VASCULAR AND METABOLIC IMPACT OF DWARFING ROOTSTOCKS ON SWEET CHERRY (*Prunus avium*, L.)

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ABSTRACT

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The availability of dwarfing rootstocks for use in sweet cherry (*Prunus avium* L.) orchards has the potential to revolutionize the industry, allowing for higher densities per unit land area, lower labor costs, and earlier crop yields compared to standard rootstocks. However, understanding of dwarfing mechanisms is limited. Many hypotheses for the mechanism of dwarfing have been proposed, including: decreased water transport caused by abnormal development of vascular tissue in the graft union; decreased plant hormone concentrations and/or hormone ratios between scion and rootstock; vascular incompatibility-induced increases in scion tissue carbohydrate concentrations, and metabolic dysfunction between scion and rootstock due to genetic differences. The research described herein examined the graft unions of three dwarfing sweet cherry scion/rootstock combinations (Rainier/Gisela 5 [R/Gi 5], Rainier/Gisela 6 [R/Gi 6], and Lapins/Gisela 5 [L/Gi 5]), and two vigorous combinations (Rainier/Colt [R/C] and Lapins/Colt [L/C]). Two hypotheses were tested: 1) that vessel characteristics would be smaller in dwarfing rootstock combinations, and 2) that carbohydrate concentrations would increase above the graft union due to physical limitations or alterations in sink/source strength. Vascular anatomy and carbohydrate concentrations of tissues within and surrounding the graft union were characterized.

Vessel lumen areas (VLA) in R/Gi 5 graft unions were smaller than in R/C, ($p < 0.05$). Vessel hydraulic diameter was reduced in graft unions of R/Gi 5. However, there were no differences in vessel frequency per mm^2, suggesting that there are a greater proportion of narrow vessels that are differentiated in the graft union of a dwarfing combination. No differences in vessel diameter in areas of maximum water transport were found between L/Gi 5 and L/C. In addition, vessels differentiated at acute angles (on a bias) to the longitudinal axis of the tree in R/Gi 5 and L/Gi 5. This, in combination with narrow vessel diameters visualized with a water soluble fluorochrome, Safranin O, was associated with reduced dye uptake through the graft union of L/Gi 5.

The pattern of starch accumulation and reallocation throughout the growing season was unique in R/Gi 5 when compared to R/C. Soluble sugar pool size in scion tissues increased in R/Gi 5 compared to R/C and tissues below the graft union. Consequently, total non-structural carbohydrate (TNC) concentration was elevated in scion tissue compared to graft union, rootstock or rootshank tissue. In L/C rootshank tissue, starch began accumulating by mid-season and continued to increase beyond terminal bud set, while there was no increase in L/Gi 5. In addition, photosynthetic rates were higher in L/Gi 5 vs. L/C. Carbohydrate storage was reduced in young ungrafted dwarfing rootstocks (1 year old) and grafted dwarfing combinations (6 months old) which may negatively affect early season scion growth. Dwarfing cherry rootstock systems differ in vascular characterization and carbohydrate profiling compared to standard root systems.

To my Father and Mother, Robert and Pranee Neumann.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

VASCULAR AND METABOLIC IMPACT OF DWARFING ROOTSTOCKS ON SWEET CHERRY (*Prunus avium* L.)

CHAPTER ONE

INTRODUCTION

Fruit tree architectures have long been manipulated to control growth and improve productivity. Grafting, in which a rootstock and scion are joined to create a composite tree, provides a method of vegetative reproduction in which desired genetic components of the scion are preserved (Table 1). Grafting also may confer benefits that a seedling tree lacks, such as increased disease resistance, reduced tree height, or increased reproductive precocity (Hartmann et al., 1997). Grafting was known to the Chinese and Greeks (Aristotle and Theophrastus) (Roberts, 1949; Shen, 1980). Renewed interest in grafting during the Renaissance increased the knowledge of grafting practices and by the sixteenth century, grafting techniques in Europe were performed with increased attention to cleanliness and proper mechanics of grafting, including matching layers of cambium to each other (Hartmann et al., 1997). This resulted in greater success in the production of grafted plants. Fundamentally, many contemporary grafting techniques are the same as those reported by Bailey (1891) in the latter 19th century.

Many fruit tree species are large (>10 m in height) in the wild and can take many years to reach reproductive maturity (e.g., 5 to 7 years for sweet cherry (*Prunus avium*) trees to produce a crop). However, the introduction of dwarfing rootstocks, most successfully in apples (*Malus*, spp.), has been very important to facilitate: reduced tree height, improved labor efficiency, and reduced pesticide

use per application, increased precocity, and increased productivity (yield efficiency). These potential advantages also apply to sweet cherry (Lang, 2000; 2001; Webster and Lucas, 1997). The possibility of increased orchard planting density and yield/ha (acre), while recouping operating costs 2 to 3 years sooner due to earlier crop production, outweigh the higher initial costs of planting dwarfing rootstocks (Seavert, 1997). In the following text, the use of 'dwarfing' and 'semi-dwarfing' are relative to the study reported. The ability of rootstocks to reduce tree size will be dependent upon soil type and climactic conditions (Perry, 1987, Perry et al., 1997).

Even though dwarfing rootstocks are now commonly used in apple production, the underlying mechanisms of dwarfing in these grafted tree systems are not well understood (Jones, 1986; Lockard and Schneider, 1981a; Rao and Berry, 1940; Robitaille and Carlson, 1976; Zhu et al., 1999). Furthermore, results attained with apple systems may or may not be extrapolated effectively to cherry trees (Jones, 1986; Lockard and Schneider, 1981a; Tukey, 1942). In apple systems, the explanation for dwarfing has rested mainly with transport restrictions in either the phloem or xylem (Atkinson et al., 2003; Bieleski, 2000; Lockard and Schneider, 1981a; Jones, 1976). However, most research on cherry scion dwarfing has focused on the production of phenolic acids at the graft union, and translocation of these compounds into the scion, where they may act as a growth inhibitor (Gebhardt and Feucht, 1982; Jones, 1986; Schmid and Feucht, 1982; Yu and Carlson, 1975). Additional hypotheses for dwarfing of scion growth have included: an alteration of hormone interaction(s) between

rootstock and scion (Lockard, 1976; Kamboj et al., 1999; Soumelidou et al., 1994a; Yadava and Dayton, 1972); a limitation in water and/or nutrient transport through the rootstock (Atkinson et al., 2000; 2001; 2003; Higgs and Jones, 1991); the existence of some level of chemical or genetic incompatibility, (Feucht et al., 1983; Lockard and Schneider, 1981a; Schmid and Feucht, 1981); or the lack of accuracy in lining up cambial tissues during grafting which then lead to the previously mentioned problems.

Relating the mechanisms of dwarfing in apple systems to cherry may be complicated by differences between the two crops in growth habit and fruit development. Cherries require only 60 to 90 days for growth and maturation of fruit compared to 120 to 180 days for apple (Tukey, 1942). Due to the relatively short fruit development period, early growth and floral development of sweet cherries is supported largely by storage reserves synthesized the previous year (Keller and Loescher, 1989). In contrast, apple fruit development is supported largely by photosynthates derived from the current season's canopy (Crane et al., 1976; Forshey et al., 1983; Fujii and Kennedy, 1985). Often, sweet cherry scion cultivars grafted on dwarfing rootstocks carry an excess number of fruit, resulting in small fruit size unsuitable for fresh market production (Lang, 2000). In addition to reducing plant height and altering precocity, dwarfing rootstocks may lead to alterations in carbon partitioning among the various parts of the tree, influencing resources available for early growth. Understanding dwarfing mechanisms could improve management in sweet cherry orchards on new dwarfing rootstocks to optimize yield and economic return to the grower.

Dwarfing Mechanisms

Grafting Techniques

One of the most important aspects of ensuring a successful scion-rootstock union is use of an appropriate grafting or budding technique. There are a number of possible grafting configurations, including cleft grafts, whip-and-tongue grafts, chip budding, and T-budding, all of which are used by commercial tree fruit nurseries (Table 2; Hartmann et al., 1997). In bud grafting of fruit trees, a physical matching of the cambium layers between the scion and rootstock tissues is not necessarily required. Rather, the continuous and protected contact between scion and rootstock tissues at the future graft union is essential. To insure this contact, the graft union area is wrapped with either polyethylene or rubber tape to hold the union in place and maintain high humidity around the union to facilitate the formation of callus tissue. Formation of callus or wound tissue originates from parenchyma cells in both the scion and rootstock tissues, and often begins to differentiate into phloem or xylem elements within two weeks of the grafting process, depending on the ratio of endogenous auxin and cytokinins (Aloni, 1988). Higher concentrations of auxin aid in the formation of xylem elements, while lower concentrations promote phloem elements (Aloni, 1995). However, formation of completely functional vascular connections between scion and rootstock may take as long as eight to ten months depending upon the compatibility of the grafted system components (Deloire and Hebant, 1983).

In apples, the grafting configuration can have a direct effect on the success of the union. For example, chip budding leads to stronger unions, more uniform trees, and increased lateral branching compared to shield budding (Howard and Skene, 1974). Additionally, time of year (fall vs. spring), temperature, maintenance of moisture at the graft union, specific plant material characteristics (i.e., age and diameter of the rootstock, carbohydrate reserve status), virus-free scion and rootstock, and genetic compatibility are all factors that influence success of a union between rootstock and scion.

Dwarfing – A Function of Incompatibility

It has been hypothesized that dwarfing is a mild form of incompatibility between two genetically different plant materials that comprise the scion and rootstock (Simons, 1982). Often, incompatibility can be indicated by the presence of certain chemical compounds in the developing graft union, like peroxidases and phenolic acids, specifically monophenols (Schmid and Feucht, 1985; Treutter et al., 1990). In dwarfing systems, these types of chemicals are often present in the graft union.

Incompatibility is defined as failure or deterioration of the graft union over time (Moore and Walker, 1981), possibly leading to tree death. Incompatibility may not be expressed at the time the graft union is undergoing the initial healing, but deterioration of union connections can take years to occur (Hartmann et al., 1997). Any discontinuity in the graft tissue, or physical or mechanical stress, can produce persistent wound periderm in which necrosis can worsen over the years

(Simons, 1982; Simons and Chu, 1984). Symptoms may not be visible until a mechanical pressure is applied and the scion breaks from the rootstock at the graft union (Waugh, 1904). For example, the sweet cherry 'Sam' on Cer W11 (Weiroot 11, *P. cerasus*) exhibited incompatibility symptoms, that resulted in death within five weeks (Schmid and Feucht, 1985). However, incompatibility in some combinations of pear (*Pyrus communis* L., cv. Bartlett) and quince (*Cydonia oblonga* Mill.) may not be detected for two years (Herrero, 1951). Incompatibility between stock and scion appears to be associated with concentrations and activities of peroxidase, acid phosphatase, and/or phenolic acids throughout the graft union. In the pear/quince (scion-rootstock) combination, the causal agent of failure was identified to be a cyanogenic glycoside (prunasin) produced by the quince rootstock. When the compound comes in contact with the pear tissue, the glycoside decomposes in a reaction with the ubiquitous plant protein arbutin (1-hydroquinone-β-glycoside), and produces toxic hydrocyanic acid (Gur et al., 1968). In *Prunus* interspecific grafts, the phenolic compound prunin can accumulate in the graft union, leading to a layer of necrotic tissue and eventual failure of the graft union (Angelica et al., 1999; Dirr et al., 1994; Usenik and Štampar, 2000).

Although peroxidases and phenols have been associated with incompatibility, they may also be involved in the dwarfing phenomenon, particularly when dwarfing is viewed as a function of incompatibility. In the incompatible sweet cherry combination of 'Sam' and Weiroot 11 (W11), reduced levels of phloem peroxidases in cambial regions were observed compared to

compatible combinations (Feucht et al., 1983). However, upon further examination of these rootstock combinations, the results reported may be due to the virus sensitivity of the rootstocks, rather than incompatible responses (Lang et al., 1997; Lang et al., 1998).

Peroxidases are involved in polymerization of cell walls and lignification of tissues where they play an important role in the reactions that crosslink individual polysaccharide units, particularly in phloem (Fry, 1982; Mader and Amberg-Fisher, 1982). Levels and/or activity of peroxidase enzymes can be an indicator of cell wall strength, which is one factor in determining the success of the developing graft union (Feucht et al., 1983). Strong reactions of peroxidase with diaminobenzidine (DAB) (Schmid and Feucht, 1980) suggest increased lignification of cell walls in compatible scion-rootstock unions. Peroxidase reactions in 'Sam'/F 12/1 (*P. avium*) remained strong in the rootstock, union, and scion of this combination as compared to low peroxidase activity in dwarfing combinations ('Sam'/W10, W11 and W 13) over a period of eight weeks (Feucht et al., 1983). However, low peroxidase activity found in phloem cells of dwarfing combinations suggested that cell walls in this region were less rigid, and prone to graft union failure when exposed to physical or mechanical stress (Schmid and Feucht, 1985). Based on the degree of lignification, compatibility of the union could be predicted, with dwarfing combinations ('Sam'/W10, W11 and W 13) exhibiting symptoms of incompatibility or virus sensitivity.

These differences in peroxidase activity between successful dwarfing and vigorous combinations may be due to the combination of genetic systems. When

homograft and heterograft systems are compared to each other, an overall reduction in peroxidase activity was found in homograft systems, with an increase in peroxidase activity above and below the graft union of heterograft systems (Feucht et al., 1983; Schmid and Feucht, 1982). Based on the fact that homografts consist of the same genetic material grafted together, while heterografts consist of different genetic material grafted onto one another, the difference in peroxidase activity may be the response of two genetically different tissues in contact with each other rather than a consistent indication of incompatibility or virus sensitivity. Of dwarfing combinations currently grown, all are heterogenetic grafts (e.g., 'Rainier' (*P. avium*)/ Gisela 5 (*P. cerasus x P. canescens*).

