trees had higher sink strength than ES of unpruned trees. Pruned trees developed more ES that were longer and that had higher LA compared with the ES of unpruned trees.

High-density sweet cherry (*Prunus avium* L.) orchards that use dwarfing rootstocks require pruning and crop load regulation to promote appropriate LA:F ratio that guarantee high-quality fruit (Ayala and Andrade, 2009; Ayala and Lang, 2004, 2015; Hansen, 1989; Kappel et al., 1996; Zhang et al., 2015). Lack of crop load regulation either by winter or summer pruning (e.g., heading-back and tipping cuts), thinning (i.e., removal of buds, flowers, or fruits), or both cause an imbalance between reproductive and vegetative growth in dwarf and semidwarf sweet cherry combinations using ‘Gisela’ rootstocks (Gutzwiler and Lang, 2001; Wang et al., 2003; Whiting et al., 2005), resulting in premature tree aging and low-quality fruit. Lack of crop load regulation reduces size, SSC, and firmness in the fruits of sweet cherry (Ayala and Lang, 2008; Bound et al., 2013; Villasante et al., 2012; Whiting et al., 2005; Whiting and Lang, 2004).

Pruning constitutes an effective and inexpensive strategy to manage crop load in highly productive sweet cherry combinations (Lang, 2005; Long, 2002; Whiting, 2005). Pruning allows adjusting the sources of photoassimilates (i.e., leaves of spurs and current season ES) and both the photoassimilates (mainly carbohydrates; CH_2O) and nitrogen (N) demands imposed by developing sinks (i.e., buds, flowers, fruits, and leaves). Spur leaves develop rapidly in spring (i.e., 30–40 d

after full bloom; DAFB), whereas ES leaves continue growing during the season (i.e., 180–200 DAFB). In addition, annual pruning allows the control of tree height, promotes vegetative growth, facilitates the renewal of fruiting wood, and optimizes light interception (Guimond et al., 1998; Lakso and Corelli-Grappadelli, 1992; Lang, 2001; Mika, 1986). Thinning of buds, flowers, or young fruits complements annual pruning in highly productive sweet cherry combinations. In this type of trees, hand thinning after pruning is required to adjust LA:F ratio, which balances the number of fruits per spur and improves photoassimilate distribution among remaining fruits (Ayala and Lang, 2004; Correa, 2008; Long, 2002; Macit et al., 2017).

The development of sweet cherry fruit and the elongation of ES occur in a short period (<60–75 d) and generate a strong demand for photoassimilates and other nutrients (Ayala et al., 2014; Ayala and Lang, 2008, 2015; Keller and Loescher, 1989; Whiting, 2005). During this period, leaves and storage reserves are sources of photoassimilates and N for the growth of reproductive and vegetative sinks (Ayala, 2004; Loescher et al., 1990; Ouzounis and Lang, 2011; Rivera et al., 2016). The distribution of carbon (C) between the organs depends on the contribution of photoassimilates, the proximity between sinks and sources, the degree of competition between the sinks and sources, and the ability of sinks to attract nutrients (Flore and Layne, 1999; Loescher et al., 1986). In studies carried out in Michigan State (United States), using the ‘Ulster’/‘GI 6’ combination, fruits were important

sinks for photoassimilates during SIII of fruit development, and ES constituted a source of C early in SI (Ayala, 2004). The correct management of sources and sinks to both promote C balance and reduce competition among sinks is critical for the growth of fruits and the development of ES in sweet cherry (Lang, 2005; Macit et al., 2017; Whiting, 2005). Whiting and Lang (2004) reported that good-quality fruit can be obtained using 250 cm^2 LA/fruit with the combination ‘Bing’/‘Gisela 5’ (‘GI 5’) in the Pacific North West (United States). Source limitations (Keller and Loescher, 1989; Marcelis, 1996; Roper et al., 1988) during fruit development must be avoided through crop load regulation strategies such as pruning; the thinning of buds, flowers, and young fruits; or both (Bound et al., 2013; Correa, 2008; Wright, 1989). Winter pruning has an important effect on sink–source balance by reducing floral structures and promoting vegetative growth, which has been reported in ‘Gisela’ rootstocks (Long, 2002, 2007; Whiting and Lang, 2004).

