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CARBON PARTITIONING IN SWEET CHERRY (*Prunus avium* L.) ON DWARFING PRECOCIOUS ROOTSTOCKS DURING FRUIT DEVELOPMENT

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CARBON PARTITIONING IN SWEET CHERRY (*Prunus avium* L.) ON DWARFING PRECOCIOUS ROOTSTOCKS DURING FRUIT DEVELOPMENT

By

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ABSTRACT

CARBON PARTITIONING IN SWEET CHERRY (*Prunus avium* L.) ON DWARFING PRECOCIOUS ROOTSTOCKS DURING FRUIT DEVELOPMENT

By

Marlene Ayala

Understanding carbon (C) partitioning is important for development of better management strategies to improve sweet cherry fruit quality on high-yielding, vigor-reducing rootstocks. To study the relative importance or temporal relationships of the primary leaf populations (i.e., fruiting spur, non-fruiting spur and current season shoot leaves) as sources of C for sweet cherry fruit and shoot development, a series of partitioning experiments using girdling, defoliation, fruit thinning and ^{13}C-labeling was established with sweet cherry trees on dwarfing/semidwarfing Gisela (GI) rootstocks. A preliminary girdling and defoliation experiment isolated fruit of 'Hedelfinger'/GI5 and 'Ulster'/GI6 from different leaf sources and indicated that leaf populations on both fruiting and non-fruiting branch segments were required for optimum fruit development. There was not a sufficient compensatory effect when one of the main leaf populations was eliminated. A second experiment used $^{13}CO_2$ to label non-fruiting spur leaves on 'Sam' /GI5 limbs with three different crop loads (quantified as leaf area to fruit ratios LA/F= 140, 75, or 40 cm^2/fruit), which indicated that fruit were stronger sinks than current season shoots during stage III of fruit development. A third experiment quantified the relative C

contribution of each leaf population to fruit and shoot development during key points throughout fruit development. Leaves on fruiting spurs, non-fruiting spurs and the new terminal shoot were exposed to $^{13}CO_2$ labeling on five representative phenological dates during fruit development. Spur and shoot leaves were significant sources of C for fruit and vegetative growth. Fruits were a priority sink vs. new shoot growth, in terms of C allocation, during the entire period of fruit development. The highest fruit sink strength was during stages I and III. Current season shoot growth provided a C source for fruit as early as stage I. Finally, a fourth experiment on 'Regina'/GI 6 labeled with $^{13}CO_2$ after terminal bud set determined the extent that storage reserves were used for spring growth, particularly fruit, and defined the transition phase during which current photoassimilates become the primary C source. In fall, the major storage organs were roots, older wood in the trunk and branches, and buds. During spring, ^{13}C-reserves were remobilized and partitioned to flowers, fruits and young leaves from before budbreak until 14 days after full bloom (DAFB). The highest ^{13}C levels in growing sinks were detected between bloom and fruit set. Reproductive organs had the strongest sink activity until 14 DAFB. Overall, these results provide a physiological foundation for canopy relationships that may help to develop specific orchard management strategies to promote a more sustainable balance between vegetative and reproductive growth in high density sweet cherry orchards on vigor-limiting rootstocks.

DEDICATION

To my parents, my brother and my husband with love

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CHAPTER I

LITERATURE REVIEW

Sweet Cherry Description and Production Trends

Sweet cherry (*Prunus avium* L.), a member of the Rosaceae family, is a temperate deciduous tree that is thought to have originated in forests close to the Caspian and Black Seas of Eastern Europe and Western Asia (Webster, 1996). Sweet cherry trees are characterized for large stature (>10 m) and strong apical dominance in natural environments (Webster, 1996; Lang, 2000). More recently, sweet cherry has become one of the most profitable tree fruits due to improvements in germoplasm breeding and selection (i.e., introduction of new varieties and rootstocks), management practices in the field, and storage and transportation.

Consequently, the international commercial production of sweet cherry is increasing. Europe and Asia are the most productive continents. In North America, >90% of the total sweet cherry production is from the United States (US) (Whiting, 2001). In 2003, the US produced 215,000 mt on 30,712 ha, with Michigan being the fourth largest US sweet cherry producer (FAOSTAT data, 2004). Among fruit commodities, sweet cherry is one of the most highly prized (Lang, 2000). A short postharvest life and limited climatic adaptability foster a profitable niche in world markets and reduce competition (Maguylo, 2003).

Recently, US sweet cherry production is in transition to high density, early fruiting orchards (Lang, 1998). Profitable orchard management of sweet cherry on vigorous rootstocks such as Mazzard (*Prunus avium* L.), Mahaleb (*Prunus mahaleb* L.) or Colt (*P.pseudocerasus* x *P.avium*) is being challenged by

inefficiencies associated with large tree size and a long establishment period before fruiting (Lang, 2000). These characteristics are undesirable due to low initial yields, delayed return on capital investment and inefficiency of orchard operations (e.g. pruning and harvest labor, pest control, etc.) (Whiting, 2001). In addition, labor costs have increased and labor availability has decreased considerably in recent years. As a consequence, sweet cherry growers are adopting dwarfing and semi-dwarfing rootstocks, which are characterized by small canopies and positive effects on precocity and yield (Weber, 2001). High density systems using dwarfing precocious rootstocks are more labor efficient and economically viable. These modern orchards are more uniform, have high and early yields, require lower production and harvest inputs, and are easier to protect against rain or bird damage.

Sweet Cherry Rootstocks and the Gisela Series

Historically, the most popular rootstocks used in commercial sweet cherry orchards, in North America and around the world, have been seedlings of Mazzard and Mahaleb, or clones of 'Colt' (Webster and Schmidt, 1996; Perry, 1987; Lang, 1998). Unfortunately, sweet cherries on these rootstocks are not convenient for highly intensive systems since trees are too vigorous and do not flower until the 6th or 7th year (Lang, 2000). However, the introduction of a new generation of more dwarfing rootstocks has great potential to promote precocity, high productivity and reduced vigor. In the 1980 s, several of the more

promising international rootstock selections were imported into the US and established under different climatic and soil conditions throughout the country (Perry et al., 1996). So far, the most promising dwarfing and semi-dwarfing rootstocks belong to the Gisela (Giessen, GI) series, particularly GI5 (148/2) and GI6 (148/1), both hybrids of *P. cerasus* x *P. canescens* (Webster and Lucas, 1997; Andersen et al., 1999; Lang, 2000; Webster, 2001). The GI rootstocks were developed in a breeding program initiated in 1965 at Justus Liebig University in Giessen, Germany (Franken-Bembenek, 1996). These rootstocks induce flowering several years earlier than usual (from the 3rd to the 5th year) and provide a size control that ranges from 50% (GI5, dwarfing) to 80% (GI6, semidwarfing) of similar trees on standard Mazzard (Lang, 2000).

Before commercial adoption of GI rootstocks by American growers becomes routine, many physiological questions must be elucidated. The extensive experience with dwarfing apple rootstocks provides some clues for intensive sweet cherry management. However, the seasonal growth and fruiting habit of sweet cherry differ from those of apple, implying that the physiological consequences of similar orchard practices might not have the same results. Despite the advantages of GI rootstocks to induce precocity and higher yield efficiency (Webster, 2001), such trees have the tendency to crop excessively beginning about the 4th or 5th year, resulting in small sized fruit (Andersen et al., 1999). In addition, GI rootstocks might have a reduced capacity for CH_2O storage due to smaller root systems and trunk and branch tissues (Lang, 2001a).

Precocious sweet cherry trees on GI rootstocks have the potential to quickly reach and imbalance between early vegetative and fruiting growth, leading a prolonged period of 'runting out' (Lang, 2001a,b). Thus, the balance between leaf area, storage reserves and crop load capacity becomes more critical in achieving high quality fruit (Lang, 2000). To overcome this situation, more precise canopy development, i.e., greater precision in management of crop loads and the different leaf populations within the canopy, is required to optimize the balance between vegetative growth and fruit quality (Lang, 2001a,b).

Reproductive and Vegetative Habits of Sweet Cherry Trees

Sweet cherry produces simple buds, which can be reproductive or vegetative. Normally, reproductive buds are initiated in the leaf axis of new or existing non-fruiting spurs; however, a few flowers also can be initiated in the leaf axis of single buds near the base of new shoots. A reproductive spur may have 1 to 6 buds, each of which may contain multiple inflorescences (Thompson, 1996). Vegetative buds form in the leaf axis on current season new shoots; in the subsequent season, each vegetative bud will become a non-fruiting spur, which in turn will initiate flowers to bloom the following year. Vegetative growth consists of extension shoots (either lateral or terminal) and spurs (short shoots with minimal internode length). Extension shoots and spurs generally emerge concomitant with bloom. In vigorous sweet cherry trees, spur leaves (fruiting and non-fruiting spurs) complete their development early in the season (~3

weeks after bloom); however, lateral and terminal shoot growth continues through harvest. During this initial post-bloom period, spur leaves constitute the primary source of C for fruit growth (Roper et al., 1987). The reproductive effort (i.e., the proportion of total biomass allocated in reproductive structures) of dwarfing trees on GI rootstocks is different from those on more standard vigorous rootstocks such as Mazzard and Mahaleb. Flowers per unit branch-size often are much more numerous on trees on GI rootstocks (Webster, 1996). Recently, Maguylo (2003) found that the number of reproductive buds and flowers of 'Hedelfinger' on either GI5 or GI6 spurs was ~4 and 3, respectively; on Mazzard, these were 0.4 and 0.8, respectively.

Healthy and abundant leaf populations, producing a continuous supply of photosynthates throughout the season, are essential for growth and storage. In many species of the Rosaceae family, including sweet cherry, spurs and extension shoot leaves are the main sources of current photosynthates for vegetative and reproductive growth (Roper et al., 1987; Corelli-Grappadelli et al., 1994; Teng et al., 2001). In most species, photoassimilate production by spur leaves is not sufficient to support optimal fruit growth and import of assimilates synthesized by leaves external to bearing spurs is required (Roper et al., 1987; Lakso and Corelli-Grappadelli, 1992; Tustin et al., 1992; Corelli Grappadelli et al., 1994; Teng et al., 1998, 2001).

In a typical two-year-old sweet cherry branch, current photoassimilates for fruit and shoot growth are provided by three distinct leaf populations. These are described below (See Figure 1):

a. ***Fruiting spur leaves***: these are located on the 2-year-old section of the branch. There are ~7 to 9 leaves at each spur. Fruit are borne on these spurs. The primary purpose of this leaf population is thought to supply CH_2O to adjacent fruit and nearby shoots (Lang, 2001b).

b. ***Non-fruiting spur leaves***: these are located acropetally to the spur fruit on 1-year-old section of the branch. There are ~6 to 8 leaves at each spur. Lang (2001b) suggested that this leaf population is a 'power house of CH_2O production' due to two reasons: (1) there is a 6- to 8-fold increase in leaf number compared to the same node during its formation the year before; (2) this segment does not have fruit to support directly. Accordingly, this leaf population might help to supply CH_2O to nearby current season shoots, developing leaves, and developing fruit on older wood.

c. ***Leaves on current season shoots***: these leaves are located acropetal to the fruit. There is one leaf at each node or single bud. Lang (2001b) suggested that these leaves may be sinks for CH_2O during active shoot extension early in spring; however, at maturity they may constitute a source of CH_2O for nearby sinks and probably for distant fruit.

If we consider summer 2004 as a reference in time, fruiting spur leaves would be located on shoot growth that was formed during 2002, non-fruiting spur leaves would be on shoot growth formed in 2003, and current season shoot leaves would be formed during 2004.

Little is known about the relative importance or temporal relationships of different leaf populations as sources of current photosynthates for fruit and shoot development in dwarfing sweet cherry trees. As indicated above, it is believed that, as in other Rosaceae species, fruiting and non-fruiting spur leaves support fruit growth from early developmental stages onwards but there is not direct evidence for this assumption. In addition, the contribution of leaves on current season shoots, as a potential C source during fruit development, has not been documented.

The Importance of Carbon Economy and Partitioning in Fruit Trees

The CH_2O economy of plants has received considerable attention during recent years. The carbon economy of a tree includes the acquisition of C by photosynthesis and subsequent utilization for biomass synthesis and maintenance (Buwalda, 1991). In this process, C has been considered a 'common currency' to asses C allocation patterns and costs in plants (Reekie and Bazzaz, 1987a,b). So far, the understanding of these processes in fruit trees is limited and only a few studies have focused on sweet cherry. Currently, one of the most

important challenges in crop physiology is to determine the mechanism governing the partitioning to and dry matter accumulation of individual sink organs (Wardlaw, 1990). Crop production is dependent not only on the ability of the plant to intercept light for C fixation, but also on the partitioning of CH_2O into economically important organs (Minchin et al., 1997). Generally, it is accepted that the majority of the historical increase in crop yield has been possible due to shifts in partitioning patterns rather than changes in photosynthetic rates or respiration (Gifford and Evans, 1981; Patrick, 1988). Therefore, future insights regarding assimilate partitioning may contribute to improvements in crop productivity by increasing total biomass production and by favoring assimilate transfer to the harvestable portion of the crop (Patrick, 1988). Indeed, this is one consequence of using dwarfing rootstocks in sweet cherry trees; fruit production is increased and vegetative growth is decreased. However, fruit quality is also of critical importance, not just biomass production.

Dry matter partitioning is the end result of a coordinated set of transport and metabolic processes governing the flux and distribution of C from source organs via a transport path to the sink organs (Patrick, 1988; Marcelis, 1996; Daudet et al., 2002). Partitioning of assimilates within the sites of synthesis (source) and between sources and various competing sites of utilization (sink), is under genetic and environmental regulation (Daie, 1989). In deciduous fruit trees, CH_2O partitioning is affected by several factors, which include: assimilate supply from photosynthesis, availability of storage reserves, canopy structure,

light interception, organ development, respiration, crop load, rootstock, cultural practices and environmental conditions (McCammant, 1988; Keller and Loescher, 1989; DeJong 1999). All of these factors must be integrated to understand the whole CH_2O economy of sweet cherry (Flore and Layne, 1999).

The balance between vegetative growth and fruiting is manipulated through horticultural practices to increase yield and/or quality and reduce management costs. Many studies have focused on the consequences of competition between organs and how this affects fruit development and quality. Fruit play a major role in biomass allocation, as they are major sinks for assimilates (Heuvelink, 1997). Biomass allocation to fruit strongly affects total fruit production, the weight of individual fruits and their quality components, which are all important determinants of the economic yield. In dwarfing sweet cherry trees, excessive flowering produces excessive crop loads, which result in small fruit (Andersen et al., 1999; Lang, 2000; Lang, 2001a,b). For fresh consumption, it is often desirable to have a smaller number of larger fruits rather than a large number of small ones, because the value per unit is much lower for small fruit than for large ones (Jackson, 1989; Stover, 2000). A high fruit yield is desirable and high biomass allocation to fruit is important; however, as the allocation to fruit is at the expense of vegetative growth, which is needed for the formation of leaf area, and hence light interception for photosynthesis, too high of an allocation of biomass to fruit will affect future production capacity negatively (Heuvelink, 1997). Enhanced fruit growth at the expense of vegetative

growth has been reported for several species (Forshey and Elfving, 1989; Kappel, 1991; Grossman and DeJong, 1995), including sweet cherry trees on dwarfing precocious rootstocks (Whiting, 2001). On the other hand, while a certain level of vigor is essential, excessive vegetative vigor reduces flowering and fruit set (Forshey and Elfving, 1989).

As indicated above, yield improvement in fruit trees involves dry matter production by various leaves populations and its partitioning and accumulation in harvested organs, i.e., fruits. A better yield is achieved by successful regulation of source-sink relationships, which influence the production and utilization of C of the whole tree (Ho, 1988; DeJong and Grossman, 1995). Minchin et al. (1997) indicate that C source-sink relationships are important in controlling fruit growth, and may ultimately determine crop yield. Assuming the competition among sink organs for CH_2O is dependent on the intrinsic ability of sink organs to control C partitioning based on their sink strength, studies on determination of sink strength may provide better strategies to improve crop productivity (Ho, 1988; Marcelis, 1993; Grossman and DeJong, 1994).

In sweet cherry trees, interactions between vegetative and reproductive sweet cherry growth change during the growing season. During early stages of development, fruits and vegetative organs compete for storage reserves (Loescher et al., 1990), while later in the season, mature leaves provide fruit and shoots with photoassimilates (Roper et al., 1987). Most of the research studies on sweet cherry C partitioning are based on the study of trees on vigorous

rootstocks; few experiments have focused on the dynamics of CH_2O partitioning on dwarfing or semi-dwarfing rootstocks, which might differ from more vigorous trees due to their reduced aerial woody structures, smaller root systems and higher harvest index. Flore and Sams (1986) indicate that in sour cherry (*Prunus cerasus* L.), photosynthesis may limit yield when crop loads are high and foliage development is low (i.e., LA/F ratios < 2). This might be the case in dwarfing sweet cherry trees, which have reduced LA/F ratios.

Carbohydrate Metabolism in Rosaceae Species

In sweet cherry, total nonstructural carbohydrates (TNC) consist mainly of starch, sorbitol, sucrose, fructose, glucose and raffinose (Keller, 1986; McCammant, 1988; Keller and Loescher, 1989). Sorbitol and sucrose are the major translocated CH_2O in several species of the Rosaceae family, especially in the subfamilies Pomoidae and Prunoidae (Gao et al., 2003). Sorbitol, a sugar alcohol, is synthesized in mature sweet cherry leaves (Keller and Loescher, 1989) and transported through the phloem to various sink tissues, where is metabolized and converted into other CH_2O (Bieleski and Redgwell, 1985). Sucrose, also considered storage CH_2O, accounts for a fourth of the soluble CH_2O in sweet cherry (Keller, 1986). Sucrose is synthesized and utilized by leaves of different ages (Loescher et al., 1982, Bieleski and Redgwell, 1985). Glucose, fructose and sorbitol are the major TNC in sweet cherry fruit (Keller, 1986).

The relative abundance of TNC in sweet cherry tissues changes qualitatively and quantitatively during the season (Keller and Loescher, 1989). The seasonal TNC changes have been described by Keller (1986) and McCammant (1988) as follows: TNC decrease before budbreak in all perennial tissues except spurs. At budbreak, fructose and glucose predominate in buds. Sorbitol is the most abundant soluble CH_2O at this time. During fruit development TNC accumulate slowly in all tissues. After harvest, TNC are accumulated at a higher rate, reaching their highest level at leaf abscission. Starch is the most abundant storage material. At the onset of dormancy, raffinose, fructose and glucose are abundant. During dormancy, interconversion of starch and soluble CH_2O occurs, with sucrose as the most predominant soluble CH_2O.

Specific enzymes are involved in synthesis or degradation of CH_2O in rosaceous species. The enzyme NADPH-dependent aldose 6-phosphate reductase (A6PR) is responsible for sorbitol synthesis in green tissues (Loescher et al., 1982; Bieleski and Redwell, 1985; Loescher and Everard, 1996; Sashanishi et al., 1998). In sink tissues, sorbitol is catabolized by the enzymes NAD-dependent sorbitol dehydrogenase (SDH), which converts sorbitol to fructose (Negm and Loescher, 1981; Loescher et al., 1982; Lo Bianco and Rieger, 2002a,b) and sorbitol oxidase (SOX), which converts sorbitol to glucose (Yamaki, 1980). On the other hand, sucrose catabolism in sink tissues occurs via sucrose synthase (SS), soluble acid invertase (AI) and neutral invertase (NI) activities (Lo Bianco et al., 1999b;

Lo Bianco and Rieger, 2002a,b). SDH, SOX and AI activity correlate positively with fruit sink strength and growth rate (Lo Bianco and Rieger, 2002b). SDH activity correlates with shoot elongation (Lo Bianco et al., 1999a)

Storage Reserves

Definition and importance

In most deciduous woody perennials, the immediate sources of CH_2O are recently synthesized photoassimilates and accumulated reserves (Oliveira and Priestley, 1988). Storage reserves are materials produced in excess of current requirements and which later may be used to support metabolism and growth (Priestley, 1960). These 'substances' (organic compounds and nutrients) are not used directly in growth and respiration but stored in the tree until required (Glerum, 1980). The use of CH_2O reserves is subject to a temporal and spatial distribution since the contents of storage reserves fluctuates, and major sites of storage may be remote from the sites of utilization (Oliveira and Priestley, 1988).

Storage reserves are important for several life processes. Reserves are used for winter survival, metabolism, respiration, defense, healing, vegetative and reproductive growth, fruit development and new growth in spring (Kandiah, 1979a,b; Oliveira and Priestley, 1988; Loescher et al., 1990; Kozlowski and Pallardy, 1997). Increased cold hardiness has been attributed to CH_2O accumulation during fall (Johnson and Howell, 1981). More vigorous trees are able to accumulate more CH_2O to heal injuries due to pathogen or insect attacks,

synthesize defensive chemicals and tolerate various environmental stresses (Kozlowski and Pallardy, 1996). Reserves also are important for 'regrowth' after pruning, premature defoliation and early season frost (McCammant, 1988; Kozlowski et al., 1991). In pecan (*Carya illinoensis* Koch.), grape (*Vitis vinifera* L.) and sweet cherry, premature defoliation reduced the accumulation of CH_2O reserves in fall (Worley, 1979; Smith et al., 1986; McCammant, 1988; Candolfi-Vasconcelos et al., 1994). Alternate bearing also has been attributed to the availability of stored reserves. In pistachio (*Pistacia vera* L. Pistah.) and pecan, increased CH_2O reserves have been observed after an 'off' year (Crane et al., 1976; Smith et al., 1986; Wood, 1995). A decrease in alternate bearing might be due to more time for the tree to accumulate CH_2O reserves before leaf fall (Stevenson and Shackel, 1998).

Several authors indicate that the initial stages of spring growth in deciduous fruit trees must depend upon mobilization of reserves accumulated the previous season, until new leaves become photosynthetically competent to provide current photosynthates (Priestley, 1960; Hansen, 1967b; Quinlan, 1969; Oliveira and Priestley, 1988). Reserves are essential for new growth because they provide energy and structural resources before root N uptake and photosynthesis occurs in spring (Cheng and Fuchigami, 2002).

Types of storage reserves

a. CH_2O reserves

Quantitatively, CH_2O constitute the predominant components of storage reserves; however, qualitatively, N and other minerals such as P, Ca, K and Mg are equally important (Tromp, 1983; Oliveira and Priestley, 1988). CH_2O reserves include soluble and insoluble forms. Starch is the main insoluble storage form in woody organs and is synthesized whenever a high level of sugars accumulates (Tromp, 1983; Kozlowski and Pallardy, 1996). In sweet cherry, starch is the most common storage material (Keller and Loescher, 1989). Small amounts of hemicelluloses also are found in storage organs (Taylor et al., 1975), but their function is primarily structural as a component of cell walls (Oliveira and Priestley, 1988). Hemicellulose is used during maturation of current season growth (Priestley, 1960). Among soluble CH_2O, sorbitol, mannitol, sucrose, glucose, fructose and raffinose have been reported as important for storage in various woody perennials (Crane et al., 1976; Loescher et al., 1990). In some species of the Rosaceae family, such as apple (*Malus domestica* Borkh.) and sweet cherry, sorbitol is the principal soluble storage CH_2O in non-photosynthetic cells (Tromp, 1983; Oliveira and Priestley, 1988; Loescher and Everard, 1996). In sweet cherry leaves, sorbitol accumulates more than starch (Roper et al., 1988), and raffinose accumulates during dormancy (Keller, 1986; Keller and Loescher, 1989). Other soluble CH_2O found in small amounts in storage organs include inositol,

xylose, rhamnose, maltose, trehalose, arabinose, ribose, mannose, galactose and stachyose (Loescher et al., 1990).

b. Nitrogenous reserves and other minerals

N reserves are also composed of soluble and insoluble fractions. Amino acids and amides, mainly arginine and asparagine, are the major soluble compounds, while proteins correspond to the insoluble fraction (Oliveira and Priestley, 1988). Mobilization and recycling of N reserves in spring is critical to support new growth shortly after budbreak, since at this time conditions for root uptake are not optimal (Habib et al., 1989). In apple, N reserves become available for new growth in spring through hydrolysis of bark and wood protein (Kennedy et al., 1975). In sweet cherry, remobilization of N reserves from roots occurs during the first 35 to 50 days after budbreak (Grassi et al., 2003).

Storage Organs

The whole perennial structure of a tree can be considered as a storage organ (Kandiah, 1979a,b; Loescher et al., 1990). In most angiosperm trees or 'hardwoods', CH_2O reserves are accumulated predominantly in living ray and axial pachenchyma cells of woody axes (i.e., branches and trunk) and roots (Oliveira and Priestley, 1988). The importance of woody axes and roots as storage organs vary among species (Tromp, 1983; Priestley, 1960; Loescher et al., 1990). Some studies indicate that there is no difference in the potential value of reserves

from different regions of the tree since no specific regions for CH_2O storage exist due to a similar distribution of CH_2O reserves above or below ground parts (Priestley 1960; Tromp, 1983; Araujo and Williams, 1988; Kandiah, 1979a,b). However, a preferential accumulation of CH_2O reserves seems to occur in roots of some woody perennials (Hansen, 1967b; Quinlan, 1969; Kandiah, 1979a,b; Keller, 1986; Loescher et al., 1990). In sweet cherry, CH_2O and N reserves in roots were higher than in other storage organs such as trunk and shoots (Loescher and Keller, 1989; Grassi et al., 2003). Roots might be the most important storage organ in sweet cherry because of their high starch content (Keller, 1986).

Seasonal pattern of storage reserves in woody perennials

Seasonal fluxes of storage reserves, mainly CH_2O, have been studied extensively in apple (Hansen, 1967b; Quinlan, 1969; Hansen and Grauslund, 1973; Hansen, 1971; Priestley, 1960; Kandiah, 1979a,b), sweet cherry (McCammant, 1988; Keller, 1986; Keller and Loescher, 1989), peach (*Prunus persica* (L.) Batsch) (Gaudillere et al., 1992; Moing and Gaudillere, 1992; Caruso et al., 1997; Inglese et al., 2002), pecan (Davis and Sparks, 1974; Worley, 1979; Lockwood and Sparks, 1978 a,b; Smith et al., 1986), grape (*Vitis vinifera* L.) (Winkler and Williams, 1945; Scholefield et al., 1978; Bains et al., 1981; McArtney and Ferree, 1999), kiwifruit (*Actinidia deliciosa* [A. Chev.] C.F. Liang et A.R. Ferguson) (Buwalda et al., 1990; Buwalda, 1991; Greaves et al., 1999), cranberry

(*Vaccinium macrocarpon* Ait) (Birrenkott et al., 1991; Hagidimitriou and Roper, 1994) and blueberry (*Vaccinium corymbosum* L.) (Maust et al., 2000).

The production, partitioning and utilization of CH_2O reserves follow specific seasonal patterns in deciduous fruit trees. Levels of reserves in perennial organs have a similar pattern of initial deposition, followed by depletion in early spring and subsequent replenishment later in summer and fall (Tromp, 1983). In spring, growing sinks attract nutrients from sources, i.e., storage organs, elsewhere in the tree. However, later in the season, new leaves become self-sufficient and sink demand changes to other organs. In late summer, shoot growth slows or ceases and nutrient accumulation in perennial tissues increases in importance, predominating in fall before leaf senescence.

Early stages of development in spring depend on reserves accumulated in the tree during the previous season (Oliveira and Priestley, 1988). Depletion of CH_2O reserves in shoots and roots of several species usually begins before budbreak and continues after bloom during early shoot growth (Priestley, 1960; Hansen, 1967b; Hansen and Grauslund, 1973; Gaudillere et al., 1992; Moing and Gaudillere, 1992; Caruso et al., 1997; Inglese et al., 2002; Scholefield et al., 1978; Bains et al., 1981; Buwalda, 1991; Lockwood and Sparks, 1978a,b; Birrenkott et al., 1991; Hagidimitriou and Roper, 1994; Teng et al., 1999). In apple, early CH_2O reserve depletion was due mainly to respiration with only a small portion (< 20%) used as building material for new growth (Hansen and Grauslund, 1973; Kandiah, 1979a,b). Labeled C fixed during the previous fall has been detected

during early spring growth of leaves, flowers, fruit and shoots of apple, grape, japanese pear (*Pyrus pyrifolia* Nakai) and pecan (Hansen, 1967b; Hansen, 1971; Scholefield et al., 1978; Teng et al., 1999; Lockwood and Sparks, 1978a,b). During early spring, root activity increases and significant amounts of CH_2O are used in metabolism, respiration, structural growth and are incorporated into amino acids (Oliveira and Priestley, 1988). Storage reserves also are used in cambial activity and phloem formation (Oliveira and Priestley, 1988). Cambial activity begins before budbreak and phloem differentiation precedes xylem formation (Evert, 1963). After reaching the lowest CH_2O levels, most species begin to accumulate storage reserves immediately. However, during fruit development and ripening, this process is slow or absent (Roper et al., 1988; Caruso et al., 1997; Inglese et al., 2002). Higher accumulation rates in permanent structures are detected after shoot extension has ceased in summer, when vegetative growth slows down and storage exceeds consumption (Chong, 1971; Kandiah, 1979a,b; Gaudillere et al., 1992; Oliveira and Priestley, 1988; Jordan and Habib, 1996; Caruso et al., 1997; Bains et al., 1981; Buwalda, 1991; Smith et al., 1986; Birrenkott et al., 1991; Hagidimitriou and Roper, 1994). After terminal bud set in late summer and before leaf fall, CH_2O reserves (mainly starch hydrolyzed to soluble transport sugars) are translocated basipetally to perennial storage organs (Priestley, 1960; Hansen, 1967b; Quinlan, 1969; Hansen and Grauslund, 1973; Kandiah, 1979a,b; Hale and Weaver, 1962; Araujo and Williams, 1988; Lokwood and Sparks, 1978; Davis and Sparks, 1974) to become part of structural growth or storage reserves,

mainly starch (Oliveira and Priestley, 1988; Loescher et al., 1990). At the beginning of dormancy, starch contents are highest (Caruso et al., 1997; Bains et al., 1981; Smith et al., 1986; Jordan and Habib, 1996; Birrenkott et al., 1991; Hagidimitriou and Roper, 1994). During winter, conversion of starch to soluble sugars occurs (Bains et al., 1981).

Seasonal pattern of storage reserves in sweet cherry

In sweet cherry, flowering often occurs before leaves are fully expanded and early stages of reproductive (flowers and fruits) and (spurs, extension shoots and roots) vegetative growth are dependent on the storage reserves accumulated the previous season (McCammant, 1988; Keller and Loescher, 1989). Other deciduous trees are less dependent on stored reserves since canopies are nearly fully expanded before anthesis (Keller and Loescher, 1989).

Seasonal nonstructural carbohydrate partitioning in sweet cherry trees on standard (vigorous) rootstocks has been studied previously (Keller, 1986; Keller and Loescher, 1989; McCammant, 1988). TNC in perennial organs of 'Bing' sweet cherry on standard rootstocks changed both qualitatively and quantitatively during the year (Keller, 1986; Keller and Loescher, 1989; McCammant, 1988). TNC declined in 1- and 2-year-old shoots and roots, beginning in mid-April and reaching a minimum in early May (McCammant, 1988; Roper et al., 1988). Shortly before budbreak, TNC decreased in all perennial organs except spurs (Keller, 1986; Roper et al., 1988; Keller and Loescher, 1989). After bloom, TNC

increased slowly until fruit harvest (Keller, 1986; Keller and Loescher, 1989; McCammant, 1988).However, the rate of accumulation slowed down during the last 4 to 6 weeks of fruit growth (Keller, 1986; Keller and Loescher, 1989). After fruit ripening and cessation of shoot extension, CH_2O reserves accumulated in different sweet cherry organs reaching a maximum at leaf abscission (Keller, 1986; Keller and Loescher, 1989; McCammant, 1988). Starch levels in current season growth, older shoots, trunk (1- to 3-year-old growth rings and bark) and roots were the greatest in fall (Roper et al., 1988). At the onset of dormancy, all soluble CH_2O increased, especially sorbitol (McCammant, 1988). During winter, interconversion of starch and soluble CH_2O in the wood of trunk and 1- and 2-year old shoots occurred. By February, sorbitol declined, while fructose and glucose began to peak in mid-April, a week before bloom (McCammant, 1988). Radioactive labeling of storage reserves in sweet cherry indicated that, at budbreak, buds had the highest ^{14}C recoveries compared to surrounding wood and bark. Shortly after leaf expansion, leaves were highly radioactive but the amount of label decreased as the shoot increased in length. When shoots were 20 to 30 cm long, expanding leaves were less radioactive than fully expanded leaves in the middle and base of the same shoot, indicating a reduced use of storage reserves.

Flow of C during early spring growth of sweet cherry trees is dependent on both storage reserves and current photosynthates. Currently, there is no information regarding the relative importance of these two components on the

dynamics of remobilization and partitioning of CH_2O reserves during spring in trees on more dwarfing rootstocks. It would be valuable to characterize the transition phase, in which storage reserves are depleted and current photosynthates become the primary source for vegetative and reproductive growth.

The Use of Labeled Carbon to Study Carbon Fluxes and Partitioning

The use radioactive carbon (^{14}C), supplied as $^{14}CO_2$ pulses, to study translocation patterns, carbon fluxes and partitioning of assimilates has been reported for several woody species. Traditional experiments in apple, peach, apricot (*Prunus armeniaca* L.), sour cherry, grape and pecan differ depending on whether the $^{14}CO_2$ was applied to whole trees, individual branches, shoots or single leaves of a shoot (Quinlan, 1969; Hansen, 1969; Corelli-Grappadelli et al., 1994; Kappes and Flore, 1989; Toldam-Andersen, 1998; Hale and Weaver, 1962; Davis and Sparks, 1974). Labeling methods vary from simple to highly sophisticated (Farrar, 1993). Few ^{14}C partitioning studies have been carried out using whole trees in full production; most considered young non-bearing trees (Quinlan, 1965; Hansen, 1967a,b; Wang et al., 1996; Wang and Quebedeaux, 1997, 1998; Bieleski and Redgwell, 1985; Kappes and Flore, 1989; Kandiah, 1979a,b). However, the use of uniform individual shoots, either attached to or excised from mature trees, has allowed a more practical study of reproductive (flowers and fruit) effects on C fluxes (Hansen, 1970, 1971; Lakso and Corelli-Grappadelli,

1992; Corelli-Grappadelli et al., 1994; Corelli-Grappadelli et al., 1996; Davis and Sparks, 1974; Génard et al., 1998; Johnson and Lakso, 1986a,b; Bepete and Lakso, 1998).

Recently, labeling with non-radioactive ^{13}C, supplied as $^{13}CO_2$, has provided a useful and environmentally friendly tool to monitor respiration and carbon fluxes in enriched sour cherry, peach, japanese pear, kiwifruit, walnut (*Juglans regia* L.) and persimmon (*Diospyros kaki* Linn. Ebenaceae) trees (Lombardini et al., 2001; Moing and Gaudillere, 1992; Teng et al., 1998; Teng et al., 1999; Teng et al., 2001; Amano et al., 1998; Maillard et al., 1994; Nakano et al., 1998).

In nature, there are two stable isotopes of carbon, ^{12}C and ^{13}C (Griffiths, 1993; Brugnoli and Farquhar, 2000). ^{12}C is the lighter and most abundant isotope, with ~98.89% of the global carbon pool, while ^{13}C is the heavier isotope in a proportion of ~1.11% (Griffiths, 1993). During photosynthetic CO_2 fixation, fractionation of stable carbon isotopes occurs, and as consequence plants are depleted in the heavier isotope ^{13}C (Brugnoli and Farquhar, 2000). In C_3 plants, fractionation occurs during diffusion of gaseous CO_2, through the boundary layer and stomata to the intercellular space. Additional fractionation steps occur during the liquid phase at the sites of carboxylation and during enzymatic reactions associated with carboxylation by ribulose-1,5-biphosphate carboxylase-oxygenase (Rubisco), dark respiration and photorespiration (Brugnoli and Farquhar, 2000).

The isotopic composition of plant inorganic material is measured by isotope ratio mass spectrometers (Griffiths, 1993). Plant tissues are converted to CO_2 by combustion and mass spectrometry analysis gives the abundance ratio R, which is defined as $R=^{13}CO_2/^{12}CO_2$. Results are traditionally expressed as $\delta^{13}C$, which is defined as $\delta^{13}C=Rp/(Rs-1)$; where Rp is the isotope ratio in plant samples and Rs is the ratio of the internationally accepted standard, Cretaceous belemnite from the Pee Dee formation in South Carolina (PDB=0.01124). ^{13}C enrichment for different plant tissues has been calculated as follows (Boutton, 1991; Vivin et al., 1996):

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

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

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

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

$$\text{New } {}^{13}C \text{ content} = (\text{Atom\% excess}/100) \times \text{Dry Matter} \times [C] \qquad \text{Eq (5)}$$

where the $\delta^{13}C$ (‰) value is calculated from the measured C isotope ratios of the sample and standard gases (Eq.1). The absolute ratio (R) of a sample is defined by Eq. 2, where $R_{PDB} = 0.0112372$. 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 according to dry mass and C concentrations (Eq. 5).

Source-Sink Relationships

Sink strength

A plant can be considered as a collection of individual sinks (reproductive and vegetative), which compete with each other (Wright, 1989; Flore and Layne, 1999). Carbon moves between sources and sinks as a function of source supply, sink demand and distance between sources and sinks (DeJong and Grossman, 1995). Sink organs are net importers of assimilates (Ho, 1988). Meristem tissues, such as developing leaves or root tips, are considered 'utilization sinks" since most of the C is used for growth and respiration. Storage organs, such as fruit, stems or roots, are considered 'storage sinks' because a substantial amount of C is stored in different forms and the storage process may be the controlling step for C imports (Ho, 1988). Sinks change their competitive ability with growth, leading to the diversion of CH_2O towards stronger sinks (Ho, 1988; Flore and Layne, 1999). The 'sink strength' of a sink organ has been defined as the ability to import assimilates and it often is measured as the product of sink size and sink activity (Ho, 1988; Zamski, 1996; Hansen, 1989). Some authors propose that the sink strength is the driving force for C transport and dry matter partitioning among sinks is regulated by the sinks themselves (Gifford and Evans, 1981; Hansen, 1989; Marcelis, 1996). However, others suggest that the term sink

strength is misleading since the distribution of assimilates is organized and coordinated at different levels by the entire source-pathway-sink plant system and is not a property of sinks alone (Minchin and Thorpe, 1993; Thornley, 1993; Stitt, 1993; Farrar, 1993; Farrar, 1996; Minchin et al., 1997). Some considerations to study sink strength in fruit trees include: (1) the distance between source leaves and active sinks since certain leaves supply CH_2O for particular sinks; (2) the sink strengths for reproductive and vegetative parts of the plant differ spatially and temporally throughout the season; (3) the direction of CH_2O translocation is dependent on phyllotaxy; and (4) the priority of fruit over vegetative growth during CH_2O distribution (Kappes, 1985; Flore and Layne, 1999). A hierarchy of sink strength in trees has been proposed by Kramer and Kowslozki (1979): fruits>young leaves and stem tips>mature leaves>cambia>roots>storage tissue. Recently, Whiting and Lang (2004) proposed a hierarchy of developmental sensitivity to low LA/F ratio for aerial organs of dwarfing sweet cherry trees ('Bing'/GI5): trunk>fruit soluble solids (stage III)>fruit growth (stage III)>LA/spur>shoot elongation>fruit growth (stages I and II)>LA/shoot.

Sink and source limitation

The C available to support maintenance and growth of sink organs depends on photoassimilates supplied by different leaf populations and storage reserves (Farrar and Williams, 1991; Grossman and DeJong, 1995; Flore and Layne, 1999; Basile et al., 2002). However, the allocation of assimilates is different

from one sink organ to another and the priority of C partitioning changes with the developmental stage (Ho, 1988). As indicated above, an order of priority exists, with developing fruits and seeds being the strongest sinks (Wright, 1989). DeJong (1999) indicates that organ growth is a consequence of the genetic potential for growth (which interacts with environmental conditions), the CH_2O availability and the inter-organ competition for resources. The growth of reproductive and vegetative sinks may be restricted by C availability, which is considered a 'source limitation', or by the inherent ability of the organ to utilize assimilates, which is a 'sink limitation' (Patrick, 1988; Basile et al., 2002). Growth and yield will be optimized when both the C source and sink activities increase simultaneously (Gifford et al., 1984). DeJong and Grossman (1995) suggest that source limitation results from insufficient C availability and/or the inability of the translocation system ('transport limitation') to deliver C to sinks. The last situation may be the result of long distance transport, high translocation resistance or competition from other sinks ('competition limitation').

