Scientia Horticulturae 124 (2010) 78–82

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Carbohydrate profiles in the graft union of young sweet cherry trees grown on dwarfing and vigorous rootstocks

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ARTICLE INFO

Article history:
Received 20 May 2009
Received in revised form 8 December 2009
Accepted 10 December 2009

Keywords:
Non-structural carbohydrates
Prunus avium L.
Starch
Sorbitol
Sucrose

ABSTRACT

In composite (scion–rootstock) dwarfing fruit trees, an overgrowth at the graft union is often observed, the severity of which is correlated with degree of dwarfing. The graft union of dwarfing sweet cherry (*Prunus avium* L.) rootstocks may limit soluble sugar transport or starch mobilization, leading to localized accumulation. Soluble sugars and starch were measured in the tissues surrounding the graft union of young 'Rainier' (2002) and 'Lapins' (2003) sweet cherry trees on 'Gisela 5' ('Gi 5'; dwarfing) and 'Colt' (vigorous) rootstocks. Separate rootstock shank, rootstock, graft union, and scion tissues were analyzed for both starch and soluble sugar content throughout the growing season in both years. Starch concentrations did not vary among locations within the graft union for 'Rainier' on either the dwarfing or vigorous rootstock, or for 'Lapins' on the dwarfing stock. However, for 'Lapins' on 'Colt', starch was highest in the rootstock shank and declined vertically (rootstock shank $\gg$ rootstock $>$ union $>$ scion). Soluble sugar concentrations were generally similar to or higher in scions on 'Gi 5' than on 'Colt', and were similar to or lower in the rootstock and rootstock shank on 'Gi 5' than on 'Colt'. Results suggest that rootstock has a significant effect of localized accumulations of carbohydrates above and within the graft union of 'Gi 5' and below the graft union of 'Colt'.

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1. Introduction

Sweet cherry (*Prunus avium* L.) trees generally are vigorous, growing over 10 m high naturally, making management and harvest difficult. On seedling rootstocks, cherry trees have low precocity, requiring 7–8 years for harvest of a profitable crop. On dwarfing rootstocks, cherry trees often produce precociously (i.e., 2–3 years to initial harvest), are more labor efficient, require less chemical usage per application, and produce more fruit per hectare than trees on seedling rootstocks. Best management practices for trees on dwarfing rootstocks have been difficult to develop in part because the underlying physiological and genetic mechanisms of dwarfing are not well understood (e.g., Zhu et al., 1999).

Dwarfing scion–rootstock plant systems often exhibit increased cross-sectional area at the graft union compared to non-dwarfing plant systems and anatomical differences have been found (Olmstead et al., 2006; Gonçalves et al., 2007) that may increase hydraulic resistance (Cohen et al., 2007). In addition, reduced polar auxin transport in dwarfing systems may reduce cambial activity and, specifically, xylem formation in the graft union region (Soumelidou et al., 1994). Graft incompatibility has been proposed as a dwarfing mechanism manifested by increased quantity and activity of phenolic compounds and peroxidases in the graft union of *Prunus* species (Deloire and Hebant, 1983). Low concentrations of starch in the rootstock also have been observed with some incompatible *Prunus* combinations (Breen, 1975; Yano et al., 2002).

In trees, smaller canopies tend to produce less wood and absolute amounts of storage carbohydrates than larger canopies (Canham et al., 1999). On dwarfing rootstocks, smaller tree canopies are manifested by shorter shoots and less leaf area (Tubbs, 1973), which decreases overall carbohydrate production for growth and storage. Stored carbohydrates are critical for allocation to new vegetative and reproductive structures in spring, particularly in sweet cherries which have a relatively short bloom-to-ripening period (60–90 days; Tukey, 1942). Smaller canopies have been associated with negative effects on storage carbohydrates and fruit quality (e.g., Whiting and Lang, 2004).

The graft union can be a barrier to transport of nutrients and water as a result of anatomical development (Simons and Chu, 1983; Cohen et al., 2007), scion/rootstock incompatibility (Schöning and Kollman, 1997), or differences in carbohydrate transport to the rootstock (Salvatierra et al., 1998). Often, swelling in the graft union area is observed in trees on dwarfing rootstocks. It is not known whether this is due to specific scion/rootstock combinations, high concentrations of carbohydrates above the union, or

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0304-4238/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.scienta.2009.12.022

additional parenchymatous tissue that may interfere with water and nutrient transport (Tubbs, 1973).

The objective of this research was to determine whether the carbohydrate profiles at the graft union could have implications for growth early in the life of the grafted tree. High carbohydrate concentrations may be associated with overgrowth at the graft union in dwarfing combinations. We hypothesized that the graft union of dwarfing cherry rootstocks limits soluble sugar transport or starch mobilization, leading to localized accumulation of sugars or starch above the graft union.

2. Materials and methods

Sixty 1-year-old nursery trees of 'Rainier' sweet cherry grafted onto 'Gi 5' (*Prunus cerasus* L. × *Prunus canescens* L., [dwarfing]) and 'Colt' (*Prunus avium* × *Prunus pseudocerasus* Lindh., [vigorous]) were used. Rootstocks were planted in May 2001 at the Horticulture Teaching and Research Center (HTRC) in East Lansing, Mich. in a randomized complete block design of three replications, consisting of three trees per replicate. The soil was a Marlette fine sandy loam, well-drained with a minor slope (2–6%). There were 2.4 m between trees and 3.0 m between rows. Herbicide strips were maintained in the tree row, and alleyways were grass, mowed periodically. Trees were irrigated by overhead sprinklers (2.5 cm per week) and fertilized at planting with a controlled-release fertilizer (Osmocote, 14-14-14, The Scotts Miracle-Gro Company, Marysville, Ohio). Meteorological data were retrieved from the Michigan Agriculture Weather Network (MAWN) station on-site. Thermal time, expressed as growing degree days (base 10 °C) was calculated using the Baskerville–Emin method (Baskerville and Emin, 1969). Flowers were removed from all trees to eliminate the influence of reproductive development on carbohydrate demand.