In sweet cherry, N uptake commonly occurs through roots (Khemira et al., 1998), although foliar applications after harvest are also important to support flower and fruit growth early in the season (Ayala et al., 2014; Rivera et al., 2016). In the combination ‘Bing’/‘GI 6’, soil and foliar N applications promote early spring growth of the buds, flowers, fruits, and young leaves of spurs and ES (Lang, 2001, 2005; Rivera et al., 2016). Ayala et al. (2014) reported that foliar application of ^{15}N -urea after harvest influenced N storage reserves in floral buds for the subsequent spring. Similarly, Ouzounis and Lang (2011) reported an increase in N level in reproductive spurs after foliar applications of urea during fall and reported a subsequent increase in leaf size during the following spring. In addition, Rivera et al. (2016) found that ^{15}N -ammonium nitrate, applied to the soil during early spring, was detected in fruit at harvest.

The aerial distribution of photoassimilates and nitrogenous compounds at SIII of fruit development in the combination ‘Bing’/‘Gisela 6’ (‘GI 6’) after a prebloom pruning has not been reported. It was hypothesized that prebloom pruning might constitute an effective practice to increase fruit quality in the sweet cherry combination ‘Bing’/‘GI 6’, as it might induce a redistribution of C and N in favor of remaining fruits. Consequently, the main objective of the study was to analyze the distribution of ^{13}C and ^{15}N during SIII of fruit development among different organs in 4-year-old sweet cherry branches of pruned and unpruned sweet cherry trees.

Materials and Methods

Plant material and location. This experiment was conducted in 2008 using 5-year-old sweet cherry trees of the semivigorous combination ‘Bing’/‘GI 6’ in Santa Cruz (34°39’ S; 71°19’ O), VI Region, Chile. The area is characterized by a Mediterranean climate with warm weather, winter rains,

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and a long dry period during summer; the average annual thermal amplitude is 13 °C and the average annual precipitation is 650 mm concentrated in winter (Gazmuri, 1990). The frost-free period occurs between September and June. The soil belongs to the 'Tuncahue' series and is characterized by medium texture and 60 cm of effective depth (Díaz, 1957). The orchard was established in a clayey soil (20.6% clay, 45.1% silt, and 34.3% sand) that had a pH of 6.0. Trees were uncovered and trained as a central axis, and scaffolds along each axis had woody sections that were 1, 2, 3, and 4 years old at the time of prebloom pruning (July 20). Pest and disease control was carried out following the commercial management. The average number of scaffolds ranged between 17 and 20 per tree. The planting distance was 2.5 m/row and 4.0 m separated each row. Standard fertigation practices were used and nitrogen was applied to the soil at a rate of 100 kg·ha⁻¹ of total urea during the season (between October and February). The pollinizer cultivar was 'Black Tartarian' at a proportion of 11%. Sweet cherry trees were not thinned by hand during the experiment. The experiment was performed in well-irrigated trees and during the free-frost period; therefore, no significant effect on pruned treatments was expected.