Source limitations during early fruit growth may decrease cell division, while limitations during late fruit development may reduce cell enlargement. Partitioning studies in peach, plum (*Prunus salicina* L.) and blueberry indicate that stages I (mainly fruit cell division) and III (mainly fruit cell elongation) of fruit development are periods of source limitation, while stage II (during endocarp lignification) is considered as a period of sink limitation (Pavel and DeJong, 1993; DeJong and Grossman, 1995; Basile et al., 2002; Swain and Darnell,

2002; Berman and DeJong, 2003). In sweet cherry, reproductive and vegetative growth occurs simultaneously during fruit development (Roper et al., 1987). This situation might generate competition between actively growing aerial sinks, i.e., fruits and extension shoots, for the available C provided by different leaf populations and storage reserves. Little information about periods of sink or source limitation during fruit development is available for sweet cherry, particularly in scion/rootstock combinations using dwarfing GI rootstocks. Source limitation affecting fruit size and vegetative growth may occur in dwarfing and semi dwarfing trees due to their lower LA/F ratios and higher harvest index. Too much fruit depresses the productivity of the whole tree since as crop load increases the fraction of dry matter partitioned to other organs decreases (Lakso et al., 1999).

Sink and source manipulation

Interactions between sink organs have several effects on trees: (1) reduction of vegetative growth by developing fruit, (2) reduction of fruit growth by developing vegetative sinks, and (3) competition between individual fruit (Wright, 1989). In several species, sink-source ratios have been manipulated experimentally by increasing or decreasing sink strength (i.e., the demand for C) or source strength (i.e., the availability of C). Reductions in sink strength by reducing crop loads (i.e., increasing LA/F ratios) have been shown to increase the C supply to other fruit and/or vegetative growth due to a reduction in sink

competition (Gucci and Flore, 1989; Grossman and DeJong, 1995; Maage, 1994). In peach and plum, fruit removal changed C distribution, which in turn increased fruit size due to a reduction in source limitation. Trees with higher fruit number had a stronger sink demand and showed limitations in C supply (Pavel and DeJong, 1993; DeJong and Grossman, 1995; Basile et al., 2002; Marsal et al., 2003). On the other hand, fruit removal in apple, peach, and blueberry increased vegetative growth indicating a source limitation to vegetative development of leaves, wood and roots (Maggs, 1963; Swain and Darnell, 2002; Grossman and DeJong, 1995; Berman and DeJong, 2003; Forshey and Elfving, 1989).

Reductions of source strength, to reduce C availability for fruit and vegetative growth, have been studied by using girdling (i.e., interruption of phloem translocation), partial defoliation and shading of vegetative and reproductive sections. Results varied depending on the timing at which source manipulation was carried out. In peach and nectarine, trunk and branch girdling induced CH_2O accumulation above the girdling (Jordan and Habib, 1996) and increased fruit size and sugar content (Allan et al., 1993), although shoot growth was decreased (Di Vaio et al., 2001). In the same species, shading reduced C export from lateral shoots to fruit (Corelli-Grappadelli et al., 1996). In apple, experiments using shading demonstrated that shoot growth was a priority over fruit growth for C partitioning since export to fruit from shoots was reduced (Corelli-Grappadelli et al., 1994; Bepete and Lakso, 1998). In raspberry (*Rubus*

idaeus L.), girdling and leaf removal resulted in lower dry weights of reproductive components (Privé et al., 1994). Similarly, in cranberry and kiwifruit, partial defoliation (i.e., removal of the new and older leaves) and girdling reduced fruit weight and number (Roper and Klueh, 1994; Buwalda and Smith, 1990; Piller et al., 1998). In japanese pear, girdling and defoliation of different age spurs indicated that fruit on young spurs import CH_2O from older spurs, while fruit on older spurs depend on their own leaves (Teng et al., 1998). Girdling of grape canes at veraison stimulated shoot growth and increased leaf area at the expense of fruit production (Novello et al., 1999). Finally, girdling of sweet cherry spurs to isolate fruit from the major sources of photoassimilates showed the deleterious effects on fruit quality; fruiting spur leaves were not the only C source to support fruit growth, and import of assimilates synthesized by leaves distal to the bearing spurs was required for optimal fruit development (Roper et al., 1987).

Clearly, manipulation of sink and source relationships constitutes a practical approach to obtain more information about the contribution of various leaf populations and storage reserves in fruit and vegetative growth during the growing season. An optimal LA/F ratio is a key factor to assure an adequate balance between fruit quality and vegetative growth, as indicated for sour cherry (> 2 leaves/fruit)(Layne and Flore, 1993), plum (6 to 10 leaves/fruit) (Maage, 1994), and peach (120-220 cm^2 LA/F) (M. Génard, personal communication) (Famiani et al., 2000), and sweet cherry (200 to 300 cm^2 LA/fruit) (Whiting, 2001).

In dwarfing sweet cherry trees, low LA/F ratios had a negative effect on fruit quality (Roper et al., 1987; Whiting, 2001) and vegetative growth; however crop load reductions improved fruit characteristics (Whiting, 2001).

Fruit as sink and shoot as sink and source

a. Fruit as a sink

In *Prunus* sp., fruit development follows a double sigmoidal pattern, which has been divided into three stages (Tukey and Young, 1939; Labreque et al., 1985; Flore, 1994; Costes et al., 1995; Berman and DeJong, 1996). Following pollination and fruit set, stage I is characterized by active cell division and rapid initial growth. Stage II or 'pit hardening' is associated with endocarp lignification, slower growth of the pericarp and rapid embryo development. Stage III or 'final swell' is a period of rapid fruit growth characterized by mesocarp cell enlargement and dry matter accumulation. Although a major period of cell division occurs early during fruit development, and cell enlargement is important during 'final swell', cell division and cell expansion are not exclusive during these stages (Tukey and Young, 1939; Scorza et al., 1991). Final fruit size depends on cell number and size. Although there is not detailed histological information for sweet cherry fruit, in sour cherry fruit, cells of the mesocarp increase in number during the pre-bloom stage and stage I, which is the period of maximum division (Tukey and Young, 1939). In addition, 50 to 80% of cherry fruit growth occurs during this stage and at maturity the largest

cells increase 25 times in diameter compared to their size in stage I (Tukey and Young 1939; Flore, 1994). In peach, differences between small and large- fruited cultivars are apparent in the ovary as early as 175 days pre-bloom (Scorza et al., 1991). The competitive ability of stone fruit and their CH_2O demand change through these three phases of sink activity (Basile et al., 2002; DeJong, 1999). In plum, stage I often is source-limited, while in peach stages I and III of fruit growth are source-limited as a result of competition from other fruit and vegetative sinks (Grossman and DeJong, 1995; Basile et al., 2002). On the other hand, stage II is usually sink-limited due to genetic factors (Berman and DeJong, 2003).

b. Extension shoots as sink and source

In several fruit tree species, vegetative development of extension shoots competes with, and seems to have a priority for CH_2O over, reproductive development early in the season during fruit cell division (Corelli-Grappadelli et al., 1994; Bepete and Lakso, 1998). However, later in the season, shoots develop enough leaf area and have the potential to support not only their own growth but also other sinks such as fruit (Johnson and Lakso, 1986a,b; Roper et al., 1987; Corelli-Grappadelli et al., 1994). Most of the information about the role of extension shoots in the C balance of whole trees or limbs has been reported in apple. Several studies indicate that an apple shoot becomes self-supporting after 5 or 6 leaves develop, with ~20% of the CH_2O used in shoot growth coming from

storage reserves (Hansen, 1967a,b; 1971). Export of current photosynthates from extension shoots began ~21 days after full bloom (DAFB) with ~10 to 16 unfolded leaves, and it increased considerably ~35 DAFB, when shoots had ~17 unfolded leaves (Johnson and Lakso, 1986a,b; Lakso and Corelli-Grappadelli, 1992; Corelli-Grappadelli et al., 1994). The upper 8 and 9 leaves exported C to the shoot tip, while mid leaves exported bidirectionally and basal leaves exported basipetally (Quinlan, 1965). In this species, short shoots, with more mature leaf area, exported more total CH_2O than long shoots during the early period of growth (Johnson and Lakso, 1986a,b). Short shoots contribute CH_2O during early fruit growth because of the reduced C investment in the supporting axis (Lauri and Kelner, 2001). It has been hypothesized that in apricot shoots, cambial growth occurring after leaf expansion is probably responsible for early shoot growth cessation leading to spur formation; crop loads seem not to affect this process (Costes et al., 2000). In sour cherry, extension shoots became net CH_2O exporters at 27% expansion, which was ~17 days after leaf emergence (Kappes and Flore, 1989; Flore and Layne, 1999). In peach, extension shoots were stronger sinks ~15 DAFB, but began exporting C to fruit ~28 DAFB (Corelli-Grappadelli et al., 1996). These authors suggested that over time, a shift in priority occurs between vegetative sinks that is related to shoot maturation. In the same species, initial shoot size has been suggested as an important determinant of final shoot growth, since as for fruit, a larger initial shoot contains more dividing cells. Longer shoots with higher leaf areas (i.e., bigger source size) have a higher

potential as source of C for fruit growth (Génard et al., 1998). Currently, there is not enough information about the impact of extension shoots on fruit and vegetative growth in less vigorous sweet cherry trees. It might be interesting to determine the timing for the shifting of extension shoots from sink status to source status for other sinks such as fruit and secondary growth.

Carbon translocation patterns: orthostichy and distance

Assimilate translocation to sink organs can be acropetal or basipetal from the source. Unidirectional and bidirectional transport from different leaf populations to different sinks have been reported for apple, sour cherry, peach, pecan, grape, cranberry and red raspberry (*Rubus idaeus* L.) among others (Quinlan, 1965; Hansen, 1969; Corelli Grapadelli et al., 1994; Kappes and Flore, 1989; Toldam-Andersen, 1998; Corelli-Grappadelli et al., 1996; Davis and Sparks, 1974; Hale and Weaver, 1962; Roper and Klueh, 1994; Privé et al., 1994). The transport of assimilates is suggested to follow a rule similar to the Münch hypothesis (Daudet et al., 2002). This mechanism assumes a viscous flow of phloem sap in response to the hydraulic pressure (turgor) gradient which is due to both the concentration in the source and the concentration gradient between regions of phloem loading (sources) and regions of phloem unloading (sinks) across transport-resistance pathways (Thornley and Johnson, 1990; Daudet et al., 2002).

Several studies using radioactive C indicate that the leaf orthostichy (i.e., 'phyllotaxy') and vascular connections between source leaves and sink organs are two factors responsible for the patterns of assimilate distribution (Ho, 1988). That is, certain leaves feed particular sinks (Flore and Lakso, 1989). In sour cherry, a 2/5 phyllotaxy influenced the direction and the onset of CH_2O export from shoot leaves. Leaves with angular distances of 144° had separate translocation paths, while leaves with distances < 72° shared some of their translocation paths (Kappes and Flore, 1986; 1989). In peach indicate that in peach, a 2/5 phyllotaxy might influence C translocation from different leaf sources (Corelli-Grappadelli et al., 1996). In apple, clear effects of phyllotaxy on C distribution and partitioning to fruit have been documented (Corelli-Grappadelli et al., 1994; Hansen, 1969). In grape, translocation patterns between leaves and fruit clusters also have been attributed to vascular connections (Hale and Weaver, 1962). Similarly in raspberry, C translocation was related to leaf phyllotaxy 75% of the time (Privé et al., 1994).

Location of sink and sources and temporal separation of growth activities seem to influence transport patterns and assimilate partitioning (Bruchou and Génard, 1999; DeJong, 1999). In several species, assimilate partitioning to fruit depends on their position relative to the leaves rather than their distance from the source (Bruchou and Génard, 1999).The importance of sink proximity to source leaves has been demonstrated in peach and kiwifruit by using girdling, partial defoliation and LA/F adjustment (Ben Mimoun et al., 1995; Buwalda and

Smith, 1990; Bruchou and Génard, 1999). In addition to positional effects on translocation, temporal separation of sink activities has been proposed. As example, in peach rapid leaf and shoot expansion occurs early in the growing season and rapid fruit enlargement during stage III occurs later in the season (DeJong, 1999).

Photosynthesis and Sink Activity

Photosynthesis is a fundamental process for plant productivity. During photosynthesis, CO_2 is converted to CH_2Os, which are transported within the tree for fruit and vegetative growth. Flore (1994) indicated that the photosynthetic potential of a fruit crop is controlled by the environment and by the sink demand of various organs. The environment influences: (a) physical and biochemical reactions, (b) leaf morphology, and (c) manufacture of the photosynthetic machinery. On the other hand, sink demand might control photosynthetic rate through a feedback signal from the sink itself.

The presence of fruit and/or increased vegetative sink strength has been associated with an increase in photosynthetic rate (A) in several fruit crops (Flore and Lakso, 1989). Reekie and Bazzaz (1987a,b) refer to this increase as 'reproductive photosynthesis'. Plants with low source : sink ratios (i.e., limiting leaf number) increase A more than plants with high source:sink ratio (i.e., limiting fruit number) (Farrar and Williams, 1991). Traditionally, the effect of crop load on A has been studied by comparing fruiting and non-fruiting plants.

In several species, the presence of fruit has been shown to have a positive effect on A. However, there are cases in which fruit had little or no effect on A. Increases in A during the period of fruit development have been reported for peach, plum, apple and sweet cherry (Fujii and Kennedy, 1985; DeJong, 1986; Gucci et al., 1991a,b; Ben Mimoun et al., 1996; Gucci et al., 1994; Wünsche and Palmer, 1997; Palmer et al., 1997). Partial defoliation has been shown to affect A similarly. In sour cherry, leaf removal resulted in A enhancement due to photosynthetic compensation (Layne, 1992). In general, the detection of a fruit sink effect on A requires a source-limiting condition, such as low LA/F ratios or severe defoliation. Source limitation to A occurs when the capacity of the reaction involved in photosynthate supply is not optimal for sink demands, while a sink limitation occurs when the rate of use of photosynthates is less than the rate of photosynthesis (Layne and Flore, 1993). Studies that have not found the fruit sink effect on A include reports on sweet cherry, sour cherry and apple (Sams and Flore, 1983; Roper et al., 1988; Giuliani et al., 1997; Flore and Layne, 1999; Whiting, 2001).

The mechanism by which fruits regulate A are unclear. High crop loads might affect A due to an increase in sink strength (Giuliani et al., 1997). On the other hand, the lack of a relationship between crop load and A , in some cases, has been associated with the presence of alternative sinks such as strong shoot growth (Gucci et al., 1991b; Giuliani et al., 1997; Palmer et al., 1997). The decline in A following fruit removal has been attributed to stomatal and non-stomatal

limitations (DeJong, 1986; Gucci et al., 1991a,b) and/or end-product inhibition. The presence of fruit increases stomatal conductance and accelerates physical and biochemical processes in leaves (Forshey and Elfving, 1989). In addition, the excessive accumulation of TNC, particularly starch, due to lack of a sink strength or excessive CH_2O supply might regulate A via end-product inhibition in leaves (Herold, 1980; Flore and Lakso, 1989; Gucci et al., 1991a; Wünsche and Palmer, 1997). Plant hormones and low orthophosphate (Pi) concentrations in the cytosol and stroma of the chloroplast also have been proposed to influence A in source leaves (Herold, 1980).

Fruits are able to photosynthesize during early stages of development and it has been suggested that the C fixed directly by fruit can impact in the C budget of individual fruits (Hansen 1970; DeJong and Walton, 1989; Kappes, 1985). In sour cherry, fruit gross photosynthesis contributed ~19%, 30% and 1.5% of the CH_2O used during stages I, II and III of fruit development, respectively; ~70% of the fixed C was incorporated into fruit dry matter, while the rest was used in dark respiration (Flore and Layne, 1999). In apple, fruit photosynthesis was < 15% of the total C supply during the season (Jones, 1981), although it may contribute to fruit growth early in the season (Lakso et al., 1999).

Respiration and Sink Activity

DeJong and Grossman (1994) indicate that the two major components of CH_2O demand in trees are growth and respiration. Two major components of

plant respiration have been described: (1) growth 'construction' respiration, which is defined as the CO_2 evolution directly related to the production of new cellular materials and (2) maintenance respiration, which supplies energy for subsistence of existing tissue (Amthor, 1984; Amthor, 1989). Maintenance respiration is assumed to have priority over vegetative and reproductive growth (Marcelis et al., 1998; Lescourret et al., 1998). C is partitioned first for maintenance of existing biomass, and the remaining C is partitioned for growth of various organs according to their respective sink strength, which depend on their relative growth rates (Buwalda, 1991). Respiration costs vary with growth rate, temperature (Q_{10} of ~1.5 to 2) and plant size or biomass (Ho, 1988; Amthor, 1984; DeJong and Grossman, 1994; Flore and Layne, 1999; DeJong, 1999). There is no information regarding respiratory costs in sweet cherry; however, respiratory demands of peach and apple trees have been documented. In peach, growth simulations indicated that daily maintenance respiration increased during the season due to increases in biomass and temperature. Of the total fixed CO_2, ~33% was utilized in maintenance respiration, while ~66% was used for growth and growth respiration (Grossman and DeJong, 1994). Fruit respiration accounted for ~16 to 20% of the total fruit CH_2O requirements, while the rest was fixed as biomass. The highest specific respiration rates in fruit were detected during early development (DeJong and Walton, 1989). In general, total CH_2O cost (dry matter plus growth respiration) of fruit growth was ~35% greater than total respiratory costs of leaf, stem and trunk growth (Grossman and DeJong, 1994). In apple,

dark respiration costs ranged between 27 to 30% of the fixed CO_2 for a full year (Lakso et al., 1999). The highest specific respiration rates occurred during spring when new leaves, shoots, fruit, stems and roots are growing most actively. Later in the season, maintenance respiration of leaves and the main perennial structures of the tree are low. In the case of fruit, respiration rates were high during cell division (~1 month) but declined during cell expansion (Lakso et al., 1999). Jones (1981) estimated that ~15% of the C imported by fruit was used in respiration, while the other 85% was accumulated as dry matter in the fruit.

Clearly, the respiratory activity of fruit trees is both qualitatively and quantitatively important in the C balance equation (Lakso, 1994). However, more information about whole-plant respiration is required for many woody species, including sweet cherry.

Rationale and objectives

Partitioning studies in sweet cherry trees on traditional vigorous rootstocks have provided insight for orchard management decisions regarding appropriate pruning, crop load regulation and other practices. However, with the move toward high-density orchards by U.S sweet cherry growers, additional research is required to understand the role of fruit sink strength and CH_2O partitioning when trees are grown on dwarfing and semi-dwarfing rootstocks such as the GI series. GI rootstocks are interspecific hybrids that have the potential to promote precocious reproductive bud formation, high yield

efficiency and reduced vegetative vigor. So far, the implementation of standard sweet cherry management practices for trees on GI rootstocks has resulted in high yields but small fruit, which is a critical problem since top quality fruit provides the best returns to growers.

Little is known about the relative importance or temporal relationships of different sweet cherry leaf populations within the canopy as sources of C for fruit and shoot development in dwarfing trees. Moreover, the partitioning of C and the effect on sink strength of fruit and shoots during fruit development has not been characterized in detail. Previous data and increasing grower experience indicates that reproductive and vegetative growth often become unbalanced after the 4th year of production on dwarfing rootstocks if the natural canopy leaf-area-to-fruit (LA:F) ratios are not altered in some way. Thus, manipulation of the reproductive and vegetative sinks may be a tool to regulate sink strength and competition among sinks during periods of resource limitation, particularly during fruit development. Adjustments in LA/F ratios through practices such as pruning to remove or stimulate leaf area or fruit and flower thinning and/or spur extinction might help to overcome the problem of overcropping and small fruit size. In this study, dwarfing and semi-dwarfing trees on GI rootstocks were used to investigate partitioning during fruit development. Results of this research provide a physiological foundation for canopy relationships that may help to develop specific orchard management strategies to promote a more

sustainable balance between vegetative and reproductive growth in high density sweet cherry orchards.

The main objectives of this study were to:

1. define the temporal importance of various leaf populations as sources of C for fruit and shoot growth during the whole period of fruit development.
2. determine the effect of reproductive and vegetative sink strengths on C partitioning during fruit development.
3. determine the importance of storage reserves as a source of C for initial fruit growth.
4. define the transition phase during which the dependence of new growth on storage reserves shifts to current photosynthate assimilation as the primary source for subsequent vegetative and reproductive development.

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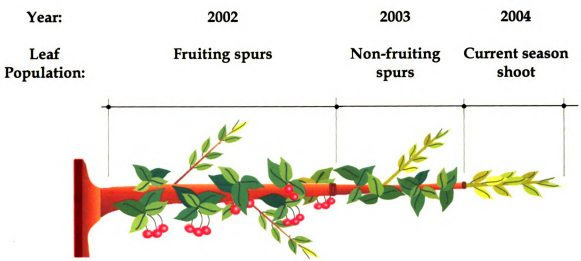

Figure 1. Leaf populations on a typical 2-year-old sweet cherry branch.

Images in this dissertation are presented in color.

CHAPTER II

EXAMINING THE INFLUENCE OF DIFFERENT LEAF POPULATIONS ON SWEET CHERRY FRUIT QUALITY

Examining the Influence of Different Leaf Populations on Sweet Cherry Fruit Quality

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Keywords: *Prunus avium* L., fruit development, shoot, carbohydrates, defoliation, girdling, source, sink, Gisela rootstock.

Abstract

Understanding sweet cherry (*Prunus avium* L.) carbohydrate (CH_2O) partitioning and source-sink relationships might lead to better management strategies for sweet cherry fruit quality on high-yielding, vigor-reducing rootstocks. Six limb treatments were established on fruiting branches of `Hedelfinger'/Gisela 5 and `Ulster'/Gisela 6 to isolate or combine two of the main leaf populations that serve as CH_2O sources for developing fruit: the leaves on the branch segment of newly-formed spurs (previous season growth having non-fruiting spurs and some new lateral shoots) and the leaves on the branch segment of previously-formed spurs (two-year-old growth having fruiting spurs, plus some previous season and new lateral shoots). At harvest, fruit weight, diameter, and soluble solids (SS) were evaluated for each treatment. For both scion/rootstock combinations, fruits from the branch treatment isolated from the rest of the tree by girdling and having a full complement of leaf populations

were larger and had higher SS than the partial leaf population treatments or the branch having a full set of leaf populations but not isolated by girdling. Fruits supplied exclusively by the leaf populations on either the fruiting spur branch segment or the non-fruiting spur branch segment were significantly smaller and had decreased SS levels. It was evident that the leaf populations most closely associated with the fruiting spur branch segment were insufficient sources of CH_2O for optimal fruit development. However, these populations were clearly important, as similar sub-optimal results were also found when only the leaf populations on the non-fruiting spur branch segment were present. Leaf populations on both fruiting and non-fruiting branch segments were required for full fruit development and there was not a sufficient compensatory effect when one of the main leaf populations was eliminated.

Introduction

Sweet cherry (*Prunus avium* L.) fruit quality, primarily size and sweetness, is highly dependent on CH_2O availability and partitioning, which in turn are dependent on the number and strength of competing sinks. Within a sweet cherry branch, the major sinks that might be considered to be competitors of developing fruit include vegetative growth (current season growth of shoots), other fruits and developing spur leaves.

Sweet cherry fruit size is dependent on cell division and enlargement. Fruit cell division occurs before anthesis and continues through the initial phase of stage I (Flore, 1994; Flore and Layne, 1999). During this period fruit constitute important sinks attracting assimilates (Ho, 1988). Later in fruit development, fruit sink strength changes to reach a maximum during stage III (or 'final swell'), when cells are actively elongating (Flore, 1994; Flore and Layne, 1999). In sour cherry (*Prunus cerasus* L.), early stage fruits act as sinks by removing photoassimilates from the translocation system. In this species, dry matter partitioning is dependent on the fruit growth stage, with the higher accumulation of carbon (C) in the fruits during final swell (Toldam-Andersen, 1998).

Loescher et al. (1986) suggested that in sweet cherry, spur (fully expanded 3 weeks after bloom) and current season shoot leaves were the primary source of CH_2O for fruit growth. Similarly, Roper and Loescher (1987) found a positive correlation between fruit quality and leaf area per fruit. These authors reported

that spur leaves alone were not able to support fruit growth during stage III and CH_2O import from other sources (i.e. non-fruiting spurs and current season growth leaves) was required. In sour cherry, shoots became net exporters of photosynthates 15 days after budbreak (DABB), while apple shoots began net export 20 to 25 DABB (Kappes and Flore, 1986; Johnson and Lakso, 1986). Thus, current season growth has the potential to provide at least some photoassimilates for fruit growth in these two species.

In sweet cherry, vegetative and reproductive growth occur simultaneously, and this can result in a strong intra-plant competition for available assimilates (Roper et al., 1987). After the previous season CH_2O reserves have been depleted in early spring, thus the sweet cherry canopy must produce current photoassimilates for the rest of the seasonal growth and a new pool of storage reserves for initial growth the next spring. Healthy and abundant leaf populations, producing a continuous supply of photosynthates throughout the season, are essential for growth and storage. Both girdling (eliminating transport via phloem tissue and importation of stored CH_2O), or defoliation (eliminating photosynthetic tissue and the availability of current photoassimilates), change source-sink relationships and the utilization of CH_2O for growth. Girdling effects have been reported for several stone fruits including sweet cherry, peach (*Prunus persica* L.) and nectarine (Roper et al., 1987; Allan et al., 1993; Jordan and Habib, 1996). In addition, the physiological responses to selective or partial defoliation have also been studied in species such as sour

cherry, cranberry (*Vaccinium macrocarpon* Ait) and pecan (*Carya illinoinensis* (Wangenh.) K. Koch.) (Layne and Flore, 1992; Layne and Flore, 1993; Roper and Klueh, 1994; Worley, 1979).

The understanding of CH_2O partitioning and sink-source relationships during fruit growth might lead to better management strategies to improve sweet cherry fruit quality on high-yielding, vigor-reducing Gisela rootstocks. Scion/rootstock combinations using Gisela (GI) stocks tend to overcrop early in the orchard life and, consequently, leaf area to fruit (LA/F) ratios become unbalanced, resulting in smaller fruits (Andersen et al., 1999). The objective of this experiment was to study the role of different leaf populations on fruit growth and development in combinations using GI stocks. We hypothesized that leaf populations on current season growth, previous season growth, and 2-year-old wood are all important to support optimal fruit growth and development. To test this hypothesis, we manipulated CH_2O availability by girdling and defoliating a 2-year-old sweet cherry branch during Stage I of fruit development. In this way, we created an artificial redistribution of non-structural CH_2O to sink organs (fruit and shoots). This was an initial approach to study the dynamics of partitioning in a sweet cherry fruiting branch on a dwarfing scion/rootstock combination.

Materials and Methods

In May 2001, at the Clarksville Horticultural Experiment Station (CHES) of Michigan State University, an experiment on 2-year-old (first shoot growth occurred in 1999) fruiting branches of 'Hedelfinger'/Gisela 5 (GI5) and 'Ulster'/Gisela 6 (GI6) sweet cherry trees was established. Six limb treatments (T) were created by using girdling and defoliation to isolate the various leaf populations that serve as CH_2O sources for developing fruits. In this way, CH_2O that should have been translocated to sink organs, such as fruits and current season growth, were restricted artificially. Limbs were either girdled and/or defoliated at different sections of the branch, depending on the treatment (See Figure 1). The treatments included:

- **C** - Untreated limb (control).
- **T1** - Branch girdled at its base, i.e., isolation from the rest of the tree.
- **T2** - Branch girdled at both sides of the wood bearing newly fruiting spurs (growth that occurred in 1999), i.e., the CH_2O source for developing fruit was limited to only the leaves of fruiting spurs and any nearby lateral shoots.
- **T3** - Branch girdled at its base and at the junction of the previous season (2000) growth and the current season (2001) growth, i.e., CH_2O source for developing fruit included both the fruiting and non-fruiting spur leaves, plus any lateral shoot leaves on these two segments of growth.

- **T4 -** Branch girdled as in T3 plus removal of all leaves associated with the branch segment containing fruiting spurs, i.e., CH_2O source was limited to leaves of non-fruiting spurs (growth that occurred in 2000) and any nearby lateral shoots.
- **T5 -** Branch girdled as in T3 plus removal of all leaves associated with the branch segment containing the non-fruiting spurs, i.e., CH_2O source was limited to leaves of fruiting spurs (growth that occurred in 1999) and any nearby lateral shoots.

Before imposing the treatments, we identified a population of 200 branches having similar vigor, crop load, length and diameter, and from these, 10 branches per treatment were selected randomly. A total of 60 branches per scion/rootstock combination were used. Initial morphological measurements for each combination included: diameter/branch, length/branch, initial fruit number/branch, fruiting and non-fruiting spur number/branch, lateral shoots/branch. Average leaf area (LA) cm^2/leaf population and average LA cm^2/branch were measured late in fruit development (Table 1). Girdling (1 cm in width at the base of the limb) and defoliation were carried out 12 days after full bloom (DAFB) early in Stage I of fruit development. Tissue removed at the girdled section included periderm, phloem and cambium. Later in fruit development (stage III), the re-growth on the girdled area was further removed to avoid phloem translocation. In the case of T4 and T5, we eliminated ~35% of

the total leaf area/branch by removing all leaves (spur and lateral shoot) from the branch segment that contained either fruiting or non-fruiting spurs, depending on the treatment.

Weight, diameter, soluble solids (SS), color and final fruit number were recorded for each branch after commercial harvest. Harvest was 65 DAFB (27 Jun) for 'Ulster'/GI6 and 67 DAFB (5 Jul) for 'Hedelfinger/GI5 .

Statistical Analysis

The data were analyzed using proc mixed SAS 8e for Windows (SAS Institute, Cary, N.C.).

Results

There were significant morphological differences between 'Ulster'/GI6 and 'Hedelfinger'/GI5. 'Ulster'/GI6 had more spurs per branch and higher total LA/branch than 'Hedelfinger'/GI5 (Table 1). In addition, initial LA/F ratios of 'Ulster'/GI6 were higher than those of 'Hedelfinger'/GI5. Although the length of branches was similar, 'Ulster'/GI6 had more fruiting and non-fruiting fruit spurs compared with those in 'Hedelfinger'/GI5. The number of lateral shoots per branch also differed significantly between combinations, being greater in 'Hedelfinger'/GI5. The fruiting and non-fruiting spur leaf populations contributed the greatest component LA to overall branch LA, with 72 and 63%, for Ulster'/GI6 and 'Hedelfinger'/GI5, respectively.

There were significant differences among treatments in both 'Ulster'/GI6 and 'Hedelfinger'/GI5 for fruit diameter, weight and SS of fruits at harvest. Differences in final fruit number occurred only in 'Hedelfinger'/GI5; however, there was high variability within treatments for this parameter. For both scion/rootstock combinations, fruits from branches that were isolated from the rest of the tree (T1) were larger and had higher SS than the other treatments, including the controls (C). Conversely, fruits for which the CH_2O source was limited to only those leaves on the branch segment having fruiting spurs (T2) or non-fruiting spurs (T4), were significantly smaller and had decreased SS levels compared to C and T1 (Table 2). 'Ulster'/GI6 fruits for which the CH_2O source was limited to only the leaves on the fruiting spur branch segment (T5) or to both the fruiting and non-fruiting spur branch segment (T3) had a reduced diameter, weight and SS, as compared to the intact control branch (C) or the otherwise intact branch that was isolated from the rest of the tree (T1). However, the same trend was not observed in 'Hedelfinger'/GI5, for which T3 (fruiting and non-fruiting spur and lateral shoot leaves) and T5 (only leaves on the fruiting spur branch segment) did not affect fruit quality as much as T2 and T4 did. Fruit color was affected positively in both rootstock/scion combinations when the entire branch was isolated by girdling (T1).

There were no significant differences in relative current season growth for either scion/rootstock combination (See Figures 2 and 3). However, current season growth on 'Ulster'/GI6 increased when girdled (T3-T5; see Figure 2),

while current season growth on 'Hedelfinger'/GI5 decreased when girdled (C and T1-T2; see Figure 3).

Discussion

Branches girdled only at their base, i.e., isolated from the rest of the tree, had fruit with greater diameter, weight, SS content and color compared to the rest of the treatments, including the untreated branches for which potential CH_2O to support developing fruit included multiple tissues throughout the tree. This suggests that the isolation of the branch from the rest of the tree prevented CH_2O export to other sink organs of the tree, thereby benefiting local fruit growth. These agree with the positive effects of girdling on peach fruit quality have been reported (De Villiers et al., 1990; Allan et al., 1993).

Fruit quality of 'Ulster'/GI6 and 'Hedelfinger'/GI5 was affected negatively as various restrictions of CH_2O sources were imposed. Thus, when branches were either girdled at both ends of the wood bearing fruiting spurs (i.e., CH_2O sources for fruit were those leaves associated with spurs and laterals on the fruiting segment of the branch), or at the base and at the junction between the previous and current season growth, with the fruiting spur (and associated lateral) leaves then removed (i.e., CH_2O sources were those leaves on non-fruiting spurs and lateral shoots), there was a detrimental effect on the fruit size and SS levels. Several previous reports illustrate the deleterious effects of girdling and defoliation on assimilate supply to sink organs, which in turn

impact final fruit growth and quality in different species including sweet cherry, apple (*Malus* x *domestica* Borkh.), japanese pear (*Pyrus pyrifolia* Nakai), kiwifruit (*Actinidia deliciosa* var.deliciosa cv. 'Hayward') and grape (*Vitis vinifera* L.) (Roper et al., 1987; Atkinson et al., 2001; Ferree and Palmer, 1982; Teng et al., 1998; Buwalda and Smith, 1990; Harrell and Williams, 1987). Restriction in C budget induces reductions in fruit size, number, weight, SS and color. Moreover, a delay in fruit maturity time has been detected (Harrell and Williams, 1987).

When the leaf populations on fruiting branch segments were the sole supply of CH_2O and import from (or export to) the rest of the tree was prevented, good fruit quality was not achieved. Thus, leaves on the 2-year-old wood were not able to support optimal development of their own fruit. These data support the observations of Roper et al. (1987) who found that in sweet cherry the isolation of fruiting spurs from other leaf populations during stage II and III had a negative impact on fruit weight, SS and color. These authors concluded that in sweet cherry, fruiting spur leaves are not the only carbon source to support fruit growth and import of assimilates synthesized by leaves external to the bearing spurs is required. In the same species, Atkinson et al. (2001) demonstrated that the isolation of each fruiting spur from the rest of the tree by using girdling reduced fruit weight. These authors concluded that individual spurs were under a source limiting situation, which in turn influenced final fruit size but not fruit number per cluster.

Similarly, when leaves on the non-fruiting branch segment were the sole CH_2O supply, optimal fruit development was not achieved either. Therefore, leaves from the non-fruiting spurs and associated laterals could not compensate for the lack of sufficient CH_2O originating from fruiting spur and lateral and terminal LA. In apple, fruit growth is supported by CH_2O produced by extension shoots and non-fruiting and fruiting spurs. Although, the contribution of these leaf population varied depending on position of source and sink organs within the canopy and their developmental stage (Hansen, 1969; Tustin et al., 1992; Corelli-Grapadelli et al., 1994).

When the branch was girdled at its base and at the junctions of the previous and current season growth (i.e., CH_2O for developing fruits was both the fruiting and non-fruiting spur leaves, plus all laterals) in 'Ulster'/GI6, fruit size, SS and color were affected negatively. This was the opposite for 'Hedelfinger'/GI5, which had good quality fruit compared with fruits of 'Ulster'/GI6. Two possibilities might be proposed to explain this difference between scion/rootstock combinations. First, Hedelfinger'/GI5 had more lateral shoots in fruiting and non-fruiting wood (i.e. more LA to supply with CH_2O) in comparison to 'Ulster'/GI6. Second, it is likely that the isolation of the terminal current season growth from the rest of the branch had different effects on the C partitioning between fruit and terminal shoots depending on the grafted combination. In the case of 'Ulster'/GI6, terminal shoots seem to be an important source of photoassimilates for fruit, while for 'Hedelfinger'/GI5 terminal shoots

might constitute a sink competing with developing fruit. When new shoot growth was girdled at its base, 'Ulster'/GI6 shoot growth increased compared to the ungirdled branches, suggesting that it was able to support its own growth and probably export some CH_2O to the rest of the branch. The opposite effect occurred in 'Hedelfinger'/GI5, which suggests that either the lower scion vigor of 'Hedelfinger' (vs. 'Ulster'), or that imposed by the more dwarfing rootstock GI5 (vs. GI6), established a physiological condition in which terminal current season shoots were insufficient to support their optimal vegetative growth, much less to contribute significantly to fruit growth. Consequently, terminal current season growth in 'Hedelfinger'/GI5 might import current photosynthates from other sources during elongation. In comparison, sour cherry shoots became net exporters of photosynthates 15 days after budbreak (DABB), while in apple shoots began net export 20 to 25 DABB (Kappes and Flore, 1986; Johnson and Lakso, 1986). Thus, sweet cherry current season growth might have the potential to provide at least some photoassimilates for fruit growth. The temporal role of current season growth, as a CH_2O source, for sweet cherry fruit development is unknown. We presume that current season growth undergoes a transition from being a competitor sink with the fruit in early stages of development to a source of CH_2O for the fruit at late stages of fruit development.

We conclude that leaf populations of either fruiting or non-fruiting branch segments alone are insufficient sources of CH_2O for optimal fruit development in sweet cherry. Both fruiting and non-fruiting spurs and lateral LA are required to

maximize fruit growth. There is not a significant compensatory effect when one of these populations is reduced or eliminated, and the contribution of current season growth to fruit development appears to vary by rootstock vigor and/or scion variety.

The use of girdling and defoliation is the first step to elucidate the fate of current photosynthates produced by the three distinct leaf populations within a 2-year-old sweet cherry branch. We demonstrated that the lack of LA reduces the availability of CH_2O for fruit growth and, as a consequence, quality is affected negatively. Questions to be answered next include the role of storage reserves in early fruit growth, the fate of CH_2O synthesized by non-fruiting sections of a 2-year-old branch, and the importance of the current season shoot growth for fruit growth and development.

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Table 1. Morphological features of 'Ulster'/GI6 and 'Hedelfinger'/GI5 branches. Means from 60 branches per combination. Measurements were recorded prior to treatment imposition and late in fruit development (65 and 67 days after full bloom).

Parameter	'Ulster'/GI6		'Hedelfinger'/GI5	
Branch length (cm)	99.0 ± 1.2	a[z]	101.0 ± 1.2	a
Branch diameter (mm)	20.0 ± 0.3	a	17.0 ± 0.3	b
Initial fruit number	54.8 ± 3.0	b	77.9 ± 3.9	a
Total spur number	37.5 ± 0.6	a	28.5 ± 0.4	b
Fruiting spur number	15.6 ± 0.4	a	13.3 ± 0.4	b
Non-fruiting spur number	21.9 ± 0.4	a	15.2 ± 0.4	b
Lateral shoot number	7.8 ± 0.3	b	11.5 ± 0.4	a
Internode length (cm)	2.2 ± 0.1	b	3.2 ± 0.1	a
Fruiting spur leaf area (cm^2)	4,623.5 ± 120.9	a	3,272.9 ± 108.5	b
Non-fruiting spur leaf area (cm^2)	4,657.9 ± 78.6	a	3,220.9 ± 85.9	b
Lateral shoot leaf area (cm^2)	3,464.5 ± 114.4	b	3,850.6 ± 133.5	a
Total leaf area per branch (cm^2)	12,745.9 ± 181.0	a	10,344.4 ± 180.6	b
Leaf area (cm^2)/fruit ratio	202.4 ± 21.1	a	164.7 ± 33.6	b

[z] Means within a row followed by the same small letter are not significantly different at $\alpha = 0.05$.

Table 2. Diameter, weight, soluble solids (SS), color and final fruit number/branch of 'Ulster'/GI6 and `Hedelfinger'/GI5 at harvest (June and July, 2001, respectively). **C** - Untreated limb (control); **T1** - Branch girdled at its base; **T2** - Branch girdled at both sides of the wood bearing newly fruiting spurs, i.e., source leaves are those associated with the branch segment that grew in 1999; **T3** - Branch girdled at its base and at the junction of the previous season growth and the current season growth, i.e., source leaves are those associated with the branch segments that grew in both 1999 and 2000; **T4** - Branch girdled as in T3 plus removal of all spur and lateral leaves on the fruiting branch segment, i.e., source leaves are those associated with the branch segment that grew in 2000; **T5** - Branch girdled as in T3 plus removal of all spur and lateral leaves on the non-fruiting branch segment, i.e., source leaves are those associated with the branch segment that grew in 1999.