Although altered levels of peroxidase activity may be involved in the dwarfing phenomenon, some level of activity is necessary for the production of a functional vascular system. A functioning vascular system is not only important for the translocation of photosynthates and water, but movement of plant hormones as well. In addition to its importance for cell wall formation, peroxidase activity parallels hormone-induced changes in tissue growth and development surrounding the graft union (Wolter and Gordon, 1975). With auxin treatments in aspen (*Populus tremuloides* Michx.), an increase in callus tissue development paralleled an increase in peroxidase activity (Wolter and Gordon, 1975). Peroxidase involvement has been demonstrated in the degradation of IAA, which is a major determinant in the balance of vascular system differentiation (Aloni and Zimmerman, 1983; Epstein et al., 1980). Thus, peroxidases may be

involved not only in determining cell wall strength, but in translocation of hormones as well.

Phenols are postulated to be involved in dwarfing mechanisms by reducing growth, and enhancing precocity, particularly in apples. Removal and transfer of the bark from a dwarfing rootstock (Malling 26; M 26) to a vigorously growing tree (Gravenstein/Malling-Merton 111; MM.111) reduced growth as a traditional interstock would be expected to do (Lockard and Schneider, 1981b). This could be the result of specific phenols present in the bark. Phenols are believed to reduce growth by interfering with plant hormone translocation, especially indole-3-acetic acid (IAA) (Gaspar et al., 1992; Volpert et al., 1995). Specifically, monophenols can enhance the catabolism of IAA (Volpert et al., 1995), whereas polyphenols assist in deterring catabolism of IAA. In heterogenetic graft combinations, as with a dwarfing rootstock, there are inherent differences in phenol concentrations between scion and rootstock (Feucht and Treutter, 1991). The role of such phenolic acids in cell metabolism is uncertain; however, they are thought to be involved by conferring resistance to pathogens (Kirkham, 1957) and insects (Levin, 1971; Ossipov et al., 2001). They are rarely translocated and act in the cell where they are produced (Bate-Smith, 1962; Whetten et al., 1998). Thus, in dwarfing combinations, the greatest activities of phenols are observed in the graft union, where scion and rootstock interact (Feucht and Treutter, 1991, Treutter et al, 1990).

The naturally occurring phenolic acid concentrations in dwarfing rootstocks have been examined as a possible mechanism of the increased

precocity that they appear to confer to the scions. Lower concentrations of phenolic acids in ungrafted M.9 apple rootstock (dwarfing) may contribute to its precocity, compared to ungrafted M.16 rootstock which develops reproductive buds four to five years later. Phenolic acids, specifically phloridzin, were found in high concentrations in vigorously expanding shoots of ungrafted M.16 that produced only vegetative buds, but at lower concentrations in the same tissues of M.9, which produced both floral and vegetative buds (Martin and Williams, 1967). Phloridzin has been suggested as a growth inhibitor to flowering (Grochowska, 1964), with increased vegetative development (Jones, 1976). Its increased presence in M.16 was confirmed by UV-spectrophotometer measurements (Martin and Williams, 1967). In shoot tips of the dwarfing apple rootstock M.7, both phloridzin and phloroglucinol enhanced shoot tip growth at levels of 1 mM (Jones, 1976). Thus, it appears that these phenolic acids may enhance vegetative development at the expense of reproductive development (Jones, 1976; Growchowska, 1964). If true for other vigorous and dwarfing rootstock combinations, this is a significant finding that commands increased research attention. However, research involving the role of phenols in dwarfing and incompatibility responses is often difficult (P. Andrews, personal communication) and monetary support for research in incompatibility responses since the early 1980's has shifted to elucidating the role of phenols for human heath benefits (e.g., Hollman, 2001; Parr and Baldwell, 2000).

The concentration at which phenolic acids actively affect plant growth has been debated; while amounts of 1 mM have promoted vegetative growth, lower

levels (between 0.1 to 0.4 mM) of phenolic compounds found in apple rootstock bark (including phloridzin and phloretin) inhibited growth in a lettuce hypocotyl bioassay (Lockard and Schneider, 1981b). These types of germination bioassays (using herbaceous species with low tolerance for secondary plant products) have been used to detect differences in the activity of phenolic acids on growth because of the difficulty of similar bioassays in trees due to large tree size (Lockard and Schneider, 1981b). It should be noted that direct comparisons between these bioassays and growth responses in woody plants may not be possible. For example, both phloridzin and phloretin were found in concentrations >20,000 ppm in the rootstock bark of both MM.111 and M.26. However, higher amounts were quantified in MM.111 (96,000 ppm; vigorous) than in M.26 (66,000 ppm; dwarfing) (Lockard and Schneider, 1981b). Additionally, in rootstock bark and new roots of M.26, numerous phenolic compounds were found at a concentration much higher (335 times) than considered lethal to lettuce hypocotyl elongation (Lockard and Schneider, 1981b). Thus, the relative amounts of phenolic acids in bark tissue of different woody species have to be considered for their potential to cause inhibition of growth.

Cell-Cell Signaling Interactions

Cell-cell interactions are involved in the formation of callus tissue and plant cell walls. In grafting scion and rootstock tissue, initial contact is important to begin the process of callus formation and wound repair. There are three

distinct events that universally occur during the creation of the graft union: 1) cohesion (union) between the rootstock and scion, 2) production of callus cells, and 3) differentiation of callus and parenchyma cells into vascular elements. Incompatibility responses may lead to necrosis at the graft union as a result of lethal cell-cell interactions (Ermel et al., 1999).

Plasmodesmata, which connect cytoplasm between living cells, play an important role in cell-cell interactions as a conduit for signaling proteins (Ghoshroy et al., 1997; Lucas, 1995), as well as numerous plant viruses (e.g., Esau, 1948; Fisher et al., 1992; Lucas and Gilbertson, 1994). There are a number of macromolecules involved in signaling, such as hydroxyproline-rich glycoproteins (HRGPs), arabinogalactan proteins (AGPs), glycine-rich proteins (GRPs), and proline-rich proteins (PRPs), in addition to others (Cassab, 1998). Many of these glycoproteins that travel through plasmodesmata (Ghoshroy et al., 1997; Lucas, 1995) are involved in specific plant morphogenetic processes at the supracellular level, rather than the cellular level (Cassab, 1998; Lucas et al., 1993). For example, HRGPs, or extensins, are involved in pollen tube growth in specific pollen-pistil interactions (Heslop-Harrison, 1987). In certain self-incompatibility (SI) responses, interactions between glycoproteins of pollen and pistil can arrest pollen tube development, prevent fertilization, and subsequently fruit development (Heslop-Harrison, 1975; McCubbin and Kao, 2000; Takayama and Isogai, 2003). The transfer of glycoproteins as a signaling molecule between scion-rootstock tissues in dwarfing rootstock systems may play an important role in determining scion growth and development.

Incompatibility in other plant processes may provide insight into proposed graft incompatibility mechanisms in woody systems. Self incompatibility in monoecious reproductive systems (perfect flowers containing functional ovaries and stamens) serves to promote outcrossing and ensure genetic variability (Heslop-Harrison, 1975; McCubbin and Kao, 2000). Through genetic and cell-cell signaling via glycoproteins, SI mechanisms prevent the self-fertilization of an ovary in a perfect flower system by arresting pollen tube growth via a signaling cascade (Heslop-Harrison, 1975; McCubbin and Kao, 2000; Takayama and Isogai, 2003). Response times can vary, but SI mechanisms are often manifested within a few minutes (Dickenson, 1995). Cell-cell recognition is associated with differences in the specificity of certain alleles, designated as '*S*' or self-incompatibility alleles (Bateman, 1955). Based on the heterogenetic nature of dwarfing scion-rootstock systems, initial contact between tissues and the transport of plant specific glycoproteins may determine compatibility responses prior to the production of callus tissue.

Physical Limitations and its Contribution to the Dwarfing Phenomenon

In addition to grafting techniques and graft incompatibility as a factor in dwarfing, the development and differentiation of tissues within the graft union are a major determinant of a successful grafted system. Callus formation results in the differentiation of cellular components involved in the transport of photoassimilates and water. However, this process can be altered negatively with the combination of a scion and a dwarfing rootstock. A number of

hypotheses have implied that a physical restriction is a component of the dwarfing phenomenon, including: alterations in phloem/xylem ratio (Beakbane and Thompson, 1939; 1947), accumulation of undifferentiated cells in dwarfing systems, discontinuity of the vascular system between the grafted components (Simons, 1982; Simons and Chu, 1984), and/or reductions in hydraulic conductivity due to restrictions of the vascular system (Atkinson et al., 2003), as well as fundamental differences in root volume between dwarfing and vigorous rootstocks (Devyatov, 1996; Zhu et al., 1999).

The Vascular System

In plants, two main systems distribute water, nutrients and macromolecules throughout the whole plant. The xylem distributes water and mineral nutrients, while the phloem and its associated elements are responsible for translocation of sugars and other macromolecules (Salisbury and Ross, 1992). Macromolecules move through the phloem system via companion cells within the sieve elements that together form sieve tubes. Sieve elements do not contain a nucleus, and thus must pair with companion cells to maintain physiological functions (Oparka and Santa Cruz, 2000). These two cells form the sieve element-companion cell complex (SE-CC complex).

Structural anomalies in these two main distribution networks, and specifically the graft union (such as development of the vascular system on a bias), can result in decreased water and nutrient status in the scion due to reduced transport volume. Healing of the graft union commences as callus

tissue generated by wounding differentiates into the vascular connections between rootstock and scion (Simons and Chu, 1983; Ussahatanonta and Simons, 1988). When scions are grafted to dwarfing rootstocks, reduced water and nutrient supply to the scion has been hypothesized as a factor in the reduced vegetative growth of scion (Jones, 1971; Olien and Lakso, 1984; 1986).

Phloem/Xylem Ratio

In support of the "phloem/xylem ratio" hypothesis, studies have found that a higher ratio of phloem to xylem tissue exists in scions grafted onto dwarfing rootstocks and in ungrafted dwarfing rootstocks than in scions grafted onto vigorous rootstocks (Simons and Chu, 1983). Thus, the physical area occupied by xylem vessel elements is reduced in terms of both size of individual cells and number of xylem cells, compared to identical scion wood grafted to vigorous rootstocks. This alteration in vascular area is hypothesized to physically limit the rate of water transport between dwarfing rootstocks and grafted scion tissues (Beakbane, 1953; 1956; Beakbane and Thompson, 1939; 1947; Rogers and Beakbane, 1957; Soumelidou et al., 1994b). Xylogenesis (the generation of xylem elements) could be affected by differences in the translocation of plant hormones between scion and dwarfing or non-dwarfing rootstocks (Kamboj et al., 1997; Lockard and Schneider, 1981a; Soumelidou et al., 1994b).

In addition to size limitation of xylem vessels in dwarfing rootstocks, size and number of phloem cells are important for carbohydrate translocation. Both size and number of sieve tube elements has been associated with capacity of the

phloem to translocate assimilates from the scion to the root (Beakbane, 1956). In apples, vigorous scion-rootstock combinations contain more functional phloem elements that are capable of translocating higher quantities of assimilates than do dwarfing combinations, which have elevated amounts of non-functioning phloem (Simons, 1982).

In addition to a higher phloem: xylem ratio, dwarfing rootstocks and/or the scion tissue grafted to them have higher amounts of parenchymatous cells in the xylem and phloem (Beakbane and Thompson, 1939; 1947). This living tissue is able to divide and grow, even when the cell is mature. Thus, it would suggest that the metabolic rate is higher in these dwarfing rootstocks than in vigorous rootstocks due to greater amount of living tissue still reproducing (Rogers and Beakbane, 1957). However, due to the alteration in plant hormone concentration (Sommelidou, 1994a, b), parenchyma cells continue to divide, but not differentiate. A decrease in the number of functional sieve tube elements in the graft union (due to limited differentiation) may limit translocation rates (Rogers and Beakbane, 1957). Alternately, the assimilate transport capacity of dwarfing rootstocks should be increased due to the greater proportion of phloem tissue that forms at the graft union and supports the metabolic requirements of differentiating cells in the developing vascular system (Lasheen and Lockard, 1972).

Discontinuity of Vascular Elements in Dwarfing Combinations

Discontinuities in any of the vascular elements, such as changes in cell orientation of vessels or sieve tubes at the graft union, can contribute further to translocation limitations (Atkinson et al., 2003), leading to patches of necrotic tissue due to accumulation of phenolic acids (Gur and Blum, 1975; Dirr et al., 1994; Ermel et al., 1999). Collectively, these limitations in water and photoassimilate transport could result in reductions of shoot growth, leading to a dwarfing effect.

Graft union characteristics of *Prunus* species (Gebhardt and Goldbach, 1988) and apples (Ussahatanonta and Simons, 1988) have been characterized with two types of microscopy to determine if incompatibility was possible to predict. Fluorescence microscopy was used to identify areas of lignification and necrosis, including vessel elements wounded during the grafting process. Instances of vascular anomalies in the graft union region were more severe in dwarfing combinations than in vigorous combinations (Ussahatanonta and Simons, 1988). In addition, development of both non-functioning and functioning phloem was reduced in the scion, as compared to the graft union and rootstock, as dwarfing capability of the rootstock increased. This indicates that carbohydrate translocation between the scion and rootstock may be reduced, either acropetally or basipetally.

Light microscopy, in conjunction with radioactive techniques, also may be used to determine development of the vascular system. Functionality of wounded vessel elements can be determined by cation transport of Rubidium

($^{86}Rb^{+}$), which mimics the transport of potassium (K^{+}) through the graft union. A reduction in transport is considered to be an indication of variation in scion-rootstock incompatibility, and thus degree of dwarfing (Gebhart and Goldbach, 1988; Gruppe, 1985). In a study examining micrografts of *Prunus cerasus* L. 'Schattenmorelle', on Weiroot 158, increased $^{86}Rb^{+}$ concentrations in leaves and stems above the graft union signified that there was a successful graft union (Gebhardt and Goldbach, 1988). However, the amount of $^{86}Rb^{+}$ translocated into the *P. cerasus* scion was significantly less than other combinations of a vigorous rootstock (*P. avium*, 'Köröser') on W158, or the homograft of W158 on W158. Although the authors did not speculate as to the cause of reduced translocation, increased lignification in wound vessel members was associated with W158 combinations, possibly reducing translocation.