Treatments. Treatments included pruned (Treatment 1; TR₁) and unpruned (Treatment 2; TR₂) 5-year-old sweet cherry trees of the combination 'Bing'/'GI 6'. In midwinter (July 20, 60 DBFB), a total of 40 trees (40 replications per TR) received annual prebloom pruning and other 40 trees remained unpruned. Prebloom pruning was made after chilling requirement was achieved and consisted of heading cuts to 3- and 4-year-old branches located at the basal and medial portions of the central axis (0.75–2.5 m in height). The heading cut was made above a fruiting spur (FS) located on a 2-year-old section, which was ≈15–20 cm from the scar that indicated the change in year between 2- and 3-year-old wood. Above 2.5 m, only tipping cuts to 2-year-old branches were made. The tipping cut was carried out above a single vegetative bud on 1-year-old wood, leaving 2/3 of the shoot. One-year-old shoots along the central axis were kept unpruned. In addition to the heading and tipping cuts, 2 or 3 whole 4-year-old branches (11% to 18% of the total number of branches) were headed back to a stub that had two spurs to promote lateral branch renewal in the spring on the 2-year-old section. Fifteen uniform 4-year-old branches headed back to a FS were selected from pruned (TR₁) and unpruned (TR₂) trees (one per tree) for double isotopic enrichment with ¹³C and ¹⁵N. Individual 4-year-old branches of pruned and unpruned trees had similar characteristics in terms of position along the axis (0.8–2.5 m), diameter (2.8–3.6 cm), length (2.0–2.8 m), the number of spurs, the number of ES (3–4 units per branch), and the number of fruits per spur (2–3 fruits per spur).

¹³C and ¹⁵N isotopic enrichment. At the beginning of SHI of fruit development, four FSs

in the middle portion of 2-year-old wood section of branches from pruned (TR₁) and unpruned (TR₂) trees were selected, and fruits from those FSs were removed. Sixty-three DAFB (Nov. 23), the leaves of those spurs were subjected to a ¹³CO₂ pulse in accordance with the protocol used by Ayala (2004). Branches were enclosed in transparent polyethylene chambers and ¹³CO₂ was applied in pulses of 15 min from 1000 to 1200 HR of a sunny day. Twenty-four hours later, the same FS leaves received a ¹⁵N-urea application following the protocol used by Ayala et al. (2014). Leaves were painted for both sides with a solution of ¹⁵N-urea (10% atom ¹⁵N; 1.5%). During labeling of FS leaves, temperatures ranged between 15 and 23 °C and solar radiation fluctuated between 900 and 1100 kW·m⁻². The average LA of spurs that were directly double-enriched was 655.0 cm²/branch.

Sample preparation. Whole double-labeled 4-year-old branches were removed at commercial harvest (70 DAFB, Dic 1) from pruned and unpruned trees. Branches were partitioned into their organs as follows: a) fruits; b) new ES (wood + leaves); c) wood (bark + phloem + xylem) tissues of 2-, 3-, or 4-year-old sections according to the TR; and d) The leaves of FSs and nonfruiting spurs (NFS). Different organs were dried in an oven (AD810L; Arquimed, Santiago, Chile) at 70 °C for 5 d to achieve a constant dry weight (DW). Total DW per organ was determined using an analytical balance (GRAM Labtech 1500, Mississauga, ON, Canada). Tissues were ground in a micromill (IKA MF 10 Basic, Staufen, Germany) using a 0.25-mm sieve. Subsequently, a 2-mg sample per organ was used for mass spectrometry analysis. ¹³C and ¹⁵N content was measured using GC-MS (PDZ Europa 20–20 mass spectrometer and ANCA-GSL sample combustion unit; PDZ Europa, Sandbach, Cheshire, UK). Additional samples were prepared from unlabeled pruned and unpruned branches for natural abundance calculations. ¹³C enrichment for different organs was calculated in accordance with the methods of Boutton (1991) and Vivin et al. (1996). ¹⁵N enrichment calculations were performed in accordance with the

methods of Weinbaum and Van Kessel (1998) and Cabrera and Kissel (1989). ¹⁵N enrichment was quantified in accordance with the methods of Millard and Neilsen (1989).

Evaluations. The length of ES and number were measured weekly for each TR. The total length of ES was measured for pruned and unpruned trees at terminal bud set (pruned trees = 101 DAFB; unpruned trees = 85 DAFB). At harvest, the total fresh weight (FW) of fruits from pruned and unpruned trees was measured. In addition, 50 fruits were randomly collected from each TR to determine SSC (%), diameter (mm), FW (g), and firmness (Durofel units). In addition, the average LA (cm²) of spurs and ES of individual 4-year-old branches of pruned and unpruned trees at terminal bud set were measured using an LA meter (LI-3100; LICOR Biosciences, Inc., Lincoln, NE).