Ulster /GI6	Diameter (mm)	Weight (g/fruit)	SS (°Brix)	Final Fruit number	Color (H°)
C	21.1 ± 0.2 b	6.1 ± 0.2 c	18.4 ± 0.1 bc	47.0 ± 6.3 a	13.0 ± 0.4 b
T1	21.9 ± 0.2 a	7.1 ± 0.2 a	21.8 ± 0.1 a	38.0 ± 7.3 a	8.8 ± 0.2 d
T2	19.8 ± 0.2 c	5.4 ± 0.2 d	15.2 ± 0.2 d	54.0 ± 9.2 a	15.3 ± 0.3 a
T3	19.3 ± 0.2 c	6.6 ± 0.2 b	18.6 ± 0.2 b	50.0 ± 10.3 a	12.9 ± 0.6 b
T4	19.2 ± 0.2 c	5.1 ± 0.3 d	17.9 ± 0.2 c	42.0 ± 4.8 a	10.8 ± 0.3 c
T5	20.9 ± 0.2 b	6.2 ± 0.2 c	18.4 ± 0.1 bc	43.0 ± 5.6 a	13.9 ± 0.4 b

Hedelfinger/ GI5	Diameter (mm)	Weight (g/fruit)	SS (°Brix)	Final Fruit number	Color (H°)
C	21.1 ± 0.2 b	7.0 ± 0.2 a	17.2 ± 0.1 b	43.0 ± 12.4 b	14.6 ± 0.5 a
T1	21.9 ± 0.1 a	6.8 ± 0.2 a	18.8 ± 0.1 a	46.0 ± 8.6 ab	11.6 ± 0.6 b
T2	20.9 ± 0.2 b	6.2 ± 0.3 b	15.0 ± 0.2 c	74.0 ± 7.9 a	15.7 ± 0.4 a
T3	21.7 ± 0.1 a	7.0 ± 0.2 a	18.3 ± 0.1 a	55.0 ± 6.6 a	10.9 ± 0.5 b
T4	20.3 ± 0.3 c	5.9 ± 0.2 b	16.7 ± 0.2 b	52.0 ± 6.6 ab	11.7 ± 0.4 b
T5	21.4 ± 0.2 ab	6.7 ± 0.2 a	15.4 ± 0.1 c	45.0 ± 6.8 b	14.9 ± 0.5 a

[z] Means within a row followed by the same small letter are not significantly different at $\alpha = 0.05$.

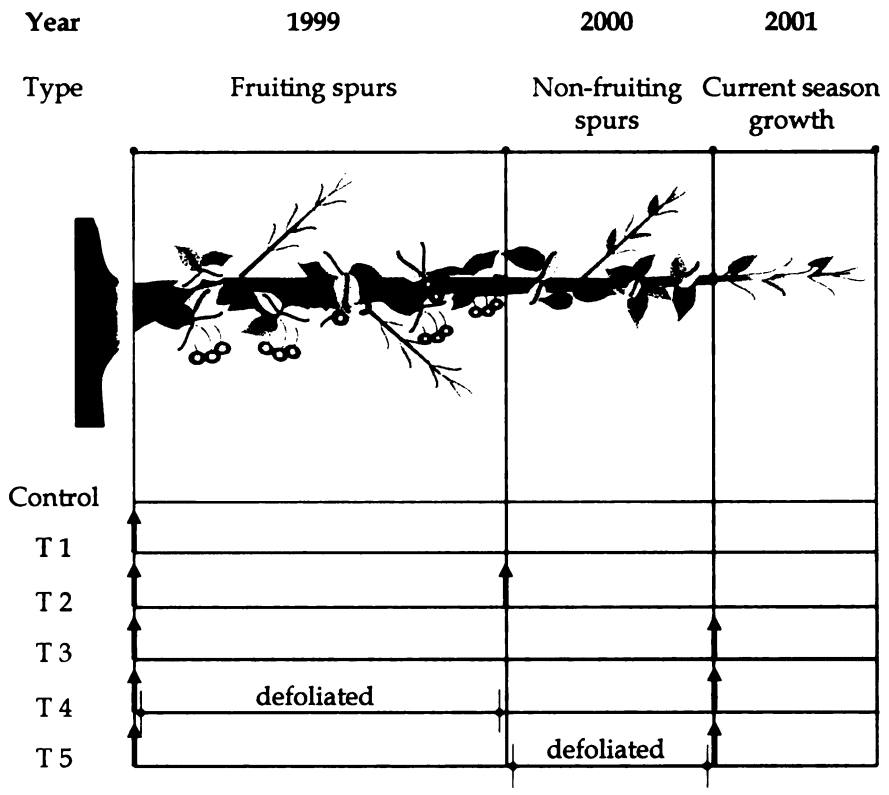

Figure 1. Sites of girdling treatments T1 to T5 imposed on 2-year-old sweet cherry branches. Black arrows indicate sites of girdling for all the treatments. Black dots indicate defoliated sections for T4 and T5.

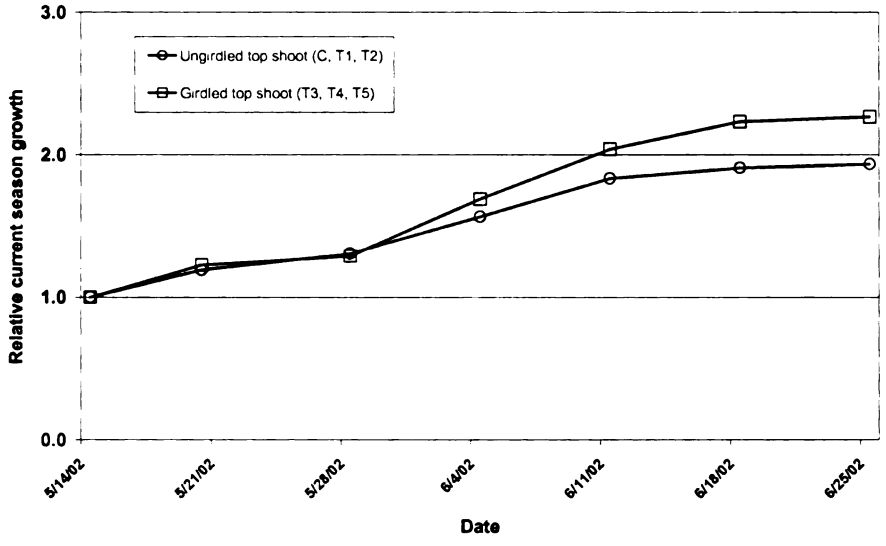

Figure 2. Relative current season growth on 'Ulster'/GI6 with (C, T1 and T2) and without girdling (T3, T4 and T5) at the junction of the previous season (2000) growth and the current season (2001) growth. Calculations were based on shoot length measured weekly for each treatment.

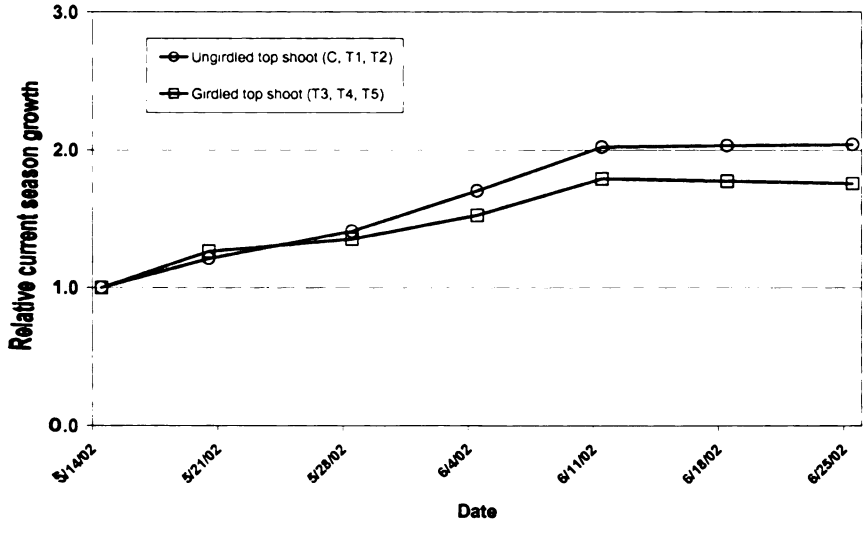

Figure 3. Relative current season growth on 'Hedelfinger'/GI5 with (C, T1 and T2) and without girdling (T3, T4 and T5) at the junction of the previous season (2000) growth and the current season (2001) growth. Calculations were based on shoot length measured weekly for each treatment.

CHAPTER III

THE EFFECT OF CROP LOAD ON ^{13}C-PHOTOASSIMILATE PARTITIONING IN SWEET CHERRY DURING STAGE III OF FRUIT DEVELOPMENT

The Effect of Crop Load on ^{13}C-Photoassimilate Partitioning from Non-Fruiting Spur Leaves in Sweet Cherry During Stage III of Fruit Development

Keywords: *Prunus avium* L., fruit growth, current season growth, carbohydrates, translocation, source, sink, partitioning, carbon, Gisela rootstock.

Abstract

Fruit quality and productivity are influenced by photoassimilate partitioning among different sink organs. In sweet cherry, 50 to 80% of fruit growth occurs during stage III of the double sigmoidal growth curve, when new shoots are still extending, likely in competition with developing fruits for current photosynthates. To study the role of the non-fruiting spur leaves as a source of assimilates for fruits and developing shoots during stage III, an experiment using 2-year-old fruiting branches of 'Sam' sweet cherry on the dwarfing rootstock, Gisela 5, was established. Three crop load treatments, based on leaf area-to-fruit ratio (LA: F) were imposed: LA : F = 140, 75, or 40 cm^2/fruit. Of the three leaf populations on the fruiting branch (fruiting spur, non-fruiting spur and new terminal shoot leaves), non-fruiting spur leaves were exposed to $^{13}CO_2$ labeling on three different dates during stage III (52, 59 and 63 DAFB). Fruits and leaves from the terminal shoot (both located in distal and proximal positions from the

labeled leaves) were sampled one and two days after labeling for analysis by gas chromatography mass spectrometry (GC-MS). ^{13}C fixed by non-fruiting spur leaves was translocated both acropetally and basipetally. For all 3 pulsing dates, fruits were more highly enriched in ^{13}C (i.e., had higher atom %) than were young leaves, and proportional enrichment ranged between ~87% to 96% of recovered ^{13}C, indicative of the stronger sink activity of fruit compared to that of shoots. There was not a consistent or significant crop load effect on ^{13}C-partitioning between fruit and shoots. However, differences in translocation between organs of the same branch, for a given treatment, were significant, as the fruits in closest proximity to the branch segment of non-fruiting spurs generally had the highest relative ^{13}C content (up to 64%, compared to more distal fruits which ranged from 26% to 40% of recovered ^{13}C). Shoot leaves had considerably lower ^{13}C contents, ranging between 1.6% and 11 % of the ^{13}C recovered.

Introduction

Fruits are major sinks for assimilates in fruit trees (Wright, 1989; DeJong and Walton, 1989; Basile et al., 2002). During fruit growth, dry matter (mainly carbon, C) and water accumulate. Dry matter accumulates in fruit as a result of C assimilation by different leaf populations and the subsequent distribution among reproductive and vegetative sinks (Teng et al., 2001). Stone fruit growth follows a double-sigmoidal curve, which can be divided into three stages (Flore, 1994; Berman and DeJong, 1996). Stage I is associated with initial growth and rapid cell division. Stage II or 'pit hardening' coincides with endocarp development and slow growth of the pericarp. Stage III or 'final swell' is characterized by rapid cell enlargement and dry matter accumulation. Sweet cherry (*Prunus avium* L.) fruit achieves between 50 to 80% of its final size during final swell (Flore, 1994).

A plant can be considered as a collection of individual sinks (reproductive and vegetative) which compete with each other (Wright, 1989; Flore and Layne, 1999). Sink strength, defined as the sink size multiplied by sink activity, is the driving force for C transport and competition between sink organs (Gifford and Evans, 1981; Hansen, 1989; Ho, 1996). The C available to support maintenance and growth of sink organs depends on photoassimilates supplied by different leaf populations and storage reserves (Grossman and DeJong, 1995; Flore and Layne, 1999; Basile et al., 2002). In sweet cherry, reproductive and vegetative growth occurs simultaneously during fruit development (Roper et al., 1987). Leaves on fruiting and non-fruiting spurs complete development early in the

season (~3 weeks after bloom). However, current season shoot growth continues through harvest in well-managed trees and during this time spur leaves constitute the primary source of C for fruit growth (Roper et al., 1987). This suggests that fruits might compete with each other and with current season shoots for available C coming from different sources. Competition between reproductive and vegetative growth, under source limiting conditions, has been reported in partitioning studies in peach (*Prunus persica* L. Batsch.) (Grossman and DeJong, 1995; DeJong and Grossman, 1995; Corelli-Grappadelli et al., 1996; Berman and DeJong, 2003).

Fruit growth potential and C availability limit final crop yield in trees (Pavel and DeJong, 1993). Source limitation results in insufficient C availability to support potential organ growth (DeJong and Grossman, 1995). Swain and Darnell (2002) indicate that periods when sources are limiting to reproductive and vegetative growth can be studied by manipulating sources (i.e., the availability of C) or sinks (i.e. the demand for C). In peach and blueberry (*Vaccinium corymbosum* L.), stages I and III of fruit development are periods of source limitation, while stage II is considered as a period of sink limitation (Pavel and DeJong, 1993; Swain and Darnell, 2002). Reductions in sink demand by reducing sink loads have been shown to increase the C supply to fruit and/or vegetative growth (Gucci and Flore, 1989; Grossman and DeJong, 1995). In peach, fruit removal increases the leaf area to fruit ratio (LA/F), which in turn increases

fruit size due to a reduction in source limitation (Pavel and DeJong, 1993; DeJong and Grossman, 1995).

In many species of the Rosaceae family including sweet cherry, photoassimilate production by spur leaves is not sufficient for optimal fruit growth, and import of assimilates synthesized by leaves external to the bearing spurs is required (Roper et al., 1987; Lakso and Corelli-Grappadelli, 1992; Corelli Grappadelli et al., 1994; Tustin et al., 1992; Teng et al., 1998, 2001; Chapter 2). In a 2-year-old sweet cherry branch, current photoassimilates for fruit and shoot growth can be provided by three distinct leaf populations: non-fruiting spur leaves (acropetal to the fruit), fruiting spur leaves where fruit are borne, and single leaves on new shoots (acropetal or basipetal to the fruit).

There are few experimental data on the movement and partitioning of assimilates in sweet cherry branches, particularly precocious and dwarfing rootstocks. So far, little is known about the importance of the non-fruiting spur leaves as a C source for fruit growth. Lang (2001) suggested that this population of leaves can help supply C to nearby new shoots or to the fruits developing farther down the branch. In the case of a high number of fruit per fruiting spur, as is common for combinations using Gisela rootstocks, C supplied by non-fruiting spurs might become extremely important for fruit growth, especially during periods of source limitation.

We hypothesized that the crop load, quantified by LA/F ratio of a whole fruiting branch unit, influences the fate and amount of C partitioned from non-

fruiting spurs to fruit and shoots during stage III. To test this hypothesis, we used sweet cherry trees on the dwarfing rootstock Gisela 5 (GI5, *Prunus cerasus* x *P.canescens*), which is precocious, productive (often 5 to 15 kg/tree in the 4th to 7th year) and generally achieves ~ 30 to 50% of the canopy volume of standard trees (Franken-Bembenek, 1996). Two-year old sweet cherry branches were labeled three separate times with ^{13}C, as $^{13}CO_2$, during stage III of fruit development. ^{13}C labeling has been used previously in other tree fruit partitioning studies (Lombardini, 1999; Nakano et al., 1998). Therefore, the main objectives of this study were to: (1) evaluate the use of ^{13}C as a non-radioactive pulse-labeling technique directly in the orchard; (2) elucidate the importance of the non-fruiting spur population as a source of C for fruit and current season growth during final fruit swell and, (3) determine whether different LA/F ratios alter C translocation patterns.

Materials and Methods

Plant material

The experiment was conducted during summer 2002 in a commercial orchard near Sparta, Michigan. Two-year-old fruiting branches on four-year-old trees of sweet cherry 'Sam' on the dwarfing the rootstock GI5 were selected. Two hundred and twenty branches having similar vigor, length and diameter were identified (Table 1). Most the branches were located in the medium and upper sections of the canopy and had comparable light exposures. Three LA/F ratio

treatments were imposed during stage III of fruit development Branches were grouped by their natural LA/F ratios and, from these groups, 7 branches per treatment were selected randomly for each ^{13}C labeling date. The crop loads of the selected branches were adjusted to the following LA/F (in cm^2/fruit): ~140 (treatment 1, T1), ~ 75 (treatment 2, T2) and ~ 40 (treatment 3, T3). These ratios corresponded to 13±3, 25±4 and 48±5 fruits/branch, respectively. Lateral current season shoots were eliminated to leave only the new terminal growth. A total of thirty branches (including three for natural baseline ^{13}C abundances for each date) were used for each pulse-labeling date. Fifty fruits were sampled to measure weight (fresh and dry), diameter, soluble solids (SS) and color. Twenty five shoots were used to measure weight (fresh and dry), length and leaf number. Fruits and shoots were sampled at each pulse-labeling date (see below for details).

^{13}C pulse-labeling

On three dates (52, 59 and 63 days after full bloom, DAFB) during stage III of fruit development, the branch section bearing non-fruiting spur leaves was enclosed in 3.7 L transparent Mylar® balloon-chambers and pulsed for 15 min with $^{13}CO_2$. A total of 1.3 mmol of $^{13}CO_2$ was injected into the chamber. $^{13}CO_2$ was generated by injecting 0.25 ml of 80% lactic acid into a 1 L plastic wash bottle containing 0.25 g of barium carbonate (98 atom% ^{13}C). The bottle was squeezed

every 2 min to pump $^{13}CO_2$ into the chamber. The labeling was carried out on sunny days between 10:00 AM and 12:30 PM.

Single leaf gas exchange of non-fruiting spur leaves on selected branches was measured prior to and during the pulse-labeling, using a CIRAS-2 infrared gas analyzer (PP-Systems Inc, Haverhill, Massachusetts, USA). The objective was to calculate the average rate of CO_2 uptake (between 9:00 AM and 12:00 PM) and carry out the pulse-labeling when assimilate rate values were positive. Net assimilation rate (A) of non-fruiting spurs varied among branches ranging between 5.0 and 18.0 $\mu mol\ m^{-2}\ s^{-1}$. Considering this natural variability, it was estimated that 15 min exposures would allow $^{13}CO_2$ uptake for all variations.

Labeling conditions were similar among labeling dates; however, light and temperature levels varied with ambient conditions and branch position within the canopy. Leaf temperature during the labeling period ranged from 23°C (9:00 AM) to 32°C (12:30 PM). Light levels were more variable and depended on the position of the branch within the canopy and the presence of clouds during labeling period. The photosynthetically active radiation (PAR) of the non-fruiting spur leaves ranged from 200 (shaded leaves) to 2705 $\mu mol\ m^{-2}\ s^{-1}$ (well exposed leaves).

Sampling and analysis

At 24 and 48 h after labeling, two 0.2 cm^2 discs per leaf and whole fruits were collected from the labeled branches and frozen immediately in liquid nitrogen for subsequent determination of ^{13}C enrichment by using gas chromatography mass spectrometry (GC-MS, model Europa Integra, PDZ Europa, United Kingdom). Additional samples were collected from unlabeled branches for natural abundance calculations. Fruit samples were collected from fruiting spurs located in the upper and lower portions of the two-year section of the branch. Leaf samples were collected from fully expanded and developing leaves on current season growth. The plant material was oven-dried at 70°C for 72 to 96 h and subsequently ground using a Wiley mill (20 and 40 mesh). ^{13}C enrichment was calculated according to Boutton (1991) and Vivin et al. (1996) as follows:

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

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

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

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

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

$$\text{Relative Partitioning (\%)} = (\text{New } {}^{13}C \text{ content in the organ})/(\text{New } {}^{13}C \text{ in all the sampled organs}) \times 100 \qquad \text{Eq (6)}$$

where the $\delta^{13}C$ (‰) value is calculated from the measured carbon isotope ratios of the sample and standard gases (Eq. 1). The absolute ratio (R) of a sample is defined by Eq. 2, where $R_{PDB} = 0.0112372$. 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 according to dry mass and C concentration (Eq. 6). As the sampling of leaves and fruits was carried out without removing the whole branch, the partitioning of new ^{13}C was expressed as a percentage of the total ^{13}C input for the sampled branch components.

Statistical Analysis

Analysis of variance was conducted by using PROC MIXED procedures of the SAS statistical analysis program (SAS Institute Inc, Cary, North Carolina). Covariance analysis indicated that there was not a significant covariate effect of total ^{13}C fixation on the ^{13}C partitioning among organs. There was not a significant effect of the sampling date on ^{13}C recoveries; therefore, statistical analyses considered the total number of observations for both sampling dates.

Results

Phenological characterization at pulsing

Most of the branches had an average of 5 non-fruiting and 11 fruiting spurs, with 97.1±3.0 and 86.9±3.7 cm^2 LA/spur, respectively. Leaf number (~7 per spur) did not differ between spur types. The three $^{13}CO_2$ pulses were imposed when fruit development was 34% (52 DAFB), 58% (59 DAFB) and 81% (63 DAFB) of final fresh weight (FW) at commercial harvest (Table 2). Between the first and the third pulse-labeling, SS and fruit diameter increased 30% and 17%, respectively. In contrast, fruit hue angle (H°) and firmness decreased 410 % and 210% respectively (Table 2). At these dates, current season shoots were 82, 89 and 98% of their final length (Table 3). At 52 DAFB, shoots had ~16 leaves (3 folded leaves) and ~ 43% of the leaves were still expanding. At the third pulse-labeling, shoots had their final leaf number present (zero folded leaves) and ~ 25% of the leaves at the tip were still expanding.

Translocation Patterns

Non-fruiting spur leaves were a source of photoassimilates for fruit and vegetative growth during stage III of fruit development. In most of the branches, ^{13}C fixed by this leaf population was translocated both basipetally (fruit) and acropetally (shoot). However, there were some branches with unidirectional translocation. In these branches, ^{13}C was only translocated basipetally to the fruit.

For the three pulse dates, most of the branches (96%) had ^{13}C enrichments in both distal and proximal fruits. In some branches (3%), ^{13}C was translocated to fruits proximal to the labeled source but no label was found in more distal fruits. The opposite was also observed in few branches (1%) for which distal fruits were labeled but proximal fruits had no label.

Most of the branches (76%) had ^{13}C enrichment in mature and developing leaves of the terminal shoot. A few branches (6%) showed no ^{13}C enrichment for either mature or developing leaves. Most of these occurred for T2 and T3 at the last pulse-labeling (63 DAFB). There were some branches (10%) in which mature leaves were ^{13}C enriched, but no label was found in developing leaves. The opposite was also observed; a similar number of branches (10%) had ^{13}C label in developing leaves but not in mature leaves.

^{13}C Partitioning

The total ^{13}C recovered (as $\mu g^{13}C/g$ DW) at 24 and 48 h after the pulse for the four sampled organs, ranged between 18 and 209 (52 DAFB), 50 and 450 (59 DAFB) and 31 and 350 (63 DAFB) $\mu g^{13}C/g$ DW/branch. Branches in which all the sampled organs had levels of enrichment close to natural abundance values (Atom % $\leq$ 1.082, leaves and $\leq$ 1.084, fruit) were discarded for lack of successful labeling.

There was not a significant LA/F ratio effect on the level of ^{13}C enrichment (atom %) of fruits and shoot leaves (Figures 1A, B and C; see

Appendix A.1 for statistics). However, the data showed two general trends across stage III. First, the highest atom % values were detected in fruits as compared to shoots. Second, the lower the LA/F ratio (i.e., the higher the crop load) the higher the atom % measured in proximal fruit compared to distal fruit. Paradoxically, there were some branches in which distal fruits had higher atom % than proximal fruits. This was particularly evident in the lowest crop load (140 cm^2 LA/fruit), for which between 33 and 86% (depending on the pulse date) of the analyzed branches had higher atom % in distal fruits than in proximal fruits. Despite some of the branches in T2 (75 cm^2 LA/F) and T3 (40 cm^2 LA/F) having distal fruits more ^{13}C enriched than proximal fruits, most had proximal fruit with higher ^{13}C contents.

Differences in partitioning of ^{13}C among sampled organs of the same branch, for a given treatment, were highly significant (Table 4). Fruit in close proximity to pulse-labeled source leaves had the highest relative ^{13}C enrichment (55 to 64%) compared to more distant fruits (28 and 40%). Mature leaves at the base of the terminal shoot, which were closer to the labeled source, had lower ^{13}C contents than developing leaves at the upper section of the shoot, but they were not significantly different. Comparisons between the total ^{13}C recovered for fruit (distal plus proximal) and shoot leaves (proximal plus distal) indicate that fruit growth is a much stronger sink than shoot growth during stage III. Considering all the labeling dates, between 87% and 96% of the recovered ^{13}C was partitioned to fruit, while between only 4% and 13% was partitioned to

current season growth. A general analysis of the data indicated that in the lowest crop load (140 cm^2 LA/F), ^{13}C was distributed more evenly between distal and proximal fruit as compared to the higher crop loads (75 and 40 cm^2 LA/F), which showed a greater difference between ^{13}C enrichments of distal and proximal fruits.

Relative carbon allocation

Initial measurements (prior to LA/F ratio adjustments) indicated that 70 to 75% of the fruit was concentrated in the medial and proximal section of the 2-year-old wood, a characteristic of sweet cherry on numerous rootstocks (Maguylo et al., 2003). Fruiting spurs on these sections had more fruits than those in the distal bottom section. After the treatments were imposed, fruiting density (fruit number/spur) decreased for T1 (140 cm^2 LA/fruit) and T2 (75 cm^2 LA/fruit). In these two treatments, fruit were distributed more evenly with an average 1.0 and 2.5 fruits/spur, respectively. The highest LA/F ratios (T3, 40 cm^2 LA/fruit) represented the natural crop load and fruiting density (averaging 4.5 fruits/spur) for 'Sam'/GI5 trees, with fruit distribution being more concentrated in the medial and proximal portions of the fruiting section. Considering the total crop load, its distribution, the ^{13}C gain/organ and the DW/organ at each labeling date, the ^{13}C relative partitioning between fruit and shoot leaves was estimated for each treatment (see Appendix A.2 for statistics). At 52 and 59 DAFB, LA/F ratios and the distribution of the crop load on the fruiting wood

significantly affected ^{13}C partitioning between distal and proximal fruit (Figures 2A and B). However, this effect was not significant at the last ^{13}C pulse-labeling (63 DAFB, Figure 2.C). For the first two labeling dates, the higher the crop load (i.e., the lower the LA/fruit ratio), the more C was translocated to proximal fruit and less to distal fruit. At the end of stage III, though this trend was similar to the other labeling dates, the differences were not significant.

Discussion

Distribution of newly-fixed C was studied in 2-year-old sweet cherry branches on 'Sam'/GI5, a dwarfing scion/rootstock combination. The manipulation of sink-source relationships by changing the LA/F ratios of individual branches and the use of stable isotope ^{13}C were used to characterize and quantify the relative partitioning of C, fixed by non-fruiting spur leaves, between rapidly growing fruit and the elongating terminal shoot during stage III.

Non-fruiting spur leaves provided a C source for both fruit and shoots during stage III. In the majority of the branches, regardless of the treatment, non-fruiting spurs exported ^{13}C basipetally to the fruit and acropetally to the terminal shoot. Bidirectional transport from non-fruiting spurs and other leaf populations has been reported for apple (*Malus domestica* Borkh.), sour cherry (*Prunus cerasus* L.), pecan (*Carya illinoensis Koch.*) and grape (*Vitis vinifera* L.), among others (Hansen, 1969; Corelli Grapadelli et al., 1994; Kappes and Flore, 1989; Toldam-Andersen, 1988; Davis and Sparks, 1974; Hale and Weaver, 1962). There were

branches in which unidirectional translocation to fruit also was observed. Unidirectional translocation has been observed in sour cherry, apple and cranberry (*Vaccinium macrocarpon* Ait.) (Kappes and Flore, 1989; Hansen, 1969; Roper and Klueh, 1996). A general analysis of the data indicated a distinct effect of orthostichy on ^{13}C translocation patterns. Phyllotactic patterns might explain the large variation between distal and proximal fruit ^{13}C enrichments on branches of a particular treatment. For example, two branches in T1 (140 cm^2 LA/F) showed a very different pattern of C partitioning between fruits. In one, 83% of ^{13}C was recovered in the distal fruit and only 10% in the proximal fruit. The opposite was observed in an otherwise similar branch, for which no ^{13}C was recovered in distal fruit but 90% was recovered in proximal fruit. The influence of vascular connections on partitioning between different organs has been described for apple, grapes, sour cherry and red raspberry (*Rubus idaeus* L.) (Hansen, 1969; Corelli Grapadelli et al., 1994; Kappes and Flore, 1986; Fernandez and Pritts, 1993; Privé et al., 1994).

LA/F ratios can affect C partitioning between fruit and vegetative growth in apple and peach (Giuliani et al., 1997; DeJong and Grossman, 1995; Berman and DeJong, 2003). In our experiment, different LA/F ratios did not affect differences in ^{13}C values recovered between fruit and shoot leaves, relative to different treatment LA/F ratios, were not significant, probably due to the shoot to shoot variability described above. However, comparisons of the relative partitioning of ^{13}C among the four analyzed organs, for a particular LA/F ratio,

indicated that rapidly enlarging fruit attracted ^{13}C more strongly than did elongating shoots (Figures 1A, B and C). On average, fruit attracted between 88 and 97% of the recovered ^{13}C vs. 4 to 12% in shoots. This strong sink demand was constant during stage III. In sour cherry and peach, fruit have priority over vegetative growth when leaves and fruit are in competition for resources (Flore and Layne, 1999; DeJong and Grossman (1995). Caruso et al. (1997), using a less vigorous rootstock in peach ('Flordaprince' on MrS 2/5), found that the reduced vegetative growth during fruit development resulted in higher dry matter partitioned to the fruit than to vegetative structures. On the other hand, Kappel (1991), using 'Lambert' sweet cherry on the vigorous seedling rootstock Mazzard (*Prunus avium* L.), found that current season growth was a greater sink for photosynthates than was fruit, suggesting that fruit on such sweet cherry trees may not be a predominant sink for C. Our results suggest that sweet cherry fruit are stronger sinks than shoots during stage III in combinations using the dwarfing rootstocks. Less vigorous vegetative growth with GI rootstocks results in shorter shoots, with earlier terminal bud set (Franken-Bembenek, 1996). In shorter shoots of apple, the onset of C export occurs earlier than in a longer shoots due to an early cessation in the sink demand of the shoot tip (Lakso and Corelli-Grappadelli, 1992). Our experiment was carried out when shoots had achieved between ~80% (~9 fully expanded leaves) and 100% (~13 fully expanded leaves) of their final length. It is highly likely that the shoots were already net exporters and, therefore, competition between fruit and shoots was

minimal. We suggest that in dwarfing sweet cherry combinations, terminal current season growth constitutes a C source for fruit growth during stage III. In apple, 35 DAFB, shoots had become a significant C source fruit growth (Lakso and Corelli Grapadelli, 1992). Similarly, in sour cherry, net export from terminal shoots began 17 days after leaf emergence, when shoots have accumulated ~27% of their final DW (Kappes, 1985).

As has been reported previously in apple and peach (Hansen, 1969; Ben Mimoun et al., 1995; Bruchou and Génard, 1999), we detected a positional effect on translocation to fruit in which those in close proximity to the labeled leaves had higher relative ^{13}C enrichments than more distal fruits. Competition between developing fruits has been reported by Wright (1989). It was not the aim of this paper to evaluate differences in fruit size at different positions of the fruiting section, but differences in fruit growth rate and size might explain this differential demand for C (Hansen, 1969). Unpublished data (G.A. Lang, personal communication) indicates that fruit size tends to vary across position, and when the LA/F increases, the variation in fruit size due to position decreases. It seems that when C is not limiting, fruit sizes tend to be similar. In contrast, under source limiting conditions, C distribution appears to be unequal and fruit size less uniform. Interestingly, the highest LA/F ratio (lowest crop load) resulted in a more even ^{13}C distribution between proximal and distal fruit. In this treatment, a greater number of branches had distal fruit with higher or similar ^{13}C enrichments compared to proximal fruit. Factors influencing the

translocation of photosynthates from certain leaf populations include: (1) vascular connections between organs; (2) LA/F ratios; (3) vigorous growth of fruit; (4) position and size of the spur within a branch; and (5) other unknown factors (Hansen, 1969).

When fruit distribution and total crop load per branch were included in the partitioning analysis, there were significant differences among different crop load treatments in terms of relative ^{13}C partitioning. At 52 and 59 DAFB, in the lower LA/F ratio treatment (i.e., ~75% of the high crop load was in the medial and proximal sections of the 2-year-old wood), most of the ^{13}C was found in proximal fruit with lower amounts partitioned to distal fruit. These higher crop loads might have imposed a source limiting condition, which generated competition between fruit at different positions. The other two treatments, which had spurs with a fairly uniform number of fruits and higher LA/F ratios, did not differ, indicating a higher C availability. According Grossman and DeJong (1995), as these treatments were thinned, fruit growth should have been sink-limited. At 63 DAFB, despite that the trend was similar to the other two pulses, fruit distribution did not significantly affect the relative partitioning among organs. It seems that in late stage III, fruit located more acropetally decrease their C demand earlier than those fruit located more basipetally. This might a consequence of the decrease in the growth rate of proximal fruit, which would promote a greater translocation to distal fruit. More information about

competition between sweet cherry fruits at different positions within a branch remains to be elucidated.

General implications of these results assist in developing concepts for precise branching and cropping in sweet cherry on dwarfing rootstocks. Greater precision in canopy development, i.e., more precise management of crop loads and the different source leaf populations, may help to optimize the balance between vegetative growth and fruit quality (Lang, 2001). It has been reported that combinations using GI5 have a serious problem with overcropping and lower quality fruit beginning about the 4^{th} or 5^{th} year. To overcome this situation and manage LA/F ratios more precisely, it is critical to have a good understanding of sink-source relationships. More knowledge about the role of the various leaf populations as sources of C for developing fruit will help to develop pruning and training strategies to manage high-density orchards.

In summary, our findings indicate that the hierarchy for C distribution within a typical sweet cherry branch on a dwarfing rootstock, during stage III, would be proximal fruit>distal fruit>mature or developing leaves on a shoot. We conclude that in 2-year-old branches: (1) non-fruiting spur leaves constitute an important C source for fruit growth during stage III; (2) fruits are a C partitioning priority over current season growth during stage III and this is relatively constant during stage III; and (3) fruits at different positions compete for C fixed in non-fruiting spur sections. These results provide a better

understanding about the link between vegetative growth and fruit development in dwarfing sweet cherry trees.

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Table 1. Morphological features of 2-year-old 'Sam'/Gisela 5 sweet cherry branches before ^{13}C pulse-labeling. Mean ± SE, n=220.

Parameter	Average or Range
Branch length (cm)	86.2 ± 2.7
Branch diameter (mm)	12.2 ± 0.5
Initial fruit number/branch [z]	10 to 65
Total spur number	16.4 ± 1.2
Fruiting spur number/branch	10.9 ± 0.9
Non-fruiting spur number/branch	5.5 ± 0.5
Total fruiting spur leaf area (cm^2)	951.9 ± 72.9
Total non-fruiting spur leaf area (cm^2)	534.2 ± 58.4

[z] Range in fruit number before thinning.

Table 2. 'Sam' sweet cherry fruit quality parameters measured at each pulse-labeling (52, 59 and 63 days after full bloom, DAFB) and at commercial harvest (67 DAFB). Mean ± SE, n=50.

DAFB	Fresh weight (g)	Dry weight (g)	S.S (°Brix)	Diameter (mm)	Color (Hue°)	Firmness (g/mm)
52	2.2 ± 0.5[z]	0.5 ± 0.1	10.0 ± 0.3	16.5 ± 0.2	73.5 ± 1.8	497.7 ± 24.2
59	3.8 ± 0.5	0.7 ± 0.1	11.1 ± 0.6	17.7 ± 0.6	30.6 ± 0.5	281.5 ± 5.9
63	5.3 ± 0.6	0.8 ± 0.1	13.7 ± 0.5	19.9 ± 0.9	17.8 ± 0.3	230.8 ± 5.4
67	6.5 ± 0.5	0.9 ± 0.1	16.4 ± 0.8	20.4 ± 0.4	8.5 ± 0.1	169.9 ± 1.2

[z] Commercial harvest (2 Jul, 2002).

Table 3. Growth and morphological measurements of current season 'Sam'/Gisela 5 sweet cherry growth at each pulse-labeling date (52, 59 and 63 DAFB) and harvest (67 days after full bloom, DAFB). Mean ± SE, n=25.

Measurement	DAFB			
	52	59	63	67[w]
Total fresh weight (g)	10.6 ± 1.0	13.0 ± 1.1	14.4 ± 0.6	15.1 ± 0.4
Total dry weight (g)	4.9 ± 0.3	5.0 ± 0.4	6.1 ± 0.5	6.2 ± 0.8
Length (cm)	20.9 ± 1.1	22.6 ± 2.3	24.9 ± 1.2	25.4 ± 1.4
Total leaves[z]	16.0 ± 1.3	17.1 ± 1.1	18.0 ± 1.1	18.4 ± 0.7
Fully expanded leaves	9.1 ± 1.1	10.7 ± 0.9	12.5 ± 1.2	13.7 ± 1.3
Developing leaves[y]	6.9 ± 1.1	6.4 ± 1.4	5.5 ± 0.9	4.69 ± 0.7
Folded leaves	3.2 ± 0.5	2.4 ± 0.4	0.2 ± 0.2	0.0 ± 0.0
Leaf area (cm^2)	288.0 ± 2.0	334.1 ± 15.2	365.8 ± 4.0	406.5 ± 7.6

[z] Total leaves include fully expanded and developing leaves.
[y] Developing leaves include folded and unfolded not fully expanded leaves.
[w] Commercial harvest.

Table 4. Relative ^{13}C enrichment for fruit and current season leaves. Mean ± SE, n=15. Calculations based on total ^{13}C-absolute (μg ^{13}C/g DW) recoveries for the four organs.

DAFB [z]	Relative ^{13}C Proportion (%)				
	Fruit		Leaves		Obtained
	Distal	Proximal	Proximal	Distal	p-value
52	30.5 ± 7.0 b[y]	61.3 ± 6.8 a	1.8 ± 0.7 c	6.6 ± 2.6 c	< 0.0001
59	34.4 ± 4.0 b	59.4 ± 8.3 a	2.3 ± 0.5 c	3.9 ± 1.2 c	< 0.0001
63	36.8 ± 4.2 b	57.7 ± 5.3 a	2.1 ± 0.6 c	3.6 ± 1.3 c	< 0.0001

[z] DAFB: days after full bloom.
[y] Means in given row followed by the same letter are not significantly different at $\alpha = 0.05$.

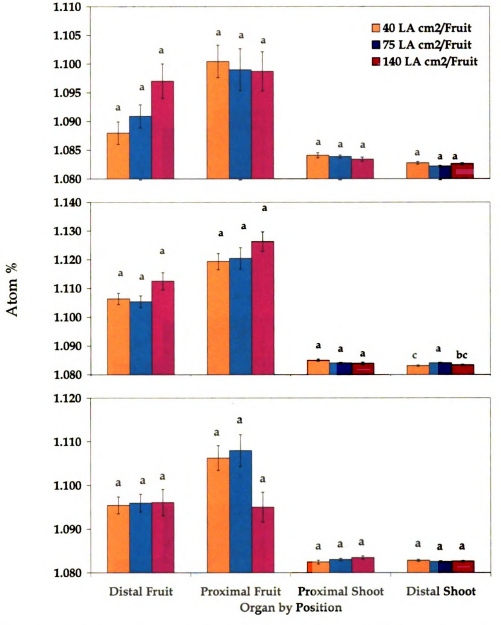

Figure 1. ^{13}C content (expressed as atom %) in fruits and current season growth leaves during stage III (52, 59 and 63 DAFB). Means ± SE are represented in colored bars for each organ within a certain treatment. Means for a certain organ followed by the same letter are not significantly different at α=0.05.