Malformation in the graft union resulting in misalignment of xylem elements (differentiated from callus tissue) suggests that water and mineral transport may be reduced (Ussahatanonta and Simons, 1988). In experiments with 'Golden Delicious' and a range dwarfing rootstocks (MM.106, M.26, M.7A, and M.9) tissue samples from each respective graft union combination (20 μm thick) were stained with safranin-O and fast green (Ussahatanonta and Simons, 1988). The configuration of 'Golden Delicious' /M.26 produced xylem misalignment, in which xylem element orientations were horizontal to the axis of the scion. Such misalignment of vascular tissues can eventually constrict transport, and lead to areas of necrotic tissue (Simons and Chu, 1980; 1984; Ussahatanonta and Simons, 1988). However, misalignments of vascular tissues

between scion and rootstock at the graft union may vary with the type of graft that is selected, as well as the rootstock combination. In the developing chip-bud graft union of 'Gala' or 'Bramley' apple on M.9, M.26, M.27 or MM.106 rootstocks, no malformation of xylem or phloem occurred in scion tissue grafted to the dwarfing rootstocks, suggesting that physical discontinuity of vascular tissues with this arrangement was less than when T-budding or cleft grafting (Table 2).

In the most severe cases of abnormal graft union development, incompatibility can result from deterioration of vascular connections. Deterioration can begin with abnormal cell development, e.g., orientation of ray cell formation during healing of the graft union (Simons, 1982). Normally, radial ray cells (xylem parenchyma) develop at right angles to the main axis of the stem (Salisbury and Ross, 1992). However, if these develop at angles deviating from ninety degrees, it could reduce the flow of water, nutrients, and carbohydrates through vascular tissues. However, studies have not been completed to verify a specific alteration of vascular tissue or how far they extend into scion tissues (Simons, 1982; Simons and Chu, 1983). Development of these ray cells at a bias can begin with early cell differentiation (3 to 4 weeks after grafting) and continue to develop throughout the life of the tree. In apples, chip budding of 'Gala' and 'Bramley' on M.9, M.26, M.27 and MM.106 rootstocks resulted in cambial and tracheary elements oriented perpendicularly across the vertical axis (Soumelidou et al., 1994b). Unfavorable cell orientation in the graft union can

result in reduced transportation capacities of both water and photoassimilates (Simons, 1982; Simons and Chu, 1980; Soumelidou et al., 1994b).

Vascular Development as Related to Hydraulic Conductivity

In general, vessel size has been related negatively to rootstock vigor (Beakbane, 1953). Scion xylem cells that developed in grafts with the most dwarfing apple rootstock, M.9, were fewer in number and smaller in size in the graft union region compared to xylem cells of 'Gala'/MM.106 (Soumelidou et al., 1994b). Although transport capacities of the vascular system were not measured, reduction in size and number of scion vessels observed in dwarfing vs. non-dwarfing combinations suggests that there could be a decrease in volume and velocity of soluble substances between rootstock and scion. Reductions in capacity could affect water potential, especially in times of water stress (Atkinson et al., 2000; Blaunsa et al., 2000; Olien and Lakso, 1986). Similar xylem exudate volumes have been collected from cut stems of both dwarfing and vigorous rootstocks (Jones 1971; 1974). However, evidence of greater hydraulic resistances for scion vascular tissue grafted to dwarfing apple rootstocks (M.9 and M.26) suggests a restriction of vascular transport compared to scion wood grafted to semi- or non-dwarfing rootstocks. Scions on semi- or non-dwarfing rootstocks had lower stem water potential at midday than did scions grafted onto dwarfing rootstocks (Olien and Lakso, 1986; Atkinson et al., 2001; 2003). In addition, graft union regions of more dwarfing combinations had higher hydraulic conductance than the same scion grafted on more vigorous

rootstocks (Atkinson et al., 2003). However, when hydraulic resistances were adjusted for leaf area, the scions grafted onto dwarfing rootstocks were more efficient at water transport than on semi-dwarfing or vigorous rootstocks (Atkinson, 2001; 2003).

Rootstock Effect on Root System Volume

Positive correlations between root system size, water transport, mineral uptake, and vigor have been found (Devyatov, 1996; Zhu et al., 1999). In plums (*Prunus domestica*), root systems of one dwarfing rootstock (VVA-1, *P. tomentosa* x *P. cerasifera*) had a higher amount of fibrous roots than did a non-dwarfing rootstocks suggesting higher transport efficiency (Myrobalan, *P. cerasifera* L.) (Devyatov, 1996). Although VVA-1 scaffold roots exhibited increased branching, they were thinner and comprised less specific mass ($g \cdot m^{-3}$) than the semi-dwarfing Manchu cherry (*P. tomentosa*) or standard Myrobalan rootstock, which can affect carbohydrate storage and reserves for future growth. However, the specific mass of VVA-1 fibrous roots near the surface was 84% more than the vigorous *P. cerasifera* rootstock in the same soil area. Vigorous plum rootstocks appeared to partition more of their growth into scaffold roots rather than fibrous roots near the surface (Devyatov, 1996). In dwarfing rootstocks, the concentration of the root system was in the top meter of the soil profile, suggesting more efficient exploitation of and reliance on surface water.

In micrografted apples (M.26, 'Gravenstein' [GR], M.26/M.26, GR/GR, M.26/GR, and GR/M.26), the relative growth rate between ungrafted, semi-dwarf

M.26 rootstock and vigorous GR scion cuttings did not differ (Zhu et al., 1999). However, micrografting reduced the relative growth rate of grafted M.26/M.26 compared to other grafted combinations suggesting that wounding was a factor in growth reduction. When multiple rootstock/scion combinations were examined, the relative growth rate of GR/M.26 was comparable to that of GR/GR and ungrafted rootstocks. However, when a reciprocal graft was tested (M.26/GR), its relative growth rate was lowest of all combinations, indicating that different genotypes can have different growth characteristics depending on their use as scion or rootstock (Zhu et al., 1999). Those combinations with decreased relative growth rates (M.26/M.26 and M.26/GR) also had the smallest root system based on root length (cm) and specific root length (m g^{-1} root dry weight). The dwarfing rootstock M.26 had the most compact root system, so it was hypothesized that dwarfing trees may dwarf the scion because of differences in root morphology (length, quantity of scaffold or branching roots, specific mass, and ability to absorb nutrients) compared to vigorous rootstocks (Zhu et al., 1999). In addition, a smaller root system may reduce scion shoot growth, resulting from a self-regulation between shoot and root growth within the tree (a 1:1 ratio).

Differences in rootstock morphology and size of rootstocks may influence nutrient uptake positively or negatively. Rootstocks can differ in their ability to absorb nutrients, which can lead to a need for alterations in fertilizer application rates (Hanson and Perry, 1989). Compact root systems of dwarfing stocks may result in localized internal deficiencies of nutrients, depending on the mobility of

the nutrient and ion type (Blaunsa et al., 2000). Rootstocks have been shown to influence nutrient uptake in sour cherry (*P. cerasus*) and nutrient content in Mazzard (*P. avium* L.) and Mahaleb (*P. mahaleb* L.) (Hanson and Perry, 1989). Sweet cherry scions grafted onto Mazzard result in a more vigorous growth habit than when grafted onto Mahaleb (Perry, 1987). When 'Montmorency' sour cherry scions were grafted onto Mazzard and Maheleb rootstocks, grown in a sandy loam site (Western Michigan) and fertilized at recommended rates (Hanson and Kesner, 1987), it was found that sour cherry trees on Mahaleb may develop deficiencies in potassium (K^+), boron (B), or manganese (Mn) compared to Mazzard (Hanson and Perry, 1989). In sweet cherries, lower concentrations of nitrogen (N), potassium (K^+) and magnesium (Mg) were quantified in scion tissue that was grafted onto semi-dwarfing or dwarfing rootstocks (Table 3) (Neilsen and Kappel, 1996; Ystaas, 1990). In apples, transport rates of ^{32}P and $^{45}Calcium$ (Ca) were higher on vigorous rootstocks than on dwarfing rootstocks (Bukovac et al., 1958). Thus, it would appear that dwarfing rootstocks have limitations in nutrient uptake. However, a consistent relationship of the differences in nutrient uptake between dwarfing and non-dwarfing rootstocks remains to be demonstrated. Recent reports using ungrafted Gi 5, Gi 6, and Mazzard, in addition to grafted cherry combinations of Rainier/Gi 5, Rainier/Gi 6, and Rainier/Mazzard, showed no difference in water and nitrogen use efficiency (Zavalloni, 2004).

Microscopy Techniques to Examine Graft Union Development in Dwarfing and Non-Dwarfing Cherry Rootstocks

Research on cell differentiation and orientation important for vascular transport has been aided greatly by advanced microscopy technology (Czymmek et al., 1994; Simons, 1972). Cell walls, protoplasts, and nuclei are observed distinctly with scanning electron microscopy, as well as with conventional microscopy in conjunction with appropriate stains and fluorochromes. Scanning electron microscopy provides a topographical image in which surface features of the cell can be observed. In scanning electron microscopy, tissue samples are placed in a fixative, cut with a rotary or freezing microtome, dehydrated, and critical point dried with liquid carbon dioxide to eliminate water contamination (Boyd et al., 1977). Samples destined for the scanning electron microscope are plated with a gold palladium alloy to facilitate the 'reflection' of the electrons (Echlin, 1981). Bombardment of the specimen with electrons results in a portion of the electrons being transmitted and reflected into an objective lens at the base of the microscope. From this information, the surface of the specimen and three-dimensional characteristics can be observed (Simons, 1972). With scanning electron microscopy, greater resolution and focal depth can be achieved than with an ordinary light microscope (Simons, 1972). With greater focal depth, uneven surfaces such as those of leaves can be detailed.

In conventional light microscopes, light is used for illumination rather than electrons. Laser confocal microscopy, an enhanced light microscopy technique, uses lasers as the illuminating source to get a fluorescent image. Confocal

microscopes are designed to remove any illuminating light not directly in the focal plane at the sample. Furthermore, samples can be optically sectioned by focusing through the sample to provide images immediately available on video monitors while using the confocal microscope. The images are illuminated by light (provided by the laser) coming from the objective focal plane directly above the sample stage of the microscope (Czymmek et al., 1994). These optical sections are similar to samples that are sectioned mechanically. Shadows from features above and below the sectioning plane do not interfere, allowing for a detailed image. Thus, optical sectioning gives enhanced resolution with the advantage of using a physically larger sample size, but still garnering detailed data from sample layers. Tissue damage or cellular disruption is minimized because the sample does not need to be embedded in any type of paraffin or wax, or (in some cases) fixed permanently to the slide. Different planes within the sample also may be explored, allowing an additional aspect of data collection not previously available (Czymmek et al., 1994). Within each plane of the sample, optical sections can be combined into an extended focus, composite picture using available computer software.

Fluorescent dyes can be used in confocal microscopy to create images. However, care must be taken when selecting the dye. Dyes that work well in conjunction with conventional light microscopes may not be suited to laser confocal microscopy due to the narrow beam of light provided by the laser (Czymmek et al., 1994). In addition, the dye must be used with the correct laser line (wavelength) for optimum fluorescence. Both electron and laser confocal

microscopy have been used in previous research dealing with grafted systems (Simons, 1972).

Laser confocal fluorescence microscopy also can be used for identification of many minerals that can accumulate in crystalline form within plant tissues, such as calcium in non-functioning sieve tube cells. These three-dimensional structures can be reconstructed using an extended focus image, in addition to the appropriate software to allow identification of unique crystalline structures (Czymmek et al., 1994). These features in the plant tissue can be examined further using x-ray spectroscopy to elucidate specific mineral components.

During the initial healing of wounds imposed by the grafting process, cells at the graft union undergo a limited necrosis, followed by a proliferation of callus tissue that undergoes cell differentiation, connecting the vascular system between rootstock and scion. Identification of the quantity and location of calcium crystals at this stage of graft healing (within the functioning and non-functioning phloem of the scion-rootstock union could indicate the extent of potential senescing or necrotic tissue development (Simons and Chu, 1980; Simons and Chu, 1984). X-ray spectroscopy of graft union tissue in dwarfing apple rootstock graft combinations shows that calcium accumulates on the rootstock side of the graft union, within functioning phloem (Simons and Chu, 1980; 1983). This is important because accumulation of Ca^{2+} crystals over time in the graft union could promote a gradual deterioration of phloem cell function, resulting in the dwarfing effect on scion tissue or eventual failure of the graft union (Simons and Chu, 1980; 1983). In apple scions grafted onto dwarfing

rootstocks, higher Ca^{2+} crystal concentrations were found in conducting phloem, compared to little or no occurrence of Ca^{2+} crystals in graft unions of scions grafted onto non-dwarfing rootstocks (Simons, 1982; Simons and Chu, 1980). Accumulation of Ca^{2+} crystals in the non-conducting phloem cells of apple scions grafted onto dwarfing rootstocks constricts vascular connections in apple (Simons and Chu, 1984). Additionally, the accumulation of a material such as Ca^{2+} could induce a physical limitation, resulting in a blockage or reduction in the transport of nutrients, sugars and/or hormones. This would negatively affect cell differentiation by reducing the amount of sorbitol or sucrose translocated from storage in the rootstock to the graft union, which is then unavailable to metabolically support differentiation of the callus tissue. Sequestration of calcium in crystal form also restricts the amount of calcium available to be utilized by the cell walls, resulting in less rigidity and strength in cell walls at the graft union, possibly contributing to eventual physical/mechanical union failure (Simons and Chu, 1984).

When the established root system of trees on dwarfing rootstocks were examined, there was an increased amount of phloem compared to xylem in roots, and this ratio increased as dwarfing capability increased (Beakbane, 1953; Beakbane and Thompson, 1939; Rogers and Beakbane, 1957). However, the percentages of functioning and non-functioning phloem were not quantified. Elevated amounts of total phloem tissue suggested the capacity of the dwarfing rootstocks to transport storage carbohydrates in root tissue was superior to

vigorous rootstocks. However, if a large portion of phloem was not functional, carbohydrate transport potential would not be increased.

Functioning phloem can be distinguished from non-functioning phloem by quantifying the amount of callose present in sieve plates (Currier, 1957). The formation of callose is often a response to wounding, senescence or dormancy, forming a barrier against the introduction of disease or pests in damaged areas, and aiding in the senescence or dormancy process by reducing the translocation of photosynthates. Callose can also block movement of macromolecules in sieve tubes (Currier, 1957). The amount of non-functioning phloem can be quantified by staining phloem tissue with aniline blue, which specifically stains callose blue, and due to its fluorescent properties, can be detected by confocal microscopy using a UV light source.