Design and statistical analysis. A completely randomized design was established for the data distribution of each pruning TR. A single tree (pruned or unpruned) was considered an experimental unit. Twenty (replications) of 40 trees (total number of experimental units per TR) were used for ¹³C and ¹⁵N enrichment of individual branches. One 4-year-old branch per tree that was pruned or not pruned was used for double isotopic enrichment with ¹³C and ¹⁵N. Analysis of variance was conducted using PROC MIXED procedures of the SAS statistical analysis program (SAS Institute, Inc, Cary, NC). The statistical model for the overall experiment considered two factors: treatment (TR = 2) and organ per individual 4-year-old branch (O = 5). As extremely high levels of ¹³C and ¹⁵N enrichment were expected in directly labeled leaves, these were not considered for statistical analysis. Means of different TRs were compared using the minimal important differences Tukey test ($P \leq 0.05$).

Results

Effect of winter pruning on vegetative growth. There was an effect of winter pruning on the growth of ES. Pruning had effect on

Table 1. Effect of winter pruning (July, 60 d before full bloom, southern hemisphere) on current season extension shoots (ES) and spur growth on individual 4-year-old branches of the sweet cherry (*Prunus avium* L.) combination 'Bing'/'GI 6' at commercial harvest (70 d after full bloom), Dec. 1; southern hemisphere, Santa Cruz, VI Region, Chile) ($n = 40$).

Type of shoot	Treatment	
	Pruned	Unpruned
Current season ES		
Leaf size per ES (cm ²)	44.2 a ^z	27.1 b
Number of leaves per ES	18.9 a	14.1 b
Number of ES per branch	3.6 a	2.4 b
Average length of ES (cm) at fruit harvest	30.2 a	7.1 b
Total length of ES (cm) per branch	108.5 a	17.3 b
Total length of ES per tree (m)	87.8 a	38.8 b
Spurs		
Number of spurs per branch	15.9 a	24.8 b
Leaf area (cm ²) per spur	203.3 a	124.2 b
Number of leaves per spur	6.7 a	5.5 b

^zMean values followed by the same letter in the same row showed no significant differences according to the Tukey test ($P = 0.05$).

the length of individual ES, the total length of ES per tree and the number of leaves and average leaf size per individual ES. Significant differences ($P \leq 0.05$) in individual and total length of ES were observed between individual 4-year-old branches of pruned and unpruned trees. The total length of ES per tree was higher in pruned trees (87.8 m) than in unpruned trees (38.8 m). The amount of FSs was 56% higher in unpruned branches as expected, but these spurs had fewer (21% less) and smaller (63% less) leaves than did FSs of pruned branches (Table 1).

Individual ES of pruned branches had significantly higher ($P \leq 0.05$) total LA (3007.4 cm²) compared with that of ES of unpruned trees (928.5 cm²) (Table 2). ES of pruned branches were longer and developed more and larger leaves than did ES of unpruned branches. In addition, significant differences ($P \leq 0.05$) were detected in terms of the number of FSs per branch, the number of leaves, and average LA per FS between 4-year-old branches of pruned and unpruned trees (Table 1). The total LA of the spur section of pruned branches (3232.5 cm²) was slightly (but no significant) higher than the LA of the spur section of unpruned branches (3080.2 cm²; Table 2).

Effect of pruning on yield and fruit quality. Winter pruning reduced fruit yield, and significant differences ($P \leq 0.05$) were observed between TRs. The yield of pruned trees (11.4 kg/tree) was 44% lower than that of unpruned trees (25.7 kg/tree). However, winter pruning had a positive effect on fruit quality on the remaining spurs of the branch. Significant differences ($P \leq 0.05$) were observed in SSC, fruit size, and fruit FW between pruned and unpruned trees. Fruits of pruned trees were larger and had a higher SSC than did fruits of unpruned trees (Table 3).