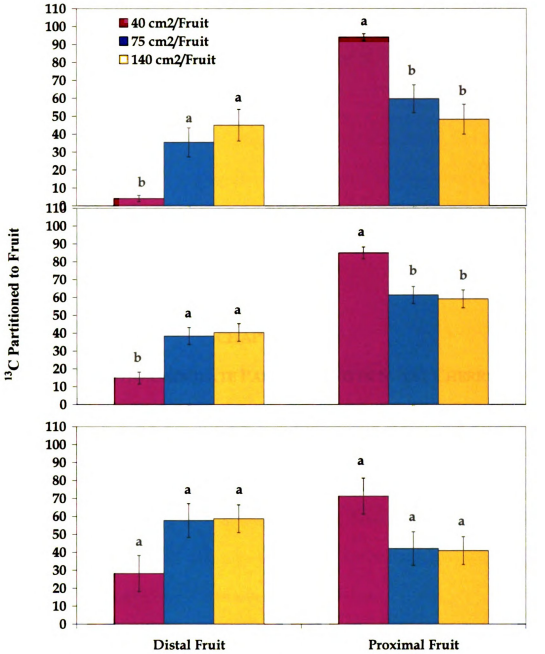

Figure 2. ^{13}C-Relative partitioning (%) in distal and proximal fruits during stage III (52, 59 and 63 DAFB). Means ± SE are represented in colored bars and vertical lines, respectively. Each treatment is represented by a different color. Colored bars within the same organ followed by the same letter are not significantly different at $\alpha = 0.05$ and $\alpha = 0.01$, respectively.

CHAPTER IV

^{13}C- PHOTOASSIMILATE PARTITIONING IN SWEET CHERRY DURING FRUIT DEVELOPMENT

^{13}C- Photoassimilate Partitioning in Sweet Cherry During Fruit Development

Keywords: *Prunus avium* L., stage I, stage II, stage III, shoot growth, leaf area, sink strength, fruit quality, carbohydrates, translocation, source, carbon, Gisela, rootstock.

Abstract

Fruit size, quality and yield are influenced by photoassimilate synthesis and subsequent partitioning among different sink organs. Little is known about the relative importance or temporal relationships of different leaf populations as sources of carbon (C) for sweet cherry fruit and shoot development, particularly on vigor-reducing rootstocks. It was hypothesized that the partitioning of C fixed by different leaf types in semi-dwarfing sweet cherry trees is influenced by reproductive and vegetative sink demands during fruit development, with the sink strength of fruit being considerably greater than that of shoots as a consequence of the higher harvest index in this type of tree. To study the contributions of different leaf populations to fruit and shoot development during stages I, II and III, an experiment using 2-year-old fruiting branches of 'Ulster' sweet cherry on the semi-dwarfing rootstock, Gisela 6 (GI6), was established. The three leaf populations on the fruiting branch, i.e., fruiting spur, non-fruiting spur

and new terminal shoot leaves, were exposed to $^{13}CO_2$ labeling on five different dates (25, 40, 44, 56, 75 days after full bloom, DAFB) during fruit development. Two days after labeling, whole branches were removed and different organs were prepared for analysis by gas-chromatography mass-spectrometry (GC-MS). Spur and shoot leaves were significant sources of C for fruit and vegetative growth. In terms of C allocation, fruits were a priority sink vs. new shoot growth during the entire period of fruit development. The highest fruit sink strength was during stages I and III. Current season (terminal) shoot growth provided a C source for fruit as early as stage I. It seems that resource limitations during stages I and III of fruit development affect final fruit size in sweet cherry on Gisela (GI) rootstocks. The source-sink relationships elucidated in this study provide a physiological foundation for the development of specific orchard management strategies to promote a more sustainable balance between vegetative and reproductive growth in high density sweet cherry orchards.

Introduction

In many tree fruits of the Rosaceae family, spur and extension shoot leaves are the main sources of current photosynthates for reproductive and reproductive growth (Teng et al., 2001; Roper et al., 1987; Corelli-Grappadelli et al., 1994). In stone fruit trees, fruit are major sinks for assimilates (DeJong and Walton, 1989). During fruit development, dry matter (mainly carbohydrates, CH_2O) and water content increase. Flore (1985) indicate that ~90% of the fruit dry weight (DW) is comprised of CH_2O. Dry matter accumulates in fruit as a result of carbon (C) assimilation by different leaf populations within the canopy and subsequent partitioning among different sinks (Teng et al., 2001). In *Prunus* sp., fruit development follows a double sigmoidal pattern, which is divided into three stages (Labreque et al., 1985; Flore 1994; Costes et al., 1995; Berman and DeJong, 1996). Following pollination and fruit set, stage I is characterized by active cell division and rapid initial growth. Stage II or 'pit hardening' is associated with endocarp lignification and slower growth of the pericarp. Stage III or 'final swell' is a period of rapid fruit growth characterized by mesocarp cell enlargement and dry matter accumulation. Fifty to 80% of cherry fruit growth occurs during this stage (Flore, 1994; Chapters 3).

A tree can be considered as a collection of individual sinks (reproductive and vegetative) that compete with each other (Wright, 1989). Reproductive and vegetative growth occurs simultaneously during sweet cherry (*Prunus avium* L.) fruit development (Roper et al., 1987). Leaves on fruiting and non-fruiting spurs

reach full leaf area early in the season. However, current season shoot growth continues developing leaf area through harvest in well managed trees. This situation might generate competition between actively growing aerial sinks, i.e., fruits and extension shoots, for the available C provided by different leaf populations. In peach (*Prunus persica* (L.) Batsch), periods of resource limitation lead to competition for photoassimilate between reproductive and vegetative organs (Grossman and DeJong, 1995). The sink demand of an organ varies with the time of year since its developmental demands change during the season (Flore and Layne, 1999). Thus, during plant growth, sinks may change in competitive ability to attract assimilates (Wright, 1989). Roper et al. (1988) indicate that since sweet cherry fruit development occurs during a short period (60 to 70 days), fruit sink effects might be highly prioritized.

The primary sink activities of fruits are growth and respiration (DeJong and Goudriaan, 1989). The C available to individual organs depends on the supply of photoassimilates from sources (leaves or storage reserves) and the demand for resources by sink organs (Basile et al., 2002). Farrar (1996) and Michin et al. (1997) suggest that the distribution of assimilates is controlled by the entire source- sink-pathway plant system and is not a property of sinks alone. In contrast, Marcelis (1996) proposed that dry matter partitioning among sinks is regulated by the sinks themselves and the effect of sources is indirect via the formation of sink organs.

Photosynthesis determines the amount of C available for plant growth (Farrar and Williams, 1991). In a 2-year-old sweet cherry branch, current photoassimilates for fruit growth can be provided by fruiting spur leaves where fruit are borne, non-fruiting spur leaves and from single leaves on new shoots (Roper et al., 1987). Fruiting spur leaves are not the only C source to support fruit growth, and import of assimilates synthesized by leaves distal to the bearing spurs is required for optimal fruit development (Roper et al. 1987; Chapter 2 and 3). Previous branch girdling and defoliation studies demonstrated the deleterious effects on fruit quality when fruit were isolated from the major sources of photoassimilates (Roper et al., 1987; Chapter 1). Leaf populations on both fruiting and non-fruiting branch segments were required for optimal fruit development (Chapter 1).

Little is known about the relative importance or temporal relationships of these different leaf populations as sources of C for sweet cherry fruit and shoot development. In this study, 2-year-old sweet cherry branches on 'Ulster'/Gisela 6 (GI6) in which fruiting spur, non-fruiting spur and current season shoot leaf populations were labeled with ^{13}C by pulsing with $^{13}CO_2$ five times during stages I, II and III of fruit development. It was hypothesized that the partitioning of C fixed by different leaf populations would be strongly and differentially influenced by reproductive and vegetative sink demands during fruit development. Accordingly, the main objectives of this study were to: (1) elucidate the temporal importance of each leaf population as a source of

assimilates for fruit and shoot growth during the whole fruit development period; (2) determine predominant translocation patterns depending on the stage of fruit development; and (3) quantify differences in the amount of C exported from different leaf sources to fruit and shoot growth at various intervals during the growing season.

Materials and Methods

Plant material

The experiment was conducted during summer 2003 at Michigan State University's Clarksville Horticultural Experimental Station (CHES), Clarksville, Michigan. Two-year-old fruiting branches on 7-year-old trees of 'Ulster' sweet cherry on the semi-dwarfing rootstock GI6 (*Prunus cerasus* L. x *Prunus canescens* L.) were selected for ^{13}C pulse-labeling during stages I, II and III of fruit development. A population of six hundred branches having similar vigor, crop load, length and diameter was identified. Ten branches per treatment were selected randomly from this population for each ^{13}C pulse-labeling date. Most the branches were located in the middle and upper sections of the canopy. ^{13}C-labeling treatments corresponded to the fruiting spur (FSP-Lf1), non-fruiting spur (NFSP-Lf2) and current season shoot (SH-Lf3) leaf populations at different stages of fruit development. A total of thirty branches (including three for natural baseline ^{13}C abundances for each date) were used for each pulse-labeling

date. Lateral current season shoots were eliminated to leave the new terminal shoot growth as the sole current season shoot source and sink.

Fruits, spurs and shoots were measured weekly. Thirty fruits were measured for weight (fresh and dry), diameter and soluble solids (SS). Thirty fruiting spurs, non-fruiting spurs and current season shoots were measured for weight (fresh and dry), leaf number and leaf area (LA).

Phenological characterization before $^{13}CO_2$ pulsing

Individual branches were measured and characterized morphologically prior to the beginning of the sequential ^{13}C labeling. Measurements included: branch length, branch diameter, fruiting and non-fruiting spur number, shoot length, shoot leaf number and fruit number. Terminal shoot growth (current season extension growth) was measured weekly from budbreak until terminal bud set for the entire population of branches.

The experimental branches were similar in vigor and morphology, but differed by position within the canopy. Branch length and diameter was similar among branches selected for a specific pulse-labeling date (Appendix B.1). At the final pulse-labeling date (75 days after full bloom, DAFB), the average diameter was greater compared to that measured at the first pulse-labeling date (25 DAFB). Most of these branches had a similar number of fruiting and non-fruiting spurs, which ranged between 12 and 14 spurs per section, respectively (Appendix B.1). Despite the similarity in the number of fruiting and non-fruiting

spurs, the length of the fruiting spur section (2001 shoot growth) was higher than that of the non-fruiting section (2002 shoot growth; Appendix B.1).

^{13}C pulse-labeling

On eight dates during fruit development, 25 DAFB (25 May), 33 DAFB (2 Jun), 40 DAFB (9 Jun), 44 DAFB (13 Jun), 51 DAFB (20 Jun), 56 DAFB (25 Jun), 63 DAFB (2 Jul) and 75 DAFB (14 Jul), fruiting spurs leaves, non-fruiting spur leaves and current season shoot leaves were enclosed as separate populations in transparent Mylar® balloon chambers of different volumes and pulsed for 15 to 20 minutes with $^{13}CO_2$. A total of 3.9 mmol of $^{13}CO_2$ was pumped into the chambers. $^{13}CO_2$ was generated by injecting 0.75 ml of 80% lactic acid into a 1 L wash bottle containing 0.75 g of Barium carbonate (98 atom% ^{13}C). The plastic bottle was agitated and squeezed every 2 minutes to pump $^{13}CO_2$ into the chamber. The labeling was carried out during sunny days between 10:00 AM and 12:30 PM.

Climatic conditions were similar among labeling dates; however localized light and temperature levels varied somewhat with ambient conditions and branch position within the canopy. The photosynthetically active radiation (PAR) varied among leaf populations depending on branch orientation, within a specific pulse-labeling date and among dates (Appendix B.2).

Single leaf gas exchange of fruiting, non-fruiting spur and current season growth leaves on selected branches was measured prior to and during the pulse-

labeling with a CIRAS-2 infrared gas analyzer (PP-Systems Inc, Haverhill, Massachusetts, USA). The average rate of CO_2 uptake for each date (between 9:00 AM and 12:30 PM) was calculated so that the pulse-labeling was carried out when assimilation rate values were positive. Net assimilation rate (A) varied among the three leaf populations and dates (Appendix B.3). Considering this natural variability, it was estimated that 15 min exposures for the three treatments would allow optimal $^{13}CO_2$ uptake among all orientations of branches and young developing shoots.

Sampling and analysis

Immediately after labeling, 1 or 3 fully expanded leaves were sampled from each treatment to estimate the initial total ^{13}C fixed by each leaf population. When fruiting spurs were the labeled population, fruit were also sampled to estimate the ^{13}C fixed due to fruit photosynthesis.

At 48 h after each pulse-labeling, whole branches were harvested destructively to measure fresh and dry weight of different organs. Measured organs included: fruiting spur leaves, non-fruiting spur leaves, current season shoot leaves, and fruit plus wood and bark of the fruiting, non-fruiting and current season shoot sections. Current season shoot growth was divided further into mature fully expanded leaves (leaves at the base of the shoot), developing leaves (leaves along the middle of the shoot), young leaves (leaves at the tip of the shoot) and wood. Fruit size and number per branch also was measured.

Branches were then prepared for ^{13}C-analysis. Analyzed organs included those indicated above. In addition, five single fruit from the fruiting spur branch, that was labeled directly, were divided into pericarp and endocarp. All plant materials were oven-dried at 70°C for 72 to 96 h and ground using a Wiley mill (20 and 40 mesh). Additional samples were prepared from unlabeled branches for natural abundance calculations. ^{13}C enrichment was measured by using gas chromatography mass spectrometry (GC-MS, PDZ Europa 20-20 mass spectrometer and ANCA-GSL sample combustion unit, PDZ Europa, Sandbach, Cheshire, United Kingdom). ^{13}C enrichment for different organs was calculated according to Boutton (1991) and Vivin et al. (1996) as follows:

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

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

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

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

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

$$\text{Relative Partitioning (\%)} = (\text{New } {}^{13}C \text{ content in the organ})/ (\text{New } {}^{13}C \text{ in all the sampled organs}) \times 100 \qquad \text{Eq (6)}$$

where the $\delta^{13}C$ (‰) value is calculated from the measured C isotope ratios of the sample and standard gases (Eq.1). The absolute ratio (R) of a sample is defined by Eq.2, where $R_{PDB} = 0.0112372$. 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 according to dry mass and C concentrations (Eq. 5). The relative partitioning of new ^{13}C was expressed as a percentage of the total ^{13}C input for the branch components (Eq. 6).

Selection of representative pulse-labeling dates for ^{13}C analysis

The eight $^{13}CO_2$ pulses were imposed when fruit dry weight (DW) was 21% (25 DAFB), 23% (33 DAFB), 24% (40 DAFB), 25% (44 DAFB), 41% (51 DAFB), 57% (56 DAFB), 78% (63 DAFB) and 100% (75 DAFB) of its final value measured at 75 DAFB (Appendix B.4). At these dates, current season shoot DW was 18%, 24%, 29%, 39%, 54%, 62%, 82% and 100% of its final value.

After analyzing the relative growth curves (Figure 1) for fruit and current season shoots for the 2003 growing season, five representative pulse-labeling dates were selected for ^{13}C analysis. Thus, stages I and II were evaluated at 25 and 40 DAFB, respectively. Stage III was evaluated at three different pulse-labeling dates at 44, 56 and 75 DAFB. The last ^{13}C-pulse was carried out late in stage III, which was 12 days after commercial harvest (2 Jul).

Climatic Data

Climatic parameters such as air temperature, PAR and growing degree days (GDD, base 4.4 °C) were recorded at CHES during the period of the

experiment (May to Aug, 2003) and were obtained from the Michigan Automated Weather Network *(MAWN)*.

Statistical Analysis

Analysis of variance was conducted by using PROC MIXED procedures of the SAS statistical analysis program (SAS Institute Inc, Cary, N.C.). The statistical model for the overall experiment was a three way factorial design with three factors: treatment (T=3), date (D=5) and organ (O=8). As extremely high levels of ^{13}C enrichment were expected in directly labeled leaves, these were not considered for statistical analysis.

Results

Growth in Two-Year-Old Branches

a. Leaf area

The LA per branch increased from budbreak to 96 DAFB. LA of individual fruiting and non-fruiting spurs increased until 54 DAFB, while shoots continued developing leaves until 96 DAFB (Figure 2; Appendix B.5). Individual non-fruiting spurs had a greater final LA than that of fruiting spurs, with 134.8 ±4.2 and 119.1±5.0 cm^2 LA/spur, respectively. Leaf number of fruiting and non-fruiting spurs did not differ. Shoot leaf area increased rapidly between 26 and 54 DAFB. At terminal bud set (96 DAFB), shoots had an average of ~852 cm^2 LA. The average leaf size by population was ~20 cm^2 for fruiting spur leaves, ~22 cm^2

for non-fruiting spur leaves and ~44 cm^2 for shoot leaves. Total LA/ branch for a certain leaf population and LA/fruit (F) ratios for branches used at the different labeling dates are provided in Appendix B.6 and C.7, respectively.

b. Fruits

Fruit set occurred between 5 and 12 DAFB (256 to 312 GDD). Stage I began 13 DAFB (319 GDD) and continued until 32 DAFB (483 GDD). Stage II occurred between 33 (492 GDD) and 46 DAFB (604 GDD). Stage III began 47 DAFB (618 GDD) and finished 75 DAFB (1135 GDD). Fruit remained on the tree and was measured from 75 DAFB until terminal bud set (96 DAFB, 1477 GDD). The total GDD accumulated during each fruit developmental stage are provided in Appendix B.8.

Sixty percent of the final DW and 50% of the SS were accumulated during stage III fruit development (Table 1). Commercial harvest was carried out 63 DAFB. At that date, fruit fresh weight (FW) and SS were 5.5 g and 17 °Brix, respectively. Between commercial harvest and terminal bud set, fruit FW and DW increased 2.7 g and 1.0 g, respectively. During the same period, SS increased from 17 to 25 °Brix.

c. Current season shoots

Current season terminal shoots began extension growth around full bloom (Figure 1). Shoots elongated rapidly between 12 and 54 DAFB (Table 2). Their

growth rate decreased when fruit began growing rapidly during stage III. Final shoot length was ~35 cm at terminal bud set. Leaf number increased until 75 DAFB. At terminal bud set, the final leaf number was ~22 per shoot. Given that sweet cherry has a 5-leaf phyllotaxy, the 2003 growth represents slightly more than 4 full phyllotaxic repetitions. Fifty percent of the final shoot DW was accumulated between 40 and 63 DAFB.

Relative FW and DW partitioning

FW relative partitioning was similar between stage I (25 DAFB) and the beginning of stage III (44 DAFB). Fruit and wood constituted most of the FW of the branch, followed by spur leaves and shoot (Figures 3; Appendix B.9 and B.10). Later in stage III the FW distribution changed, with 60% to 70% of the total FW partitioned to the fruit. FW partitioned to the shoot fluctuated between 3 and 6% of the total FW. As in the case of FW partitioning, DW was partitioned mostly to fruit and wood (Figures 4; Appendix B.11 and B.12). However, between stage I and beginning of stage III, the relative partitioning favored wood. This situation changed during stage III since most (40% to 52%) of the DW of the branch was partitioned to fruit. Partitioning to extension growth of terminal shoots fluctuated between 4 and 7% of the total DW per branch.

Total FW and DW of 2-year-old fruiting branches increased 61% and 64%, respectively, between 25 and 75 DAFB. The greatest change in DW was detected in the fruit and shoots, which increased DW by 84% and 82%, respectively,

between 25 and 75 DAFB. Non-fruiting wood DW increased more than that of fruiting wood, 46% vs. 30%, respectively. Fruiting and non-fruiting spur leaf DW increased with time, but such changes were minor compared to those detected in other organs of the branch.

Translocation patterns in two-year-old branches

By 48 h after ^{13}C labeling of fruiting spur, non-fruiting spur and terminal shoot leaves, there were differences in the ^{13}C translocation patterns depending on leaf population (Tables 3 and 4). A large proportion of the labeled C remained in the pulsed leaves. In addition, significant amounts of ^{13}C were found in wood of different sections, indicating active ^{13}C translocation to different organs at the moment of branch removal.

Total ^{13}C in leaves and fruit immediately after pulsing

All (100%) branches, for all leaf populations and pulse-labeling dates, were labeled successfully. The total ^{13}C content in fruiting, non-fruiting and current season leaves immediately after the labeling did not differ significantly (Table 5). Moreover, the amount of ^{13}C fixed by these three leaf populations did not show significant differences along labeling dates (Table 5). Despite the lack of differences in total ^{13}C among leaf populations, it is important to note that at 25 DAFB, the amount of ^{13}C in terminal shoot leaves was considerably lower than

that detected for the fruiting and non-fruiting spur leaves. At this date, shoots were 9 cm in length and had only 10 leaves (Table 2).

Fruit from fruiting spur leaves (FSP-Lf1) were directly exposed to $^{13}CO_2$. Individual fruit fixed ^{13}C at all the pulse-labeling dates; however, ^{13}C content varied significantly among labeling dates (Table 6). At 25 DAFB, fruit fixed the highest amounts of ^{13}C. At 40 and 44 DAFB, fruit continued fixing ^{13}C but in lower quantities. No significant differences were detected among these two dates. At 56 and 75 DAFB, the amounts of ^{13}C recovered in fruit were lowest and no significant differences were detected between these dates.

Total ^{13}C recoveries 48 hours after pulsing

Forty-eight h after ^{13}C labeling of fruiting spur, non-fruiting spur and terminal shoot leaves, there were differences in the ^{13}C translocation patterns depending on leaf population (Tables 3 and 4). A large proportion of the labeled C remained in the pulsed leaves. In addition, significant amounts of ^{13}C were found in wood of different sections, indicating active ^{13}C translocation to different organs at the moment of branch removal.

Across all labeling dates, the total amount of ^{13}C recovered in the branches 48 h after labeling was lower than the amount of ^{13}C fixed initially (Table 5); due to unexplained tree variability, 4% of the branches were an exception to this result. The lowest ^{13}C values per branch were recovered when shoot leaves were pulsed. The largest differences were measured 25 DAFB, when total ^{13}C

recoveries from pulsed shoot leaves, were ~60% lower than those measured in either of the pulsed spur leaf treatments, which were similar during fruit development. At 75 DAFB, ^{13}C recoveries after 48 h were similar for all the treatments.

Absolute and relative partitioning of ^{13}C 48 hours after pulsing

By 48 h after pulsing, there were significant differences in ^{13}C content among different organs of the branch, depending on pulsed leaf population and labeling date. For all three source leaf populations, the greatest proportion of ^{13}C was detected in fruit. This predominant partitioning to fruit was constant for stages I, II and III. However, there were significant differences among treatments regarding the amount of ^{13}C partitioned to fruit at each pulse-labeling date. The highest ^{13}C levels in fruit were detected when fruiting spur leaves were the labeled source, and this was evident on all the labeling dates. The second most important source for fruit was the non-fruiting spur leaves. The lowest ^{13}C recoveries in fruit were found when extension shoot leaves were the labeled source. However, for the last pulse labeling (75 DAFB), shoot leaves were equally as important as non-fruiting spur leaves for C partitioning to the fruit.

a. Fruiting spur leaves as ^{13}C source

Fruiting leaves were a source of current photoassimilates for fruit and vegetative growth during stages I, II and III of fruit development. In all the

pulse-labeling dates, ^{13}C fixed by this leaf population was translocated predominantly to fruit and wood subtending the labeled leaves (Tables 3 and 4). In most (86%) of the branches, acropetal translocation to non-fruiting spur leaves, non-fruiting wood, and current season wood and leaves was detected. A few branches (13%) did not show translocation to either non-fruiting leaves or different sections of the shoot. This was particularly evident 44 DAFB, when 100 % of the branches did not translocate ^{13}C to any of the organs located acropetally to fruiting spur leaves.

There were significant differences in the absolute amount of ^{13}C recovered for each organ (Tables 3 and 4). Most of the translocated ^{13}C was partitioned to fruit, followed by fruiting spur wood. Lower ^{13}C contents were detected in the rest of the organs. The highest ^{13}C recoveries in fruit were detected 56 DAFB (stage III), followed 75 (stage III), 44 (stage III) and 25 (stage I) DAFB (Table 7). The lowest ^{13}C levels were recovered at 40 DAFB (stage II). At 25 and 56 DAFB, the amounts of ^{13}C recovered in 2-year-old-wood were considerably lower compared to those found on the rest of the labeling dates. When fruit were exposed directly to $^{13}CO_2$ labeling, they constituted an additional source of C as indicated previously (Table 6).

The relative partitioning indicated that between 18% and 36% of the ^{13}C recovered was 48 h later in fruiting spur leaves (Figure 5; Appendix B.13). The rest of the ^{13}C was recovered in different organs in the following order: fruit (57 to 79%), fruiting spur wood (3 to 9%), non-fruiting spur wood (0 to 1%), non-

fruiting spur leaves (0 to 1%) and shoot (0 to 0.1%). The highest percentage of ^{13}C partitioned to fruit occurred at 56 DAFB, followed by the 25 DAFB pulse. The lowest was detected 75 DAFB. The lowest ^{13}C recovery in fruiting spur leaves was at 56 DAFB. Greater detail for the minimal ^{13}C partitioning in shoots is shown in Appendix B.14.

b. Non-fruiting spur leaves as ^{13}C source

Non-fruiting leaves also were a source of current photoassimilates for fruit and vegetative growth during stages I, II and III of fruit development. In all the pulse-labeling dates, ^{13}C fixed by this leaf population was translocated predominantly basipetally to fruit and fruiting wood (Tables 3 and 4). Significant amounts of ^{13}C were detected in the wood subtending the labeled leaves. Acropetal ^{13}C translocation to current season wood and leaves also was observed. A few branches (12%) did not show translocation to either fruiting leaves or different sections of the shoot. The majority of these branches were from the 44 DAFB pulse.

There were significant differences in the absolute amount of ^{13}C recovered for each organ (Tables 3 and 4). Most of the translocated ^{13}C was partitioned to fruit, followed by fruiting spur and non-fruiting spur wood. Lower ^{13}C recoveries were found in fruiting spur leaves and the terminal shoot. The highest ^{13}C recoveries in fruit were detected at 56 DAFB, followed by 25, 75 and 44 DAFB (Table 8). The lowest ^{13}C recovery was found at 40 DAFB. Partitioning of ^{13}C to

non-fruiting and fruiting wood followed an opposite pattern to that of fruit, with wood ^{13}C contents lowest on those dates in which recoveries in fruit were the highest, i.e., 56 and 25 DAFB.

The relative partitioning indicated that between 20% and 50% of the ^{13}C recovered 48 h later was in non-fruiting spur leaves (Figure 6; Appendix B.15). The rest of the ^{13}C was recovered in different organs in the following order: fruit (31 to 71%), fruiting spur wood (5 to 17%), non-fruiting spur wood (3 to 8%), shoot (0 to 0.2%) and fruiting spur leaves (0 to 1%). The highest percentage of ^{13}C partitioned to fruit occurred at 56 DAFB, followed by the 25 DAFB pulse. The rest of the pulse-labeling dates showed a similar partitioning. The lowest ^{13}C recovery in non-fruiting spur leaves was at 56 DAFB. Greater detail for the minimal ^{13}C partitioning to shoots is shown in Appendix B.16.

c. Terminal shoot leaves as ^{13}C source

Current season shoot leaves were an additional source of photoassimilates for fruit and vegetative growth during stages I, II and III of fruit development. In all the pulse-labeling dates, ^{13}C fixed by this leaf population was translocated basipetally to non-fruiting spur leaves, non-fruiting spur wood, fruiting spur leaves, fruiting spur wood and fruit (Tables 3 and 4). Several branches (16%) did not translocate ^{13}C to either fruiting or non-fruiting leaves. Significant amounts of ^{13}C were detected in leaves and wood of current season shoots. However, a

preferential translocation to fully expanded leaves and developing leaves in the basal and medial position of the shoot was detected at all labeling dates.

There were significant differences in the absolute amount of ^{13}C recovered for each organ (Tables 3 and 4). Most of the translocated ^{13}C was partitioned to fruit, followed by either non-fruiting spur wood or fruiting spur wood (Tables 3 and 4). Lower ^{13}C recoveries were found in fruiting spur and non-fruiting spur leaves. The highest ^{13}C contents in fruits were found at 56 DAFB, followed by 75 DAFB (Table 9). At these dates, shoots were ~30 cm (~20 leaves) and ~34 cm (~22 leaves) in length (Table 2). The lowest recovery was detected at 25 DAFB, when shoots were ~10 cm in length and had only ~10 leaves. ^{13}C recoveries in fruiting and non-fruiting wood were lowest at 56 DAFB.

The relative partitioning indicated that between 31% and 69% of the ^{13}C recovered 48 h later was in terminal shoot leaves (Figure 7; Appendix B.17). The rest of the ^{13}C was found in different organs in the following order: fruit (18 to 59%), non-fruiting spur wood (4% to 16%), fruiting spur wood (5 to 11%), fruiting spur leaves (0 to 1%) and non-fruiting spur leaves (0 to 1%). The highest percentage of ^{13}C partitioned to fruit occurred at 56 DAFB, followed by 75 and 25 DAFB pulses. The lowest was measured 44 DAFB. The lowest ^{13}C recoveries in shoot leaves and wood were found at 56 DAFB. More detailed relative ^{13}C partitioning in shoots is shown in Figures 8 and 9.

Partitioning of ^{13}C from distal leaf populations

In most of the organs and tissues analyzed for ^{13}C content, translocation from the leaf populations distal from the organ of interest did not differ significantly (Tables 10, 11 and 12). Two exceptions were fruit and non-fruiting spur wood. As indicated above, fruit attracted more ^{13}C translocated from fruiting spur leaves (Table 10). However, at 56 DAFB, non-fruiting spur contributed as much as fruiting spur leaves to fruit growth. In addition, non-fruiting spur and terminal shoot leaves contributed similar amounts of ^{13}C at 75 DAFB. With regard to non-fruiting spur wood tissues, their ^{13}C content varied significantly depending on the pulsed leaf population and fruit developmental stage (Table 11). When fruiting spur leaves were pulsed, the amount of ^{13}C recovered in non-fruiting spur wood was lower than when non-fruiting or terminal shoot leaves were pulsed. For the latter treatments, the highest ^{13}C levels recovered in non-fruiting wood were at 40 and 75 DAFB, while the lowest were measured at 25 and 56 DAFB.

^{13}C partitioning in individual fruit

At 48 h after ^{13}C labeling, fruit pulsed directly with $^{13}CO_2$ were highly ^{13}C enriched at all pulse-labeling dates. The highest ^{13}C contents in individual fruits were at 25 and 56 DAFB, with 218 and 221 µg ^{13}C/fruit (Table 13). The lowest contents were at 40 and 75 DAFB, with 116 and 119 µg ^{13}C/fruit.

The absolute partitioning between pericarp (flesh) and pit (seed) changed significantly between dates (Table 13). At 25 and 40 DAFB, 75 and 82% of the total ^{13}C in the fruit was partitioned to the pit (Figure 10; Appendix B.18). At the beginning of stage III (44 DAFB), the partitioning between fruit tissues was similar, although more ^{13}C was partitioned to the pit. At 56 and 75 DAFB, 78% and 92% of the total ^{13}C recovered was in the pericarp. Across pulse-labeling dates, the highest ^{13}C content in the pericarp was detected at 56 DAFB (stage III), while the lowest occurred at 40 DAFB (stage II). In the case of the pit, the highest ^{13}C content was found at 25 DAFB, while the lowest occurred at 75 DAFB.

Discussion

The partitioning of newly-fixed C during fruit development was studied in 2-year-old sweet cherry branches of 'Ulster'/GI6, a semidwarfing scion/rootstock combination. ^{13}C was used as a tracer to characterize and quantify the relative partitioning of ^{13}C, fixed as $^{13}CO_2$, by the three major photosynthetic sources of assimilates within a 2-year-old limb. Branch sections containing the fruiting spur, non-fruiting spur, and terminal shoot leaf populations were labeled with ^{13}C during stages I, II and III of fruit development. Our objective was to elucidate the contribution of each leaf population as a source of current photoassimilates for fruit and shoot growth during the entire period of fruit development.

Translocation patterns differed depending on the leaf population that was pulsed. Fruiting spur, non-fruiting spur and shoot leaves exported ^{13}C-photoassimilates to both fruit and shoot growth during stages I, II and III. Fruiting spur leaves exported ^{13}C to their own fruit and wood. These leaves also translocated ^{13}C to non-fruiting spurs and terminal shoots. However, a few branches did not translocate ^{13}C out of the fruiting section. Non-fruiting spur leaves exported ^{13}C bidirectionally to fruit and terminal shoots. The predominant translocation of these leaves was basipetally to the fruit. A few branches did not translocate ^{13}C to the shoot. These results are in agreement with our prior study carried out on 'Sam'/Gisela 5 limbs in which non-fruiting spur leaves translocated most of the labeled C to fruit during final swell (Chapter 2). Current season growth leaves translocated ^{13}C basipetally to the non-fruiting and fruiting sections. As in the case of spur leaves, the predominant ^{13}C export was towards fruit. When either non-fruiting spur or terminal shoot leaves were labeled, the wood located basipetally from these sources was highly enriched in ^{13}C. Unidirectional and bidirectional transport from different leaf populations have been reported for apple (*Malus domestica* Borkh.) (Hansen, 1969; Corelli Grappadelli et al., 1994), sour cherry (*Prunus cerasus* L.) (Kappes and Flore, 1986; Toldam-Andersen, 1998), pecan (*Carya illinoensis* Koch.) (Davis and Sparks, 1974), grape (*Vitis vinifera* L.) (Hale and Weaver, 1962), cranberry (*Vaccinium macrocarpon* Ait.) (Roper and Klueh, 1996), and red raspberry (*Rubus idaeus* L.)

(Privé et al., 1994) among others. In these species the predominant translocation is either to fruit or to current season shoots.

^{13}C distribution among different organs varied, depending on the labeled source (leaf population) and the developmental stage. The ^{13}C relative partitioning indicated a predominant ^{13}C distribution to fruit regardless of the photoassimilate source and the stage of fruit development. Fruit were a stronger sink than current season shoots and had the highest ^{13}C enrichments. The highest fruit sink strength was detected 56 DAFB (stage III). In peach (*Prunus persica* (L.) Batsch), fruit were a stronger sink for photoassimilates than were stems (Grossman and DeJong, 1995). In sour cherry, the highest fruit sink strength was during stage III (Flore and Layne, 1999). During stage I, sink activity of small fruit was an important factor to attract ^{13}C assimilates for cell division since the highest ^{13}C atom % excess per unit basis was detected at this time. Increased sink activity of fruit promotes the uptake of assimilates, which in turn accelerates its growth rate (Hansen, 1987). As in peach (DeJong and Grossman, 1995), the competitive ability of sweet cherry fruit varies during development by changing individual sink activity and/or total sink strength. In sour cherry, small fruit act strongly as sinks by removing C from the translocation system, which is explained by their high specific growth rate in young fruit (Toldam-Andersen, 1998). Similarly in grape, sink activity of the immature small berry was important for DW accumulation during the first week of growth when cell expansion is slow (Coombe, 1989).

There were significant differences among ^{13}C sources regarding the amount of ^{13}C partitioned to fruit. The more distant the ^{13}C source, the lower the amount of ^{13}C that was detected in fruit. On average, fruiting spur leaves contributed more ^{13}C (60 to 80%) to fruit than did non-fruiting spur (30 to 70%) and shoot leaves (18 to 60%). This was the trend for stage I, stage II and the beginning and end of stage III. The exception was at 56 DAFB (mid-stage III, ~812 GDD), during rapid cell enlargement and dry matter accumulation, when the amounts of ^{13}C partitioned to fruit were significantly higher than those detected from any of the ^{13}C-sources at other pulse labeling dates. The lowest ^{13}C contents in pulsed leaves of all sources were found in stages I (25 DAFB) and III (56 DAFB). In addition, all wood of different sections had reduced ^{13}C levels during mid-stage III. The presence of fruit actively demanding photoassimilates has been reported to reduce CH_2O levels in sweet cherry leaves on shoot, non-fruiting spur and fruiting spur wood (Roper et al., 1988). Similarly, in Japanese pear (*Pyrus pyrifolia* Nakai) the ^{13}C content of spur leaves decreased during the period of rapid fruit growth (Teng et al., 2001).

At 48 h after pulsing, the amount of ^{13}C recovered per branch was 11% and 44% lower than the initial ^{13}C fixation across all leaf sources. The highest recoveries (62% to 89% of the ^{13}C fixed initially) were at 25 and 56 DAFB. Differences in recoveries may have been a consequence of either export out of the limb or respiratory costs of different sink organs. In a previous experiment using the same scion/rootstock combination (Chapter 2), girdling at the base of 2-year-

old branches increased fruit size and shoot length, indicating CH_2O export out of the limb. Thus, examiningboth studies, it appears some ^{13}C was exported out of the branch during fruit development. In peach, branch autonomy with respect to C partitioning during stage III of fruit development was not absolute, indicating C translocation from other sources (Marsal et al., 2003). In addition, some ^{13}C must have been used in respiration. Respiration costs are greatest for growth of new organs such as developing fruit and shoots and roots (Lakso et al., 1999). Loescher et al. (1986) estimated that 16 to 23% of the total CH_2O requirements for sweet cherry fruit growth were used in respiration, while in peach 16 to 20% of the seasonal CH_2O requirements were used by developing fruit (DeJong and Walton, 1989). A high respiration rate in persimmon (*Diospyrus khaki* L.) fruit was important for maintaining sink strength during final swell (Nakano et al., 1998).

Fruit were able to photosynthesize $^{13}CO_2$ during stages I, II and III. Fruit photosynthesis has been reported for sour cherry (Kappes and Flore, 1986; 1989). The highest ^{13}C fixations were detected during stages I and II. It is likely that the ^{13}C fixed directly by fruit had some impact in the C budget of individual fruits (Hansen, 1970; DeJong and Walton, 1989; Kappes, 1985). In sour cherry, fruit gross photosynthesis contributed 19%, 30% and 1.5% of the CH_2O used during stages I, II and III of fruit development, respectively; ~70% of the CH_2O was incorporated into fruit dry matter, while the rest was used in dark respiration (Flore and Layne, 1999). In apple, fruit photosynthesis is < 15% of the total C supply during the season (Jones, 1981), although it may contribute to fruit

growth early in the season (Lakso et al., 1999). In this study, 2 days after labeling, ^{13}C partitioning between pericarp (flesh) and pit (seed) varied significantly depending on the fruit developmental stage. The highest ^{13}C contents in single fruits were detected during stage I (25 DAFB) and the peak of final swell (56 DAFB), while the lowest ^{13}C contents were measured during stage I and at the end of stage III (75 DAFB). During stages I and II, more ^{13}C (74 to 80%) was partitioned to the pit. However, late in stage III (56 DAFB and 75 DAFB), most of the total ^{13}C (77% to 83%) was recovered in the pericarp. Teng et al. (2001) reported that Japanese pear fruit accumulated most of the ^{13}C in its flesh during the period of active growth. Similar results have been reported for peach fruit (Corelli-Grappadelli et al., 1996).

Current season growth was not a strong sink for assimilates during sweet cherry fruit development. Minimal amounts of ^{13}C (< 1%) were found in shoots when fruiting and non-fruiting spur leaves were labeled. This trend was similar among the 3 fruit developmental stages. Kappel (1991), using 'Lambert' sweet cherry on *Prunus avium* L. seedling, a vigorous rootstock, reported that current season growth had a greater sink strength for photosynthates than fruit since more DW accumulated in shoots. This would imply that differences in source-sink relationships and relative partitioning might depend on the genotype of the rootstock (Moing and Gaudillere, 1992; Caruso et al., 1997). In the current study, when terminal shoots were labeled directly, their mature basal leaves and developing medial leaves had higher ^{13}C enrichments than those of young apical

leaves and wood. Young apical leaves imported minimal amounts of ^{13}C from spur leaves. More mature leaves at the base of the terminal shoot must have synthesized adequate CH_2O to be partitioned between the shoot tip and fruit. These results agree with those found in a prior experiment using 'Sam' on Gisela 5, for which young leaves on current season growth did not constitute a strong sink for ^{13}C photoassimilates during stage III of fruit development (Chapter 3). Interestingly, shoots exported ^{13}C to fruit, even during very early stages of development (i.e., with only ~10 cm in length and ~10 leaves). In sour cherry, shoots become net CH_2O exporters 17 days after budbreak (Kappes, 1985). Apple extension shoots begin C export with ~9 to 17 leaves (Lakso and Corelli-Grappadelli, 1992; Corelli-Grappadelli et al., 1994), while peach extension shoots begin exporting to fruit 30 DAFB (Corelli-Grappadelli et al., 1996).