The Role of Plant Hormone Transport in the Dwarfing Mechanism

Plant hormones may be involved in root to shoot communication that ultimately coordinates the balance of root and shoot growth (Davies, 1995; Jackson, 1993). In many studies, alterations in plant hormone concentration and/or ratio in scions grafted onto dwarfing vs. non-dwarfing rootstocks have been suggested as a mechanism for dwarfing (Jones, 1986; Kamboj et al., 1997; Robitaille and Carlson, 1971; Robitaille and Carlson, 1976; Tukey, 1986). Numerous research studies have focused on plant hormones implicated in either shoot elongation (e.g., cytokinins or GA; Ibrahim and Dana, 1971; Jones, 1973;

Robitaille and Carlson, 1971; 1976) or shoot suppression (e.g., IAA; Hrotkó, 1996).

Plant hormones acting in concert with each other, may ultimately affect the success of a graft union. Graft unions on dwarfing rootstocks tend to have a higher phloem to xylem ratio than on standard rootstocks, possibly because lower concentrations of auxin in these graft unions favor differentiation of phloem cells (Aloni, 1988; 1995; Rogers and Beakbane, 1957). Increased phloem to xylem ratios also are observed in the scion when grafted on dwarfing rootstocks, resulting in thicker bark around the graft union region. In some cases, low concentrations of auxin can cause induction of phloem without xylem in the graft union, provided optimal cytokinin concentrations are present (Aloni, 1987). This higher ratio of phloem to xylem may favor auxin degradation by transporting substances, such as phenolic acids, that oxidize auxin during polar transport (Gur et al., 1968). Conversely, in apple scion-rootstock combinations, higher auxin levels were found in the phloem of scion tissue grafted on dwarfing rootstocks than in scion tissue grafted on vigorous rootstocks during the growing season, suggesting a possible role for auxin by suppressing growth when concentrations reach inhibitory levels (Grochowska and Buta, 1984).

Auxin or indole-3-acetic acid (IAA) is synthesized in shoot apices and translocated basipetally to the root system via an intercellular pathway in cambial tissues. Auxin is the major plant hormone involved in apical dominance of shoots (Phillips, 1975), has a role shoot elongation (Went, 1939), and an important role in vascular differentiation (Aloni, 1987; Dengler, 2001; Digby and Wareing, 1966;

Mattsson et al., 1999). IAA is the most abundant naturally occurring form of auxin; however, it is also found as indole-3-butyric acid (IBA), phenyl acetic acid, and 4-chloro-IAA (Normanly et al., 1995). When conjugated to amino acids, peptides, or carbohydrates, IAA is biologically inactive and believed to play a role in IAA storage (Kende and Zeevaart, 1997). Intercellular vascular movement of auxin involves an extravascular, polar transport system, which regulates the influx and efflux of auxin between cells, aided by carrier proteins. Auxin is carried through this transport system to the root system, where it affects the production of other plant hormones, such as cytokinins (Jackson, 1993; Kende and Zeevaart, 1997; Torrey, 1976). Once auxin has been transported to the root system, it can be redistributed acropetally to the shoot system via polar transport, resulting in normal growth rates (Hertel, 1987; Jones, 1998; Goldsmith, 1977).

In dwarfing rootstocks, polar transport of auxin may be limited due to deactivation of the auxin efflux carrier (Kamboj et al., 1997; Soumelidou et al., 1994a). Thus, it has been proposed that the dwarfing of scion growth occurs because of an imbalance in hormone levels; reduced basipetal efflux of auxin at the graft union affects the ratio of other hormones like cytokinins (Kamboj et al., 1997; Soumelidou et al., 1994a). Malfunctions in the efflux carrier protein can increase the concentration of auxin within the cells of the graft union, causing an imbalance that affects vascular differentiation in the cambium (Kamboj et al., 1997). Malfunction of the rootstock protein carriers may increase auxin levels to growth-inhibiting concentrations at the graft union. In general plant tissues, either grafted or non-grafted, vessel structure and number are affected by auxin

concentration; high levels of auxin lead to higher densities of vessels, whereas low concentrations of auxin produce lower densities of vessels (Aloni, 1995; Aloni and Zimmerman, 1983; Chong and Andrews, 2003). However, local increases in concentration due to structural or physiological obstructions may increase the number of vessels formed in cambial areas, as indicated by radioactive labeling of auxin ([^{3}H]-IAA) in the scion (Davies, 1995; Kamboj et al., 1997; Soumelidou et al., 1994a). This is important, since in the graft union region continuous formation of callus during healing and formation of vascular connections differentiates into vascular elements.

High concentrations of auxin in scion tissue above graft unions on dwarfing rootstocks can lead to abnormal graft union formation and overgrowth, due to excess callus and parenchymatous tissue. Cell development in this region of increased auxin is often associated with abnormal xylem and phloem configurations (Simon and Chu, 1980; 1984; Soumelidou, et al., 1994b). Such abnormal vascular configurations in the graft union growth could delay movement of water and nutrients across the union, because of vascular misalignment, in addition to increased amounts of parenchymatous tissue (Jones, 1986).

Cytokinins, in combination with auxin, are active in cell differentiation and are a primary signal for initiating root development (Davies, 1995; Kende and Zeevaart, 1997). A balance or ratio of hormone concentrations must exist within the tissues of roots and shoots for normal growth to occur. Cytokinins are synthesized in the root and translocated acropetally, which promotes vascular

differentiation and shoot growth (Beck and Wagner, 1994; Aloni, 1995; Jones, 1973).

Extracted xylem root exudates applied to apple shoots revealed cytokinin-like activity (Jones 1973, 1974). This suggests that cytokinins are transported in the xylem (Beck and Wagner, 1994; Chen et al., 1985; Jones, 1973). Vessel differentiation and xylem formation is favored at high concentrations of cytokinins and auxin (0.03% to 1.0% w/w), whereas low levels of auxin favor phloem formation (<0.03% w/w) (Aloni and Zimmerman, 1983; Aloni, 1995). Cytokinins also increase tissue sensitivity to auxin (Aloni, 1995). For example, *Coleus* tissues that were treated with cytokinins were sensitive to low levels of auxin and produced high ratios of phloem/xylem (Aloni, 1980).

In apple rootstocks, higher concentrations of total cytokinins (zeatin and zeatin riboside) were found in scions grafted onto vigorous rootstocks such as MM.106 than on dwarfing rootstocks such as M.27 and M.9 (Kamboj et al., 1999). It was suggested that higher concentrations of cytokinins in vigorous rootstocks could be due to the ability of these rootstocks to synthesize cytokinins, load cytokinins into the xylem, or to differences in the number of roots synthesizing cytokinins. Similarly in peaches, it was found that the most dwarfing rootstock, cv. Armking, had the lowest concentration of cytokinins and auxin when used as a rootstock or as a scion compared to GF 677 (*P. persica* x *P. amygdalus* B. hybrid) (Sorce et al., 2002). Due to the role auxins may play in controlling the amount of cytokinins synthesized in the roots (by suppressing cytokinin concentrations and shoot growth), an overall dwarfed tree would result.

Gibberellic acid (GA) is associated primarily with actively dividing tissue, which makes up a large portion of the developing graft union. Gibberellins are associated with stimulating shoot elongation and leaf expansion; however they also may be found in the root system (Davies, 1995). The active form, GA_3, has been the GA examined most often in dwarfing rootstock studies (Carr et al., 1964; Robitaille and Carlson, 1971; 1976). Transport of GA has been detected in the transpiration stream of both apples and pears (Jones and Lacey, 1968). In the 1970's, one or more gibberellin-like substances, most likely GA_3, were implicated in studies of dwarfing apple rootstocks. Root xylem exudate from dwarfing rootstocks had the least effect on shoot growth of pea seedlings and the least expansion of leaf discs compared to a 100% increase in growth with the application of exogenous GA (Ibrahim and Dana, 1971). Small amounts of root xylem exudates (10 μl) from dwarfing EM 9 rootstocks failed to stimulate leaf expansion of apple, unlike extracts from vigorous rootstocks (EM 1 and EM 25). The results from these bioassays suggest that decreased levels of GAs were produced or transported in dwarfing rootstocks (Ibrahim and Dana, 1971).

Additional work using exogenous GA_3 suggests that dwarfing of the scion can be explained partially by decreased gibberellin transport from roots to shoots (Carr et al., 1964; Jones and Lacey, 1968; Robitaille and Carlson, 1971). Injections of 2.79^{-3} mM or 10 ppm concentrations of GA_3 resulted in increased terminal shoot growth on EM.9 compared to EM.7 or MM.111 (Robitaille and Carlson, 1971). In addition, the dwarfing apple rootstock EM 9 was more sensitive to GA_3 at the lower concentration (2.79^{-3} mM) than either EM 7 or MM

111. Injections were accomplished using 20 ml syringes, with the needle placed directly into scion wood of 'Red Prince Delicious' on EM 9, EM 7, or MM 111.

In addition to lower concentrations of GA in dwarfing apple rootstocks, GA may be broken down more quickly in the phloem due to the increased degradation that is possible with increased phloem in the graft union of dwarfing systems (Ibrahim and Dana, 1971; Robitaille and Carlson, 1976). Elevated concentrations of GAs may reduce the level of IAA oxidase in vigorous rootstocks, thereby increasing auxin activity and growth (Robitaille and Carlson, 1976). For example, when GA synthesis was prevented in apple rootstocks (M.27 [least vigorous], M.9, and M.7 [most vigorous]) by applications of paclobutrazol, flurprimidol, or uniconazole, dwarfing effects were exaggerated in all three rootstocks, depending on initial dwarfing abilities (M.7>M.9>M.27) (Tukey, 1986). These compounds halt GA production, which results in shorter internodes and shorter stems.

An additional factor maybe that the bark of dwarfing rootstocks contain higher concentrations of growth-inhibiting substances [ρ-coumaric acid and abscisic acid (ABA)] than growth-promoting substances (GA). These compounds can be transferred to the scion when it is grafted onto a dwarfing rootstock (Ibrahim and Dana, 1971; Robitaille and Carlson, 1976). Application of paclobutrazol and TIBA (IAA transport inhibitor) in the graft union region of sour cherries reduced shoot growth, but only paclobutrazol decreased shoot length in sweet cherries (Grochowska and Hodun, 1997). This suggested that shoot

length, and consequently tree height, may be controlled by different endogenous hormones and pathways within the same species.

The concentration and the balance of plant hormones may affect plant growth and development by influencing sucrose distribution. Sucrose distribution is effected by the relative distribution of IAA and cytokinins in some plants by the increased sink strength due to cell development (Gersani et al., 1980). The graft union in woody plants represents an additional sink demand due to continual development of callus tissue and cell differentiation. This area is a zone of cell regeneration and development, in which ratios of IAA to cytokinin have an important role. Alterations in hormone ratios can influence development of undifferentiated tissue into xylem or phloem elements, the major pathway through which water and photoassimilates travel. Excess production of non-functional phloem may decrease carbohydrate translocation. The reduction in carbohydrate translocation between scion-rootstock tissues could impact growth, from increased precocity of scions on dwarfing rootstock, to early cessation of shoot growth compared to scions grafted on standard rootstocks.

Another theory suggests that a growth inhibitor, identified initially as phloridzin or phloroglucinol, is present in dwarfing rootstocks and is translocated within the graft union to the scion, thereby reducing leaf area and shoot growth (Beakbane, 1956; Davison, 1965; Lockard and Schneider, 1981a). Phloridzin (a dihydrochalcone) and phloroglucinol, produced from phloridzin, are phenolic compounds, often found in varying concentrations above and below the graft unions of apples in dwarfing systems (Beakbane, 1956). These substances are

involved in the lignification of vascular elements in the graft union region (Carlson, 1974) and enhance IAA breakdown through oxidative decarboxylation, thus decreasing growth (Zenk and Muller, 1963). This increased degradation of IAA by phloridzin and phloroglucinol has been proposed to lead to the dwarfing effect on scion growth.

Examining Carbohydrate Translocation through the Graft Union Region of Dwarfing and Non-dwarfing Rootstocks

Macromolecules such as sugars and proteins, wound-induced biochemicals (callose), and pathogens are loaded into the sieve tubes for long-distance transport within the plant both actively (symplastically) and passively (apoplastically) through companion cells and sieve elements (SE-CC complex). Apoplastic movement involves the transport of fluid through the free space of the vascular system, whereas symplastic movement occurs via plasmodesmata and plasma membranes within the cell, requiring energy (Münch, 1930; Henton et al., 2002). Münch's pressure-flow hypothesis is generally accepted to explain apoplastic and symplastic solute movement within the phloem based on the concept that the concentration of solutes determines the direction of flow, with water moving from areas of low solute concentration to areas of high concentration (Münch, 1930). Solutes thus move within the phloem according to an osmotic gradient, with positive gradients existing near source tissue and diminishing towards sink tissue because of assimilate use (Milburn, 1974).

Macromolecules, specifically sugars and proteins, are loaded into the SE-CC complex via active transport, although unloading of macromolecules may be passive near sink tissues (Oparka and Santa Cruz, 2000; Viola et al., 2001). Active transport within the SE-CC complex is facilitated by transmembrane carriers in apoplastic-loading plant species, and by intermediary cells in symplastic-loading plant species (van Bel, 1993). Macromolecule mobility within the phloem depends on where and when the molecule is synthesized within the organ. For example, plasmodesmata that connect sieve elements and companion cells (collectively referred to as pore plasmodesma units or PPUs) have a large size exclusion limit (>40 kDa), allowing large macromolecules to pass freely into the translocation stream, compared to plasmodesmata that connect non-phloem cells (Fisher et al., 1992; Imlau et al., 1999). Studies of loading and unloading in the phloem are possible using jellyfish green fluorescent proteins (GFP; 27 kDa) (Imlau, et al., 1999), fluorescent tracers (F-Ficoll, 400 kDa) (Fisher and Cash-Clark, 2000) or fluorescent labeled virus (Roberts et al., 1997). Recently, carboxyfluorescein has been used to trace phloem sap translocation and loading/unloading of macromolecules because it is not membrane-permeable at pH levels in the phloem (pH 6.3). Thus, it is strictly confined to the phloem, is stable for long periods (>4 days), and parallels ^{14}C transport in plant organs (Grignon et al., 1989).