Effect of pruning on ¹³C recovery. ¹³CO₂-pulsed leaves of FSs on 2-year-old sections of pruned and unpruned 4-year-old branches could fix and translocate ¹³C-photoassimilates to acropetal and basipetal fruits and ES. Significant differences ($P \leq 0.05$) in the ¹³C concentration (expressed as µg ¹³C/g DW) among different organs of branches of pruned and unpruned trees were detected (Table 4). In both TRs, ¹³C concentration in fruits was the highest, followed by ES, wood, and spurs of the same branch. No significant differences ($P > 0.05$) in terms of ¹³C concentration in individual organs were observed between TRs except for ES. ¹³C concentration was higher in ES of pruned branches than ES of unpruned branches.

In relation to the total ¹³C content per organ, significant differences ($P \leq 0.05$) were observed in 4-year-old branches of pruned and unpruned trees. In both TRs, fruits registered the highest ¹³C content (expressed as relative distribution in %) compared with that in the other organs of the branch (Fig. 1). Although most of the recovered ¹³C was detected in fruits, significant differences ($P \leq 0.05$) in terms of ¹³C content in the ES of pruned and unpruned branches were detected. The ES of 4-year-old

pruned branches had a higher ¹³C content than did ES of unpruned branches (Fig. 1).

Effect of pruning on ¹⁵N recovery. Significant differences ($P \leq 0.05$) in the ¹⁵N content among different organs of individual 4-year-old branches of pruned and unpruned trees were observed (Table 5). In both TRs, ¹⁵N content in fruits was the highest, followed by wood, ES, and spurs of the same branch. ES, wood, and spurs (FSs + NFs) had similar and lower ¹⁵N contents in pruned and unpruned trees.

When TRs were compared for ¹⁵N content (nitrogen derived from fertilizer; %) in individual organs, significant differences ($P \leq 0.05$) were observed in current season ES. The growth of the ES of the 4-year-old branches of pruned trees showed higher ¹⁵N content than did the ES of the branches of unpruned trees (Fig. 1).

In terms of nitrogen use efficiency [NUE (%)], the highest values were registered in the directly enriched leaves of FSs, followed by fruits. The other organs showed similar and lower NUE (Table 6). Significant differences ($P \leq 0.05$) in NUE for individual organs between TRs were observed only for ES. The growth of the ES of the 4-year-old branches of pruned trees showed higher NUE than did the ES of the branches of unpruned trees.

Discussion

Effect of pruning on yield, fruit quality, and vegetative growth. In the combination 'Bing'/'GI 6', winter pruning had a positive effect on fruit quality but reduced final yield by 44% (data not shown). Reductions in yield and increases in fruit quality after pruning in sweet cherry have been reported previously (Long, 2002; Villasante et al., 2012; Whiting, 2005; Whiting and Lang, 2004). Pruned trees registered an increase in the LA of ES as there were more, longer, and larger developed leaves. In addition, individual FSs of pruned trees showed higher LA because of the larger leaves, although the number of spurs per branch was higher in the unpruned treatment. The effect of pruning on increased fruit quality and vegetative vigor has previously been reported for highly advanced dwarf combinations using 'Gisela' rootstocks (Lang, 2000; Vercammen and Vanrykel, 2009; Whiting et al., 2005).