In April 2003, 165 one-year-old trees of 'Lapins' sweet cherry on 'Gi 5' or 'Colt' were planted in a completely randomized design with six replicates and 10 trees per replicate at HTRC. There were 1.8 m between trees and 3.0 m between rows, with herbicide strips, grass alleyways, fertilization, and irrigation as described above. All flowers were removed.

In 2002, terminal shoot length of 'Rainier' was measured weekly from budbreak until leaf fall, using the uppermost meristem on the tree, while in 2003, measurements in 'Lapins' were limited to the period of maximum shoot growth. Additional measurements in 2003 included lateral shoot length, trunk diameter at three locations (above, at, and below the graft union), and number of vegetative and reproductive nodes that formed. Trunks were assumed to be circular.

Trees were destructively harvested and sampled eight (2002, $n = 3$) and twelve (2003, $n = 10$) times during the growing season between budbreak and leaf fall (Day of Year [DOY] 121–304, 2002; DOY 119–213, 2003), roughly twice monthly during the period of active shoot growth, and once monthly thereafter. Concentrated sampling of 'Lapins' occurred during the period of maximum shoot elongation in 2003. Cross sections ~4 cm thick were taken from four regions around the graft union:scion (2 cm above the graft union), graft union, trunk (from 2 cm below the graft union), and rootstock (from 2 cm below the soil surface). Samples were placed immediately into liquid nitrogen for transfer to a −80 °C freezer for storage. Tissues were lyophilized, ground in a Wiley Mill (40 mesh screen; Thomas Scientific, Swensboro, NJ), and stored with desiccant prior to carbohydrate analysis.

2.1. Non-structural carbohydrate extraction

Lyophilized tissue (0.2 g dry mass) was extracted four times with 3 ml of 80% (v/v) ethanol on ice, per the method by Robbins and Pharr (1987). Briefly, iced extracts were ground with a homogenizer (Brinkmann/KINEMATICA Polytron, Westbury, NY). The homogenate was held in a boiling water bath for 5 min, immediately cooled in an ice bath and centrifuged at 9600 × *g* for 10 min to obtain ethanol-soluble and ethanol-insoluble fractions. The procedure was repeated three additional times. The final pellet (ethanol-insoluble fraction) was retained for starch determination. All supernatant (ethanol-soluble fraction) was collected for soluble sugar analysis.

2.2. Starch determination

The ethanol-insoluble fraction was enzymatically hydrolyzed using dialyzed amyloglucosidase (MWCO 3500, Fisher Scientific, Hampton, NH; Sigma–Aldrich Chemical Co., A-9913, St. Louis, MO) prior to glucose analyses as previously described (Robbins and Pharr, 1987). Released glucose was analyzed using a glucose-6-phosphate dehydrogenase (G6PDH) enzymatic linked assay (Robbins and Pharr, 1987). The reduction of $NADP^+$ by G6PDH was determined spectrophotometrically at 340 nm (Unicam UV 300, ThermoSpectra, San Jose, CA).

2.3. Soluble sugar determination

Soluble sugars were determined by the method of Roper et al. (1988). In brief, ethanol was evaporated off in the ethanol-soluble fractions and rehydrated to 1 ml. A fraction (200 μl) of this aqueous sample was dehydrated prior to derivatization using hexamethyldisalizane and trifluoroacetic acid (Sweeley et al., 1963; Williams and Martin, 1967). Derivatized samples and sugar standards were then analyzed by Gas Chromatography-Flame Ionization Detector (GC-FID; HP 5890 II, Agilent Technologies Inc., Palo Alto, CA). GC-FID conditions are explained in detail by Roper et al. (1988). External standard (sorbitol, fructose, glucose, myo-inositol, and sucrose; Sigma–Aldrich Chemical Co., St. Louis, MO) calibration method was used for quantification and identification.

2.4. Statistical analysis

Data were analyzed using General Linear Models (GLM) and analysis of variance adjusted for repeated measures as appropriate for the data set with alpha levels set *a priori* at 0.05. Where data were not distributed normally, a logarithmic transformation was applied. Repeated measures were back-transformed. Mean separation was accomplished using Tukey's HSD as appropriate (SAS Institute, Inc., Cary, NC).

3. Results

Terminal shoots of 'Rainier' and lateral shoots of 'Lapins' on 'Gi 5' were shorter than on 'Colt' (Figs. 1 and 2), resulting in dwarfed phenotype. Growth rates in 2002 did not differ between combinations during the period of maximum shoot elongation (DOY 143–DOY 175) as a result of high variation in the growth rate ($p > 0.05$; $n = 6$). In 2003, 'Lapins' on 'Gi 5' lateral shoots consistently had lower growth rates than 'Lapins' on 'Colt' ($p = 0.01$, $n = 10$). Terminal shoots on 'Rainier' and lateral shoots on 'Lapins' reached maximum lengths within the same 2-week period in 2002 (Fig. 1) and 2003 (Fig. 2). In addition, 'Lapins' on 'Gi 5' had more growing points (nodes) than 'Lapins' on 'Colt' ($p > 0.05$; data not shown), indicating a potential for increased carbohydrate demand for growth in the dwarfing combination. In both cultivars, rootstock did not have an effect on the timing of cessation of rapid shoot growth ($p > 0.05$).

In 2003, stem cross-sectional area (A_{stem}) for each scion/rootstock combination and tissue section increased only slightly