Macromolecules such as mRNA (Xoconstle-Càzares et al., 1999), p-proteins (Golecki et al., 1999) and sugars are believed to be involved in long-distance transport of messaging signals, genetic and hormonal, that regulate

both vegetative and reproductive development. mRNA molecules are responsible for transporting encoded base sequences to the designated cell, and are translated by tRNA into specific proteins that function in the cell. The mRNA molecule sucrose transport 1 (*SUT1*) has been located in the SE-CC plasmodesmata, with the corresponding protein confined to the sieve element, indicating that RNA can travel through phloem tissue (Kühn et al., 1997). P-proteins are 'phloem specific' proteins that are translocated and can accumulate in sieve elements, specifically at sieve plates (Golecki et al., 1999). P-proteins are species-specific and may act as long-distance messengers within the phloem, impacting growth and development (Evert, 1977; Golecki et al., 1999). During wounding, P-proteins accumulate in the form of filaments at sieve plates and block translocation by forming protein plugs. These protein plugs have been used as an indicator of incompatibility in *Prunus* graft unions (Schmid and Feucht, 1981).

Transport of macromolecules (carbohydrates and proteins) may be affected in heterogenetic grafts because the exact structures may be species or cultivar specific (Evert, 1977; Bieleski, 2000). This is an important point, as macromolecules from different species or cultivars may have size and/or conformational differences that could hinder transport in heterogenetic grafts (Bieleski, 2000). Should this be the case, any physical misalignment or physiological abnormality between scion and rootstock may induce a transport limitation, resulting in increased carbohydrates at the graft union (Bieleski, 2000). Thus instead of being translocated to the root system for utilization in root

growth, development, and storage, carbohydrates may accumulate in graft union tissues. In a known incompatible combination of 'Fay Elberta' peach (*P. persica*) scion and 'Marianna 2624' plum rootstock (*P. cerasifera x P. munsonianna*), the starch concentration of the scion increased in mid-summer, when translocation to the root system occurs (Breen, 1975). These increases in scion bark starch, sugar, and/or polyol concentrations were attributed to current season photosynthate (supplied by scion leaves), but were depleted quickly by late winter in the peach scion wood. Carbohydrate concentrations in the plum rootstock were 50-60% lower than concentrations in the scion bark. It was hypothesized that transport limitations were imposed at the graft union, which eventually led to death of the root system (Breen, 1975). Examination of the sweet cherry 'Frogmore' on a sour cherry rootstock F.250 revealed that there was an accumulation of starch above the graft union and little, if any, was translocated through the graft union (Herrero, 1951).

Soluble sugars present in the phloem sap are important for translocation to source or sink organs. In higher plants, sucrose is the predominate translocatable sugar, and is present in the highest quantities in phloem tissues. However, in *Rosaceae*, sorbitol (a sugar alcohol) is the predominate transport sugar, with sucrose transported in lesser concentrations (Loescher et al., 1982; 1985). Sorbitol is synthesized in source tissues via an NADPH-dependent aldose-6-phosphate reductase (A6PR) and sorbitol-6-phosphate phosphatase (Grant and ap Rees, 1981), which catalyzes the following reaction:

[1] Glucose-6-Phosphate + NADPH + H^+ $\longleftrightarrow$ Sorbitol-1-Phosphate + $NADP^+$

The amount of A6PR present in active form to catalyze the reaction illustrated above will depend in part on the sink demand of various plant organs. Once sorbitol reaches sink tissues, it is catabolized via sorbitol dehydrogenase (SDH) to fructose by the following reaction (Yamaki and Ishikawa, 1986):

[2] Sorbitol + NAD^+ $\longleftrightarrow$ Fructose + $NADH^+$ + H^+

Other enzymes are reported to catabolize sorbitol as well, such as sorbitol oxidase (SOX) (Yamaki, 1980), sorbitol-1-phosphate (Redgwell and Bieleski, 1978), and $NADP^+$-dependent sorbitol dehydrogenase (SDH) (Yamaki, 1984). SOX breaks down sorbitol directly to glucose, whereas NAD^+-dependent SDH converts sorbitol to fructose and its activity can fluctuate with sucrose accumulation, principally present during fruit development (Moriguchi et al., 1990). These two enzymes are often concentrated in sink tissue and are considered to play a minor role compared to other sorbitol metabolizing enzymes (Loescher et al., 1982).

Evaluation of the dynamics of carbohydrate pool size of translocatable sugars (i.e., sorbitol and sucrose), as well as other nonstructural carbohydrates (glucose, fructose, and starch) in grafted cherry scion-rootstock combinations, may illuminate whether there is a reduction of carbohydrate flow to the root systems of dwarfing rootstocks (Turgeon, 1989, Turgeon and Webb, 1975). Differences in carbohydrate pools between scion and rootstock could be influenced by physical anomalies of the phloem in the developing graft union,

minor incompatibilities (non-functioning or necrotic cells within the newly generated vascular system), and/or differences in genetically-regulated metabolic pathways, resulting in an accumulation of carbohydrates above the graft union (Gaudillére et al., 1992; Rao and Berry, 1940). Accumulation of carbohydrates above the graft union has the potential to decrease the photosynthetic rate of the scion through feedback inhibition, and ultimately reduce the quantity of assimilates available to support scion growth (Edin et al., 1996; Gucci et al., 1991; Layne and Flore, 1992; Looney, 1968; Schechter et al., 1991). Additionally, dwarfing rootstocks may reduce mobilization and transport of stored carbohydrates to sinks from roots in the late winter or early spring due to a reduced number and/or area of xylem vessels (and consequently water transport) in these rootstocks (Colby, 1935).

In addition to differences in carbohydrate translocation in dwarfing systems, initial research suggests that dwarfing apple rootstocks may be more efficient at water uptake and transport (Atkinson et al., 2001; 2003); this suggests that although carbohydrate translocation resistance may be higher, the transport of water may be more efficient than in non-dwarfing rootstocks. Limitations in transport could lead to a reduction in reserve carbohydrates. Clearly, this dichotomy is an important question to explore when researching mechanisms of dwarfing and their effects on tree physiology.

Carbohydrate Partitioning in Dwarfing and Vigorous Sweet Cherry Rootstocks—Analysis of Reserve Carbohydrates Levels

Demand for carbohydrates is high in deciduous fruit tree systems, from bloom in the spring through fruit growth, for the storage of reserve carbohydrates for the following growing season (Loescher et al., 1990; Whiting and Lang, 2004). Reserve carbohydrate levels are especially important in cherry production because all early growth depends on these reserves. Initial shoot growth, is dependent upon the previous years' reserve carbohydrate levels (Keller and Loescher, 1989; Tukey, 1942). Additionally, until new shoot growth is photosynthetically competent to export assimilates to sink tissues, flower and fruit development is dependent exclusively on carbohydrate reserves (Loescher et al., 1990; Westwood, 1978).

Dwarfing rootstocks may have a profound affect on carbohydrate partitioning in grafted fruit tree systems. This partitioning may affect growth, especially in early in the season. A reduced root system in some dwarfing rootstock systems could reduce the amount of storage carbohydrates (Devyatov, 1996; Zhu et al., 1999). In peaches, shoots from trees on a vigorous rootstock, 'Nemaguard', initiated growth sooner than shoots from trees on dwarfing rootstocks (i.e., K-146-44 and K-146-43) (Weibel et al., 2003). It was hypothesized that vigorous rootstocks contained higher concentrations of stored carbohydrate reserves than dwarfing rootstocks.

Research in sweet cherries on a dwarfing rootstock ('Bing'/ Gisela 5) suggested that crop load indirectly impacted whole canopy net CO_2 exchange

rates (NCER) (Whiting and Lang, 2001a). Trees from which all fruit had been removed prior to bloom had NCER rates as high as trees with either a high or modest crop load. After harvest, photosynthesis dropped in all treatments. However, toward the end of the growing season, NCER rates in treatments where there had been no crop load were significantly lower than in the other two crop load treatments (Whiting and Lang, 2001a). The trees which had a crop load until harvest continued to photosynthesize at a higher rate until leaf drop. These data suggest that trees without a crop load may supply their carbohydrate reserve needs more quickly during the growing season than trees with heavy or modest crop loads (Whiting and Lang, 2001b). Heavily cropped trees that do not fully re-supply carbohydrate reserve levels by leaf fall may enter the following season with depleted reserve support for early growth. If crop loads remain at a consistently high level over successive seasons without sufficient storage of carbohydrate reserves, it is necessary to implement intensive pruning management (Lang 2000, 2001), or the tree may over-crop to the point of death (Sanderson, 1999).

Hypotheses and Objectives

Given the extensive body of research on potential dwarfing mechanisms in fruit tree systems, it is not unlikely that a number of different factors may contribute to the dwarfing growth response in sweet cherry grafted onto specific rootstocks. The hypothesis to be tested is that the vessel elements that develop in a sweet cherry grafted onto a dwarfing rootstock contribute to increased metabolic

resistances. Specifically, increased frequency of vascular discontinuities between scion and rootstock decrease hydraulic conductance due to misalignment of xylem and phloem cells. Furthermore in dwarfing systems, the region above the graft union is likely to accumulate starch and soluble carbohydrates, which may be due to the reduced flow of water and nutrients through fewer, narrow vessel elements.

The vascular development subsequent to initial healing of the graft union was examined using various microscopy techniques. In addition, a number of different approaches were utilized to determine carbohydrate translocation in grafted trees on dwarfing and non-dwarfing rootstocks. Objectives included: 1) examine xylem vessel number to verify increased xylogenesis in the graft union region of different scion/dwarfing rootstocks combinations that could lead to swelling at the graft union, 2) examine xylem vessel number and area to determine if there is an anatomical limitation to water transport through the graft union that could affect carbohydrate translocation between rootstock and scion tissue, 3) determine if variations exist in starch and other non-structural carbohydrates surrounding and including the graft union regions of vigorous and dwarfing scion-rootstock combinations; 4) assess healing responses in the graft union to verify if healing mechanics predispose genetically different tissues to induce early growth responses that result in dwarfing.

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Table 1. Definition of terms contained in manuscript.

Term	Definition
Grafting	The act of combining (joining) two different plant stems (genus or species) in order to form one contiguous plant.
Plant growth regulator	Exogenous compounds, including synthetic chemicals that alter growth and development of plant organs.
Plant hormone	Endogenous compounds produced by the plant that alter growth and development of plant organs.
Precocity	The ability of a plant system to produce flowers and/or fruit at an unusually early age.
Rootstock	Root system upon which a scion variety is grafted in order to withstand conditions such as drought, nutrient deficiency, or confer tree size and architectural attributes.
Scion	Aerial portion of a tree or plant under which a rootstock is grafted; fruit is usually harvested for sale from this portion of the tree or plant.
Sink	Plant tissues which are not photosynthetically competent and whose growth and basic metabolism are dependent on carbohydrates which are preferentially partitioned to them, such as flowers, fruit, new leaf growth, roots, storage organs, and bark.
Source	Plant organs that have reached full photosynthetic competency and produce photosynthate product in excess of metabolic needs. This is then available for export to sink organs.

Table 2. Grafting techniques commonly used in the production of fruit trees (Hartmann et al., 1997).

Term	Definition
Cleft graft	A technique in which wedge-shaped scion tissue is inserted into a slot in the central sections of stock tissue.
Whip and tongue graft	This technique is used for small material, and maximizes vascular cambium contact. Both the scion and rootstock are cut to form a 'W' and placed together.
Chip budding	A single bud is cut from stock material and placed on a section of the rootstock in which cambial tissue has been cut away to match the external bud. This budding technique usually results in more rapid union development than in T-budding.
T-budding	A bud from scion material is inserted into a bud shield made from a 'T' incision through the cambial tissue on the rootstock material.

Table 3. A list of cherry rootstocks examined in nutrient uptake research with common and scientific names (Neilsen and Kappel, 1996; Ystaas, 1990). Vigor levels are assigned for local performance at research sites and will vary according to soil classification and climactic conditions.

Vigorous	
Mazzard	***Prunus avium* L.**
Mahaleb	***P. mahaleb* L.**
Colt	***P. avium* L. x *P. pseudocerasus* Lind.**
Semi-dwarfing	
MxM 2, MxM 60	***P. avium* L. x *P. mahaleb* L.**
GM 79 (Camil)	***P. canescens* L.**
GI 196/4	***P. canescens* L. x *P. avium* L.**
GI 195/1	***P. canescens* L. x *P. cerasus* L.**
Dwarfing	
GM 61/1 (Damil)	***P. x dawyckensis***
GM 9 (Inmil)	***P. incisa* Thunb. x *P. serrula* Franch.**
MxM 46	***P. avium* L. x *P. mahaleb* L.**
GI 148/1 (Gi 6)	***P. cerasus* x *P. canescens***

CHAPTER TWO

Vessel Characterization of Sweet Cherries (*Prunus avium* L.) Grafted Onto Dwarfing and Non-Dwarfing Rootstocks

ABSTRACT

Sweet cherries (*Prunus avium* L.) are one of a number of fruit species that can benefit from the use of dwarfing rootstocks. Recent research has suggested that hydraulic resistance is increased at the graft union of apples on dwarfing rootstock systems.

This research examined vessel characteristics of sweet cherry scion-dwarfing rootstock combinations. These combinations included 'Rainier' (*P. avium* L.) on vigorous (Colt and F 12/1), semi-dwarfing (Gisela 6; Gi 6), and dwarfing (Gisela 5; Gi 5) rootstocks. Mean vessel element length, vessel frequency per mm^2, diameter (VD) and lumen area (VLA) were measured. Vessel hydraulic diameters (VHD) were calculated from vessel diameters to estimate theoretical water conductance.

Vessel area decreased in graft union sections of Rainier/Gi 5 compared to Rainier/Colt. Most grafted treatments exhibited smaller VLA in the scion compared to the graft union, except for Rainier/F 12/1 and Gi 6/F 12/1, for which scion VLA increased compared to the graft union. There was no significant decrease in VLA in graft union tissues compared to rootstock tissues of combinations with 'Rainier' as the scion; however, vessels in the graft union of Rainier/Gi 5 were narrower than in Rainier/F 12/1. Similar observations were recorded in reciprocal grafts.