The highest ^{13}C export from terminal shoots (60% of the total C recovered per branch) to other organs was detected at 56 DAFB. At this time, fruit were rapidly accumulating dry matter and the terminal shoot (~ 30 cm in length and 20 leaves) began decreasing its growth rate. The lowest ^{13}C export from shoots was detected at the beginning of stage III (44 DAFB, 570 GDD), when shoots were elongating rapidly. According to these results, the terminal shoot not only supported its own growth, but was a C source for fruit growth as well. Roper et al. (1987) proposed that during stage III, part of the photoassimilates used for fruit growth might come from single leaves on shoots since spur leaves were not able to support optimal fruit growth. As mentioned above, fruit was always the

strongest aerial sink for current photoassimmilates translocated from different leaf sources. The priority of the fruit growth over vegetative growth is further supported by the fact that current season shoots began exporting ^{13}C to fruit as early as 25 DAFB. It is likely that fruit growth had a detrimental effect on shoot development in sweet cherry combinations on dwarfing and semi-dwarfing rootstocks. Grossman and DeJong (1995) reported that the presence of fruit decreased stem length and DW accumulation in a peach cultivar with a short fruit growth period, suggesting competition for C between vegetative growth and fruit.

Significant amounts of ^{13}C were found in structural wood of directly labeled and unlabeled sections, indicating active translocation at the moment of branch removal. A portion of this ^{13}C must have been utilized for primary growth of shoots and secondary growth of older wood as observed in apricot (Costes et al., 2000). When fruiting and non-fruiting spur leaves were ^{13}C labeled, wood subtending fruit was the most ^{13}C enriched. When shoot leaves were labeled, non-fruiting spur wood had higher ^{13}C levels than terminal shoot wood. For this leaf population, the lowest ^{13}C partitioning to non-fruiting wood was at 56 DAFB, when levels were considerably lower than at other pulse-labeling dates. During stage II (40 DAFB) and late stage III (75 DAFB), translocation from the shoot to fruit was reduced since ^{13}C content in non-fruiting spur wood increased. These observations might suggest the presence of sink limiting condition at stage II and late during fruit development. Sink limitations due to

resource restrictions and decreased transport and phloem unloading capacities have been documented for late maturing peach and plum (*Prunus salicina L.*) cultivars (Pavel and DeJong, 1993; DeJong and Grossman, 1995). In sweet cherry, reductions in translocation might influence C partitioning to fruit in the short term (Michin et al., 1997), although the vascular system responds is able to generate a higher translocation rates to keep the fruit growth rate constant (Bustan et al., 1995; Heuvelink, 1996).

In this study, LA/F ratios were low and varied between 33 and 60 cm^2 LA/F (< 1 to 3 leaves/fruit), which indicates a persistent source limitation during fruit development. Stage I (319-483 GDD) and mid-stage III (753-874 GDD) were periods characterized by stronger resource restrictions in C availability. At stage I, the canopy was still developing and leaves of spurs and extension shoots were competing for CH_2O with young developing fruit (Chapter 5), while at mid-stage III fruit were accumulating dry matter rapidly. Failure to grow to full size potential is assumed to be a consequence of source limitations, i.e., insufficient CH_2O for dry matter accumulation (Berman and DeJong, 1996). However, growth limitations also seem to be determined by processes in the sink (fruit) itself and by genetic constraints (Starck, 1983; Marcelis, 1996; Basile et al., 2002). In highbush blueberry (*Vaccinium corymbosusm* L.), high crop loads during stage I and III imposed source limitations, which affected fruit and vegetative development (Swain and Darnell, 2002). Reduction in CH_2O availability to support potential growth might lead to competition

among individual organs (DeJong and Grossman, 1994; DeJong, 1999). In peach and apple the maximum potential DW accumulation of an individual fruit is achieved when most of the fruit are removed early during development (Grossman and DeJong, 1995; Basile et al., 2002; Palmer, 1992). On the other hand, in kiwifruit (*Actinidia deliciosa* (A.Chev.) C.F. Liang et A.R. Ferguson), shoot elongation during fruit growth had a negative effect on partitioning of ^{13}C into fruit (Amano et al., 1998). In sour cherry, LA/F ratios < 2 are indicative of source limitation, which is especially important during stage III, the period of maximum sink strength (Flore, 1985; Layne and Flore, 1993). In sweet cherry, a higher LA/F ratio has been postulated to be necessary for fresh market fruit quality (Whiting and Lang, 2004). In our study, a low fruit DW at commercial harvest reflected a restriction in photosyntathes to support optimal fruit growth. Fruit loads accounted for more than 50% of the total DW for an individual branch at commercial harvest, indicating high biomass allocation to fruit. It is likely that fruit vs. fruit and fruit vs. shoot competition resulted in smaller fruit and reduced shoot growth, which is a common characteristic of trees on GI roostocks.

The results provide additional information about the contribution of the various leaf populations in sweet cherry canopies as sources of C for developing fruit and vegetative growth. Clearly, the natural balance between reproductive and vegetative growth is not optimal for production of premium quality fruit in high-density sweet cherry orchards with dwarfing and semi-dwarfing GI

rootstocks. To overcome this situation, a more precise management of LA/F ratios is critical to achieve not only an optimal crop load, but also an optimal development of leaf area during the productive years of the orchard (Lang, 2001 a,b).

In summary, in 2-year-old sweet cherry branches, on a semi-dwarfing scion/rootstock combination: (1) spur and shoot leaves constitute a significant sources of C for fruit and vegetative growth; (2) current season (terminal) shoot growth provides a C source for fruit as early as stage I; (3) fruits are a priority sink vs. new shoot growth, in terms of C allocation during the entire period of fruit development; (4) the highest fruit sink strength is during stage III; and (5) resource limitations during fruit development affect final fruit size in semi-dwarfing combinations (i.e., with rootstocks of the GI series). These results provide a physiological foundation for canopy relationships that may help to develop specific orchard management strategies to promote a more sustainable long-term balance between vegetative and reproductive growth in high density sweet cherry orchards.

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Table 1. Fruit quality parameters measured weekly between stages I and III on 'Ulster'/Gisela 6 sweet cherry branches (19 May to 4 Aug, 2003). Fruit remained on the tree until 96 DAFB. Mean ± SE, n= 30.

Developmental stage	DAFB[z]	Fresh Weight (g)	Dry Weight (g)	Diameter (mm)	SS (°Brix)
Stage I	19	0.6±0.1	0.06 ± 0.02	9.7 ± 0.3	3.4 ± 0.2
	26	1.1 ±0.1	0.1 ±0.02	11.7 ± 0.1	6.6 ± 0.4
Stage II	33	1.2 ±0.1	0.2 ± 0.03	12.6 ±0.1	8.6 ± 0.1
	40	1.4 ±0.1	0.3 ± 0.02	13.2 ± 0.1	7.9 ± 0.1
Stage III	47	2.2 ±0.1	0.5 ± 0.01	15.1 ±0.3	10.6 ± 0.3
	54	2.8 ±0.1	0.6 ± 0.01	16.0 ± 0.3	12.5 ± 0.3
	61	5.4 ± 0.2	1.3 ± 0.3	20.4 ±0.3	17.4 ± 0.3
	68	6.4 ± 0.2	1.4 ± 0.2	21.3 ±0.3	19.0 ± 0.3
Terminal bud set	75	7.3 ± 0.2	1.8 ± 0.1	22.4 ± 0.2	22.1 ± 0.3
	82	7.9 ± 0.1	1.8 ± 0.2	23.1 ±0.1	23.2 ± 0.4
	89	7.9 ± 0.2	2.2 ± 0.5	22.0 ±0.2	25.0 ± 0.5
	96	8.2 ± 0.2	2.3 ± 0.3	23.2 ±0.2	25.0 ± 0.3

[z] DAFB: days after full bloom. Full bloom occurred on 30 Apr, 2003.

Table 2. Length and leaf number of current season shoots on 2-year-old 'Ulster'/Gisela 6 sweet cherry branches between full bloom and terminal bud set (30 Apr to 4 Aug, 2003). Mean ± SE (n=170).

Developmental Stage	DAFB[z]	Shoot Length (cm)	Leaf Number	
			Total	Unfolded
Full Bloom	0	0.5 ± 0.1[y]	3.1 ± 0.2	0.7 ± 0.2
Fruit set	5	0.7 ± 0.1[y]	8.5 ± 0.2	6.8 ± 0.3
	12	2.5 ± 0.1	9.7 ± 0.2	7.2 ± 0.2
Stage I	19	6.2 ± 0.1	10.8 ± 0.1	9.3 ± 0.1
	26	9.4 ± 0.2	11.6 ± 0.1	10.0 ± 0.1
Stage II	33	13.9 ± 0.2	13.1 ± 0.1	11.2 ± 0.1
	40	17.9 ± 0.3	15.1 ± 0.2	13.3 ± 0.1
Stage III	47	23.7 ± 0.4	17.3 ± 0.2	15.4 ± 0.1
	54	28.2 ± 0.5	19.1 ± 0.2	17.6 ± 0.2
	61	31.7 ± 0.7	20.4 ± 0.3	19.6 ± 0.2
	68	33.2 ± 0.8	21.2 ± 0.3	20.7 ± 0.3
Terminal bud set	75	33.9 ± 0.9	21.7 ± 0.4	21.8 ± 0.4
	82	34.5 ± 0.9	21.8 ± 0.4	21.8 ± 0.4
	89	34.6 ± 0.9	21.9 ± 0.4	21.9 ± 0.4
	96	34.7 ± 0.9	21.9 ± 0.4	21.9 ± 0.4

[z] DAFB: days after full bloom. Full bloom occurred on 30 Apr, 2003.

[y] At these dates, a few rudimentary shoots were emerging from apical buds.

Table 3. ^{13}C content in different organs of a 2-year-old 'Ulster'/Gisela 6 sweet cherry branch during stages I and II of fruit development (25 DAFB, 25 May and 40 DAFB, 9 Jun). Mean ± SE, n=5.

Leaf population	µg ^{13}C/Organ or Tissue								
	Fruit	FS leaves	NFS leaves	TS wood	TS basal leaves	TS medial leaves	TS apical leaves	FS wood	NFS wood
Stage I									
FS[z] leaves	13253.0 a[y] ± 1457.4	8484.5[x] ± 496.3	165.7 b ± 87.6	3.6 b ± 1.7	10.1 b ± 7.1	15.1 b ±11.5	1.3 b ± 0.4	802. b ± 199.0	138.4 b ± 47.8
NFS[z] leaves	10977.0 a ± 1218.1	33.9 c ± 10.4	9992.6[x] ± 1204.7	6.7 c ± 2.9	16.6 c ± 5.6	13.9 c ±3.7	2.5 c ± 0.6	2055.3 b ± 472.7	999.1 bc ± 166.7
TS[z] leaves	2621.7 a ± 501.7	22.1 c ± 15.4	35.9 c ±13.1	493.5[x] ± 146.5	1568.7[x] ± 294.5	2368.3[x] ±301.0	784.4[x] ± 18.7	746.3 bc ± 129.7	752.3 b ± 149.9
Stage II									
FS leaves	10422.0 a[y] ± 683.2	6404.8 ± 762.7	47.2 d ± 4.7	1.5 d ± 0.7	5.0 d ± 2.4	0.8 d ±0.4	0.6 d ± 0.4	1789.4 b ± 385.6	121.2 cd ± 87.2
NFS leaves	6274.3 a ± 1134.8	138.4 d ± 78.7	8405.4[x] ± 735.8	10.4 d ± 4.3	17.4 d ± 10.7	3.5 d ±1.3	8.6 d ± 6.2	3355.2 b ± 534.8	1519.0 c ± 106.3
TS leaves	3951.9 a ± 3951.9	3.8 c ± 0.4	21.9 c ± 9.4	1078.3[x] ± 307.2	2952.7[x] ± 343.9	4169.8[x] ±603.3	1330.4[x] ± 259.6	1766.0 b ± 405.3	3019.3 ab ± 761.9

[z] FS: fruiting spur; NFS: non-fruiting spur; TS: terminal shoot.

[y] Means within a row followed by the same small letter are not significantly different at $\alpha = 0.05$ and $\alpha = 0.01$.

[x] Leaf population that was directly labeled with $^{13}CO_2$. Organ/tissue was not considered in statistical analysis.

Table 4. ^{13}C content in different organs of a 2-year-old 'Ulster'/Gisela 6 sweet cherry branch during stage III of fruit development (44 DAFB, 13 June; 56 DAFB, 25 Jun; 75 DAFB, 14 Jul). Mean ± SE, n=5.

Leaf	µg ^{13}C/Organ								
Population	Fruit	FS leaves	NFS leaves	TS wood	TS basal leaves	TS medial leaves	TS apical leaves	FS wood	NFS wood
44 DAFB[z]									
FS[y] leaves	12506.0 a[x] ± 1234.6	9123.7[w] ± 527.2	0.0 c ± 0.0	0.3 c ± 0. 3	0.0 c ± 0.0	0.0 c ± 0.0	0.0 c ± 0.0	1825.8 b ± 391.2	0.0 c ± 0.0
NFS[y] leaves	7241.6 a ± 1562.4	0.0 c ± 0.0	10684.0 ± 1034.4[w]	46.2 c ± 34.0	1.6 c ± 1.5	29.1 c ± 25.3	39.7 c ± 3.5	3585.7 b ± 1415.1	1747.2 bc ± 366.9
TS[y] leaves	3475.8 a ± 533.4	9.6 d ± 1.0	0.0 d ± 0.0	1764.8[w] ± 327.9	4960.6[w] ± 761.9	4247.1[w] ± 585.6	2724.2[w] ± 368.7	927.9 c ± 103.2	1853.0 b ± 366.6
56 DAFB									
FS leaves	28531.0 a[x] ± 969.0	6581.6 ± 327.9	116.5 b ± 74.5	10.0 b ± 3.2	17.1 b ± 3.8	7.6 b ± 2.1	5.4 b ± 1.1	935.3 b ± 242.6	54.8 b ± 9.1
NFS leaves	24451.0 a ± 932.0	187.3 c ± 128.2	68 92.3[w] ± 1392	26.3 c ± 4.3	29.2 c ± 8.4	24.4 c ± 5.9	13.0 c ± 3.7	1767.5 b ± 290.3	1136.3 bc ± 198.7
TS leaves	12073.0 a ± 3368.9	69.2 b ± 19.0	71.4 c ± 31.2	512.9[w] ± 59.4	1774.0[w] ± 380.0	1733.2[w] ± 105.3	1335.1[w] ± 321.6	887.7 b ± 101.0	660.6 b ± 87.4
75 DAFB									
FS leaves	14685.0 a[x] ± 2007.3	10259.0[w] ± 1689.6	55.1 ± 8.2 b	22.5 b ± 7.7	19.4 b ± 2.7	24.3 b ± 9.2	7.6 b ± 2.3	1469.2 b ± 339.4	110.0 b ± 11.2
NFS leaves	8949.5 a ± 1923.2	48.4 c ± 25.8	13092.0[w] ± 1371.8	42.7 c ± 16.8	51.1 c ± 22.1	36.8 c ± 13.4	17.4 c ± 6.2	3017.5 b ± 783.7	1297.9 bc ± 213.0
TS leaves	7916.1 a ± 1782.7	12.2 c ± 4.5	43.5 c ± 10.3	1518.1[w] ± 175.9	5073.5[w] ± 832.3	4494.7[w] ± 559.8	2653.7[w] ± 774.1	2969.0 b ± 745.6	2587.4 b ± 229.8

[z] DAFB: days after full bloom. Full bloom occurred on 30 Apr, 2003.

[y] FS: fruiting spur; NFS: non-fruiting spur; TS: terminal shoot.

[x] Means within a row followed by the same small letter are not significantly different at $\alpha = 0.05$ and $\alpha = 0.01$.

[w] Leaf population that was directly labeled with $^{13}CO_2$. Organ/tissue was not considered in statistical analysis.

Table 5. Total ^{13}C content (mg ^{13}C) in pulsed fruiting spur, non-fruiting spur and terminal shoot leaves at 0 h after each ^{13}C pulse-labeling, and total ^{13}C content recovered for the whole branch at 48 h after each ^{13}C pulse-labeling. Calculations are based on total DW of organs. Mean ± SE, n=5.

Leaf Population	mg ^{13}C/branch											
	DAFB[y]											
	25			40			44			56			75		
0 h after pulse												
FS[z] leaves	42.9 ± 5.5	a[x]	A[w]	34.9 ± 3.2	a	A	36.8 ± 5.6	a	A	47.1 ± 6.0	a	A	46.0 ± 5.3	a	A
NFS[z] leaves	34.5 ± 5.2	a	A	31.8 ± 4.5	a	A	38.1 ± 2.7	a	A	38.9 ± 3.4	a	A	42.7 ± 5.0	a	A
TS[z] leaves	13.5 ± 3.5	a	A	32.7 ± 4.5	a	A	35.2 ± 8.3	a	A	30.8± 5.8	a	A	44.7 ± 6.5	a	A

48 h after pulse	25	40	44	56	75
FS leaves	26.4 ± 2.3[v]	20.8± 1.9	26.9 ± 2.6	36.9 ± 1.5	28.3 ± 4.5
NFS leaves	24.1 ± 3.1	19.7 ± 2.6	23.4 ± 4.5	34.5 ± 2.3	26.6 ± 4.3
TS leaves	9.4 ± 1.8	18.3 ± 3.3	20.0 ± 3.1	19.1 ± 4.5	27.3 ± 5.1

[z] FS: fruiting spur; NFS: non-fruiting spur; TS: terminal shoot.

[y] DAFB: days after full bloom. Full bloom occurred on 30 Apr, 2003.

[x] Means within a column followed by the same small letter are not significantly different at $\alpha = 0.05$.

[w] Means within a row followed by the capital letter are not significantly different at $\alpha = 0.05$.

v Value correspond to the total ^{13}C recovered per branch for each leaf population.

Table 6. ^{13}C content in fruit sampled immediately (0 h) after labeling of the fruiting spur leaves at each pulse-labeling date. Mean ± SE, n=5.

Developmental Stage	DAFB[z]	µg ^{13}C/g DW	
Stage I	25	188.3 ± 64.8	a[y]
Stage II	40	69.3 ± 12.5	b
Stage III	44	98.0 ± 17.6	b
	56	8.4 ± 2.7	c
	75	11.6 ± 3.7	c

[z] DAFB: days after full bloom. Full bloom occurred on 30 Apr, 2003.

[y] Means within a column followed by the same small letter are not significantly different at α = 0.05. Obtained p-value < 0.0001.

Table 7. ^{13}C content in different organs of a 2-year-old 'Ulster'/Gisela 6 sweet cherry branch 48 h after pulsing of the fruiting leaf population with $^{13}CO_2$. Mean ± SE, n=5.

DAFB[z]	µg ^{13}C/organ								
	Fruit	FS[w] leaves[x]	NFS[w] leaves	TS[w] basal leaves	TS medial leaves	TS apical leaves	TS wood	FS wood	NFS wood
25	13253.0 ± 1457.4 bc[y]	8484.5[x] ± 496.3	165.7 ± 87.6 a	10.1 ± 7.1 ab	15.1 ± 11.5 a	1.3 ± 0.4 b	3.6 ± 1.7 b	802.0 ± 199.0 b	138.4 ± 47.8 a
40	10422.0 ± 683.2 c	6404.8[x] ± 762.7	47.2 ± 4.7 a	5.0 ± 2.4 b	0.8 ± 0.4 b	0.6 ± 0.4 b	1.5 ± 0.7 b	1789.4 ± 385.6 a	121.2 ± 87.5 ab
44	12506.0 ± 1234.6 bc	9123.7[x] ± 527.2	0.0 ± 0.0 a	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	0.3 ± 0.3 b	1825.8 ± 391.2 a	121.3 0.0 ± 0.0 b
56	28531.0 ± 969.9 a	6581.6[x] ± 327.9	116.5 ± 74.5 a	17.1 ± 3.8 ab	7.6 ± 2.1 a	5.4 ± 1.1 a	10.0 ± 3.2 b	935.3 ± 242.6 ab	54.8 ± 9.1 ab
75	14685.0 ± 2007.3 b	10259.0[x] ± 1689.6	55.1 ± 8.2 a	19.4 ± 2.7 a	24.3 ± 9.2 a	7.6 ± 2.3 a	22.5 ± 7.7 a	1469.2 ± 339.4 a	110.0 ± 11.2 ab

[z] DAFB: days after full bloom. Full bloom occurred on 30 Apr, 2003.

[y] Means within in a column followed by the same small letter are not significantly different at $\alpha = 0.01$. Obtained p-value < 0.0001.

[x] Leaf population that was pulsed with $^{13}CO_2$. These leaves were not considered in the statistical analysis.

[w] FS: fruiting spurs; NFS: non-fruiting spurs; TS: terminal shoot.

Table 8. ^{13}C content in different organs of a 2-year-old 'Ulster'/Gisela 6 sweet cherry branch 48 h after pulsing of the non-fruiting spur with $^{13}CO_2$. Mean ± SE, n=5.

DAFB[z]	µg ^{13}C/organ								
	Fruit	FS[w] leaves	NFS[w] leaves	TS[w] basal leaves	TS medial leaves	TS apical leaves	TS wood	FS wood	NFS wood
25	10977.0 b[y] ± 1218.1	33.9 a ± 10.4	9992.6[x] ± 1204.7	16.6 ab ± 5.6	13.9 a ± 3.7	2.5 a ± 0.6	6.7 a ± 2.9	2055.3 a ± 472.7	999.1 b ± 166.7
40	6274.3 c ± 1134.8	138.4 a ± 78.7	8405.4[x] ± 735.8	17.4 ab ± 10.7	3.5 a ± 1.3	8.6 a ± 6.2	10.4 a ± 4.3	3355.2 a ± 534.8	1519.0 a ± 106.3
44	7241.6 bc ± 1562.4	0.0 a ± 0.0	10684.0[x] ± 1034.4	1.6 b ± 1.5	29.1 a ± 25.3	39.7 a ± 3.5	46.2 a ± 34.0	3585.7 a ± 1415.1	1747.2 a ± 366.9
56	24451.0 a ± 932.0	187.3 a ± 128.2	6892.3[x] ± 773.9	29.2 ab ± 8.4	24.4 a ± 5.9	13.0 a ± 3.7	26.3 a ± 4.3	1767.5 a ± 290.3	1136.3 ab ± 198.7
75	8949.5 bc ± 1923.2	48.4 a ± 25.8	13092.0[x] ± 1371.8	51.1 a ± 22.1	36.8 a ± 13.4	17.4 a ± 6.2	42.7 a ± 16.8	3017.5 a ± 783.7	1297.9 ab ± 213.0

[z] DAFB: days after full bloom. Full bloom occurred on 30 Apr, 2003.

[y] Means within in a column followed by the same small letter are not significantly different at $\alpha = 0.01$. Obtained p-value < 0.0001.

[x] Leaf population that was pulsed with $^{13}CO_2$. These leaves were not considered in the statistical analysis.

[w] FS: fruiting spurs; NFS: non-fruiting spurs; TS: terminal shoot.

Table 9. ^{13}C content in different organs of a 2-year-old 'Ulster'/Gisela 6 sweet cherry branch 48 h after pulsing of the current season shoots. Mean ± SE, n=5.

DAFB[z]	µg ^{13}C/organ								
	Fruit	FS[w] leaves	NFS[w] leaves	TS[w] basal leaves	TS medial leaves	TS apical leaves	TS Wood	FS wood	NFS wood
25	2621.7 ± 501.7 c[y]	22.1 ± 15.4 b	35.9 ± 13.1 ab	1568.7[x] ± 294.5	2368.3[x] ± 301.0	784.4[x] ± 218.7	493.5[x] ± 146.5	746.3 ± 129.7 b	752.3 ± 149.9 bc
40	3951.9 ± 640.6 bc	3.8 ± 0.4 b	21.9 ± 9.4 b	2952.7[x] ± 343.9	4169.8[x] ± 603.3	1330.4[x] ± 259.6	1078.3[x] ± 307.2	1766.0 ± 405.3 b	3019.3 ± 761.9 a
44	3475.8 ± 533.4 bc	9.6 ± 1.0 b	0.0 ± 0.0 b	4960.6[x] ± 761.9	4247.1[x] ± 585.6	2724.2[x] ± 368.7	1764.8[x] ± 327.9	927.9 ± 103.2 b	1853.0 ± 366.6 ab
56	12073.0 ± 3368.9 a	69.2 ± 19.0 a	71.4 ± 31.2 a	1774.0[x] ± 380.0	1733.2[x] ± 105.3	1335.1[x] ± 321.6	512.9[x] ± 59.4	887.7 ± 101.0 b	660.6 ± 87.4 c
75	7916.1 ± 1782.7 ab	12.2 ± 4.5 b	43.5 ± 10.3 ab	5073.5[x] ± 832.3	4494.7[x] ± 559.8	2653.7[x] ± 774.1	1518.1[x] ± 175.9	2969.0 ± 745.6 a	2587.4 ± 229.8 a

[z] DAFB: days after full bloom. Full bloom occurred on 30 Apr, 2003.

[y] Means within in a column followed by the same small letter are not significantly different at $\alpha = 0.01$. Obtained p-value < 0.0001.

[x] Leaf population that was pulsed with $^{13}CO_2$. Leaves and wood were not considered in the statistical analysis.

[w] FS: fruiting spurs; NFS: non-fruiting spurs; TS: terminal shoot.

Table 10. ^{13}C content in fruit, wood and fruiting spur leaves on 2-year-old 'Ulster'/Gisela 6 sweet cherry branches 48 h after pulsing the fruiting spur, non-fruiting spur and terminal shoot leaf populations with $^{13}CO_2$. Mean ± SE, n=5.

	µg ^{13}C/organ		
Pulsed Leaf Population:	Fruiting spurs	Non-fruiting spurs	Shoot
DAFB[z]			
FS[y] FRUIT			
25	13253.0 ± 1457.4 a[x]	10977.0 ± 1218.1 b	2621.7 ± 501.7 c
40	10422.0 ± 683.2 a	6274.3 ± 1134.8 b	3951.9 ± 640.6 c
44	12506.0 ± 1234.6 a	7241.6 ± 1562.4 b	3475.8 ± 533.4 c
56	28531.0 ± 969.0 a	24451.0 ± 932.0 b	12073.0 ± 3368.9 c
75	14685.0 ± 2007.3 a	8949.5 ± 1923.2 b	7916.1 ± 1782.7 b
FS WOOD			
25	802.0 ± 199.0 a[x]	2055.3 ± 472.7 a	746.3 ± 129.7 a
40	1789.4 ± 385.6 a	3355.2 ± 534.8 a	1766.0 ± 405.3 a
44	1825.8 ± 391.2 a	3585.7 ± 1415.1 a	927.9 ± 103.2 a
56	935.3 ± 242.6 a	1767.5 ± 290.3 a	887.7 ± 101.0 a
75	1469.2 ± 339.4 a	3017.5 ± 783.7 a	2969.0 ± 745.6 a
FS LEAVES			
25	8484.5 ± 496.3 [w]	33.9 ± 10.4 a[x]	22.1 ± 15.4 a
40	6404.8 ± 762.7 [w]	138.4 ± 78.7 a	3.8 ± 0.4 a
44	9123.7 ± 527.2 [w]	0.0 ± 0.0 a	9.6 ± 1.0 a
56	6581.6 ± 327.9 [w]	187.3 ± 128.2 a	69.2 ± 19.0 a
75	10259.0 ± 1689.6 [w]	48.4 ± 25.8 a	12.2 ± 4.5 a

[z] DAFB: days after full bloom. Full bloom occurred on 30 Apr, 2003.
[y] FS: fruiting spur.
[x] Means within a row followed by the same small letter are not significantly different at $\alpha = 0.05$.
[w] Fruiting spur leaves were pulsed directly with $^{13}CO_2$. Leaves were not considered in statistical analysis

Table 11. ^{13}C content measured in non-fruiting spur leaves on 2-year-old 'Ulster'/Gisela 6 sweet cherry branches 48 h pulsing the fruiting spur, non-fruiting spur and terminal shoot leaf populations with $^{13}CO_2$. Mean ± SE, n=5.

	µg ^{13}C/organ		
Pulsed Leaf Population:	Fruiting spurs	Non-fruiting spurs	Shoot
DAFB[z]			
NFS[y] WOOD			
25	138.4 ± 47.8 b[x]	999.1 ± 166.7 a	752.3 ± 149.9 ab
40	121.2 ± 87.2 c	1519.0 ± 106.3 b	3019.3 ± 761.9 a
44	0.0 ± 0.0 b	1747.2 ± 366.9 a	1853.0 ± 366.6 a
56	54.8 ± 9.1 b	1136.3 ± 198.7 a	660.6 ± 87.4 ab
75	110.0 ± 11.2 c	1297.9 ± 213.0 b	2587.4 ± 229.8 a
NFS LEAVES			
25	165.7 ± 87.6 a[y]	9992.6 ± 1204.7[w]	35.9 ± 13.1 a
40	47.2 ± 4.7 a	8405.4 ± 735.8[w]	21.9 ± 9.4 a
44	0.0 ± 0.0 a	10684.0 ± 1034.4[w]	0.0 ± 0.0 a
56	116.5 ± 74.5 a	6892.3 ± 13092.0[w]	71.4 ± 31.2 a
75	55.1 ± 8.2 a	13092.0 ± 1371.8[w]	43.5 ± 10.3 a

[z] DAFB: days after full bloom. Full bloom occurred on 30 Apr, 2003.
[y] NFS: non-fruiting spur.
[x] Means within a row followed by the same small letter are not significantly different at $\alpha = 0.05$.
[w] Non-fruiting spur leaves were pulsed directly with $^{13}CO_2$. Leaves were not considered in the statistical analysis.

Table 12. ^{13}C content measured in basal, medial and apical leaves and wood of terminal shoots on 2-year-old 'Ulster'/Gisela 6 sweet cherry branches 48 h after pulsing the fruiting spur, non-fruiting spur and terminal shoot leaf populations with $^{13}CO_2$. Mean ± SE, n=5.

	μg ^{13}C/organ		
Pulsed Leaf Population:	Fruiting spurs	Non-fruiting spurs	Shoot[y]
DAFB[z]			
TS[y] BASAL LEAVES			
25	10.1 ± 7.1 a[x]	16.6 ± 5.6 a	1568.7 ± 294.5[w]
40	5.0 ± 2.4 a	17.4 ± 10.7 a	2952.7 ± 343.9[w]
44	0.0 ± 0.0 a	1.6 ± 1.5 a	4960.6 ± 761.9[w]
56	17.1 ± 3.8 a	29.2 ± 8.4 a	1774.0 ± 380.0[w]
75	19.4 ± 2.7 a	51.1 ± 22.1 a	5073.5 ± 832.3[w]
TS MEDIAL LEAVES			
25	15.1 ± 11.5 a	13.9 ± 3.7 a	2368.3 ± 301.0[w]
40	0.8 ± 0.4 a	3.5 ± 1.3 a	4169.8 ± 603.3[w]
44	0.0 ± 0.0 a	29.1 ± 25.3 a	4247.1 ± 585.6[w]
56	7.6 ± 2.1 a	24.4 ± 5.9 a	1733.2 ± 105.3[w]
75	24.3 ± 9.2 a	36.8 ± 13.4 a	4494.7 ± 559.8[w]
TS APICAL LEAVES			
25	1.3 ± 0.4 a	2.5 ± 0.6 a	784.4 ± 218.7[w]
40	0.6 ± 0.4 a	8.6 ± 6.2 a	1330.4 ± 259.6[w]
44	0.0 ± 0.0 a	39.7 ± 3.5 a	2724.2 ± 368.7[w]
56	5.4 ± 1.1 a	13.0 ± 3.7 a	1335.1 ± 321.6[w]
75	7.6 ± 2.3 a	17.4 ± 6.2 a	2653.7 ± 774.1[w]
TS WOOD			
25	3.6 ± 1.7 a	6.7 ± 2.9 a	493.5 ± 146.5[w]
40	1.5 ± 0.7 a	10.4 ± 4.3 a	1078.3 ± 307.2[w]
44	0.3 ± 0.3 a	46.2 ± 34.0 a	1764.8 ± 327.9[w]
56	10.0 ± 3.2 a	26.3 ± 4.3 a	512.9 ± 59.4[w]
75	22.5 ± 7.7 a	42.7 ± 16.8 a	1518.1 ± 175.9[w]

[z] DAFB: days after full bloom. Full bloom occurred on 30 Apr, 2003.

[y] TS: terminal shoot.

[x] Means within a row followed by the same small letter are not significantly different at α = 0.05.

[w] Leaves were pulsed directly with $^{13}CO_2$. Leaves were not considered in statistical analysis.

Table 13. ^{13}C content in pericarp (flesh) and endocarp (pit) of 'Ulster'/Gisela 6 sweet cherry fruit 48 h after labeling to fruiting spur leaves at each date. Mean + SE, n=5.

		^{13}C content (µg ^{13}C/g DW)				
Tissue	DAFB[z]	25	40	44	56	75
Pericarp		55.9 ± 5.5 b BC[y]	21.1 ± 4.0 b C	43.5 ± 7.3 a BC	171.9 ± 48.1 a A	109.3 ± 50.0 a AB
Endocarp		161.6 ± 15.0 a A[x]	95.1 ± 20.5 a AB	90.1 ± 13.7 a B	49.2 ± 16.6 b BC	9.5 ± 1.5 b C

[z] DAFB: days after full bloom. Full bloom occurred on 30 Apr, 2003.

[y] Means within in a column followed by the same small letter are not significantly different at $\alpha = 0.05$. Obtained p-value < 0.0001.

[x] Means within in a row followed by the same capital letter are not significantly different at $\alpha = 0.05$. Obtained p-value < 0.0001.

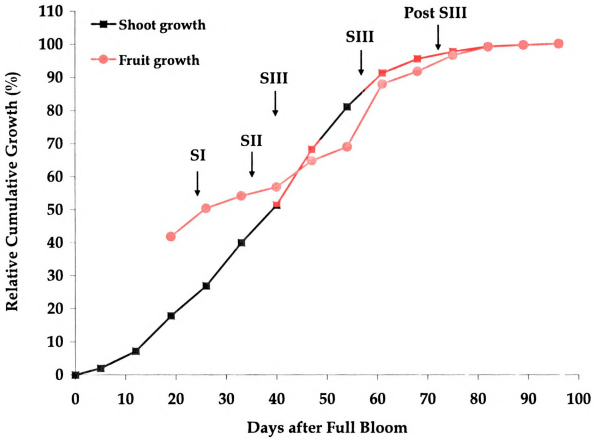

Figure 1. Relative cumulative growth of the terminal shoot and fruit on 2-year-old 'Ulster'/Gisela 6 sweet cherry branches. Calculations are based on weekly measurements of shoot length (cm) and fruit diameter (mm). Mean ± SE, n=30. SI: Stage I, SII: Stage II, SIII: Stage III, Post SIII: Post Stage III.

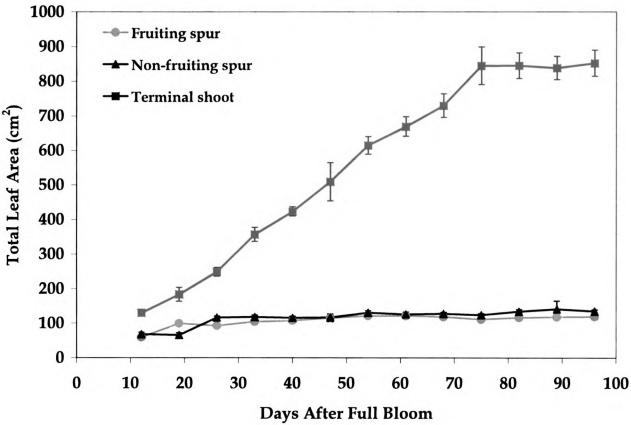

Figure 2. Cumulative leaf area of individual spurs, non-fruiting spurs and terminal shoots of 2-year-old 'Ulster'/Gisela 6 sweet cherry branches. Mean ± SE, n=30.

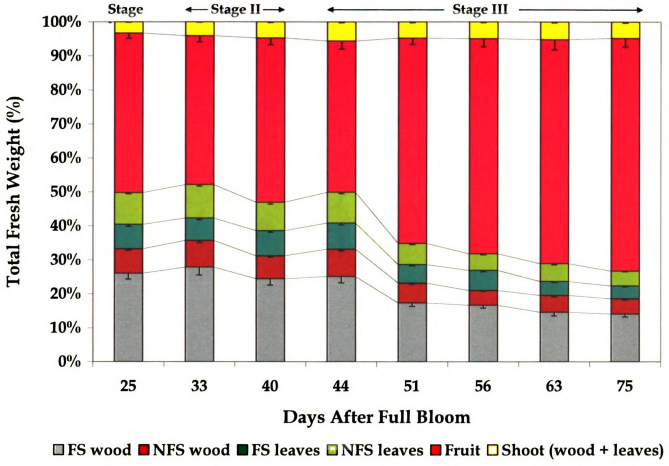

Figure 3. Relative fresh weight partitioning on 2-year-old 'Ulster'/Gisela 6 sweet cherry branches at each pulse-labeling date (May to Jul, 2003). Calculations are based on absolute total FW for each organ (Appendix B.10). Mean ± SE, n=30. FS: fruiting spur; NFS: non-fruiting spur.

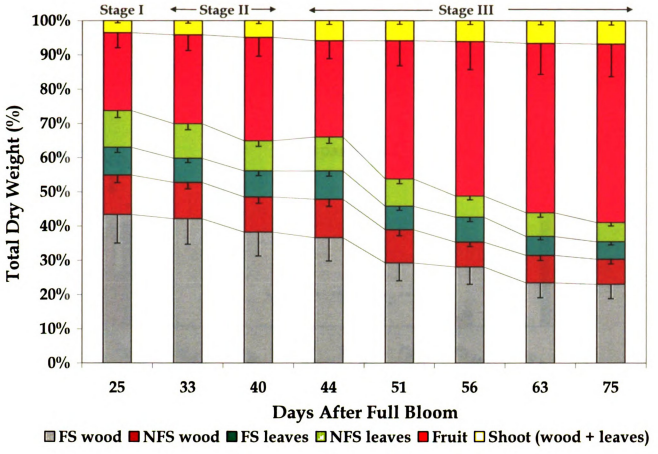

Figure 4. Relative dry weight partitioning in 2-year-old 'Ulster'/Gisela 6 sweet cherry branches at each pulse-labeling date (May to Jul, 2003). Calculations are based on absolute total DW for each organ (Appendix B.12). Mean ± SE, n=30. FS: fruiting spur; NFS: non-fruiting spur.