In this study, winter pruning increased the LA of ES and spurs, improving the LA:F ratio in the combination 'Bing'/'GI 6'. Pruning practices reduce crop load in highly productive sweet cherry combinations (Gutzwiller and Lang, 2001; Lang, 2005; Whiting and Lang, 2004) and optimize LA:F ratios, which in turn increases fruit quality. Pruned 'Bing'/'GI 6' trees had an average LA:F ratio of 48.33 cm²/fruit, whereas unpruned trees had an average LA:F ratio of 21.6 cm²/fruit (data not shown). Suboptimal LA:F ratios due to the lack of pruning reduced fruit quality and the growth of ES in 5-year-old 'Bing'/'GI 5' trees (Correa, 2008). An adequate balance between sources (i.e., ES and spur leaves) and fruit sink demands for photoassimilates is required to

Table 2. Average leaf area (cm²) of spurs and current season extension shoots (ES) of an individual 4-year-old branch of pruned and unpruned trees of the sweet cherry combination 'Bing'/'GI 6' at terminal bud set [pruned trees = 101 d after full bloom (DAFB); unpruned trees = 85 DAFB; southern hemisphere, Santa Cruz, VI Region, Chile] ($n = 40$).

Leaf area (cm ²)	Treatment	
	Pruned	Unpruned
Complete branch	6,239.9 a ^z	4,008.7 b
Extension shoots per branch	3,007.4 a	928.5 b
Total spurs per branch	3,232.5 a	3,080.2 a

^zMean values followed by the same letter in the same row showed no significant differences according to the Tukey test ($P = 0.05$).

Table 3. Effect of winter pruning (July, 60 d before full bloom, southern hemisphere) on the yield and fruit quality of the sweet cherry (*Prunus avium* L.) combination 'Bing'/'GI 6' at commercial harvest (70 d after full bloom, Dec. 1; southern hemisphere, Santa Cruz, VI Region, Chile) ($n = 40$).

	Treatment	
	Pruned	Unpruned
Yield per tree (kg)	11.4 a ^z	25.7 b
Fruit fresh weight (g)	9.8 a	8.2 b
Soluble solids content (%)	26.5 a	23.9 b
Diameter (mm)	26.1 a	24.6 b
Firmness (Durofel units, 0–100)	82.4 a	82.8 a

^zMean values followed by the same letter in the same row showed no significant differences according to the Tukey test ($P = 0.05$).

Table 4. ¹³C concentration (µg of ¹³C/g dry weight) in the organs of 4-year-old branches of pruned and unpruned sweet cherry (*Prunus avium* L.) trees of the combination 'Bing'/'GI 6' at commercial harvest (70 d after full bloom, Dec. 1; southern hemisphere, Santa Cruz, VI Region, Chile) ($n = 20$).

Organ	Treatment	
	Pruned	Unpruned
Spurs	3.2 ± 0.3 A ^z b ^y	4.3 ± 0.2 A b
Wood	2.2 ± 0.1 A b	2.2 ± 0.2 A b
Fruit	22.4 ± 1.0 A a	32.6 ± 1.3 A a
Extension shoots	9.4 ± 0.6 A b	3.5 ± 0.3 B b
P value	0.002	0.0015
Enriched spurs leaves ^x	74.0 ± 2.1 A a	101.2 ± 3.0 A a

^zMean values followed by the same uppercase letter in the same row showed no significant differences according to the Tukey test ($P = 0.01$).

^yMean values followed by the same lowercase letter in the same column showed no significant differences according to the Tukey test ($P = 0.05$).

^xEnriched spurs received ¹³CO₂ and ¹⁵N-urea foliar application directly on their leaves.

obtain premium fruit quality and to promote more vegetative vigor in dwarf sweet cherry combinations that use 'Gisela' rootstocks (Whiting, 2005; Whiting and Lang, 2004).

In mature trees, pruning allows the renovation of reproductive structures and promotes new shoot development (Mika, 1986). In this experiment, 'Bing'/'GI 6' trees that were not pruned had higher yield, but they

Fig. 1. Relative distribution of ¹³C content and nitrogen derived from fertilizer (%) in the organs of 4-year-old branches of pruned and unpruned sweet cherry (*Prunus avium* L.) trees of the combination 'Bing'/'GI 6' at commercial harvest (70 d after full bloom, Dec. 1; southern hemisphere, Santa Cruz, VI Region, Chile) (n = 20).