In both years, VHD calculations confirmed an estimated reduction in water transport. Combinations with Gi 5 as the rootstock in homograft or heterograft combinations had a lower scion HVD; similar results were obtained in

heterografts and reciprocal heterografts. In dwarfing combinations, the incidence of vascular anomalies increased.

Ungrafted Gi 6 had more vessels per mm^2 than ungrafted Gi 5, Colt, or . F 12/1. Vessel area of ungrafted Gi 6 was significantly and consistently greater than Gi 5. However, VLA of ungrafted Gi 6 was greater than Colt in 2001, but less than F 12/1 in 2002. In ungrafted Gi 5, VHD was significantly less in 2001 than in either ungrafted Gi 6 or Colt, while in 2002 it was less than F 12/1 but greater than Gi 6. Vessel element length did not differ between any of the treatments.

In grafted systems, vessels per mm^2 in graft union tissues decreased when the scion was paired with a vigorous rootstock, and was highest in Rainer/Gi 5. However, in homograft combinations, Gi 6/Gi 6 had the highest vessels per mm^2. Vessel frequency per mm^2 was highest overall in rootstock sections, with a decrease in the graft union of heterograft and reciprocal treatments. Homograft combinations, including Rainier/F 12/1, exhibited no difference in vessel frequency per mm^2 between scion and graft union sections. Gi 5 rootstocks produced a large number of narrow vessels, whereas Gi 6 and F 12/1 rootstocks tended to produce fewer vessels with larger VLA. Based on these results, the combination of narrower and fewer vessels in scion and graft union tissues of dwarfing sweet cherry combinations may increase hydraulic resistance in the graft union region.

INTRODUCTION

Several hypotheses have been proposed to explain the reduced (dwarfed) growth effects of some rootstocks on scion tissue. These include: alterations in concentrations or ratios of plant hormones (Jones, 1986; Kamboj et al., 1997; 1999; Sommelidou et al., 1994a); partial incompatibility of rootstock and scion genotypes (Gur et al., 1968), necrotic cell formation in the graft union (Gur et al., 1968), and decreased water conductivity between rootstock and scion tissues as a result of vascular anomalies (Gur and Blum, 1975). However, a single mechanism or unifying hypothesis has not emerged. This research was initiated to examine characteristics of the vascular transport system in sweet cherry scion-dwarfing rootstock combinations.

In apples, it has been established that there are genetically controlled differences in the growth of dwarfing root systems and individual cell components of the rootstock vascular system compared to vigorous root systems. For example, a lower proportion of xylem to phloem in roots of dwarfing vs. vigorous rootstocks was observed than in vigorous rootstocks (Beakbane and Thompson, 1937; 1947). Rootstocks that are more dwarfing also have smaller vessel elements than vigorous rootstocks (Beakbane and Thompson, 1937; 1947; McKenzie, 1961). Both of these characteristics ultimately may regulate the vascular transport within the plant system (Jones, 1974).

Vascular transport may play a major regulatory role in tree growth and productivity. The rate of water transport is based upon the hydraulic architecture of the tree, consisting of functional xylem (tracheids, vessels, and rays) and

secondary xylem (wood; Tyree and Ewers, 1991). Measurements of hydraulic conductivity, which consists of the ratio of water flow through stem or branch tissue and the pressure causing water movement, provide an indication of water transport and sap flow quantity. These suggest there are inherent differences in transport between non-grafted and grafted plant systems (Atkinson et al., 2001; 2003). Functional xylem elements may decrease in an acropetal direction, especially in grafted combinations that utilize dwarfing rootstocks (Atkinson et al., 2003).

For ungrafted apple rootstocks, root hydraulic conductivity was lowest in the dwarfing (M.27) compared to the semi-vigorous (MM.106; Atkinson et al., 2003). Similar results were observed when stem sections and leaves from both rootstocks were compared. This suggests that water conduction in dwarfing tissues inherently may be limited due to both size and number of functional vessel elements that determine vascular transport rates throughout the plant. Sap-flow measurements (another indicator of vascular transport rate) were reduced in grafted versus non-grafted systems, with the most dwarfing rootstock/scion combinations having the lowest rate of sap flow (Hussein and McFarland, 1994). Thus, development of vascular tissue in grafted combinations with dwarfing rootstocks may have an additive effect, reducing already genetically-predetermined lower transport rates.

Within the graft union, development and differentiation of the vascular tissue is not distributed uniformly. In fact, scions grafted onto dwarfing rootstocks have shown that vessel diameters decrease in the graft union, with the potential

to negatively affect water transport and overall hydraulic conductivity (Poniedzialek et al., 1979). This has been associated with alterations in the auxin/cytokinin ratio (Soumelidou et al., 1994a,b).

Restriction of transport within grafted systems may also be due to misalignment of vascular tissues (Simons and Chu, 1980). It has been suggested that structural anomalies in the anatomy of vascular elements profoundly restrict xylem and phloem transport of water nutrients, plant hormones, and/or photoassimilates (Atkinson et al., 2003, Gur and Blum, 1975; Sommelidou et al., 1994a). Ultimately, restriction of transport may be as simple as misalignment of vascular tissues (Simons and Chu, 1980), or may involve more complex interactions involving differentiation in the graft union, the rootstock, and the scion. Currently vessel development and differentiation within the initial stages of graft wound healing have not been characterized in functional dwarfing rootstock combinations. Thus, the specific effects on vessel differentiation are not well understood in these systems.

A series of experiments were designed to test the hypothesis that vessel number and size differ in vigorous and dwarfing cherry rootstocks. It was further hypothesized that these genetic differences in vascular capacity ultimately influence transport between rootstock and scion, and thus indirectly control growth of the scion in grafted combinations. Thus, the objectives of these experiments were first to characterize the size and number of vessels in non-grafted vigorous and dwarfing rootstocks, and then to determine if these vascular attributes were perpetuated in heterografts (traditional scion-rootstock

combinations), reciprocal heterografts (inverted scion and rootstock combinations), and homografts (rootstock bud tissue grafted to identical rootstock).

MATERIALS AND METHODS

2001 Confocal Laser Scanning Microscopic Analysis

In March 2001, thirty-six 'Rainier' (*Prunus avium* L.) sweet cherry trees grafted onto three rootstocks were planted in 8.8 L pots and placed in a lathe house at the Horticulture Teaching and Research Center (HTRC) at Michigan State University, East Lansing, Mich. The three rootstocks were classified by their dwarfing effects: dwarfing (Gisela 5; Gi 5), semi-dwarfing (Gisela 6; Gi 6), and vigorous (Colt) cherry rootstocks. All three rootstocks are clonal hybrids (Gisela series- *P. cerasus* × *P. canescens*; Colt- *P. pseudocerasus* × *P.* avium) and were propagated commercially. Ungrafted rootstocks were used to determine vessel characteristics as non-wounded controls (Table 1). Each of the three cherry rootstocks was chip-budded with the scion `Rainier` in August 2001 to create heterograft combinations. Homograft combinations, in which a vegetative bud from the rootstock is grafted back onto the stock, were included to differentiate the wounding effect of grafting from the response of grafting genetically different scion-rootstock combinations (Table 1). Trees were moved into a greenhouse in September to prolong growth of the developing graft union. Day/night temperatures were 24/10 °C, respectively, and supplemental lighting

(~1750 μmol/$m^{-2}s^{1}$ PPFD) was supplied with high pressure sodium lamps to achieve a 14 h day /10 h night regime.

Sections from each scion-rootstock combination were collected monthly, beginning one month after grafting through six months of growth. Samples consisted of 2 mm transverse segments of scion tissue, graft union, and rootstock tissue (Figure 1). Ungrafted rootstock samples were taken from the same region of the rootstock (~8 mm from soil level).

Tissue samples were placed directly into a formalin-acetic acid-alcohol solution (10:5:50 FAA; Ruzin, 1999) and immersed for one week, after which the solution was changed to 50:50 ethanol:water for storage until sectioning at room temperature (25°C). Longitudinal sections (20μm) were made with a sliding microtome, placed on glass slides, and rehydrated with distilled water before staining. Transverse sections were embedded in a water-soluble wax (Carbowax 1500, Dow Chemical Company, Midland, Mich.), sectioned (15 μm) with a rotary microtome, and placed in an oven for 6 hours at 55°C to flatten and remove the wax from sections (Steedman, 1960).

As a preliminary test and prior to staining all sections, two water-soluble fluorescent stains, safranin O and acridine orange, were evaluated on both transverse and longitudinal sections to determine which was most effective in defining sweet cherry xylem elements. Safranin O is a fluorescent dye that excites with blue light (c.a. 530 nm) and emits in the yellow wavelength range (Lillie, 1977). Acridine orange is a metachromatic fluorescent dye that excites at 500 nm and emits with peaks in both green (526 nm), and red (650 nm) ranges

(Lillie, 1977). Unstained samples of the same tissue were observed using the same microscopic parameters to ensure that the fluorescence signal was not from autofluorescence. Fluorescent images were captured using a confocal laser scanning microscope (CLSM; 40x dry; Zeiss 210, Germany) with digital image storage capability. Fluorescent images were obtained using a 488nm argon laser line for excitation and a 520 nm long pass filter for emission. Upon initial visual analysis, safranin O stained cell walls clearly due to its single absorption peak. Based on this initial analysis, all samples were stained using safranin O.

2002 Scanning Electron Microscopic Analysis

In April 2002, 225 rootstocks each of Gisela 5 (Gi 5), Gisela 6 (Gi 6), and three hundred F 12/1 (*P. avium*) were planted into 13.2 L pots and placed in a greenhouse with maximum temperatures of 25 °C and ambient radiation (maximum=1385 $\mu mol/m^{-2}s^{1}$ PPFD). After one month of growth, trees remained ungrafted or were chip-budded to create seven different combinations, representing heterografts, reciprocal heterografts, and homografts (Table 1). Rainier/F 12/1 was utilized as both a homograft (*P. avium/P. avium*) and a heterograft (scion/vigorous rootstock) due to the unavailability of both F 12/1 budwood and/or own-rooted 'Rainier'. Reciprocal grafts were included to examine the effect of the rootstock grafted onto tissue that is commercially designated as scion material.

Samples were harvested monthly for six months after completing the bud-grafting of the combinations. Ungrafted rootstocks were harvested at the same time. Based on results of preliminary analyses of 2001 tissues, samples for the 2002 study were divided into three physical locations for sectioning: scion tissue, graft union, and rootstock tissue. Samples were fixed and stored as described for the 2001 experiment. Six month samples were sectioned into transverse and longitudinal sections (120 μm) with a sliding microtome, and placed into 2 ml microcentrifuge tubes for dehydration in preparation for scanning electron microscopy (SEM). Sections were dehydrated in a graded ethanol series and dried via critical point drying (Flegler et al., 1993). To further facilitate drying, samples were kept in a desiccator at ambient temperatures for one week before examination. They were then coated with gold for 9 min. at a rate of 3 $nm \cdot min^{-1}$. Images were captured to measure vessel number (x 100) and vessel lumen area (x 150) within current year growth. Composite images were constructed using Adobe Photoshop 6.0 (Adobe Systems Inc., San Jose, CA, USA). Images were layered, matching overlapping features, to construct a continuous cross-sectional image from pith to bark.

Image Analysis

Images were analyzed using the trace measurement tool in Sigma Scan Pro 5.0 (Systat, Richmond, Calif.). Images in this dissertation are presented in color. The image was calibrated to a defined dimension, and then the desired image feature was measured. The trace measurement tool allows the user to

define, within a specific scale, two points on an image and measure the feature using the software, which presents data in a spreadsheet.

Vessel cell differentiation and development were followed during the first six months of graft wound healing. Vessel diameter, frequency per mm^2, lumen area, and length were measured to determine if vessel anatomy could contribute to reductions in water translocation throughout the graft union leading to the reduced growth of the scion (i.e., dwarfing).

Vessel diameter (VD) was determined as two measurements perpendicular to each other across the widest part of the vessel lumen, and the sample mean (i.e., Σ[(horizontal + vertical)/2]/n) was calculated to obtain mean vessel diameter (N>25). Maximum vessel diameter (MVD) was reported as an indicator of the largest single vessel measured in each treatment. Vessel lumen area (VLA) was calculated using the mean diameter, determined as the area of an ellipse, given by π^*ab, where *a* equals the longest diameter and *b* equals the shortest diameter. Vessel frequency per mm^2 were counted visually from images taken in 2001 (field of view equaled 534.2 μm^2 at 10x), while in 2002, vessels per mm^2 were counted from the pith to vascular cambium of each section. Vessel element length was determined from CLSM images and measured end to end, delineated by the perforation plate of each vessel element.

Mean vessel hydraulic diameters (VHD) were calculated by dividing Σd^5 / Σd^4 where *d* is vessel diameter to determine the hydraulic efficiency of vessels in scion, graft union, and rootstock material. This calculation results in a hydraulically weighted mean of vessel diameter that accounts for variation in the

vessel area measured, with wider vessels more important to water flow (Pockman and Sperry, 2000). It reflects the conductive potential of a vessel population, allowing a cursory prediction of the effect of vessel size on water transport.

Statistical Analysis

Each experiment was a split plot, completely randomized design, with the main treatment being rootstock type. Failed bud unions were not included in the statistical analysis. General linear models (GLM) were used as appropriate, with alpha levels set at 0.05, *a priori*. Data were assumed to be normal and continuous with homogeneous variances. When normality was not satisfied, data were transformed logarithmically to gain normality before statistical analysis. Mean separation was accomplished using Tukey's HSD and Fisher's LSD as appropriate (SAS; Cary, N.C.).

Results

2001 Vessel Lumen Area. In 2001, a pattern of vessel differentiation and regeneration was observed in ungrafted samples (Figure 2). In ungrafted Colt and Gi 6 trees, there was an increase in vessel lumen area (VLA), first at 2 months, decreasing around 3 months, and then additional area increase at 5 months. In fact, Gi 6 trees showed their greatest VLA increase between 4 and 5 months after grafting. For ungrafted Gi 5, there was a shift in the pattern of vessel differentiation, with the decrease in VLA occurring one month later than in

Gi 6 or Colt. In the last two sampling periods of 5 and 6 months after grafting, Gi 5 had significantly smaller vessels than either Gi 6 or Colt, with the difference in size being clearly visible in images from confocal laser microscopy (Figure 3B, 3C). During the early sampling period (1 and 2 months), Colt VLA was significantly higher than Gi 5 or Gi 6 ($p \leq 0.05$); however, Gi 6 VLA was not significantly different than Colt at six months (Figure 2; $p > 0.05$).