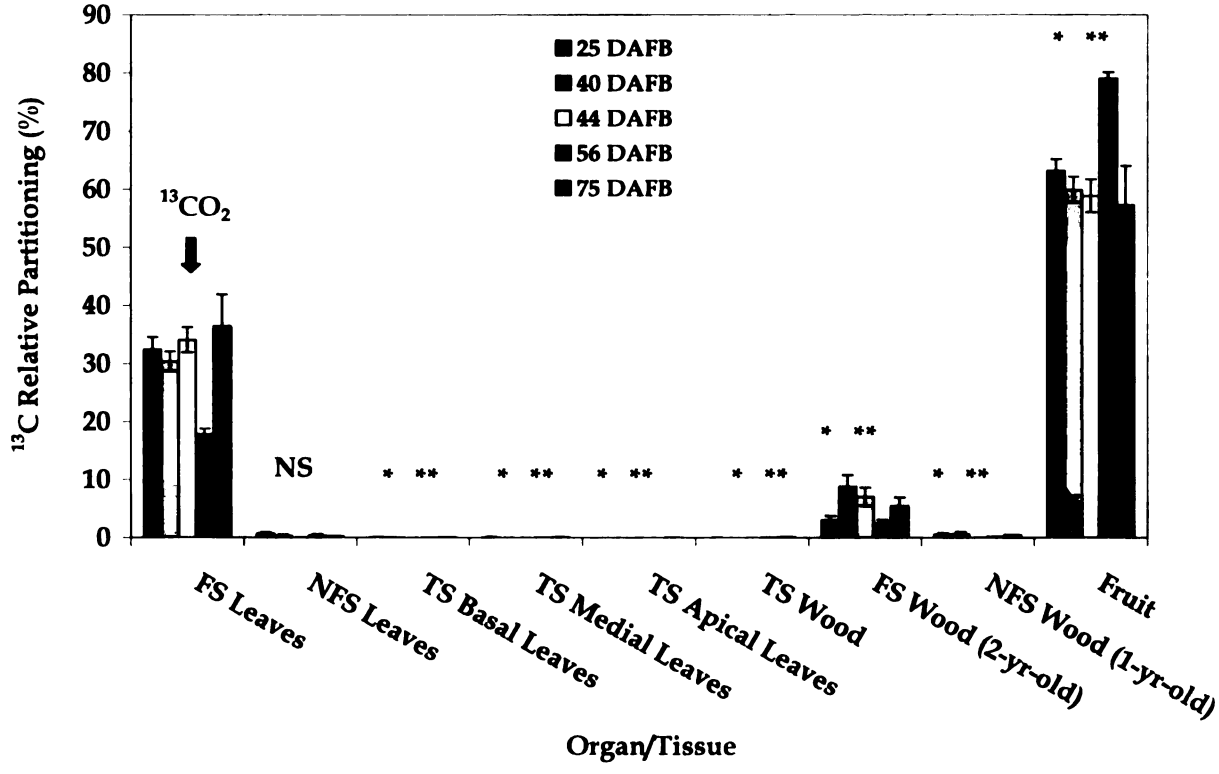

Figure 5. ^{13}C-Relative partitioning among different organs on 2-year-old 'Ulster'/Gisela 6 sweet cherry branches. Calculations are based on absolute amounts of ^{13}C recovered for each organ 48 h after each $^{13}CO_2$ pulse of the fruiting spur leaves. For statistics, see Table 7. Mean ± SE, n=5. FS: fruiting spurs; NFS: non-fruiting spurs; TS: terminal shoot.

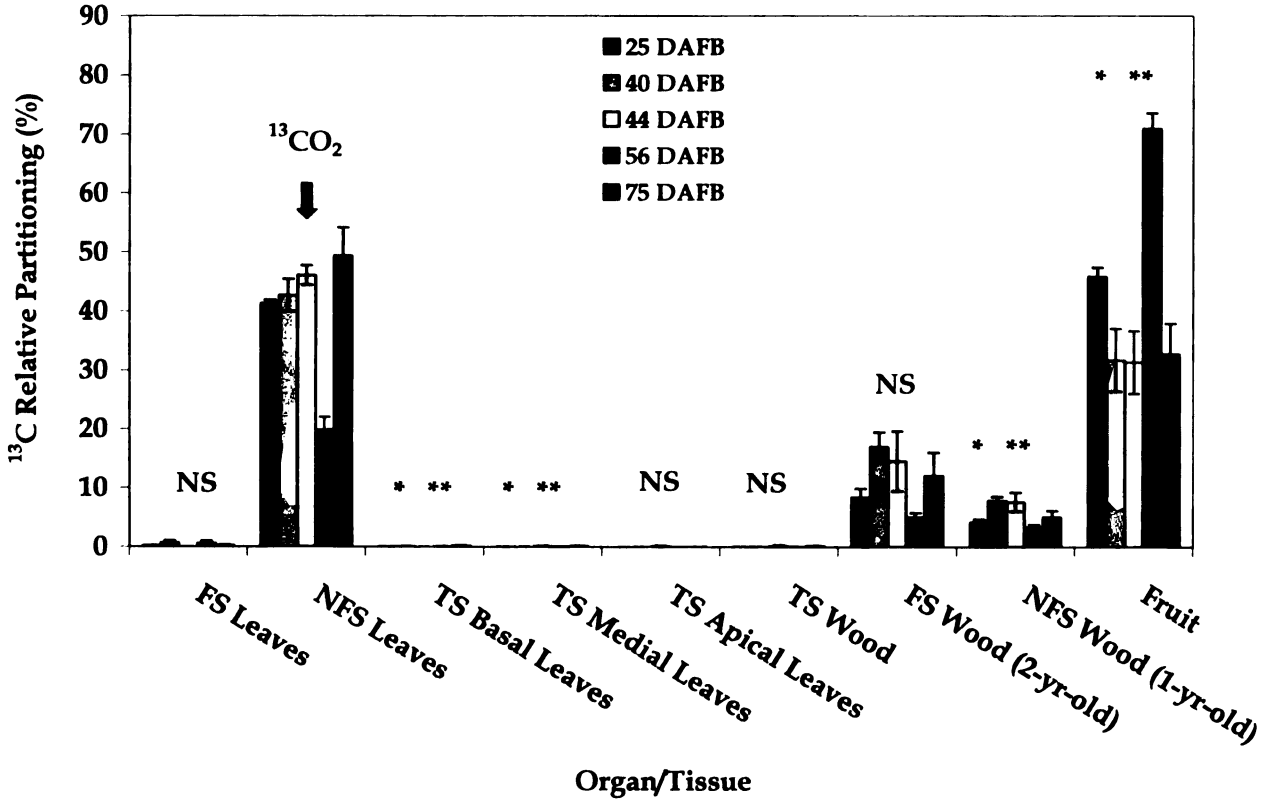

Figure 6. ^{13}C-Relative partitioning among different organs on 2-year-old 'Ulster'/Gisela 6 sweet cherry branches. Calculations are based on absolute amounts of ^{13}C recovered for each organ 48 h after each $^{13}CO_2$ pulse of the non-fruiting spur leaves. For statistics, see Table 8. Mean ± SE, n=5. FS: fruiting spurs; NFS: non-fruiting spurs; TS: terminal shoot.

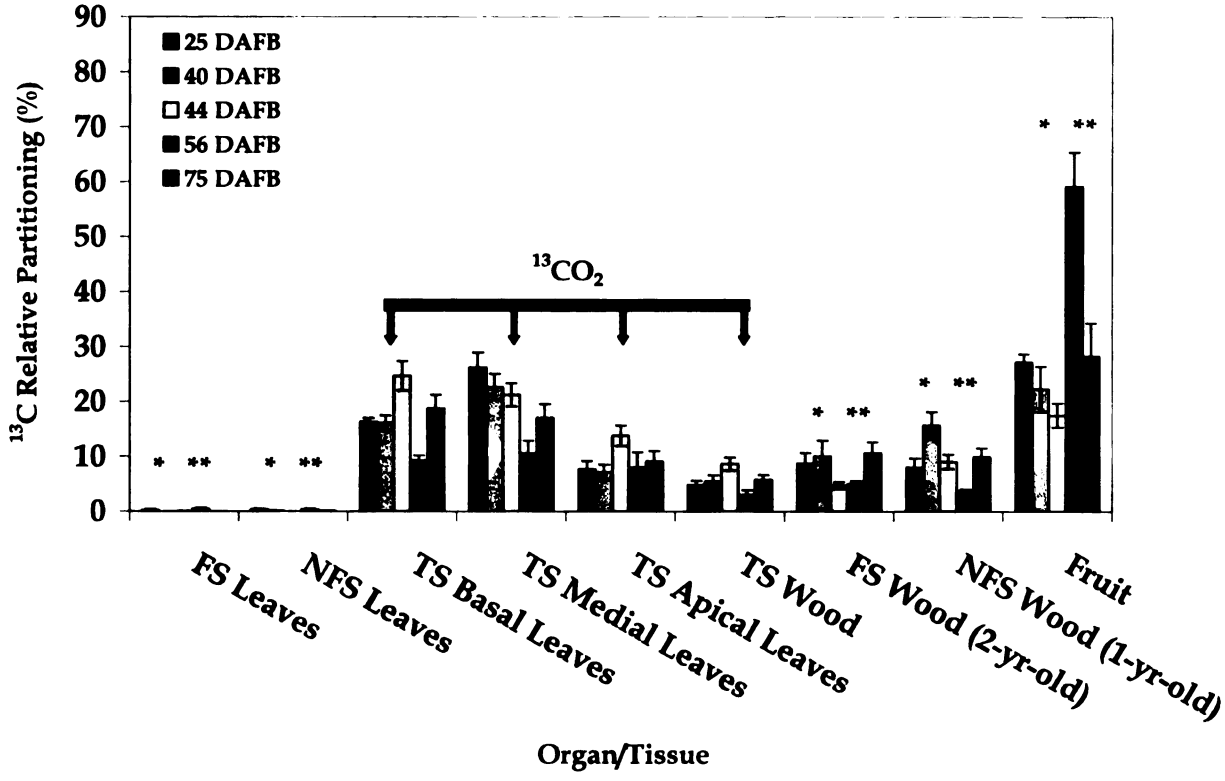

Figure 7. ^{13}C-Relative partitioning among different organs on 2-year-old 'Ulster'/Gisela 6 sweet cherry branches. Calculations are based on absolute amounts of ^{13}C recovered for each organ 48 h after each $^{13}CO_2$ pulse-labeling to terminal shoots. For statistics see Table 9.Mean ± SE, n=5. FS: fruiting spurs; NFS: non-fruiting spurs; TS: terminal shoot.

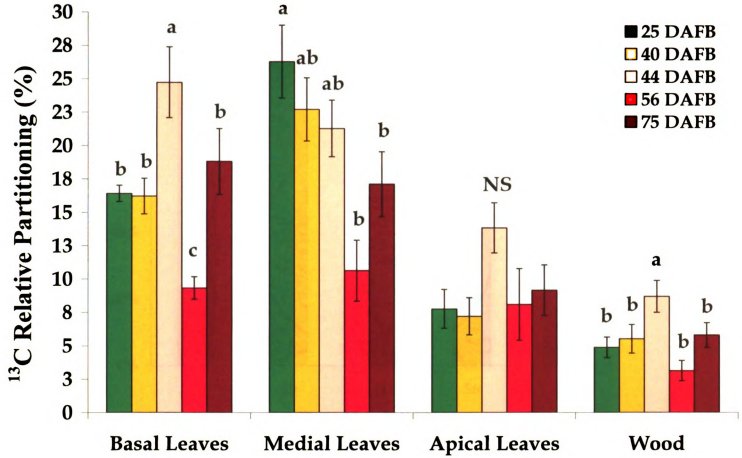

Figure 8. ^{13}C-Relative partitioning among leaves and wood of current season shoots on 2-year-old 'Ulster'/Gisela 6 sweet cherry branches. Calculations are based on absolute amounts of ^{13}C recovered in shoots pulsed directly with $^{13}CO_2$ at each date. Mean ± SE, n=5. FS: fruiting spurs; NFS: non-fruiting spurs; TS: terminal shoot.

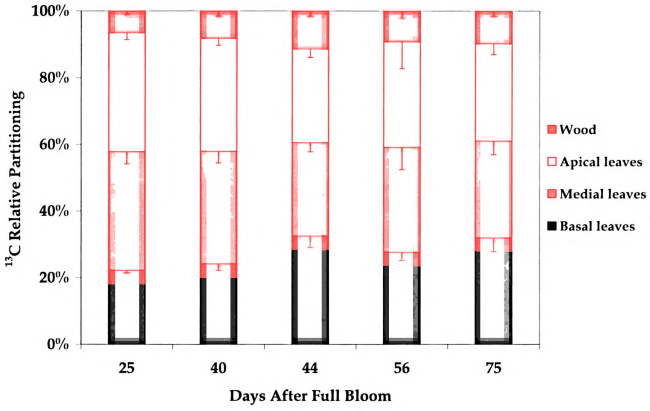

Figure 9. ^{13}C-Relative partitioning among leaves and wood of terminal shoots on 2-year-old 'Ulster'/Gisela 6 sweet cherry branches. Calculations are based on absolute amounts of ^{13}C recovered in shoots pulsed directly with $^{13}CO_2$ at each date. Mean ± SE, n=5.

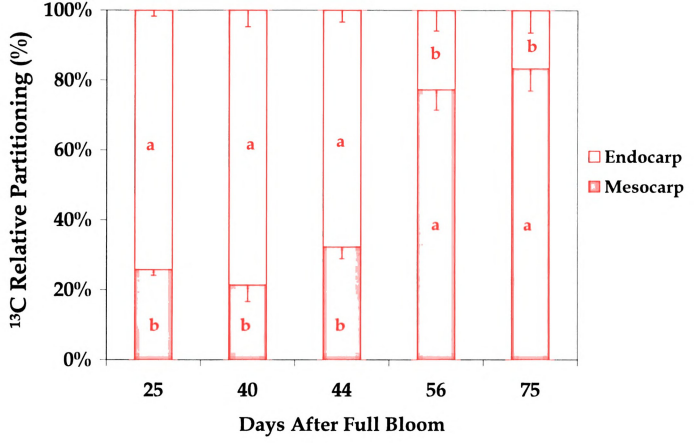

Figure 10. Relative ^{13}C partitioning between pericarp and endocarp of fruit from 2-year-old 'Ulster'/Gisela 6 sweet cherry branches 48 h after $^{13}CO_2$ pulsing of fruiting spur leaves. Calculations are based on absolute amounts of ^{13}C recovered in both tissues at five dates. For statistics, see Table 13. Mean ± SE, n=5.

CHAPTER V

^{13}C- PHOTOASSIMILATE PARTITIONING IN SWEET CHERRY DURING EARLY SPRING

^{13}C- Photoassimilate Partitioning in Sweet Cherry During Early Spring

Keywords: Prunus avium L., storage reserves, transition, spring remobilization, sink, Gisela rootstock, stage I, fruit set.

Abstract

In deciduous fruit trees, storage reserves accumulate during fall and are used for early spring growth. In sweet cherry (*Prunus avium* L.), stored reserves are critical for early growth and there is a transition phase during which current photoassimilates become the primary source for support of reproductive and vegetative sinks. As little is known about this transition, an experiment using 4-year-old 'Regina' sweet cherry on the semidwarfing rootstock, Gisela 6, was established. Using whole canopy exposure chambers, five trees were pulse-labeled with high levels of $^{13}CO_2$ three times during fall (Aug-Sep). At leaf drop, leaves, buds, wood, bark and roots were sampled for gas chromatography-mass spectrometry (GC-MS) analysis of pre-winter storage reserves. The major storage organs (i.e., those with the highest ^{13}C atom % excess) were roots, older wood in the trunk and branches and buds. During spring, newly developing organs (flowers, fruits and leaves) were sampled weekly from bloom to stage III of fruit development for additional GC-MS analysis. The ^{13}C-reserves were

remobilized and partitioned to flowers, fruits and young leaves from before budbreak (side green) until 14 days after full bloom (DAFB). The highest ^{13}C levels in growing sinks were detected between bloom and fruit set. The isotopic composition of new organs differed significantly among organs and phenological stages. Reproductive organs had the strongest sink activity until 14 DAFB, but in terms of total dry matter, non-fruiting spurs had the highest sink strength.

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 materials or substances (organic compounds and nutrients) produced in excess of current requirements and which may be used later in support of metabolism and growth (Priestley, 1960; Glerum, 1980). Carbohydrates (CH_2O), in the form of starch and soluble sugars, are the major component of reserve materials in the tree, but nitrogen (N), in the form of proteins and amino acids, and other minerals also are important (Tromp, 1983). Reserves accumulate in various organs including buds, leaves, branches, stems, roots, seeds and fruits (Kozlowski and Pallardy, 1997).

Storage reserves are important for several life processes such as winter survival, metabolism, respiration, defense, healing, vegetative and reproductive growth, fruit development and new growth in spring (Kandiah, 1979a,b; Flore et al., 1983; Oliveira and Priestley, 1988; Loescher et al., 1990; Kozlowski and Pallardy, 1997). Several authors indicate that the initial stages of spring growth of deciduous fruit trees must depend upon mobilization of reserves accumulated the previous season, until new leaves become photosynthetically competent to provide new assimilates (Priestley, 1960; Quinlan, 1969; Hansen, 1967; Hansen, 1971; Oliveira and Priestley, 1988). Storage reserves are utilized in new growth and respiration to provide energy and cellular structure materials before root N

uptake and photosynthesis occurs in spring (Hansen 1967; Cheng and Fuchigami, 2002).

In sweet cherry (*Prunus avium* L.), flowering usually occurs before leaves are fully expanded, and early stages of reproductive (flower and fruit) and vegetative (spur, extension shoot and root) growth are dependent on the storage reserves accumulated the previous fall (McCammant, 1988; Keller and Loescher, 1989). Thus, early fruit growth seems to be solely dependent upon stored CH_2O reserves (Lang, 2001a). Other deciduous trees such as apple are less dependent on stored reserves since canopies are developed more fully before bloom (Keller and Loescher, 1989; Hansen, 1971). An intermediate situation has been described for Japanese pear (*Pyrus pyrifolia* Nakai), which usually has ~30% of the 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). In sweet cherry, storage reserves, mainly starch, accumulate in different organs after fruit ripening and cessation of shoot extension, reaching a maximum concentration at leaf abscission (Keller, 1986; Keller and Loescher, 1989; McCammant, 1988). In spring, activated meristems draw upon assimilates from storage organs throughout the tree (Tromp, 1983). This continues until new leaves become competent sources of photoassimilates and other parts of the tree require nutrients for metabolism (Tromp, 1983). Premature leaf abscission might result in CH_2O storage limitation; therefore, any type of biological stress (e.g., leaf damage due to pests and

diseases) should be avoided since it might reduce the amount of storage CH_2O available for new growth the next year (Flore, 1994). In sweet cherry and pecan (*Carya illinoensis* Koch.), premature defoliation reduced the accumulation of storage reserves in fall (Worley, 1979; McCammant, 1988). In grapes (*Vitis vinifera* L.), premature defoliation altered the natural translocation pattern of storage reserves and dry matter partitioning (Candolfi-Vasconcelos et al., 1994).

The hypothesis that storage reserves are a source of carbon (C) for initial fruit growth during stage I before current photoassimilates become the major C source was tested in sweet cherry. C partitioning in fall and remobilization of reserves in spring were studied in young, fruiting 'Regina' sweet cherry on the semi-dwarfing rootstock, Gisela 6 (*Prunus cerasus* x *P. canescens*). ^{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 current photosynthates produced during the following spring by newly expanded leaves. ^{13}C constitutes a useful physiological technique since C-3 plants 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).

The main objectives of this study were: (1) to study the distribution of ^{13}C-reserves among organs during early spring following $^{13}CO_2$ assimilation the previous fall; (2) to elucidate whether storage reserves constitute a C source for initial fruit growth during stage I; and (3) to define the C source transition phase,

during which the dependence of new growth on storage reserves shifts to current photosynthate assimilation as the primary source for vegetative and reproductive development.

Materials and Methods

Plant material

The experiment was conducted at Michigan State University's Clarksville Horticultural Experimental Station, Clarksville, Michigan. Five orchard grown 4-year-old sweet cherry trees of 'Regina' on the semi-dwarfing rootstock Gisela 6 (GI6) were selected for pulse-labeling with high $^{13}CO_2$ levels in fall 2002. Trees were trained to a central leader and had similar height, trunk cross-sectional area (TCSA) and leaf area (LA, ~11.3±0.5 m^2). During spring 2003, 2-year-old limbs on 2- and 3-year-old trunk sections bloomed for the first time. Trees were not pruned during the experiment. Trees were fertilized and microsprinkler irrigated following standard commercial practices.

^{13}C labeling

After terminal bud set in 2002, each tree was enclosed in a transparent polyethylene balloon (volume 6.3 m^3) and pulsed for 20 min with $^{13}CO_2$. A total of 5.1 mmol of $^{13}CO_2$ was injected into each balloon. $^{13}CO_2$ was generated by adding 5.0 ml of 80% lactic acid to each of two 1 L plastic bottles containing 5 g of barium carbonate (98 atom% ^{13}C). As the reaction generated $^{13}CO_2$, the plastic

bottles were pumped manually into the balloon via plastic tubing. The labeling was carried out during the morning of sunny days between 10:00 AM and 12:00 PM. The first pulsing was done on 12 Sep and was repeated on 25 Sep and 12 Oct to assure adequate labeling of reserves. Labeling conditions were similar between labeling dates; however, light and temperature levels varied with ambient conditions. Net assimilation rate (A) was measured at each labeling date with a CIRAS-2 infrared gas analyzer (PP-Systems Inc, Haverhill, Massachusetts, USA) and ranged from 3.0 to 14.1 μmol m^{-2} s^{-1} on 12 Sep, 2.2 to 9.3 μmol m^{-2} s^{-1} on 25 Sep, and 0 to 8.6 μmol m^{-2} s^{-1} on 12 Oct.

Growth measurements

Growth of target sinks (flowers, fruits, spurs and current season shoots) was quantified weekly during spring 2003. Four representative shoots per tree were measured for extension growth and leaf number (total folded and unfolded leaves). A sample of 25 fruits was measured for fresh weight (FW), diameter and soluble solids (SS). A sample of 10 fruiting spurs, 10 non-fruiting spurs, 10 shoots, 50 flowers and 50 developing fruit, were collected weekly for FW and dry weight (DW) determinations.

The total number of apical and lateral meristems (fruiting spurs, non-fruiting spurs, and single buds) were counted soon after budbreak (Apr, 2003) and at subsequent terminal bud set (Aug, 2003). This included potential fruits counted during bloom and at fruit set (see Appendix C.1).

^{13}C Sampling and analysis

Two sets of plant tissues were sampled from the labeled trees. The first consisted of trunk, branch and root sections (Appendix C.2), which were collected soon after leaf abscission (2 Nov, 2002) and at budbreak ('side green' stage) of bud development (19 Apr, 2003). Small (5x5 cm) patches of wood and bark, from 2002 growth, were removed from each tree at different locations along the trunk and branches. Wood samples consisted of the xylem tissue. Bark samples included periderm, phloem and cambium. Fruiting spurs and single buds were collected randomly throughout the canopy. Roots were collected in the first 60 cm of the root zone below the surface by excavating at four points around the trunk between and within rows. Roots then were separated according to size into fine (< 1mm), medium (1-5 mm) and coarse (> 5 mm).

The second set of plant tissues were from actively growing vegetative and reproductive aerial organs sampled during spring and summer (May to Jul) of 2003. These included fruiting spurs, non-fruiting spurs, single buds, single flowers, spur flowers, young leaves at the tip of current season growth and fruits (Appendix C.3). Additional samples of the same organs were collected from three unlabeled trees for natural abundance calculations.

Samples were frozen immediately in liquid nitrogen for subsequent ^{13}C enrichment determination by gas chromatography mass spectrometry (GC-MS). The plant material was oven-dried at 70°C for 72 h and subsequently ground

using a Wiley mill (20 and 40 mesh). ^{13}C enrichment was calculated according to Boutton (1991) and Vivin et al. (1996) as follows:

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

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

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

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

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

where the $\delta^{13}C$ (‰) is calculated from the measured carbon isotope ratios of the sample and standard gases Eq. (1). The absolute ratio (R) of a sample is defined by Eq. 2, where $R_{PDB} = 0.0112372$. ^{13}C abundance in the sample is expressed as ^{13}C atom% excess. This value 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 (atom % approx. 1.108%) prior to the $^{13}CO_2$ pulse (Eq. 3 and 4). The new ^{13}C content is calculated for the different organs according to dry mass and ^{13}C concentrations. The absolute amount of recovered ^{13}C for each organ was expressed as μg ^{13}C (Eq. 5).

Statistical Analysis

Analysis of variance was conducted by using PROC MIXED procedures of the SAS statistical analysis program (SAS Institute Inc, Cary, NC). Covariance analyses were conducted for repeated measurements during spring.

Results

Phenological characterization

During spring 2003, 2-year-old limbs (growth of 2001) on 2- and 3-year-old trunk sections bloomed for the first time. The rest of the canopy was comprised of vegetative growth, which included 1-year-old shoots (growth of 2002) with non-fruiting spurs and current season growth (growth of 2003) with single leaves.

The first visual sign of budbreak ('side green' stage) was observed 15 days before full bloom (DBFB), after an accumulation of 148 growing degree-days (GDD, base 4.4°C). At first or early bloom (~ 4 to 6 DBFB; 239-263 GDD), only single and spur flowers and non-fruiting spurs were growing actively. Single flower buds at the upper section of the limb (at the base of 2002 growth) bloomed earlier than spur flower buds (those on 2001 growth). At full bloom (264-287 GDD), growth was evident for the organs described above plus fruiting spurs and young leaves of growing shoots. Fruit set occurred (~ 4 to 7 days after full bloom (DAFB) 288-342 GDD). Stages I, II and III of fruit development occurred

from ~ 8 to 26 DAFB (343-522 GDD), 27 to 38 DAFB (523-680 GDD) and 39 to 63 DAFB (681-1102 GDD), respectively (see Appendix C.4).

The sequential order in which organs began exhibiting visual signs of growth was: single flower, spur flower and non-fruiting spur meristems, terminal shoot meristems, and finally fruiting spur meristems. Dry matter increased for all organs until 35 DAFB. At that point, foliar growth of fruiting and non-fruiting spurs ceased, but shoots and fruits continued accumulating DW (Appendix C.5 and C.6).

Current season shoot length and leaf number increased rapidly from 14 DAFB until 49 DAFB (Table 1). On the other hand, fruit showed a rapid increase in growth beginning 42 DAFB, with 40% of final size achieved during stage III (Table 2). The sigmoidal and double sigmoidal growth curves of current season shoots and fruits, respectively, are shown in Appendix C.7.

^{13}C-labeled storage reserves at leaf abscission

The levels of ^{13}C in all organs at leaf abscission (Nov, 2002) were above natural abundance. However, ^{13}C varied significantly among organs (Figure 1; Appendix C.8 for statistics). The highest ^{13}C levels, expressed as higher ^{13}C atom % excess, were detected in 2- and 3-year-old wood (grown in 1999 and 2000) of the trunk, roots (coarse and medium) and vegetative buds. Significant ^{13}C levels also were found in younger wood (2001) of the trunk, as well as branches, fruiting buds and fine roots. Current season growth (2002) and bark from

sections of various age had significantly lower ^{13}C atom % excess. At leaf abscission, ^{13}C content in leaves was ~74% lower than that measured in leaves immediately after labeling. The ^{13}C loss due to leaf abscission was ~14% of the ^{13}C fixed in fall.

^{13}C-Reserve partitioning at budbreak

The levels of ^{13}C in all organs collected at side green (Apr, 2003) remained above natural abundance (Figure 2; Appendix 8 for statistics). However, ^{13}C-reserves were either remobilized or utilized during the period between leaf abscission and budbreak. The highest ^{13}C atom % excess values at budbreak were detected in fruiting buds, non-fruiting buds and coarse roots. Significant ^{13}C levels also were detected in medium roots, fine roots and wood grown in 2001 (trunk and branch). The rest of the organs had lower ^{13}C atom % excess values that were not different from each other.

In most of the organs, ^{13}C atom % excess detected at budbreak was lower or similar to those values measured at leaf abscission (Figure 3; Appendix 8 for statistics). 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 buds. The greatest reductions in ^{13}C atom % excess were detected in 2- and 3-year-old wood of the trunk, followed by those of most of the bark sections. ^{13}C levels of roots of

all sizes and 1-year-old wood (grown in 2001) did not show a significant change between leaf abscission and budbreak.

^{13}C-Reserve partitioning during early spring

^{13}C levels significantly higher than the natural abundance were detected from first bloom (~ 6 DBFB) until the beginning of stage I (~14 DAFB). Significant differences in ^{13}C atom % excess were detected among aerial organs within specific developmental stages, indicating differences in the level of dependency on storage reserves (Figure 4; Appendix 9 for statistics). 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, indicating either a decline in dependence on, or a steady depletion of, ^{13}C storage reserves.

^{13}C atom % excess values were highest during first and full bloom (Figure 5; Appendix 9 for statistics). During first bloom, spur flowers had the highest ^{13}C levels followed by single flowers and non-fruiting spur leaves. At this stage, fruiting spur leaves and shoot leaves had not yet begun to grow. At full bloom, similar ^{13}C levels were detected in flowers (single and spur clusters) and young shoot leaves, followed by non-fruiting spur leaves. Fruiting spurs had the lowest ^{13}C content. At this stage, shoots were 0.3 cm in length and had 3 small developing leaves (Table 1).

Between full bloom and fruit set, a dramatically lower ^{13}C atom % excess (i.e., lower ^{13}C enrichments) was observed in all organs. However, ^{13}C levels were still higher than natural abundance values. At fruit set (~7 DAFB), fruiting spur leaves and tiny fruits (0.3 g FW) had the highest ^{13}C contents, followed by non-fruiting spur leaves. In contrast to full bloom, fruiting spur leaves had the highest, and shoots the lowest, ^{13}C gains. 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 fruiting spur leaves. Non-fruiting spur leaves and shoots had the lowest ^{13}C levels. Later in stage I (~21 DAFB), ^{13}C levels reached their lowest point. For the first time, non-fruiting spurs had the highest ^{13}C atom % excess compared to the rest of the organs. The ^{13}C levels in shoots (4.8 cm in length and 10 leaves) were closest to natural abundance values. After this, relatively constant ^{13}C levels for all organs, especially for shoots, indicated minimal additional contributions from ^{13}C-reserves.

As indicated above, ^{13}C content varied not only among organs but also among developmental stages. Reproductive and vegetative tissues, collected at leaf abscission and at and after budbreak, had a distinct ^{13}C seasonal fluctuation pattern (Figure 6). At leaf abscission, vegetative meristems were even more highly enriched with ^{13}C than reproductive buds. However, this was reversed at budbreak through bloom, indicating remobilization of ^{13}C-reserves from other storage organs to flower buds. ^{13}C contents of reproductive organs (flowers and

fruits) also were higher than those of vegetative buds at 14 DAFB. During later stages of fruit development, this situation was again reversed, with higher ^{13}C contents in vegetative buds.

Relative ^{13}C-reserve partitioning throughout the canopy during spring

Considering the ^{13}C gain and the total number of units (i.e., total DW) for a particular organ at each developmental stage, the total ^{13}C partitioning (expressed as μg ^{13}C) was calculated for each of the aerial organs sampled through 28 DAFB. After that, ^{13}C levels of all organs remained close to natural abundance levels and did not vary considerably. These calculations provide a better understanding of the absolute amount of ^{13}C that was partitioned to all units of a particular organ at specific stages. Clearly, spurs, flowers and shoots compete for ^{13}C reserves during bloom. However, the greatest relative partitioning was to non-fruiting spurs (Figure 7; Appendix 10 for statistics). The highest recovery for this organ type occurred at full bloom, when spur leaves were actively growing. Single and spur flowers used ^{13}C reserves in low amounts relative to those partitioned to non-fruiting spurs. In terms of flower types, spur flowers had a greater relative ^{13}C demand than single flowers.

Between fruit set and 28 DAFB, the pattern of partitioning among organs was consistent. Non-fruiting spur leaves attracted most of the labeled reserves, while partitioning to fruit, fruiting spur leaves and shoot leaves resulted in low and similar ^{13}C contents. Fruit did not attract an important amount of ^{13}C and the

highest gains were detected at 7 and 14 DAFB, when fruit DW were only 33 and 150 mg, respectively.

Discussion

Carbon partitioning in fall and remobilization of reserves in spring were studied in 4-year-old sweet cherry trees on a semi-dwarfing 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 current photosynthates produced during the following spring by newly expanded leaves. The main objective was to study the partitioning of CH_2O reserves among organs in spring, and to characterize the transition phase, in which storage reserves are depleted and current photosynthates become the primary source for vegetative and reproductive growth.

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

At leaf abscission, the ^{13}C content in leaves was ~74% lower than that measured immediately after labeling, indicating either ^{13}C translocation to other organs, respiratory loss or both. ^{13}C loss due to leaf abscission was ~14% of the ^{13}C fixed in fall. Basipetal translocation of CH_2O and other nutrients from leaves to perennial storage organs, after terminal bud set and prior to leaf drop, have been reported in apple (Kandiah, 1979a,b; Quinlan, 1969), Japanese pear (Teng et al. 1999), grape (Hale and Weaver, 1962; Araujo and Williams, 1988), pecan (Lockwood and Sparks, 1978a,b; Davis and Sparks, 1974) and sweet cherry (Loescher et al., 1990) to become part of structural growth or storage reserves (Oliveira and Priestley, 1988). The higher ^{13}C accumulations were detected in older wood of the trunk (1999 and 2000), coarse roots and vegetative buds. Less important storage organs were younger wood in trunk and branches, fruiting buds and fine roots. Bark from different sections did not store much ^{13}C compared with the other organs. This was in contrast to high CH_2O accumulation in wood of the trunk and older branches has been found in sweet cherry and apple (Keller and Loescher, 1989; Greer et al., 2002).

At budbreak, the pattern of ^{13}C distribution throughout the tree was different from that at leaf drop. The ^{13}C content of wood and bark from older sections (1999 and 2000) had decreased significantly. However, fruiting buds had ^{13}C contents that were dramatically higher than those at leaf drop, indicating that ^{13}C-reserves were remobilized from other storage organs during the period between dormancy and budbreak. It is likely that ^{13}C-reserves were translocated,

prior to bloom, from wood and bark of the trunk to the reproductive meristems, based on fluctuations in ^{13}C levels between leaf drop and budbreak.

Reproductive meristems had the strongest sink activity for the remobilized ^{13}C-assimilates even before budbreak, and these continued being a priority for ^{13}C partitioning until 14 DAFB. Remobilization and utilization of storage reserves for metabolism during dormancy as been reported previously (Tromp, 1983; Priestley, 1981; Oliveira and Priestley, 1988). It was not the aim of this research to develop a comprehensive accounting of ^{13}C-reserve use, as by non-cropping-related sink activities (such as those of roots, phloem and cambial growth), but it is possible that before budbreak, some of the ^{13}C reserves already had been used for these additional sink activities as reported by Oliveira and Priestley (1988). Keller and Loescher (1989) indicate that aboveground sweet cherry tissues begin to utilize CH_2O in late winter and interconversions of starch to soluble sugars in wood and bark occur during dormancy. Similar reductions in the amount of reserves and remobilization from roots and stems to meristematic regions over winter have been reported for apple (Hansen, 1967; Quinlan, 1969; Priestley 1981). In this species, the first indication of phloem differentiation appears in early April, preceding xylem development by ~6 weeks (Evert, 1963). Certainly, some of the ^{13}C-reserves were used for respiration over the course of fall and winter as well. 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; Ogrën, 2000).

Interestingly enough, ^{13}C-content of roots, considered the major storage organ in sweet cherry (Loescher et al., 1990), did not vary at least until budbreak. These results are in agreement with those reported by Keller and Loescher (1989) which that indicate that in sweet cherry, root CH_2O reserves do not decrease until budbreak. It is possible that in sweet cherry, ^{13}C-reserves in roots may constitute a source of C for aerial sinks later in spring; that is, the first reserves used may be those closest to the sites of sink activity, with distant storage sites such as roots being remobilized only as more localized reserves are depleted, forming a gradient of sorts as reported in kiwifruit (*Actinidia deliciosa* (A.Chev.) C.F. Liang et A.R. Ferguson) and peach (*Prunus persica* (L.) Batsch) (Greaves et al., 1999; Jordan and Habib, 1996). The utilization of sweet cherry and apple root CH_2O reserves is soil temperature dependent, with little depletion at temperatures <10°C (McCammant, 1988; Greer et al., 2002). However, it may be possible that after leaf abscission, ^{13}C continued being translocated to roots from aerial storage organs, thereby, offseting any losses to respiration and/or root growth. If so, the utilization or remobilization of ^{13}C reserves in roots may not be noticed in early spring. It is also interesting that in fine roots ^{13}C contents did not decreased between leaf abscission and budbreak. Fine roots of sweet cherry have been suggested as storage organs by Keller and Loescher (1989). 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 (Loescher et al., 1990; Priestley, 1981). In peach, rootstock vigor, crop load and the time of ripening affected the extent of CH_2O reserve utilization among cultivars (Inglese et al., 2002). In prune (*Prunus domestica* L.), the rootstock genotype modified the kinetics of CH_2O mobilization and interconversion in the dwarfing rootstock 'Pixy' (Gaudillere et al., 1992). Additional information is required to elucidate the role of roots as a storage organ for more dwarfing combinations.

Various studies have reported that storage reserves are important to support early spring growth (flowers, leaves, shoots and fruits) in deciduous species (Quinlan, 1969; Hansen and Grauslund, 1973; Lockwood and Sparks, 1978a, b; Tromp, 1983; Oliveira and Priestley, 1988; Loescher et al., 1990; McArtney and Ferree, 1999). In our study, we confirmed that early spring growth of sweet cherry flowers, fruits, spur leaves and shoots was supported by reserves accumulated the previous fall. Mobilization of stored ^{13}C to new aerial growth was detected before budbreak and continued until 14 DAFB, when 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, 1979a,b), pecan (Lockwood and Sparks, 1978a, b) grape (Scholefield et al., 1978), apricot (*Prunus armeniaca* L.) (Costes et al., 1995) 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 spring. The importance of budbreak phenologies for potential partitioning effects among cultivars has been reported previously for two Southern highbush blueberry (*Vaccinium corymbosum* L.) cultivars, 'Misty' and 'Sharpblue' (Maust et al., 2000). In 'Sharpblue', floral and vegetative budbreak occurs simultaneously, while vegetative budbreak in 'Misty' occurs several weeks after floral budbreak. Root starch concentrations decreased ~65% between dormancy and bloom in 'Sharpblue', indicating a strong mobilization of reserves before budbreak. In contrast 'Misty' root starch concentrations decreased only ~35%. The increased rate of starch depletion in 'Sharpblue' during the period prior to bloom resulted in a greater rate of leaf development relative to 'Misty', which in turn resulted in an increase in newly-synthesized CH_2O to supply developing fruit and replenish root CH_2O. In japanese pear, initial growth of leaves and shoots is more dependent on storage reserves than are organs that develop later (Teng et al., 1999). Similarly, in apple extension shoots, leaves developed earlier in spring were more dependent on storage reserves than were upper (later developing) leaves (Quinlan, 1969).

The greatest dependence of sweet cherry 'Regina' on storage reserves was at bloom; after this, utilization of ^{13}C-reserves declined. At fruit set, ^{13}C levels of different growing organs were lower but the competition continued. The demand of individual organs varied during this period, which was reflected in their ^{13}C concentrations. Accordingly, the highest sink activity was detected in

reproductive organs (flowers and fruit). However, considering the total number of units for a particular organs type or tissue, vegetative structures had the highest sink strength for ^{13}C-reserves. At 14 DAFB, the ^{13}C concentration in all organs decreased, indicating a decline in dependence or depletion of ^{13}C-reserves. Keller and Loescher (1989) demonstrated that CH_2O in sweet cherry roots, wood and bark decline rapidly during full bloom. Similar reductions in storage reserves during spring, especially in roots, have been reported for apple (Priestley, 1960; Quinlan, 1969; Hansen, 1967; Kandiah, 1979a,b), pecan (Lockwood and Sparks, 1978 a,b) and japanese pear (Teng et al., 1999). Depletion of storage reserves after bloom has been attributed to the abscission of floral tissues and unfertilized flowers (Hansen, 1971; Teng et al., 1999). Other reports indicate that reserves decrease after budbreak primarily due to respiratory loss, with a small portion used for new reproductive and vegetative growth (Hansen, 1967; Hansen and Grauslund, 1973; Kandiah, 1979a,b). In apple, most of the fruit growth depends on current 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 of flowers and shoots come from storage reserves until flowers show color and 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 since the current results indicate that of total ^{13}C fixed, only between 3 to 11% was

partitioned to new aerial organs until 14 DAFB, when extension shoots had ~ 5 leaves and fruit were 12 mm in diameter.

In sweet cherry, final fruit size is dependent on cell division (stage I) and subsequent cell elongation at final swell (stage III). There is not detailed histological information for sweet cherry fruit, but in sour cherry (*Prunus cerasus* L.) fruit, cells of the mesocarp increase in number during the pre-bloom stage and stage I, which is the period of maximum division (Tukey and Young, 1939). Scorza et al. (1991) indicate that in peach, cell number in the mesocarp and not cell size is the major difference between small- and large-fruited peach cultivars and this difference is detected early during the growth of the ovary (~175 days before full bloom). Lang (2001a) proposed that N and CH_2O reserves are critical for final flower development, bloom and fruit set in sweet cherry. At this time, cell division is taking place rapidly in young shoots and fruits, defining final fruit potential size and spur leaf area. These findings confirm that storage reserves are the major C source for reproductive organs during bloom and early stages of fruit cell division. At these early stages, competing sinks are source limited because the canopy is not fully expanded. Source limitation results in insufficient C availability to support potential organ growth (DeJong and Grossman, 1995). A period of extreme source limitation might occur in sweet cherry using dwarfing and semi-dwarfing rootstocks, which bloom heavily and begin cropping excessively about the 4^{th} or 5^{th} leaf, resulting in reduced final fruit size. The timing of reserve utilization by reproductive meristems, in competition with

other sinks through 7 to 14 DAFB, suggests that reserve levels may be a potential determinant for variation in fruit set and final fruit size in less vigorous sweet cherry trees.