Table 5. Nitrogen derived from fertilizer (% mg/organ) in the organs of 4-year-old branches of pruned and unpruned sweet cherry (*Prunus avium* L.) trees of the combination 'Bing'/'GI 6' at commercial harvest (70 d after full bloom, Dec. 1; southern hemisphere, Santa Cruz, VI Region, Chile) (n = 20).

Organ	Treatment	
	Pruned	Unpruned
Spurs	0.6 ± 0.0 A ^a b ^y	0.8 ± 0.0 A b
Wood	1.6 ± 0.0 A b	1.3 ± 0.0 A b
Fruit	7.2 ± 0.3 A a	7.3 ± 0.3 A a
Extension shoots	0.8 ± 0.0 A b	0.1 ± 0.0 B b
P value	0.001	0.002
Enriched spurs leaves ^x	9.1 ± 0.2 A a	7.8 ± 0.2 A a

^aMean values followed by the same uppercase letter in the same row showed no significant differences according to the Tukey test (P = 0.01).

^yMean values followed by the same lowercase letter in the same column showed no significant differences according to the Tukey test (P = 0.05).

^xEnriched spurs received ¹³CO₂ and ¹⁵N-urea foliar applications directly on their leaves.

Table 6. Nitrogen use efficiency (%) in the organs of the 4-year-old branches of pruned and unpruned sweet cherry (*Prunus avium* L.) trees of the combination 'Bing'/'GI 6' at commercial harvest (70 d after full bloom, Dec. 1; southern hemisphere, Santa Cruz, VI Region, Chile) (n = 20).

Organ	Treatment	
	Pruned	Unpruned
Spurs	1.6 ± 0.3 A ^a b ^y	2.0 ± 0.5 A b
Wood	4.3 ± 0.8 A b	3.5 ± 0.5 A b
Fruit	19.4 ± 0.8 A a	19.7 ± 0.9 A a
Extension shoots	2.1 ± 0.1 A b	0.3 ± 0.0 B b
P value	0.001	0.003
Enriched spurs leaves ^x	24.8 ± 0.5 A a	21.3 ± 0.4 A a

^aMean values followed by the same uppercase letter in the same row showed no significant differences according to the Tukey test (P = 0.01).

^yMean values followed by the same lowercase letter in the same column showed no significant differences according to the Tukey test (P = 0.05).

^xEnriched spurs received ¹³CO₂ and ¹⁵N-urea foliar applications directly on their leaves.

also had smaller fruit size and very reduced shoot growth and LA development. Fewer and shorter ES in unpruned trees indicate not

only low LA:F ratios but also a reduced capacity to renew fruiting wood and maintain stable fruit quality, yield, and vegetative vigor over the years. On the other hand, the length and LA of the ES of pruned trees were higher than those in unpruned trees. At terminal bud set, the ES of pruned 4-year-old branches were four times larger than were the ES of unpruned branches. Pruning of sweet cherry using vigor-reducing rootstocks is recommended to promote vegetative vigor and the redistribution of C and N among remaining sinks (Lang, 2005; Whiting et al., 2005). In this study, a positive correlation between fruit quality parameters (i.e., size, SSC, and FW) and the growth of ES (i.e., length and LA) in pruned 'Bing'/'GI 6' trees was observed, confirming the need for pruning to balance the LA:F ratio and to promote new shoot growth and LA development.