In homograft treatments, VLA decreased over the six month sampling period in all three rootstocks (Figure 2B). The minimum area in homograft combinations occurred at two and six months for Gi 5/Gi 5, three and five months for Gi 6/Gi 6, and at five months for Colt/Colt (Figure 2B). Gi 6/Gi 6 VLA decreased most significantly within the first three months, with both Gi 5/Gi 5 and Colt/Colt having stable, although decreasing vessel area measurements (Figure 2B).

In heterograft combinations, Rainier/Gi 5 had the lowest VLA in the graft union four to six months after grafting ($p < 0.05$; Figure 2C). In contrast and unexpectedly, Rainier/Gi 6 graft union tissue had the largest VLA throughout most of the six month interval (Figure 2C). However, by six months after grafting, Rainier/Gi 6 VLA was the same as Rainier/Colt.

Bud development progressed as expected with a large number of callus cells at the scion-rootstock interface (Figure 3A). Differences in vessel area between ungrafted Gi 5 and Colt are visible in CLSM images, with numerous smaller vessels in ungrafted Gi 5 (Figure 3B, 3C). In graft union tissue of Rainier/Colt (Figure 3D) and Rainier/Gi 5 (Figure 3E), there are a greater number

of smaller vessels in Rainier/Gi 5 (Figure 3E) than in Rainier/Colt graft union sections (Figure 3D).

2001 Vessel Diameter. Vessel diameter (VD) at six months indicated that vessels derived from ungrafted Gi 5 were smaller than vessels from ungrafted Colt, with Gi 6 having the largest vessels of all ungrafted rootstocks (Table 2). VD in ungrafted Gi 5 was significantly greater than scion sections of Gi 5/Gi 5, and greater than graft union sections of Rainier/Gi 5 ($p < 0.05$). In fact, VD in graft union tissue of Rainier/Gi 5 had the smallest vessels ($p < 0.01$). Similarly, ungrafted Gi 6 VD was greater than Gi 6/Gi 6 scion and graft union tissue and Rainier/Gi 6 graft union tissue ($p < 0.01$). Ungrafted Colt MVD was higher than in graft union tissues of Colt/Colt while essentially the same in tissues of Rainier/Colt ($p < 0.01$). In combinations containing Gi 5 as a component, maximum vessel diameter (MVD) was smallest in scion tissues of Gi 5/Gi 5 and nearly the smallest in graft union tissues of Rainier/Gi 5, indicating that combinations containing Gi 5 had an overall reduction in vessel size.

2001 Vessel Hydraulic Diameter. Vessel hydraulic diameter (VHD) followed a similar pattern as VD in ungrafted samples, with ungrafted Gi 5 VHD significantly smaller than ungrafted Colt and Gi 6 ($p < 0.01$) (Table 2). The VHD was smallest for vessels in Gi 5/Gi 5 scion tissue and Rainier/Gi 5 graft union tissue. Ungrafted Gi 6 and Colt VHD were the same statistically (Table 2). Compared to homograft combinations, ungrafted Gi 6 and Colt rootstocks had larger mean hydraulic diameters than scion and graft union tissues ($p = 0.05$). Overall, both MVD and VHD for ungrafted and rootstock tissue were higher than in scion and

graft union tissue ($p < 0.05$). Of heterograft combinations, Rainier/Gi 5 was the sole treatment that exhibited a reduction in VHD in graft union tissue compared to both scion and rootstock tissue (Table 2).

2001 Vessel Frequency per mm^2. All ungrafted rootstocks had more vessels in 2001 than their respective grafted combinations (Table 3; $p < 0.001$). Only Rainier/Gi 5 exhibited differences in vessels per mm^2, with increased more vessels compared to Rainier/Colt ($p < 0.05$). Sampling at months two and three resulted in the highest number of vessels per mm^2 overall ($p = 0.01$) (data not shown), which coincided with the first peak in vessel area increase of ungrafted rootstocks (Figure 2A). In addition, ungrafted rootstocks tended to have more vessels than in grafted combinations (Table 3). In all treatments, vessel element length did not differ significantly, either between ungrafted rootstocks or grafted combinations (data not shown) ($p \geq 0.05$).

Vessel frequency per mm^2 in grafted systems varied depending upon the rootstock treatment. In treatments of genetically identical grafted material, Gi 6/Gi 6 had the most vessels in the graft union region (Table 3). The graft union region of Gi 5/Gi 5 and Colt/Colt had less vessels than in ungrafted controls (Table 3). Overall, the treatment of Gi 6/Gi 6 had significantly more vessels than Colt/Colt ($p < 0.05$). In 2001, Rainier/Colt exhibited a lower vessel frequency per mm^2 in graft union tissues than in graft union tissues for Rainier/Gi 5 ($p < 0.05$), with Rainier/Gi 6 intermediate in vessel frequency of graft union tissues. In all homograft and heterograft treatments and locations, vessel element length did

not differ significantly within the six month sampling period ($p > 0.05$) (data not shown).

2002 Vessel Lumen Area. Ungrafted rootstocks had increasing VLA according to the vigor of the rootstock (Figure 4A). Gi 5 had the smallest VLA, with Gi 6 and F 12/1 having successively larger vessel areas (Figure 4A, Figure 5A, 5B). In grafted treatments, vessels were smaller in scion tissue than in the graft union, except for Rainier/F 12/1 and Gi 6/F 12/1 (Figure 4A). There were no differences in VLA between rootstock and graft union tissues of homografts or heterografts with the scion 'Rainier' compared to those with the scion Gi 5 or Gi 6 (Figure 4A). This is contrary to the trend observed in 2001 in which dwarfing rootstocks had smaller VLA in graft union tissues of homografts and heterografts; however, Colt rootstock was used instead of F 12/1 (Figure 2A, 2B, Figure 4A). SEM images of graft union tissue in Rainier/Gi 5 suggest that in addition to smaller vessel size, tissue growth occurs at a bias in the graft union that is not observed in Rainier/Colt graft union (Figure 5C, 5D).

Rainier/Gi 6 demonstrated the highest VLA in rootstock and graft union sections of those combinations with 'Rainier' as the scion (Figure 4A). There was little callus formation and cell differentiation in the graft union region due to the high number of failed bud unions (data not shown) and little change in VLA from rootstock to graft union tissue.

Reciprocal grafts displayed a similar pattern, with scion tissue in Gi 5/F 12/1 having significantly lower VLA than in Gi 6/F 12/1 scion tissue (Figure 4A). In Gi 5/F 12/1, VLA in scion tissue was significantly lower than in the graft union,

unlike Gi 6/F 12/1, where VLA increased (Figure 4A). However, in both reciprocal treatments, scion tissue had larger vessels compared to Rainier/F 12/1.

2002 Vessel Diameter. Ungrafted F 12/1 had a larger MVD than all other combinations, except Rainier/F 12/1 rootstock tissue (Table 4). Ungrafted Gi 5 and GI 6 were similar in VD; however, ungrafted Gi 5 VD was smaller than ungrafted Gi 6 VD. Scion tissue in all combinations had reduced VD compared to graft union and rootstock tissues.

In homograft combinations, Gi 6/Gi 6 scion and Rainier/F 12/1 scion tissue had essentially the same VD. Gi 5/Gi 5 scion VD was larger than any other homograft combination. In heterograft combinations, there were no differences in either Rainier/Gi 5 or Rainier/Gi 6 between graft union and rootstock tissue (Table 4). Conducting vessel elements in scion tissues were smaller in Rainier/Gi 5 than in Rainier/F 12/1 ($P \leq 0.05$). In addition, maximum vessel diameter was smallest in Rainier/Gi 5 scion tissue.

2002 Vessel Hydraulic Diameter. Measurement of VHD indicated significant differences among ungrafted rootstocks, with Gi 5 exhibiting the lowest VHD followed by Gisela 6 and F 12/1 (Table 4). VHD of ungrafted F 12/1 was the largest of all combinations. VHD of ungrafted Gi 5 was larger than scion tissues of Gi 5/Gi 5 and Rainier/Gi 5, but not larger than graft union or rootstock tissue. However, in Gi 5/F 12/1, VHD was higher in graft union and rootstock tissues than ungrafted rootstock tissue.

In grafted treatments, graft union VHD values were lower than in rootstock tissues, with the exception of Gi 6/Gi 6 and Rainier/Gi 5 (Table 4). In Rainier/Gi 5, there was a 77% reduction in VHD between graft union and scion tissues that was not observed in any other homograft, heterograft, or reciprocal heterograft treatment (Table 4). In two homograft treatments, Gi 6/Gi 6 and Rainier/F 12/1, there was at least a 40% reduction in VHD between graft union and scion tissues. In most combinations, there was a decrease between rootstock and graft union tissues, with the exception of Rainier/Gi 6 which did not have a successful bud union (Table 4).

2002 Vessel Frequency per mm^2. In 2002, vessel frequency per mm^2 in ungrafted rootstocks were significantly higher than in all grafted combinations, with Gi 6 having the greatest vessels per mm^2 ($p < 0.05$), followed by Gi 5 and F 12/1, which produced essentially the same numbers of vessels (Figure 4B). However, visual examination of SEM micrographs suggest that there are more secondary xylem vessels in ungrafted Gi 5 (Figure 5A) compared to ungrafted F 12/1 (Figure 5B). There was an increase in total frequency of vessels per mm^2 in ungrafted rootstocks during the 2002 experiment due to examination of the entire stem radius.

In grafted combinations, rootstock tissues had the greatest vessel frequency per mm^2 (Figure 4B). In heterografts (Rainier/Gi5 and Rainier/Gi 6), there was a significant reduction of vessels per mm^2 in the graft union compared to rootstock tissue, with a further decrease in scion tissues of Rainier/Gi 5. In addition, there was a significant reduction in vessel frequency in the graft union

of homografts compared to rootstock tissue ($p < 0.05$) (Figure 4B). Reciprocal heterografts had stable vessel numbers between scion and rootstock tissues, with a reduction in graft union tissues only in Gi 6/F 12/1. Combinations utilizing Gi 5 or Gi 6 as the rootstock had fewer vessels in the graft union than if F 12/1 was the rootstock, suggesting that dwarfing rootstocks have an effect on vessel number.

Vascular Anomaly Presence. Examination of SEM micrographs revealed that as expected, ungrafted F 12/1 had a wider radius than ungrafted Gi 5 rootstock (Figure 6). However, when the rootstocks were grafted with the scion 'Rainier', the radius of each combination was almost identical, due to the higher proportion of non-functional phloem and undifferentiated callus tissue in Rainier/Gi 5. In fact, there was a 64% increase in callus and non-functional phloem in Rainier/Gi 5 compared to Rainier/F 12/1 (Figure 6). Development of callus tissue into vascular elements resulted in a whorl of tissue throughout the radius of Rainier/Gi 5 compared to the linear development of xylem rays in Rainier/F 12/1 (Figure 6).

Within the graft union of Rainier/Gi 5, callus tissue in this region developed vascular anomalies that were evident upon higher magnification (Figure 7). There were multiple xylem vessels that developed on a bias (acute angle) to the longitudinal axis of the tree. In addition, there were a greater number of these vascular anomalies in Rainier/Gi 5 than in any other combination.

Discussion

Confocal laser fluorescence microscopic techniques offered advantages and disadvantages. Although staining afforded a fairly clear image of vessel anatomy, the embedding and sectioning procedure proved challenging with wood tissue. Scanning electron microscopic techniques provided a more detailed image, with the additional advantage of acquiring composite images from pith to bark.

In ungrafted rootstocks, a distinct pattern of increasing and decreasing VLA was observed during growth and development. This pattern is most likely caused by vessel maturation and cell differentiation, which displaces older cells to the inner portions of the stem. Immature vessel cells derived from the vascular cambium (secondary xylem) where new cells are continuously generated, exhibit fluctuations in cell diameter and area. In 2001, the pattern of fluctuating VLA was delayed in ungrafted Gi 5, suggesting that ungrafted Gi 5 exhibits an inherently different growth and development of the vascular system. In spite of this, all rootstocks exhibited a fluctuation in VLA affected by vessel maturation near the vascular cambium in 2001.

The decrease in VLA of the Rainier/Gi 5 graft union during the last three months of the experiment was especially significant (Figure 4A). This decreased VLA implies decreased potential for water and nutrient movement through the graft union region when scions are grafted onto Gi 5 rootstocks. This decreased vessel area supports the hypothesis that hydraulic resistances are increased at the graft union of dwarfing systems (Atkinson et al., 2003).

Comparisons of VD between ungrafted and their respective grafted combinations revealed that a wounding effect exists. In most cases, VD in graft union tissues of grafted combinations were reduced compared to their ungrafted counterparts, possibly resulting in reduced xylem transport in this region. Calculations of VHD based on VD measurements confirmed this in 2001. VHD reduction in the graft union of Rainier/Gi 5 suggests that this section of the grafted system, in particular, could lead to increased hydraulic resistance and reduced transport rates. Other grafted treatments (heterograft and homograft) showed a slight increase or stable values throughout the graft union region for HVD, suggesting a pathway of little resistance within the grafted tree for these scion-rootstock combinations.

Both ungrafted Gi 5 and Gi 6 rootstocks had greater numbers of vessel per mm^2 than in Colt. This suggests that although scions grafted on Gi 5 and Gi 6 exhibit a dwarf growth habit, more vessels are produced than in a vigorous ungrafted rootstock. Fluctuations in VLA extended to vessel frequency per mm^2 as well in 2001, with the highest frequency of vessels observed between the second and third month. However, no overall differences were observed between treatments throughout the six month experiment.

A wounding effect in homografts was found, with a significant reduction ($P < 0.01$) in vessel frequency per mm^2 of grafted combinations compared to ungrafted rootstocks (Table 3). Furthermore, Gi 6/Gi 6 tended to have the highest average vessel number per mm^2, while overall Gi 5/Gi 5 average vessel frequency per mm^2 was intermediate between Gi 6/Gi 6 and Colt/Colt (Table 3).