It is important to point out that reserve partitioning in 4-year-old trees was evaluated, which bloomed for the first time during the experiment. Therefore, the crop load was the same as would be for a tree in full production since most of the tree was in a vegetative stage. This may explain the fact that in terms of sink strength, more ^{13}C-reserves were partitioned to vegetative growth, especially non-fruiting spurs. This partitioning pattern cannot be extrapolated to mature trees; however, unpublished data (M. Ayala, personal observation) indicate that the same trees bloomed extensively one year later and the vegetative growth (expressed as shorter current shoots) was reduced. Such a situation may be more likely to promote the partitioning of most of the storage reserves to reproductive organs, which would compete strongly among vegetative sinks and other growth early in the season.

In summary, this study indicates that the hierarchy for stored C distribution among aerial organs of a 4-year-old sweet cherry tree is dynamic from budbreak through stage I of fruit growth. During this period, reproductive organs have the highest sink activity for storage reserves until 14 DAFB; however, a strong competition between flowers, fruits and different leaf populations occurred. Late in stage I, with shoots of ~5 cm in length and 10 leaves and fruit of ~0.2 mg DW, storage reserves do not constitute the main

source of assimilate and new expanded leaves become the major source of C for fruit and shoot growth.

These results advance the understanding of the importance of storage reserves for early spring growth in sweet cherry using dwarfing and semidwarfing rootstocks, which are known for increased precocity and high yields. Practical implications of this research include: (1) the maintenance of healthy photosynthetic sources during the previous fall to promote optimal reserve accumulation in storage sites, (2) a more precise manipulation of aerial growing centers to achieve a more balanced partitioning of reserves during early spring, (3) the selection of scion/rootstock genotypes for optimal CH_2O accumulation and distribution during the postharvest period, and (4) 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 early spring.

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Table 1. Current season growth (shoot) measurements of 'Regina'/Gisela 6 sweet cherry trees between bloom and terminal bud set (2003). Mean ± SE, n=40.

Developmental Stage	Days relative to full bloom	Shoot Length (cm)	Leaf Number		
			Total	Folded	Unfolded
First white/first bloom[z]	-6	0.3 ± 0.01[x]	3.1 ± 0.2	2.5 ± 0.1	0.6 ± 0.1
Full bloom	0	0.5 ± 0.01	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

[z] Developmental stages overlapped during this week.
[y] Fruit was kept on the tree after commercial harvest.
[x] At this date a few rudimentary shoots emerging from terminal buds.

Table 2. 'Regina'/Gisela 6 sweet cherry fruit growth measurements from fruit set through stage III. Mean ± SE, n= 25.

Developmental Stage	DAFB[z]	GDD[y]	Fresh Weight (g)	Dry Weight (g)	Diameter (mm)
Fruit set	7	342	0.3 ±0.02	0.03 ±	6.2 ± 0.2
Stage I	14	405	1.2 ±0.1	0.15 ±	11.5 ± 0.5
	21	469	1.5 ±0.1	0.20 ±	13.0 ± 0.2
	28	533	1.7 ±0.1	0.31 ±	13.6 ± 0.2
Stage II	35	618	1.9 ±0.1	0.48 ±	14.6 ± 0.2
	42	722	2.5 ±0.1	0.70 ±	16.2 ± 0.2
Stage III	49	843	4.8 ±0.3	1.04 ±	19.8 ± 0.4
	56	968	8.8 ±0.5	1.98 ±	25.4 ± 0.6
	63	1087	11.2 ±0.7	2.73 ±	26.0 ± 4.9

[z] DAFB: days after full bloom.
[y] GDD: growing degree days.

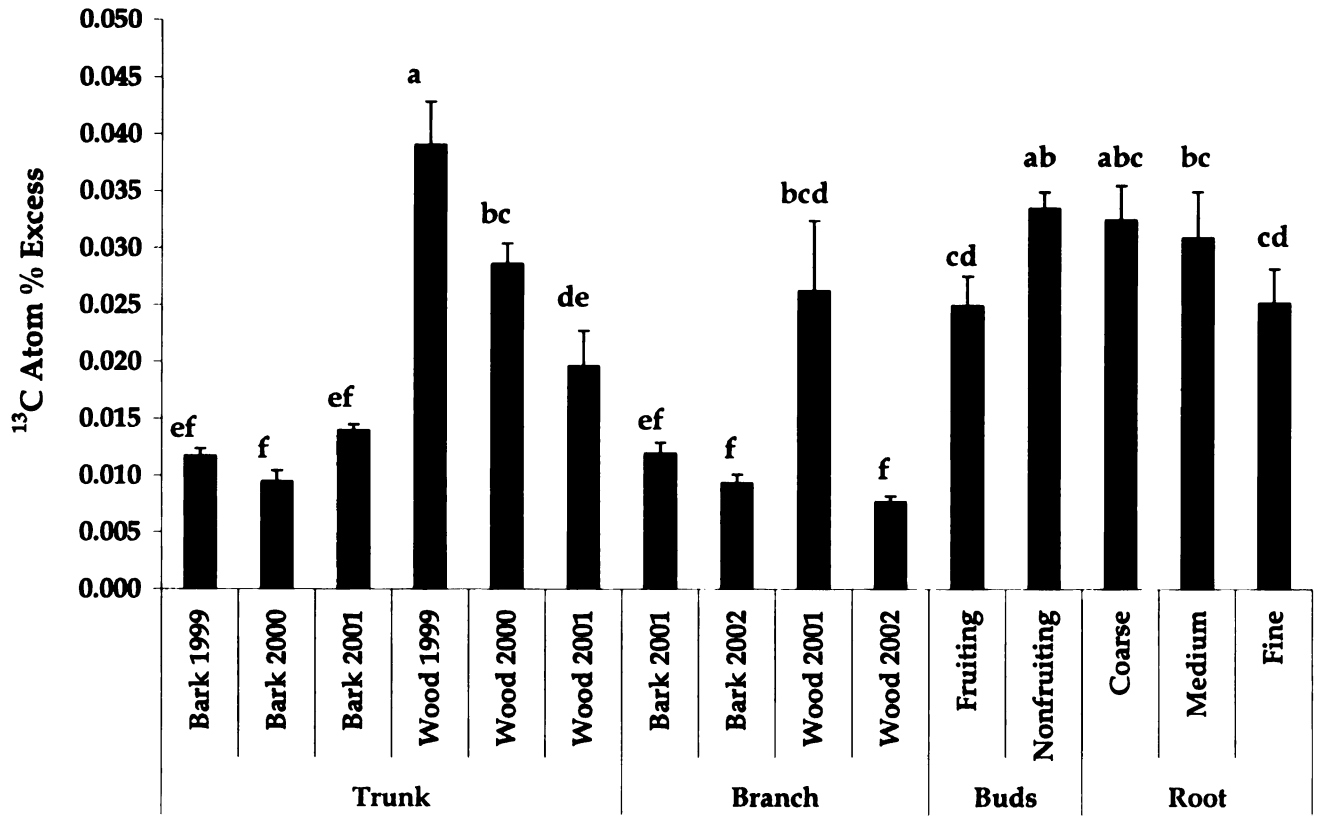


Figure 1. ^{13}C atom % excess in different organs of 'Regina'/Gisela 6 sweet cherry at leaf abscission (Nov, 2002). Colored bars indicate mean for each organ. Vertical lines indicate SE, n=5. Means followed by the same small letter are not significantly different at $\alpha = 0.05$ and $\alpha = 0.01$. Obtained p-value < 0.0001.

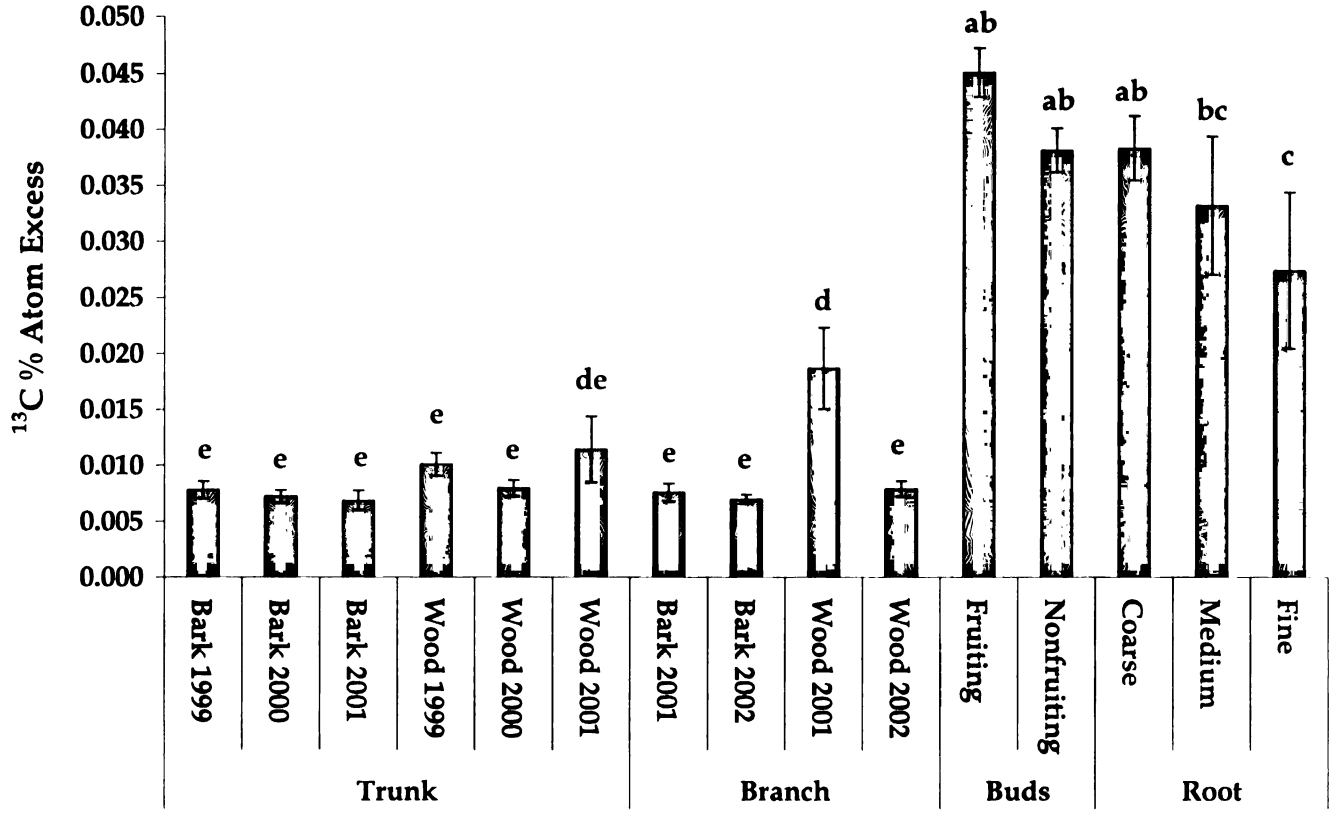


Figure 2. ^{13}C atom % excess in different organs of 'Regina'/Gisela 6 sweet cherry at budbreak (Apr, 2003). Colored bars indicate mean for each organ. Vertical lines indicate SE, n=5. Means followed by the same small letter are not significantly different at $\alpha = 0.05$ and $\alpha = 0.01$. Obtained p-value < 0.0001.

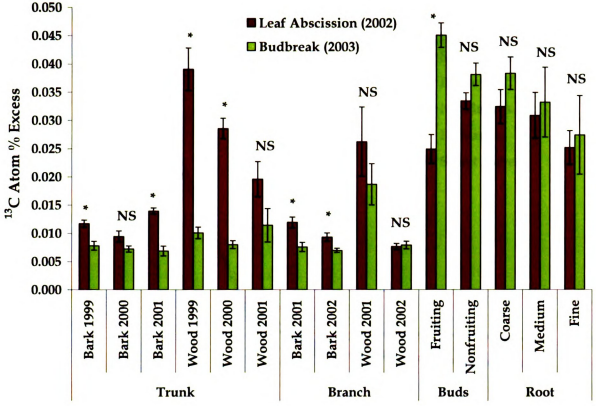


Figure 3. ^{13}C atom % excess in different organs of 'Regina'/Gisela 6 sweet cherry at leaf abscission (Nov, 2002) and budbreak (Apr, 2003). Colored bars indicate mean for each organ. Vertical lines indicate SE, n=5. Asterisk and NS indicate presence or absence of significant differences between sampling dates, respectively at $\alpha = 0.05$. Obtained p-value < 0.0001.

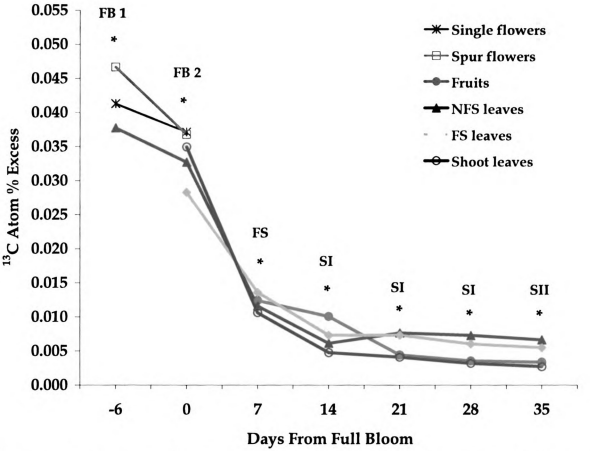


Figure 4. . ^{13}C atom % excess detected in aerial organs of 'Regina'/Gisela 6 sweet cherry during spring (May to Jun, 2003). Asterisks indicate presence of significant differences among organs at each developmental stage at $\alpha = 0.05$. FB1: first bloom; FB2: full bloom; FS: fruit set; SI: stage I; SII: stage II.

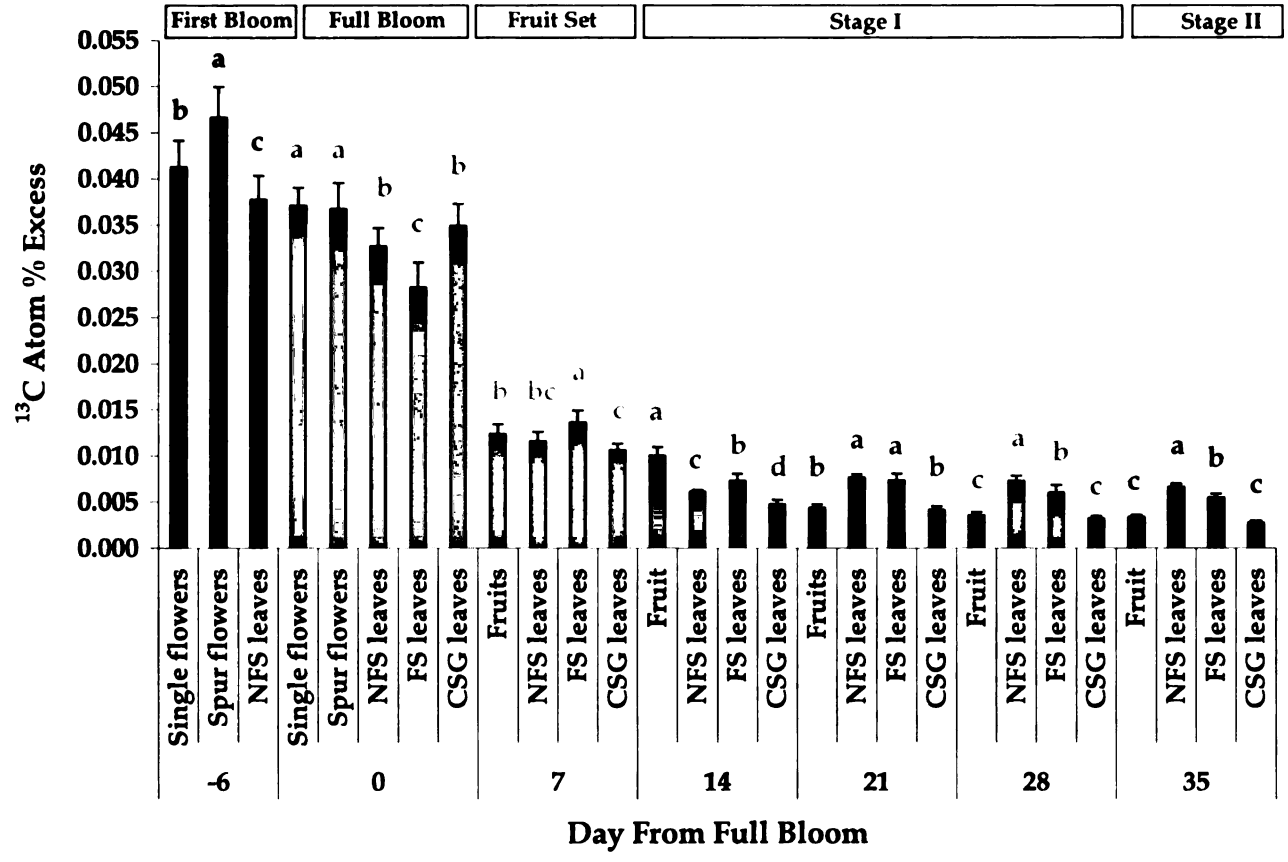


Figure 5. ^{13}C atom % excess for different aerial organs of 'Regina'/Gisela 6 sweet cherry during spring (May to Jun, 2003). Colored bars indicate mean for each organ. Vertical lines indicate SE, n=5. Means within a given sampling period followed by the same small letter are not significantly different at $\alpha = 0.05$. FS: fruiting spur, NFS: non-fruiting spur; CSG: current season growth.

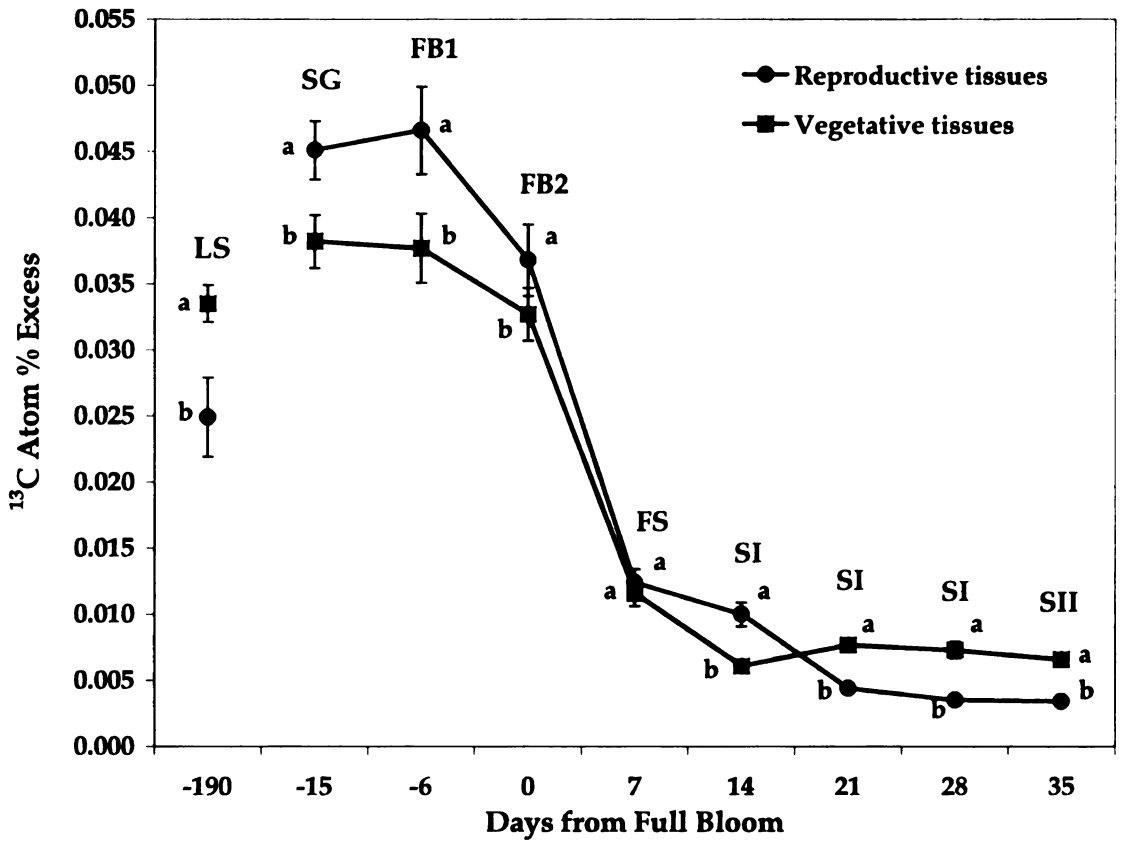


Figure 6. ^{13}C atom % excess values in reproductive and vegetative tissues of fall-pulsed 'Regina'/Gisela 6 sweet cherry trees during fall (Nov, 2002) and spring (Apr to Jun, 2003). Colored lines indicate mean for each organ. Vertical lines indicate SE, n=5. Means within a given stage period followed by the same small letter are not significantly different at α = 0.05. LA: leaf abscission; SG: side green; FB1: first bloom; FB2: full bloom; FS: fruit set; SI: stage I; SII: stage II.

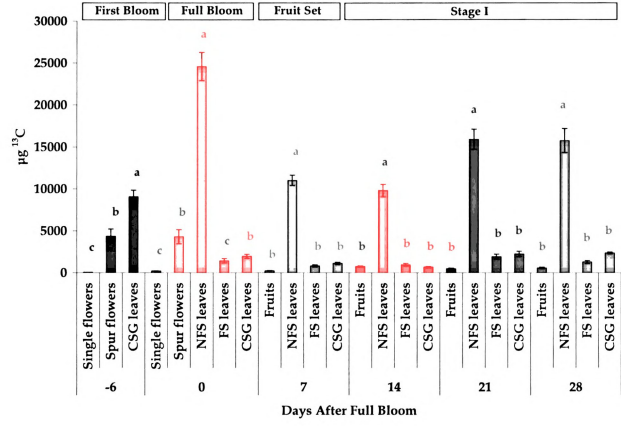


Figure 7. Total ^{13}C content (µg ^{13}C) for different organs of 'Regina'/Gisela 6 sweet cherry trees during spring (May to June, 2003). Colored bars indicate mean for each organ. Vertical lines indicate SE, n=5. Means within a given stage followed by the same letter are not significantly different at $\alpha = 0.05$. FS: fruiting spur, NFS: non-fruiting spur; CSG: current season growth.

CHAPTER VI

DISSERTATION PROJECT SUMMARY

Summary

Interest is high among U.S sweet cherry growers to adopt dwarfing and semi-dwarfing precocious rootstocks, such as the Gisela (GI) series, which are characterized by small canopies and positive effects on precocity and yield. High density systems using dwarfing precocious rootstocks are more labor efficient and economically viable when yields and fruit quality can be maintained or improved. These modern orchards are more uniform, have high and early yields and require lower production and harvest inputs. Before commercial adoption of GI rootstocks by American growers becomes routine, however, many physiological questions must be elucidated. Additional research is required to understand the role of fruit sink strength and carbohydrate (CH_2O) partitioning when trees are grown on dwarfing and semi-dwarfing precocious rootstocks, particularly of the GI series selections that are currently available. So far, the implementation of standard sweet cherry management practices for trees on GI rootstocks has resulted in high yields but small fruit, which is a critical problem since top quality fruit provides the best returns to growers.

As little was known about the relative importance of different sweet cherry leaf populations (i.e., fruiting and non-fruiting spurs and current season shoot leaves) and storage reserves as sources of carbon (C) for fruit and shoot development in dwarfing trees, this study focused on the following objectives: (1) to define the temporal importance of various leaf populations as sources of C

for fruit and shoot growth during the whole period of fruit development, (2) to determine the effect of reproductive and vegetative sink strengths on C partitioning during fruit development, (3) to determine the importance of storage reserves as a source of C for initial fruit growth, and (4) to define the transition phase during which the dependence of new growth on storage reserves shifts to current photosynthate assimilation as the primary source for subsequent vegetative and reproductive development. Accordingly, a series of partitioning experiments using girdling, defoliation, fruit thinning and ^{13}C-isotopic labeling of different leaf populations and storage reserves was established with sweet cherry trees on dwarfing (Gisela 5, GI5) and semidwarfing (Gisela 6, GI6) GI rootstocks.

A preliminary girdling and defoliation experiment isolated fruit of 'Hedelfinger'/GI5 and 'Ulster'/GI6 from different leaf sources. Results indicated that fruits supplied exclusively by the leaf populations on either the fruiting spur branch segment or the non-fruiting spur branch segment were significantly smaller and had decreased SS levels. Leaf populations on both fruiting and non-fruiting branch segments were required for full fruit development and there was not a sufficient compensatory effect when one of the main leaf populations was eliminated.

A second experiment used $^{13}CO_2$ to label non-fruiting spur leaves on 'Sam' /GI5 limbs with three different crop loads quantified as leaf area (LA) to fruit (F) ratio (LA/F = 140, 75, or 40 cm^2/fruit) 3 times during stage III of fruit

development. Results indicated that ^{13}C fixed by non-fruiting spur leaves was translocated both acropetally and basipetally. For all 3 pulsing dates, fruits were more highly enriched in ^{13}C than were young leaves, suggesting that the sink activity of fruit was stronger compared to that of shoots. There was not a consistent or significant crop load effect on ^{13}C-partitioning between fruit and shoots. However, differences in translocation between organs of the same branch, for a given treatment, were significant, as the fruits in closest proximity to the branch segment of non-fruiting spurs generally had the highest relative ^{13}C content (up to 64%, compared to more distal fruits which ranged from 26% to 40% of recovered ^{13}C). As crop load increased, this trend for preferential partitioning became more pronounced. Shoot leaves had considerably lower ^{13}C contents (ranging between 1.6% and 11 % of the ^{13}C recovered).

A third experiment quantified the relative C contributions of different leaf populations on 'Ulster'/GI6 limbs to fruit and shoot development during stages I, II and III of fruit development. The three leaf populations on the fruiting branch, i.e., fruiting spur, non-fruiting spur and new terminal shoot leaves, were exposed to $^{13}CO_2$ labeling on five representative phenological dates (25, 40, 44, 56, 75 days after full bloom, DAFB) during fruit development. Results indicated that spur and shoot leaves were significant sources of C for fruit and vegetative growth. ^{13}C fixed by different leaf sources was translocated acropetally, basipetally or both. In terms of C allocation, fruits were a priority sink vs. new shoot growth during the entire period of fruit development. However, the more

distant was the ^{13}C source, the lower the amount of ^{13}C detected in fruit. Fruit photosynthesized some ^{13}C in early stages of development (stages I and II). The highest fruit sink strength was during stages I and III, while the highest shoot sink strength was during rapid elongation. The terminal current season shoot provided a C source for fruit as early as stage I.

Finally, a fourth experiment on 'Regina'/GI6 trees labeled with $^{13}CO_2$ after terminal bud set determined the extent of storage reserve use of for spring growth, particularly fruit, and defined the transition phase during which current photoassimilates become the primary C source. In fall, the major storage organs were roots, older wood in the trunk and branches, and buds. During spring, ^{13}C-reserves were remobilized and partitioned to flowers, fruits and young leaves from before budbreak (side green) until 14 DAFB. The highest ^{13}C levels in growing sinks were detected between side green and fruit set. The isotopic composition of new organs differed significantly among organs and phenological stages. Reproductive organs had the strongest sink activity until 14 DAFB, but in terms of total dry matter, non-fruiting spurs had the highest sink strength as a function of being the predominant aerial tissue type in the 4-year-old tree.

Previous data and increasing grower experience indicates that reproductive and vegetative growth often become unbalanced after the 4^{th} or 5^{th} year of production on dwarfing and semidwarfing GI rootstocks if the natural canopy LA/F ratios are not altered in some way. Thus, manipulation of the reproductive and vegetative sinks may be a tool to regulate sink strength and

competition among sinks during periods of resource limitation, particularly during fruit development. So far, chemical thinning of flowers or fruit is not a common practice in sweet cherry. Therefore, adjustments in LA/F ratios through practices such as pruning to remove or stimulate leaf area, or fruit and flower thinning and/or spur extinction might help to overcome the problem of overcropping and small fruit size. The appropriate timing for each of these practices will depend on the final objective. For instance, removal of some of the current season growth after leaf abscission (once storage reserves have been accumulated) or during winter might be a possible strategy to control excessive crop load 2 years later, and additionally stimulate more vigorous vegetative growth (i.e., more leaf area to support fruit growth) the year after pruning. Growers should be encouraged to begin this type of management soon after establishment, by 4^{th} or 5^{th} leaf or as crop load become a significant sink, to achieve and maintain more balanced trees, in terms of crop load and LA. Pruning current season shoots during the period of fruit development might be detrimental if it is not done precisely since, as demonstrated in this study, this leaf population constitutes a C source for fruit as early as stage I, and its removal could negatively affect fruit quality.

Another alternative to control excessive crop loads might be spur extinction (i.e., selective spur removal) during summer or after terminal bud set. Selective removal of reproductive spurs every season would reduce future crop loads. However, a disadvantage of this technique is that it does not promote

additional LA development on current season shoots. Thus, because spur leaf area is not sufficient for optimal fruit development, extinction would not promote the supplemental LA contributed by extension shoots, which might become a limitation to keep optimal LA/F ratios in more dwarfing scion/rootstocks combinations. In the future, it would be interesting to explore how the mix of shoot pruning and spur extinction may shift source-sink relationships depending on the inherent vigor and precocity of the scion/rootstock combination. For instance, scion/rootstocks combinations using GI5, a dwarfing rootstock, might require more pruning to promote extension shoot growth than combinations using GI6, a semidwarfing rootstock. Extension programs should emphasize the importance of distinct leaf populations as C sources for fruit and shoot development. Sweet cherry growers using dwarfing and semidwarfing rootstocks can use the results of this dissertation to promote more leaf area development and protect leaves not only during fruit development, but also after harvest when storage reserves are being accumulated. Overall, results of this study provide a physiological foundation for the canopy relationships that may guide to develop specific orchard management strategies to promote a more sustainable balance between vegetative and reproductive growth in high density sweet cherry orchards on vigor-limiting rootstocks.

APPENDIX A

Appendix A.1. ^{13}C content (expressed as atom %) in 'Sam'/Gisela 5 sweet cherry fruits and current season shoot leaves during stage III (52, 59 and 63 days after full bloom, DAFB). Mean ± SE. For calculations see Material and Methods.

LA cm^2/F	^{13}C Atom %			
	Distal Fruit	Proximal Fruit	Proximal Shoot	Distal Shoot
52 DAFB				
140 (T1)	1.097 ± 0.003 a[z]	1.099 ± 0.003 a	1.083 ± 0.001 a	1.083 ± 0.001 a
75 (T2)	1.091 ± 0.002 a	1.099 ± 0.004 a	1.084 ± 0.001 a	1.082 ± 0.001 a
40 (T3)	1.088 ± 0.002 a	1.101 ± 0.003 a	1.084 ± 0.001 a	1.083 ± 0.001 a
Significance	NS (0.0559)[y]	NS (0.9365)	NS (0.5089)	NS (0.1273)
59 DAFB				
140 (T1)	1.113 ± 0.004 a	1.126 ± 0.006 a	1.084 ± 0.001 a	1.083 ± 0.001 bc
75 (T2)	1.105 ± 0.003 a	1.121 ± 0.005 a	1.084 ± 0.001 a	1.084 ± 0.001 a
40 (T3)	1.106 ± 0.007 a	1.119 ± 0.006 a	1.085 ± 0.001 a	1.083 ± 0.001 c
Significance	NS (0.6202)	NS (0.7443)	NS (0.6322)	* (0.0198)
63 DAFB				
140 (T1)	1.096 ± 0.002 a	1.095 ± 0.002 a	1.084 ± 0.001 a	1.083 ± 0.001 a
75 (T2)	1.096 ± 0.002 a	1.108 ± 0.005 a	1.083 ± 0.001 a	1.083 ± 0.001 a
40 (T3)	1.095 ± 0.003 a	1.106 ± 0.005 a	1.082 ± 0.001 a	1.083 ± 0.001 a
Significance	NS (0.9845)	NS (0.0694)	NS (0.2774)	NS (0.8054)

[z] Means within a column followed by the same small letter are not significantly different (NS) at $\alpha = 0.05$.

[y] Obtained p-value.

Appendix A.2. Relative ^{13}C partitioning (expressed as %) between fruits and current season shoot leaves during stage III (52, 59 and 63 days after full bloom, DAFB). Means ± SE. Calculations based on absolute ^{13}C recoveries (μg ^{13}C) and total DW for each organ.

		Relative ^{13}C Partitioning (%)			
LA cm^2/F		Distal Fruit	Proximal Fruit	Proximal Shoot	Distal Shoot
	52 DAFB				
140 (T1)		45.0 ± 8.8 a[z]	48.2 ± 8.3 b	6.6 ± 2.2 a	0.20 ± 0.09 a
75 (T2)		35.5 ± 8.1 a	59.7 ± 7.8 b	4.8 ± 1.5 a	0.04 ± 0.03 a
40 (T3)		4.1 ± 1.7 b	94.0 ± 1.9 a	1.8 ± 0.5 a	0.06 ± 0.02 a
Significance		* ** [z]	* **	NS	NS
		(0.0061) [y]	(0.0034)	(0.1744)	(0.3928)
	59 DAFB				
140 (T1)		40.4 ± 5.0 a[z]	59.1 ± 5.0 b	0.3 ± 0.01 a	0.19 ± 0.01 a
75 (T2)		38.3 ± 4.7 a	61.3 ± 4.7 b	0.2 ± 0.01 a	0.21 ± 0.01 a
40 (T3)		14.8 ± 3.4 b	85.0 ± 3.3 a	0.2 ± 0.01 a	0.05 ± 0.01 b
Significance		* **	* **	NS	*
		(0.0015)	(0.0015)	(0.4549)	(0.0347)
	63 DAFB				
140 (T1)		58.7 ± 7.7 a [z]	40.9 ± 7.7 a	0.3 ± 0.2 a	0.00 ± 0.00 a
75 (T2)		57.7 ± 9.4 a	42.1 ± 9.3 a	0.2 ± 0.1 a	0.03 ± 0.01 a
40 (T3)		28.2 ± 9.0 a	71.2 ± 9.0 a	0.3 ± 0.3 a	0.25 ± 0.1 a
Significance		NS	NS	NS	NS
		(0.1521)	(0.1588)	(0.8539)	(0.4195)

[z] Means within a column followed by the same small letter are not significantly different (NS) at α = 0.05.

[y] Obtained p-value.

APPPENDIX B

Appendix B.1. Diameter, length and number of spurs (fruiting and non-fruiting) of 2-year-old branch sections of 'Ulster'/Gisela 6 sweet cherry trees measured at each pulse-labeling date. Mean ± SE, n=30.

Developmental Stage	DAFB[z]	Diameter[y] (mm)	Length (cm)	Section length (cm)		Spur number	
				Fruiting	Non-fruiting	Fruiting	Non-fruiting
I	25	13.8 ± 0.4	99.2 ± 2.1	56.6 ± 1.9	42.6 ± 1.6	14.3 ± 0.4	12.4 ± 0.5
II	33	13.8 ± 0.5	99.5 ± 2.2	58.8 ± 1.6	40.7 ± 1.4	13.7 ± 0.4	13.5 ± 0.6
	40	13.5 ± 0.5	97.4 ± 2.6	58.3 ± 1.7	39.1 ± 1.3	13.6 ± 0.5	12.2 ± 0.5
III	44	12.6 ± 0.3	100.4 ± 2.5	57.6 ± 2.0	42.8 ± 1.4	12.0 ± 0.3	13.3 ± 0.5
	51	13.6 ± 0.4	96.8 ± 2.0	55.5 ± 1.4	41.3 ± 1.2	12.4 ± 0.5	13.6 ± 0.5
	56	13.9 ± 0.3	100.8 ± 4.1	64.0 ± 3.6	36.8 ± 1.6	13.4 ± 0.5	12.7 ± 0.6
	63	15.2 ± 0.5	97.4 ± 2.6	56.7 ± 1.2	40.7 ± 1.7	13.0 ± 0.3	13.9 ± 0.7
Terminal bud set	75	16.1 ± 1.5	99.4 ± 2.0	58.5 ± 4.9	40.9 ± 1.2	13.4 ± 0.5	13.4 ± 0.5

[z] DAFB: days after full bloom. Full bloom occurred on 30 Apr, 20003.

[y] Diameter was measured 5 cm above the junction between the limb and the scaffold or the trunk.

Appendix B.2. Ranges of air temperature and photosynthetically active radiation (PAR) measured between 9:00 AM and 13:00 PM during each pulse-labeling date (25, 40, 44, 56 and 75 days after full bloom, DAFB). Data obtained from the Michigan Automated Weather Network *(MAWN)*.

DAFB	Air temperature (°C)	PAR (KJ/m^2)
25	8.8 - 13.4	173.5-1620.8
40	11.9 - 18.3	327.9-2559.4
44	14.1 - 18.0	291.4-1367.4
56	24.3 - 29.9	1072.7-2774.0
75	20.0 - 26.3	1080.4-2366.6

Appendix B.3. Ranges of net photosynthesis (A), photosynthetically active radiation (PAR) and leaf temperature (T°) of each leaf population within the canopy at each pulse-labeling (25, 40, 44, 56 and 75 days after full bloom, DAFB). n=5.

DAFB	Leaf population	PAR ($\mu mol\ m^{-2}s^{-1}$)	Leaf T° (°C)	A ($\mu mol\ CO_2\ m^{-2}s^{-1}$)
25	Fruiting spurs	80 - 282	21.3 - 22.5	2.9 - 5.9
	Non-fruiting spurs	216 - 780	20.8 - 27.7	7.4 - 12.7
	Terminal shoot basal	183 - 403	22.1 - 23.5	4.6 - 8.4
	Terminal shoot medial	117 - 296	21.5 - 23.2	0.9 - 5.5
	Terminal shoot apical	76 - 212	21.2 - 23.5	-0.7 - -8.2
40	Fruiting spurs	1096 - 1832	25.1 - 27.7	8.7 - 11.6
	Non-fruiting spurs	923 - 1480	25.1 - 27.8	8.6 - 16.9
	Terminal shoot basal	109 - 1440	23.6 - 26.9	1.0 - 16.3
	Terminal shoot medial	106 - 1301	21.8 - 27.0	-0.8 - 15.0
	Terminal shoot apical	87 - 1235	23.1 - 28.7	-0.4 - 2.8
44	Fruiting spurs	183 - 307	24.6 - 25.9	5.9 - 9.8
	Non-fruiting spurs	535 - 707	26.1 - 27.6	7.4 - 12.8
	Terminal shoot basal	447 - 524	25.8 - 26.3	7.9 - 11.3
	Terminal shoot medial	557 - 681	26.0 - 26.9	7.2 - 7.5
	Terminal shoot apical	568 - 641	27.3 - 28.1	-3.0 - -6.3
56	Fruiting spurs	447 - 1396	31.8 - 33.6	8.0 - 19.7
	Non-fruiting spurs	923 - 1708	33.3 - 34.9	8.2 - 19.2
	Terminal shoot basal	905 - 1565	34.0 - 35.5	9.1 - 15.0
	Terminal shoot medial	773 - 1352	34.5 - 35.4	10.4 - 12.6
	Terminal shoot apical	894 - 1425	36.3 - 36.8	-2.7 - 2.2
75	Fruiting spurs	183 - 1700	27.5 - 30.5	7.1 - 19.9
	Non-fruiting spurs	76 - 1444	26.7 - 32.8	1.4 - 13.9
	Terminal shoot basal	846 - 1788	30.0 - 33.3	14.1 - 22.5
	Terminal shoot medial	417 - 1612	30.7 - 32.9	11.0 - 20.4
	Terminal shoot apical	527 - 1656	31.9 - 32-8	-8.9 - 18.9

Appendix B.4. Fruit and terminal current season shoot dry weight (DW) measured on 2-year-old 'Ulster'/Gisela 6 sweet cherry branches at each labeling date. Mean ± SE, n=30.