Effect of pruning on ¹³C distribution. Fruits were priority sinks for ¹³C-photoassimilate compared with other organs of the branches in pruned and unpruned trees of the combination 'Bing'/'GI 6'. Similar results were reported by Ayala (2004) for 'Ulster'/'GI 6', in which fruits were the strongest sink for ¹³C-photoassimilates during SIII. Fruits are important sinks for photoassimilates in sweet cherry. Similarly, in studies in sour cherry (*Prunus cerasus*), fruits near ES show strong sink strength throughout the whole period of fruit growth, and the demand for photoassimilates is related to endocarp and cell wall development (Toldam-Andersen, 1998). Fruits influence the translocation patterns of carbohydrates (CH₂O), inducing competition with vegetative organs (Ayala and Lang, 2008; Roper et al., 1987). New ES are sinks in early developmental stages, and competition for photoassimilates occurs with young fruits until ES become net exporters (Flore and Layne, 1999). The importance of knowing more about the relative sink activity of fruits and shoots during the season has been treated by Reyes et al. (2016). In this experiment, the fruits of pruned and unpruned sweet cherry 'Bing'/'GI 6' trees registered the highest sink activity (¹³C concentration) and sink strength (total ¹³C content per organ) compared with ES, wood, and spurs. However, new ES of pruned branches had a higher ¹³C total recovery than did the ES of unpruned branches, indicating a higher sink strength of the former.

Winter pruning influenced ¹³C distribution between fruits and ES at SIII of fruit development. Although greatest amount of ¹³C was detected in fruits at 70 DAFB in both TRs, more ¹³C was observed in the ES of pruned branches than in unpruned branches. According to Ayala (2004), terminal ES provide photoassimilates for fruit growth at SIII in the combination 'Ulster'/'GI 6'. In the present experiment, 'Bing'/'GI 6' pruned trees developed higher LA in ES, which may have constituted an additional source of photoassimilates for fruits during final fruit swelling. Winter pruning promoted the development of more growth of ES, which may have increased the availability of photoassimilates for fruits during final swelling, but competition for ¹³C-photoassimilates between fruits and ES was detected. During final swelling, fruit growth and shoot elongation occur simultaneously; consequently, there is demand for CH₂O by developing fruits and ES, which could influence C distribution between both organs (Ayala, 2004; Lakso and Corelli-Grappadelli, 1992; Roper et al., 1988). It is highly possible that the leaves of ES become a source of C for fruits at a later stage, after the leaves of ES become net exporters (Flore, 1994; Flore and Layne, 1999; Hansen, 1989; Keller and Loescher, 1989).

Effect of pruning on ¹⁵N distribution. FS leaves on 4-year-old branches in the combination 'Bing'/'GI 6' exported ¹⁵N to fruits, ES, wood, and other spurs after a foliar ¹⁵N-urea application at SIII. Fruits were priority sinks for foliar ¹⁵N-urea applied 63 DAFB in pruned and unpruned branches. ES, wood, and spurs of pruned and unpruned branches registered lower sink demand for ¹⁵N. However, in pruned trees, ES registered higher ¹⁵N recovery than did the ES of trees without pruning. Pruned branches showed higher growth of ES than did unpruned branches, suggesting a stronger demand for ¹⁵N at SIII. Below the pruning cut, individual branches developed between 2.5 and 3.6 new ES during the spring, and these ES grew concomitantly with the fruits. Fruits and ES imported ¹⁵N from FS leaves, but the fruits were stronger sinks than were ES at SIII. N demand by developing sweet cherry fruits at SI was described by Ayala et al. (2014) and Rivera et al. (2016) in the combination 'Bing'/'GI 6' after foliar and soil applications. In the present study, the highest recovery of foliar ¹⁵N applied to pruned 4-year-old branches during SIII was mostly observed in fruits; however, the ES of these branches imported more ¹⁵N than did the ES of unpruned branches, which suggests some degree of competition for this nutrient (Khemira et al., 1998; Millard and Neilsen, 1989).

Conclusions

Prebloom pruning alters the distribution of ¹³C and ¹⁵N in 4-year-old sweet cherry branches during SIII of fruit development and constitutes an effective practice for

increasing fruit quality in the combination 'Bing'/'Gisela 6'. The results of this study contribute to the physiological basis for improving crop regulation strategies and foliar nutritional programs in highly productive sweet cherry combinations using 'Gisela' rootstocks. However, additional research comparing sweet cherry orchards with different climatic conditions is required to better understand the physiological effect of prebloom pruning from year to year.

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