Developmental stage	DAFB[z]	Dry weight (g)	
		Fruit	Terminal shoot
I	25	0.35 ± 0.07	2.5 ± 0.1
II	33	0.37 ± 0.01	3.3 ± 0.1
	40	0.39 ± 0.01	4.0 ± 0.2
III	44	0.40 ± 0.01	5.4 ± 0.2
	51	0.67 ± 0.01	7.4 ± 0.4
	56	0.94 ± 0.02	8.6 ± 0.4
	63	1.28 ± 0.04	11.3 ± 0.6
Terminal bud set	75	1.64 ± 0.05	13.7 ± 0.9

[z] DAFB: days after full bloom. Full bloom occurred on 30 Apr, 2003.

Appendix B.5. Leaf area and leaf number for individuals fruiting spurs, non-fruiting spurs and current season shoots on 2-year-old 'Ulster'/Gisela 6 sweet cherry branches measured weekly between fruit set and terminal bud set. Mean ± SE, n=30.

Developmental Stage	DAFB[z]	Fruiting Spur		Non-fruiting spur		Current season shoot	
		Leaf area (cm^2)	Leaf number	Leaf area (cm^2)	Leaf number	Leaf area (cm^2)	Leaf number
Fruit set	12	59.2 ± 5.2	4.3 ± 0.3	68.1 ± 5.3	3.7 ± 0.2	129.9 ± 9.7	8.1 ± 0.5
Stage I	19	99.4 ± 13.5	4.0 ± 0.3	65.9 ± 6.2	3.0 ± 0.3	183.5 ± 19.9	8.4 ± 0.6
	26	92.5 ± 6.7	4.0 ± 0.2	116.2 ± 4.6	5.4 ± 0.2	248.7 ± 12.9	8.7 ± 0.3
Stage II	33	104.5 ± 25.7	5.8 ± 0.2	118.0 ± 6.0	5.7 ± 0.2	357.2 ± 20.2	12.3 ± 0.6
	40	107.4 ± 7.5	5.6 ± 0.2	116.0 ± 4.6	5.4 ± 0.2	423.6 ± 13.4	14.8 ± 0.3
Stage III	47	115.8 ± 11.6	5.4 ± 0.3	116.9 ± 8.0	5.6 ± 0.4	509.4 ± 55.2	15.3 ± 0.8
	54	121.1 ± 5.0	5.6 ± 0.3	130.6 ± 5.6	6.1 ± 0.2	614.3 ± 25.8	17.0 ± 0.7
	61	121.0 ± 5.1	5.8 ± 0.2	126.1 ± 6.2	6.0 ± 0.2	668.9 ± 28.3	17.8 ± 0.5
	68	118.2 ± 4.4	5.8 ± 0.1	127.6 ± 4.1	6.0 ± 0.2	729.6 ± 34.1	17.8 ± 0.6
Terminal bud set	75	111.0 ± 2.4	5.8 ± 0.2	124.1 ± 4.0	6.0 ± 0.2	844.5 ± 54.2	19.2 ± 0.5
	82	116.0 ± 5.9	5.6 ± 0.2	134.4 ± 5.2	6.0 ± 0.1	844.9 ± 37.1	19.0 ± 0.6
	89	117.8 ± 7.2	5.7 ± 0.2	126.8 ± 23.2	6.0 ± 0.1	838.3 ± 33.4	19.1 ± 0.7
	96	119.1 ± 5.0	5.6 ± 0.2	134.8 ± 4.2	6.0 ±0.1	852.1 ± 37.7	19.1 ± 0.1

[z] DAFB: days after full bloom. Full bloom occurred on 30 Apr, 2003.

Appendix B.6. Total leaf areas for the fruiting, non-fruiting and terminal shoot sections of 2-year-old 'Ulster'/Gisela 6 sweet cherry branches at each pulse-labeling date. Mean ± SE, n=30.

Developmental Stage	DAFB[z]	Total Leaf Area per Leaf Population (cm^2)		
		Fruit Spurs	Non-fruiting spurs	Terminal shoot
I	25	1322.3 ± 41.1	1443.5 ± 60.1	248.7 ± 12.9
II	33	1427.7 ± 45.2	1467.5 ± 63.7	357.2 ± 20.2
	40	1456.7 ± 51.4	1411.4 ± 55.1	423.6 ± 13.4
III	44	1385.2 ± 38.2	1551.2 ± 55.5	509.4 ± 55.2
	51	1503.5 ± 54.4	1776.1 ± 64.7	614.3 ± 25.8
	56	1623.5 ± 62.9	1610.3 ± 73.5	668.9 ± 28.3
	63	1537.2 ± 40.2	1756.9 ± 95.3	729.6 ± 34.1
Terminal bud set	75	1523.6 ± 78.3	1600.3 ± 82.7	844.5 ± 54.2

[z] DAFB: days after full bloom. Full bloom occurred on 30 Apr, 2003.

Appendix B.7. Total leaf area, fruit number and leaf area to fruit (LA/F) ratios for the 2-year-old 'Ulster'/Gisela 6 sweet cherry branches measured at each pulse-labeling. Mean ± SE, n=30.

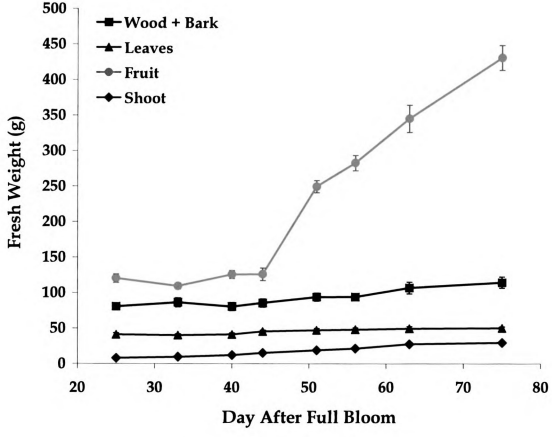
Developmental stage	DAFB[z]	Leaf area/branch (cm^2)	Fruits/branch	LA/F ratio (cm^2/fruit)
Stage I	25	2984.3 ± 67.0	94.5 ± 3.3	32.6 ± 1.3
Stage II	33	3252.4 ± 82.2	79.8 ± 3.3	42.7 ± 2.0
	40	3291.6 ± 82.8	79.8 ± 3.4	43.5 ± 2.2
Stage III	44	3445.8 ± 61.9	67.1 ± 3.6	54.9 ± 2.6
	51	3908.3 ± 97.4	77.1 ± 3.0	53.1 ± 2.2
	56	3899.6 ± 80.0	68.7 ± 3.0	60.0 ± 2.6
	63	3980.4 ± 101.5	68.3 ± 3.3	60.7 ± 2.9
Terminal bud set	75	3994.4 ± 126.0	66.1 ±3.2	60.9 ± 3.0

[z] DAFB: Days after full bloom on 30 Apr, 2003.

Appendix B.8. Growing degree day (GDD, base 4.4°C) accumulation for stages I, II and III of fruit development at CHES (May 1 to Aug 4, 2003).

Developmental Stage	DAFB[z]	GDD
Fruit Set	5 to 12	256.0 - 312.4
Stage I	13 to 32	319.4 - 483.2
Stage II	33 to 43	492.1 - 604.7
Stage III	44 to 75	618.5 - 1134.8
Post Stage III	76 to 96	1152.9 - 1476.6

DAFB: days after full bloom. Full bloom occurred on 30 Apr, 2003.



Appendix B.9. Fresh weight cumulative increment in different organs and/or tissues on 2-year-old 'Ulster'/Gisela 6 sweet cherry branches during each pulse-labeling date (May to Jul, 2003). Mean ± SE, n=30.

Appendix B.10. Fresh weight for different organs on 2-year old 'Ulster'/Gisela 6 sweet cherry branches at each pulse-labeling. Mean ± SE, n=30.

	Fresh weight (g)							
DAFB[z]	25	33	40	44	51	56	63	75
FS[y] section[x]	63.4 ± 4.1	69.8 ± 5.7	62.2 ± 4.8	65.9 ± 4.8	70.4 ± 4.3	74.9 ± 3.7	76.9 ± 5.6	87.9 ± 5.9
NFS[y] section[x]	17.6 ± 1.1	19.7 ± 1.5	17.2 ± 0.9	21.2 ± 1.4	23.7 ± 1.3	19.6 ± 1.1	26.2 ± 2.2	27.7 ± 1.9
FS leaves	17.4 ± 1.3	16.4 ± 1.2	18.8 ± 1.2	20.2 ± 1.6	22.1 ± 1.6	26.6 ± 1.9	21.5 ± 1.6	23.5 ± 1.6
NFS leaves	22.8 ± 1.0	24.8 ± 1.2	21.2 ± 1.4	23.8 ± 1.1	25.3 ± 1.4	22.1 ± 1.4	27.6 ± 1.8	27.2 ± 1.4
Shoot[w]	8.0 ± 0.3	10.0 ± 0.4	12.0 ± 0.5	14.6 ± 0.6	19.1 ± 1.1	22.0 ± 0.9	27.0 ± 1.4	29.6 ± 1.8
Fruit	114.2 ± 3.6	109.7 ± 4.5	123.0 ± 5.1	117.0 ± 6.1	245.2 ± 7.5	284.9 ± 10.8	347.2 ± 15.4	426.7 ± 16.3
Total[v]	243.5 ± 7.6	250.3 ± 9.9	254.4 ± 9.8	262.8 ± 9.8	405.9 ± 12.9	450.0 ± 13.2	526.4 ± 23.1	622.5 ± 18.5

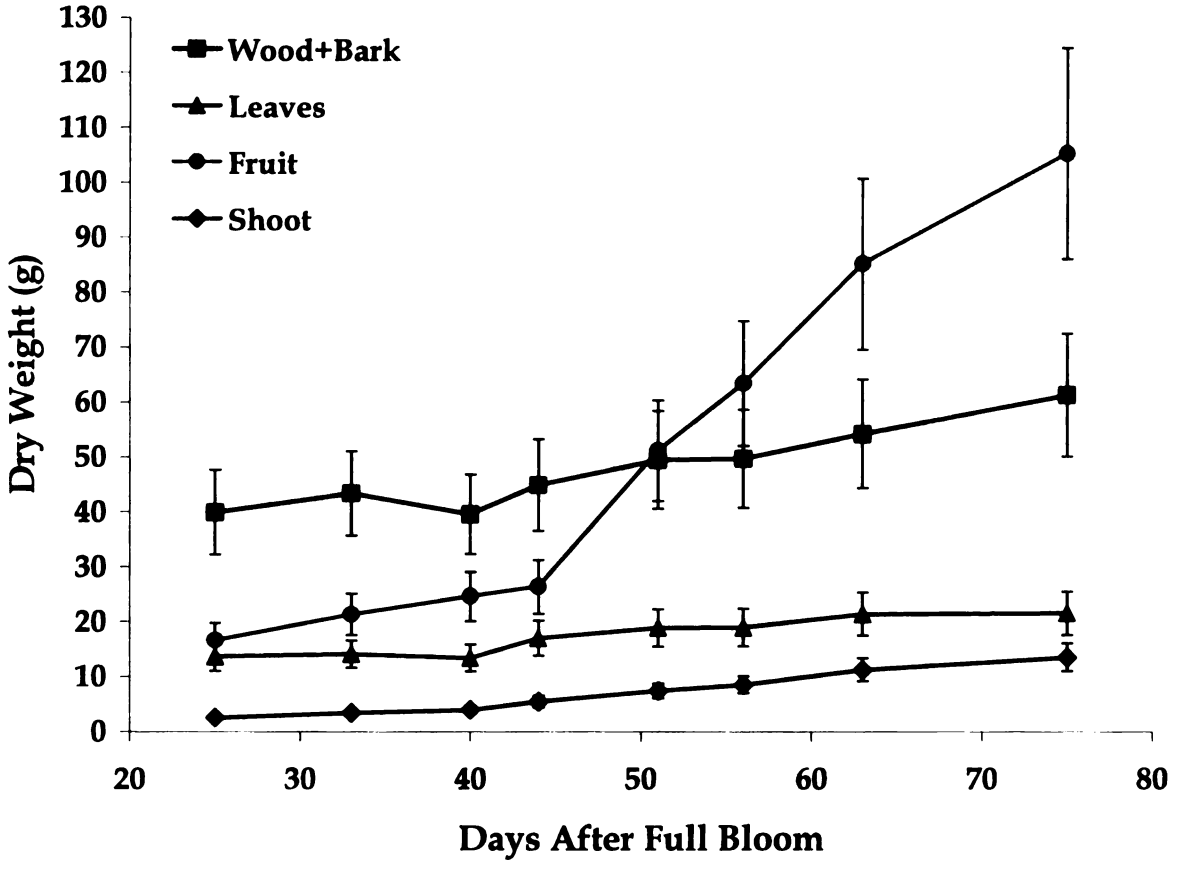
[z] DAFB: days after full bloom. Full bloom occurred on 30 Apr, 2003.

[y] FS: fruiting spur; NFS: non-fruiting spur.

[x] FS and NFS sections include bark and wood.

[w] Shoot includes current season wood and leaves.

[v] Total fresh weight per branch.



Appendix B.11. Dry weight cumulative increment in different organs and/or tissues on 2-year-old 'Ulster'/Gisela 6 sweet cherry branches during each pulse-labeling date (May to Jul, 2003). Mean ± SE, n=30.

Appendix B.12. Dry weight for different organs on 2-year old 'Ulster'/Gisela 6 sweet cherry branches at each pulse-labeling. Mean ± SE, n=30.

	Dry weight (g)							
DAFB[z]	25	33	40	44	51	56	63	75
FS[y] section[x]	31.7 ± 1.9	34.0 ± 2.7	31.2 ± 2.3	33.5 ± 2.5	37.2 ± 2.2	39.5 ± 1.9	40.3 ± 2.8	45.9 ± 3.3
NFS [y] section[x]	8.0 ± 0.5	8.6 ± 0.6	8.4 ± 0.4	10.5 ± 0.7	12.3 ± 0.6	10.2 ± 0.6	13.9 ± 1.2	14.8 ± 1.0
FS leaves	6.3 ± 0.5	5.7 ± 0.4	6.2 ± 0.4	7.8 ± 0.6	8.7 ± 0.6	10.2 ± 0.7	9.4 ± 0.8	10.6 ± 0.8
NFS leaves	7.6 ± 0.4	8.2 ± 0.4	7.2 ± 0.4	9.1 ± 0.4	10.1 ± 0.5	8.7 ± 0.5	12.0 ± 0.8	11.5 ± 0.5
Shoot[w]	2.5 ± 0.1	3.3 ± 0.1	4.0 ± 0.2	5.4 ± 0.2	7.4 ± 0.4	8.6 ± 0.4	11.3 ± 0.6	13.7 ± 0.9
Fruit	16.7 ± 0.6	21.1 ± 0.7	24.6 ± 0.9	26.6 ± 1.3	51.1 ± 1.4	63.4 ± 2.4	85.9 ± 3.9	105.9 ± 4.0
Total[v]	72.8 ± 2.8	81.0 ± 3.7	81.5 ± 3.5	92.9 ± 3.8	126.9 ± 4.1	140.6 ± 4.0	172.8 ± 7.7	202.3 ± 6.8

[z] DAFB: days after full bloom. Full bloom occurred on 30 Apr, 2003.
[y] FS: fruiting spur; NFS: non-fruiting spur.
[x] FS and NFS sections include bark and wood.
[w] Shoot includes current season wood and leaves.
[v] Total fresh weight per branch.

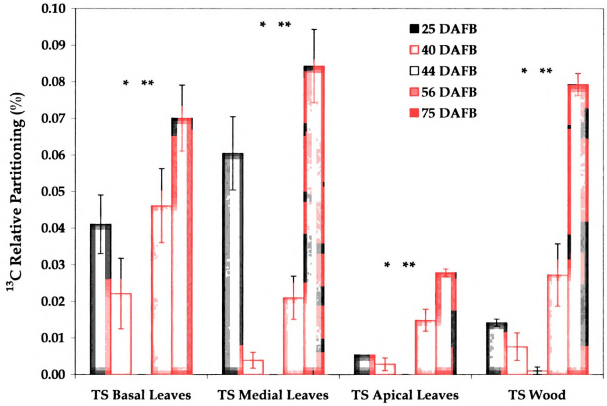
Appendix B.13. Relative ^{13}C partitioning among different organs on 2-year-old 'Ulster'/Gisela 6 sweet cherry branches at 48 h after $^{13}CO_2$ pulsing of fruiting spur leaves. Calculations are based on absolute amounts of ^{13}C recovered for each organ at each pulse-labeling (See Materials and Methods). Mean ± SE, n=5.

Organ	Relative ^{13}C Partitioning (%)				
	DAFB[x]				
	25	40	44	56	75
FS[z] leaves[y]	32.5 ± 2.1	30.4 ± 1.7	34.1 ± 2.2	17.9 ± 0.9	36.4 ± 5.5
NFS[z] leaves	0.7 ± 0.2	0.2 ± 0.2	0.0 ± 0.0	0.3 ± 0.2	0.2 ± 0.0
TS[z] basal leaves	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0
TS medial leaves	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0
TS apical leaves	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
TS wood	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0
FS wood	3.0 ± 0.7	8.8 ± 2.0	7.0 ± 1.6	2.5 ± 0.6	5.4 ± 1.4
NFS wood	0.5 ± 0.2	0.6 ± 0.4	0.0 ± 0.0	0.1 ± 0.0	0.4 ± 0.0
Fruit	63.2 ± 1.9	59.9 ± 2.2	58.9 ± 2.8	79.1 ± 1.1	57.3 ± 6.7

[z] FS: fruiting spur; NFS: non-fruiting spur; TS: terminal shoot.

[y] Fruiting spur leaves were pulsed directly with $^{13}CO_2$.

[x] DAFB: days after full bloom. Full bloom occurred on 30 Apr, 2003.



Appendix B.14. ^{13}C-Relative partitioning among leaves and wood of current season growth on 2-year-old 'Ulster'/Gisela 6 sweet cherry branches. Calculations are based on absolute amounts of ^{13}C recovered for each organ 48 h after each $^{13}CO_2$ pulse to fruiting spur leaves. For statistics, see Table 7. Mean ± SE, n=5. FS: fruiting spurs; NFS: non-fruiting spurs; TS: terminal shoot.

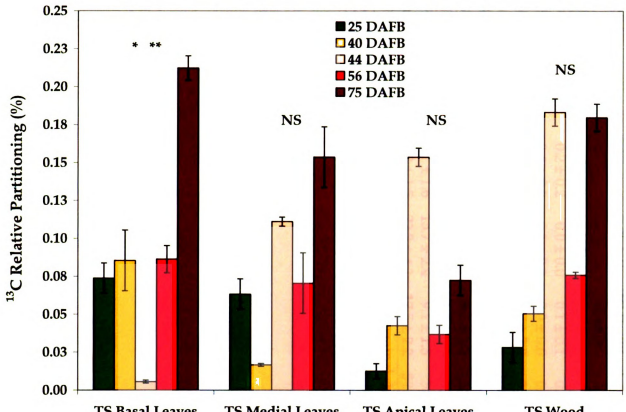
Appendix B.15. Relative ^{13}C partitioning among different organs on 2-year-old 'Ulster'/Gisela 6 sweet cherry branches measured at 48 h after $^{13}CO_2$ pulsing of the non-fruiting spur leaves. Calculations are based on absolute amounts of ^{13}C recovered for each organ at each pulse-labeling. Mean ± SE, n=5.

Organ	Relative ^{13}C Partitioning (%)				
	DAFB[x]				
	25	40	44	56	75
FS[z] leaves	0.2 ± 0.0	0.7 ± 0.4	0.0 ± 0.0	0.5 ± 0.4	0.2 ± 0.1
NFS[z] leaves[y]	41.3 ± 0.6	42.7 ± 2.7	46.1 ± 1.7	19.9 ± 2.1	49.4 ± 4.8
TS[z] basal leaves	0.1 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.0	0.2 ± 0.1
TS medial leaves	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.1
TS apical leaves	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1	0.0 ± 0.0	0.1 ± 0.0
TS wood	0.0 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.2 ± 0.1
FS wood	8.4 ± 1.5	16.9 ± 2.5	14.5 ± 5.1	5.1 ± 0.7	12.1 ± 3.9
NFS wood	4.2 ± 0.5	7.8 ± 0.7	7.6 ± 1.6	3.3 ± 0.5	5.0 ± 1.1
Fruit	45.8 ± 1.5	31.7 ± 5.3	31.3 ± 5.3	70.9 ± 2.6	32.7 ± 5.2

[z] FS: fruiting spur; NFS: non-fruiting spur; TS: terminal shoot.

[y] Non-fruiting spur leaves were pulsed directly with $^{13}CO_2$.

[x] DAFB: days after full bloom. Full bloom occurred on 30 Apr, 2003.



Appendix B.16. ^{13}C-Relative partitioning among leaves and wood of current season growth on 2-year-old 'Ulster'/Gisela 6 sweet cherry branches. Calculations are based on absolute amounts of ^{13}C recovered for each organ 48 h after each $^{13}CO_2$ pulse to non-fruiting spur leaves. For statistics, see Table 8. Mean ± SE, n=5. FS: fruiting spurs; NFS: non-fruiting spurs; TS: terminal shoot.

Appendix B.17. Relative ^{13}C partitioning in 2-year-old 'Ulster'/Gisela 6 sweet cherry branches at 48 h after $^{13}CO_2$ pulsing of terminal shoots. Calculations are based on absolute amounts of ^{13}C recovered for each organ at each pulse-labeling. Mean ± SE, n=5.

Organ	Relative ^{13}C Partitioning (%) DAFB[x]				
	25	40	44	56	75
FS[z] leaves	0.2 ± 0.2	0.0 ± 0.0	0.1 ± 0.1	0.5 ± 0.2	0.0 ± 0.0
NFS[z] leaves	0.4 ± 0.2	0.1 ± 0.1	0.0 ± 0.0	0.4 ± 0.2	0.2 ± 0.0
TS[z] basal leaves[y]	16.4 ± 0.6	16.2 ± 1.3	24.7 ± 2.7	9.3 ± 0.8	18.8 ± 2.5
TS medial leaves[y]	26.3 ± 2.7	22.7 ± 2.4	21.3 ± 2.1	10.6 ± 2.3	17.1 ± 2.4
TS medial leaves[y]	7.8 ± 1.5	7.2 ± 1.4	13.8 ± 1.9	8.1 ± 2.7	9.2 ± 1.9
TS wood[y]	4.9 ± 0.8	5.5 ± 1.1	8.7 ± 1.2	3.1 ± 0.8	5.8 ± 0.9
FS wood	8.8 ± 1.9	10.1 ± 2.9	4.8 ± 0.6	5.0 ± 0.6	10.7 ± 2.1
NFS wood	8.1 ± 1.6	15.7 ± 2.4	9.1 ± 1.3	3.7 ± 0.4	10.0 ± 1.6
Fruit	27.2 ± 1.5	22.3 ± 4.1	17.5 ± 2.2	59.2 ± 6.2	28.3 ± 6.0

[z] FS: Fruiting spur; NFS: Non-fruiting spur; TS: terminal shoot.

[y] Current season leaves and wood were pulsed directly with $^{13}CO_2$.

[x] DAFB: days after full bloom. Full bloom occurred on 30 Apr, 2003

Appendix B.18. Relative ^{13}C partitioning between pericarp and endocarp of 'Ulster'/Gisela 6 sweet cherry fruit 48 h after $^{13}CO_2$ pulsing of fruiting spur leaves at five datess. Calculations are based on absolute amounts of ^{13}C recovered for each fruit tissue at each pulse-labeling date. Mean + SE, n=5.

		Relative ^{13}C Partitioning (%)				
Tissue	DAFB[z]:	25	40	44	56	75
Pericarp		25.8 ± 1.7	21.4 ± 4.7	32.3 ± 3.4	77.4 ± 5.9	83.4 ± 6.4
Endocarp		74.2 ± 1.7	78.6 ± 4.7	67.7 ± 3.4	22.6 ± 5.9	16.6 ± 6.4

[z] DAFB: days after full bloom. Full bloom occurred on 30 Apr, 2003

APPENDIX C

Appendix C.1. Total number of aerial organs on 4-year-old 'Regina'/Gisela 6 sweet cherry trees at budbreak and post- budset (2003).

Tree	Side green bud stage		Terminal bud set	
	Fruiting spurs[z]	Non fruiting spurs[y]	Shoots[x]	Single bud[w]
1	173	860	95	1554
2	46	959	135	1749
3	74	759	77	992
4	94	782	100	1260
5	77	597	66	773
Average	93	791	95	1266

[z] Number of fruiting spurs on 2-year-old limb sections (2001 growth).
[y] Number of non-fruiting spurs on 1-year-old limb sections (2002 growth).
[x] Number of extension shoots that grew in 2003.
[w] Total number of single buds on extension shoots grown in 2003.

Appendix C.2. Tissues collected from 'Regina'/Gisela 6 sweet cherry tree at leaf abscission (Nov, 2002) and at budbreak (side green) stage (Apr, 2003) for ^{13}C analysis.

Tree section	Organ/Tissue	Year of growth
Trunk	Bark	1999[z], 2000[y], 2001[x]
	Wood	1999, 2000, 2001
Branch	Bark	2001, 2002[w]
	Wood	2001, 2002
	Spur buds	2001
	Single buds	2002
Root	Coarse (> 5 mm)	-
	Medium (1-5 mm)	-
	Fine (< 1mm)	-

[z] Growth of 1999 corresponds to 3-year-old-wood in fall 2002.
[y] Growth of 2000 corresponds to 2-year-old-wood in fall 2002.
[x] Growth of 2001 corresponds to 1-year-old-wood in fall 2002.
[w] Growth of 2002 corresponds to current season growth in fall 2002.

Appendix C.3. Aerial organs of 'Regina'/Gisela 6 sweet cherry trees sampled at different developmental stages during spring and summer (2003) for ^{13}C analysis.

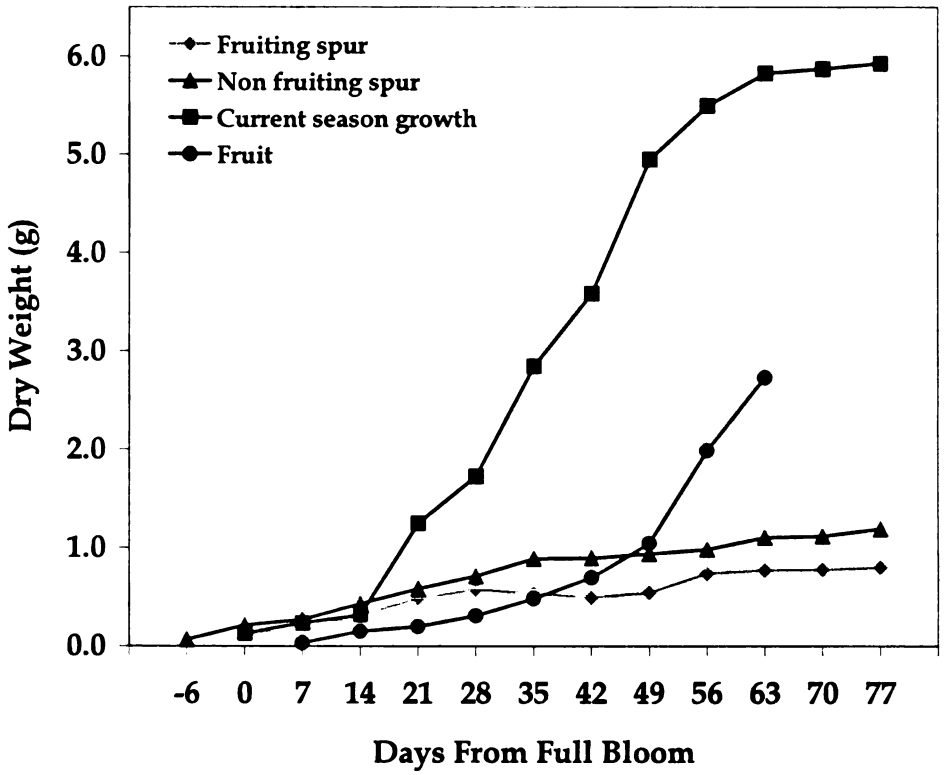
Stage	Organs						
	Buds[y]	Single flowers	Spur flowers	Fruiting spurs	Non-fruiting spurs	Shoots[x]	Fruits
Side green	x						
First bloom[z]		x	x		x		
Full bloom		x	x	x	x	x	
Fruit set				x	x	x	x
Stage I				x	x	x	x
Stage II				x	x	x	x
Stage III				x	x	x	x

[z] 'First white' and 'first bloom' stages overlapped during the same week.
[y] Includes vegetative and reproductive buds.
[x] Current season growth (2003).

Appendix C.4. Growing degree days (GDD) accumulated at each developmental stage and sampling substages for 'Regina'/Gisela 6 sweet cherry trees. Base temperature: 4.4 °C.

Developmental Stage	Days relative to full bloom	Accumulated GDD
Side green	- 15 to -12[y]	148-166
	-13[x]	163
First bloom[z]	- 4 to - 6	239 - 264
	-5	245
Full bloom[z]	0	264 - 319
	1	287
Fruit set	4 to 7	320 - 342
	7	342
Stage I	8 to 26	343- 533
	14	405
	21	469
	28	533
Stage II	29 to 42	534 - 722
	35	618
	42	722
Stage III	43- 64	723 - 1102
	49	843
	56	968
	63	1087

[z] Overall bloom period lasted ~ 10 to 12 days.
[y] Number of days at a specific developmental stage considering full bloom (9 May, 2003) as reference date.
[w] Sub-stage at which organs were sampled within each main developmental stage.



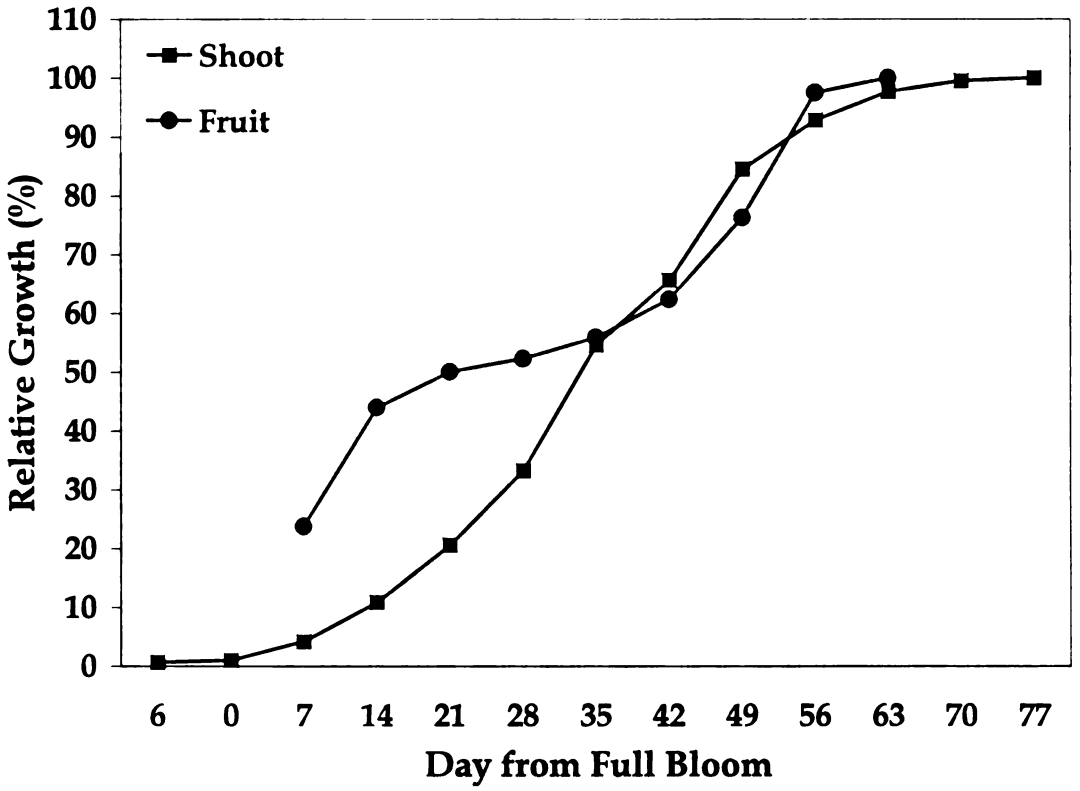
Appendix C.5. Accumulation of dry weight (DW) matter in 'Regina'/Gisela 6 sweet cherry spurs, shoots and fruit. Period between first bloom and terminal bud set (May to Jul, 2003). Weekly measurements, n=10. Fruit were eaten by raccoons 63 DFFB.

Appendix C.6. Dry weight (DW) of different organs at each developmental stage for 'Regina'/Gisela 6 sweet cherry trees, n=10.

Stage	Days relative to full bloom	DW (mg)							
		Bud	Single flower	Flower cluster	Spur flower	Fruiting spurs	Non-fruiting spurs	Shoot	Fruit
Side Green	-15	add							
First bloom	-6	-[y]	4.6	228.3	0.0	0.0	66.7	21.0	-
Full bloom	0	-	24.4	-	25.0	123.0	208.8	129.0	-
Fruit set	7	-	-	-	-	149.6	269.0	232.2	33.3
Stage I	14	-	-	-	-	315.5	425.9	316.5	148.2
	21	-	-	-	-	482.3	582.7	1249.3	196.6
	28	-	-	-	-	573.0	608.7	1726.0	307.4
Stage II	35	-	-	-	-	533.4	885.0	2847.9	478.0
	42	-	-	-	-	493.7	934.3	3588.4	696.2
Stage III	49	-	-	-	-	543.3	891.5	4951.9	1042.0
	56	-	-	-	-	738.0	978.8	5496.6	1984.8
	63	-	-	-	-	766.6	1492	5826.4	2731.8

[z] DFFB: days from full bloom.

[y] dash indicates that the organ was not sampled at a certain date.



Appendix C.7. Cumulative relative growth of 'Regina'/Gisela 6 sweet cherry shoots and fruits between first bloom and terminal bud set (May to Jul, 2003). n= 25 (fruit) and n=15 (shoots). Fruit was eaten by raccoons 63 DFFB.

Appendix C.8. ^{13}C atom % excess in different organs of 'Regina'/Gisela 6 sweet cherry at leaf abscission (Nov, 2003) and budbreak ('side green'; Apr, 2003). Data within the same sampling period (column) followed by the same small letter are not significantly different at $\alpha = 0.05$. Data for the same organ type (row) followed by the same capital letter are not significantly different at $\alpha = 0.05$ and $\alpha = 0.01$, respectively. Mean ± SE (n=5). For calculations see Materials and Methods.

Organ		^{13}C atom % excess						Obtained p-value[y]
		Leaf abscission			Side Green			
Trunk	Bark 1999	0.0117 ± 0.0006	ef	A	0.0078 ± 0.0008	e	B	0.0045
	Wood 1999	0.0391 ± 0.0040	a	A	0.0101 ± 0.0011	e	B	<0.0001
	Bark 2000	0.0095 ± 0.0010	ef	A	0.0072 ± 0.0006	e	A	0.0828
	Wood 2000	0.0286 ± 0.0018	bc	A	0.0080 ± 0.0007	e	B	<0.0001
	Bark 2001	0.0140 ± 0.0005	ef	A	0.0069 ± 0.0009	e	B	<0.0001
	Wood 2001	0.0196 ± 0.0031	de	A	0.0114 ± 0.0030	de	A	0.1146
Branch	Bark 2001	0.0119 ± 0.0010	ef	A	0.0076 ± 0.0008	e	B	0.0083
	Wood 2001	0.0262 ± 0.0061	bcd	A	0.0187 ± 0.0034	d	A	0.3219
	Bark 2002	0.0093 ± 0.0007	ef	A	0.0070 ± 0.0004	e	B	0.0222
	Wood 2002	0.0077 ± 0.0005	ef	A	0.0080 ± 0.0007	e	A	0.7958
Buds	Fruiting	0.0249 ± 0.0030	cd	B	0.0451 ± 0.0022	a	A	0.0003
	Non-fruiting	0.0335 ± 0.0014	ab	A	0.0382 ± 0.0020	ab	A	0.0909
Roots	Coarse	0.0325 ± 0.0030	abc	A	0.0383 ± 0.0029	ab	A	0.1970
	Medium	0.0309 ± 0.0040	bc	A	0.0332 ± 0.0062	bc	A	0.7596
	Fine	0.0252 ± 0.0030	cd	A	0.0274 ± 0.0070	c	A	0.7756
Obtained p-value[z]			< 0.0001			< 0.0001		

[z] p-value for comparison among different organs within the same stage (column).

[y] p-value for same organ compared between stages (row).

Appendix C.9. ^{13}C atom % excess for different organs during spring through different phenological stages (May to June, 2003). Data within the same sampling period (column) followed by the same small letter are not significantly different at $\alpha = 0.05$. Data for the same organ type (row) followed by the same capital letter are not significantly different at $\alpha = 0.05$. Mean ± SE, n=5. For calculations see Materials and Methods.

	^{13}C atom % excess																			
Stage:	First Bloom		Full Bloom		Fruit Set		Stage I								Stage II					
DFFB:	-6		0		7		14		21		27		34							
Single flower	0.0413 b ± 0.0028	A	0.0371 a ± 0.0019	B	-		-		-		-		-							
Spur flower	0.0466 a ± 0.0033	A	0.0368 a ± 0.0027	B	-		-		-		-		-							
Fruit	-[y]		-		0.0124 b ± 0.0010	A	0.0100 a ± 0.0009	B	0.0044 b ± 0.0003	C	0.0035 c ± 0.0004	C	0.0034 c ± 0.0002	C						
FS[z] leaves	-		0.0283 c ± 0.0027	A	0.0136 a ± 0.0013	B	0.0073 b ± 0.0007	C	0.0073 a ± 0.0007	D	0.0060 b ± 0.0008	D	0.0054 b ± 0.0004	D						
NFS[z] leaves	0.0377 c ± 0.0026	A	0.0327 b ± 0.0020	B	0.0116 bc ± 0.0010	C	0.0061 c ± 0.0002	D	0.0077 a ± 0.0003	D	0.0073 a ± 0.0006	D	0.0066 a ± 0.0004	D						
CSG[z] leaves			0.0350 b ± 0.0024	A	0.0106 c ± 0.0007	B	0.0048 d ± 0.0005	C	0.0041 b ± 0.0004	D	0.0032 c ± 0.0003	D	0.0027 c ± 0.0002	D						

[z] FS: fruiting spur; NFS: non-fruiting spur; CSG: current season shoot.

[y] Days from full bloom.

[x] Organ was not sampled at this stage.

Appendix C.10. Total ^{13}C content (µg ^{13}C) in different organs of 'Regina'/Gisela 6 sweet cherry trees during spring (May to Jun, 2003). Data within the same stage (column) followed by the same small letter are not significantly different at α = 0.05. Data for the same organ type (row) followed by the same capital letter are not significantly different at α = 0.05. Mean ± SE. n=5. For calculations see Materials and Methods.

	µg ^{13}C																					
Stage:	First Bloom [y]			Full Bloom			Fruit Set			Stage I									Stage II			
DFFB:	-6 [x]			0			7			14			21			27			34			
Single flower	42.9 ± 3.5	c	A	198.3 ± 12.1	c	A	-			-			-			-			-			
Spur flower	4359.0 ± 871.0	b	A	4306.4 ± 834.1	b	A	-			-			-			-			-			
Fruit	- [w]			-			223.9 ± 32.5	b	C	750.5 ± 81.4	b	A	463.3 ± 78.8	b	B	572.3 ± 100.0	b	B	786.2 ± 170.7	c	A	
FS [z] leaves	-			1422.1 ± 255.1	c	A	811.2 ± 131.4	b	A	953.9 ± 157.7	b	A	1912.4 ± 340.2	b	A	1277.3 ± 179.9	b	A	1186.5 ± 238.0	c	A	
NFS [z] leaves	9074.5 ± 773.3	a	E	24594.2 ± 1682.4	a	A	11012.7 ± 615.8	a	D	9805.0 ± 734.1	a	E	15920.8 ± 1216.0	a	C	15755.8 ± 1438.8	a	C	20769.7 ± 1008.5	a	B	
CSG [z] leaves	-			1959.4 ± 250.4	bc	B	1102.6 ± 144.7	b	B	673.6 ± 83.3	b	B	2244.6 ± 306.2	b	B	2348.7 ± 117.4	b	AB	3534.0 ±532.7	b	A	

[z] FS: fruiting spur; NFS: non-fruiting spur; CSG: current season growth (shoot).
[x] DFFB: days from full bloom.
[w] Organ was not sampled at this stage.