In 2001, vessel characteristics of ungrafted Gi 6 and Colt were similar. Thus, although Gi 5 and Gi 6 are of the same genetic background, they affect growth and development differently. This corroborates previous reports in apple rootstock systems. Grafting inverted apple bark rings (cv. Macintosh) onto the same scion wood produced greater generation of callus into phloem cells than xylem cells, with initial differentiation occurring at the basipetal portion of the graft union (Poniedzialek et al., 1979). Not only was there a reduction in the xylem: phloem tissue, but xylem cell diameter and number decreased progressively in the basipetal portion of the graft union. The reduction in xylem cell capacity as a result of callus differentiation during the healing process suggests a specific wounding response associated with grafting that could be exacerbated when dwarfing rootstocks are used in graft combinations. Recall that dwarfing rootstocks are predisposed to reduced xylem element number and size.

In 2002, restricting measurement of vessels to the same cell lineage (i.e. growth ring) prevented the fluctuations recorded for VLA as in 2001 (data not shown). In addition, an adjustment was made to accommodate the species vascular architecture. *Prunus* trees exhibit semi-ring porous architecture; thus measurements were made to include mature vessels, expected to have the highest conductance (Zimmerman and Jeje, 1981). Semi-ring porous architecture identifies differences in VLA, with larger vessels produced early in the growing season (earlywood) that is followed by production of smaller vessels later in the growing season (latewood). Thus, measurements were made within

the current season's growth ring, specifically the earlywood, stabilizing fluctuations in vessel area.

There was an overall reduction in VLA of scion tissue compared to graft union tissue. Young scions have reduced overall diameter in comparison to the rootstock due to their development from a dormant bud. As the grafted system develops, the diameter of the functioning vessels should mature and be comparable to vessel diameter throughout the stem. Exceptions to this observation were those of Rainier/F 12/1 and Gi 6/F 12/1 where vessel area in the graft union was reduced or similar to scion and rootstock tissue. The reciprocal graft combination of Gi 5/F 12/1 was the only combination containing F 12/1 where VLA in scion tissue was smaller than in the graft union. This suggests that Gi 5 may have smaller VLA regardless of whether it is grafted as scion or rootstock.

The VHD data suggests that scion tissues of genetically mixed grafted systems (heterografts) utilizing a dwarfing component can reduce theoretical water conduction in scion tissue. During 2001, VHD tended to be lowest in grafted combinations containing Gi 5 as a component. Similar results were obtained in Rainier/Gi 5 in 2002. An overall wounding effect on VHD was observed in scion tissue having consistently lower VHD than graft union tissue.

Ungrafted rootstock VHD indicated that Gi 5 and Gi 6 were similar; however, Gi 6 was significantly lower than Gi 5. In addition, homografts of Gi 5/Gi 5 had the highest overall VHD compared to Gi 6/Gi 6 and F 12/1, suggesting that although there is a general wounding effect, the combination of Gi 5/Gi 5

may not exhibit characteristics of a dwarfing rootstock system. In fact, dwarfing rootstocks may not always exhibit dwarfing characteristics, depending upon soil type and climactic conditions. For example, rootstock trials (NC-140 regional rootstock research project) indicated that Gi 6 was semi-dwarfing in eastern states, but in the west, the reduction in tree size was moderated, and in some cases tree size was comparable to vigorous rootstocks (Perry et al., 1997).

Ungrafted Gi 6 exhibited a tendency to produce a high frequency of vessels that were intermediate in area compared to Gi 5 and F 12/1. However, Gi 5 tended to produce an intermediate to high number of vessels with small vessel area, and F 12/1 produced low numbers of vessels with large VLA. Thus, Gi 5 rootstocks have a greater frequency of narrow vessels, whereas more vigorous rootstocks like Gi 6 and F 12/1 tend to produce lower numbers of vessels with larger VLA.

In grafted combinations examined in 2002, there was a significant decrease in vessel frequency per mm^2 between graft union and scion tissue of combinations with Gi 5 or Gi 6 as the rootstock. However, in two of the three grafted combinations containing F 12/1 as the rootstock, there were no significant differences in vessel frequency per mm^2 between graft union and scion tissue (Figure 5A). This could be attributed to the moderating effect of the vigorous rootstock on scion growth, influencing vessel differentiation.

One other hypothesis to explain reductions in water transport has been that of vascular anomalies in the graft union when there is a mixture of genetically different plant material (Simons and Chu, 1980). Vascular anomalies,

such as whorls of parenchyma and xylem rays on a bias to the longitudinal axis of the tree, were more prevalent in the dwarfing combination of Rainier/Gi 5 than in Rainier/F 12/1 (Figure 7). In addition, there was an ~ 64% increase in the amount of callus and non-functioning phloem present in the graft union of Rainier/Gi 5 compared to the same section of Rainier x F 12/1 (Figure 7). This substantiates early reports of the increased phloem to xylem ratio found in dwarfing apple roots and bud union (Beakbane and Thompson, 1939; 1947; Sommelidou et al., 1994b). The development of vessels in the graft union at interfering angles to the existing vascular system may lead to a reduction in water transport, simply because of the length of conduit that must be traveled between rootstock and scion.

Conclusions

Vessel area was smaller in graft union tissue of Gi 5 combinations, giving a good indication that a hydraulic restriction may occur in the graft union with the highest proportion of genetically mixed tissue (scion + rootstock). MVD values of all ungrafted rootstocks were greater compared to graft union sections of respective homograft and heterograft combinations. Calculation of VHD supports the hypothesis that Rainier/Gi 5 graft union tissue may exhibit reduced water conduction compared to scion or rootstock tissues, and was the only treatment to exhibit a decrease in VHD of the graft union. In addition, VHD indicated that a wounding effect took place with VHD decreases in scion tissues compared to graft union tissue.

Vessel number in dwarfing rootstocks was not higher in Gi 5 compared to Colt or F 12/1, suggesting that there is a greater proportion of narrow vessels produced in Gi 5 rootstock combinations. Vessel length was not significant and there was no significant pattern among treatments.

From these experiments, both vessel area and vessel number were reduced in dwarfing combinations, which may negatively affect water and nutrient translocation as indicated in previous studies (Atkinson et al., 2003; Gur and Blum, 1975; Sommelidou et al., 1994a). Ungrafted rootstocks produced greater vessel numbers, larger in size than in their grafted counterparts, and thus these differences were probably due to a wounding effect. In addition, dwarfing combinations tended to produce a greater number of small vessels with reduced hydraulic efficiency, most likely a factor that could influence scion growth. As in many plant systems, it is likely that a number of processes are affecting growth in grafted systems. From this research, it appears that the combination of a larger proportion of narrow vessels with reduced hydraulic efficiency and increased incidences of vascular anomaly greatly contributes to the dwarfed scion habit Gi 5 is used as part of a dwarfing tree system.

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Table 1. 2001 and 2002 graft combinations for vascular anatomy studies of dwarfing and vigorous rootstocks with `Rainier` sweet cherry scion.

2001	***Graft Combinations***
Controls	Gi 5, Gi 6, Colt ungrafted trees
Homografts	Gi 5/Gi 5, Gi 6/Gi 6, Colt/Colt
Heterografts	Rainier/Gi 5, Rainier/Gi 6, Rainier/Colt
2002	
Controls	Gi 5, Gi 6, F 12/1 ungrafted trees
Homografts	Rainier/F 12/1, Gi 5/Gi 5, Gi 6/Gi 6
Heterografts	Rainier/Gi 5, Rainier/Gi 6, Rainier/F 12/1
Reciprocal Heterografts	Gi 5/F 12/1, Gi 6/F 12/1

Figure 1. Samples for confocal laser scanning and scanning electron microscopy of xylem. The graft union was divided and designated as a 6 mm section between scion and rootstock. Tissue sections from ungrafted rootstocks were harvested in approximately the same location as those treatments that were bud-grafted for comparison.

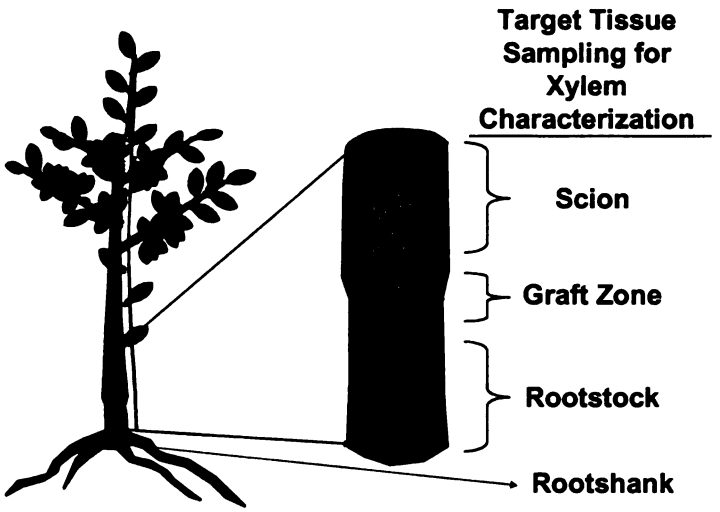


Figure 2. Vessel lumen area (VLA) of individual vessel elements as measured from confocal laser microscopy images in 2001. Ungrafted rootstocks (A) were used as controls, and sampling locations were harvested as to approximate locations in grafted combinations. Graft union sections of grafted combinations are presented in homografts (B) and heterografts (C) over a six month sampling period. Destructive sampling commenced one month after bud-grafting. Error bars denote one SE of the mean (n=3).

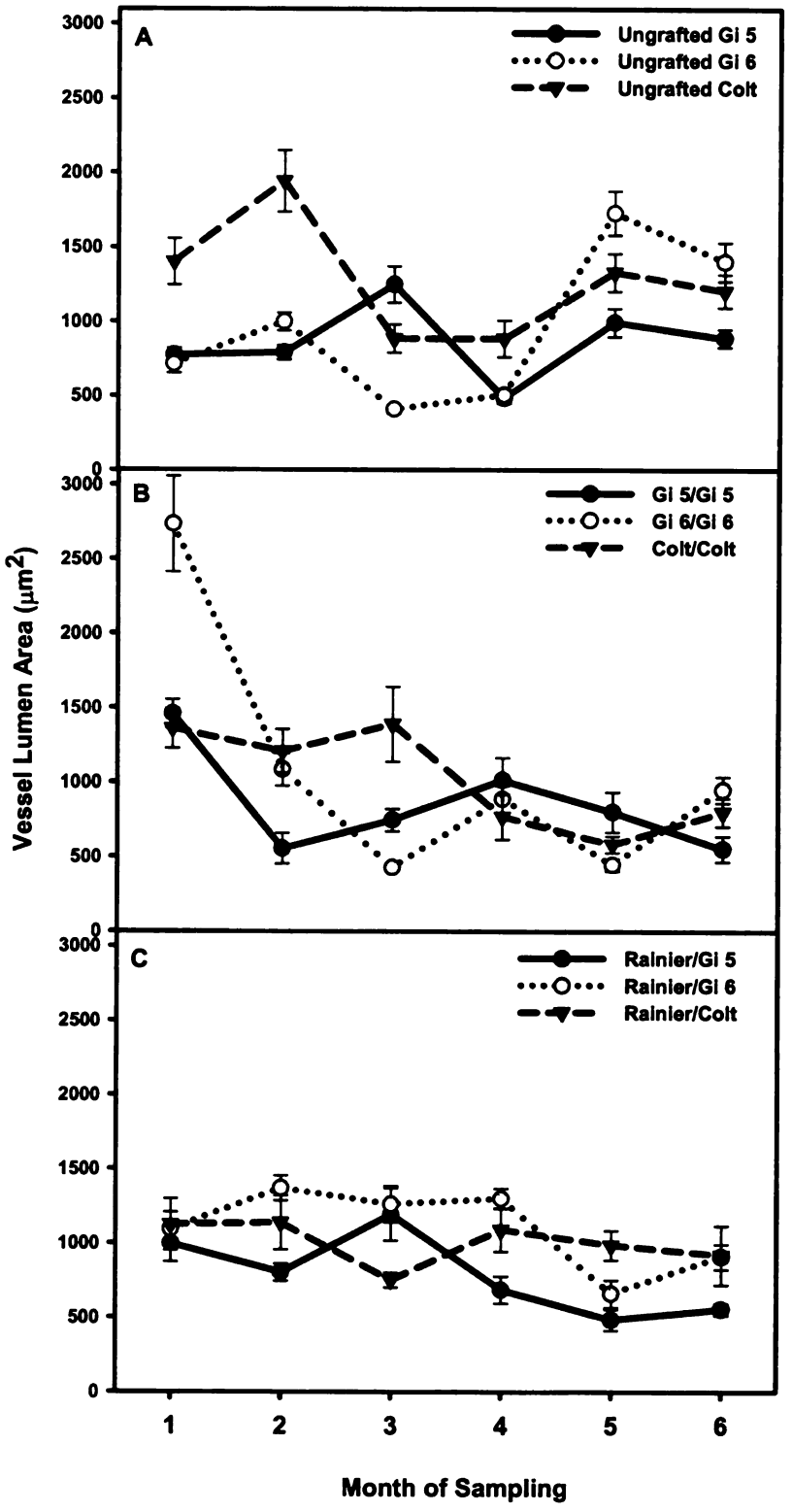

A
Ungrafted Gi 5
Ungrafted Gi 6
Ungrafted Colt
B
Gi 5/Gi 5
Gi 6/Gi 6
Colt/Colt
C
Rainier/Gi 5
Rainier/Gi 6
Rainier/Colt
Vessel Lumen Area (μm2)
0
500
1000
1500
2000
2500
3000
1
2
3
4
5
6
Month of Sampling

Figure 3. Fluorescence images captured using confocal laser microscopy of Rainier/Colt grafted bud union with differentiating vessels (A); vessel elements in ungrafted rootstocks of Colt (B) and Gisela 5 (C); and in central graft union sections of Rainier/Colt (D) and Rainier/Gi 5 (E). All images are of samples six months after bud-grafting combinations. Sections of ungrafted rootstock were taken in approximately the same location as in grafted combinations. In image (A), vc: vascular cambium; cc: callus cells in the developing bud; xy: xylem tissue. In images (B-E), ve: vessel element; xr: xylem ray. Magnification in A: 10x, B-E: 40x.

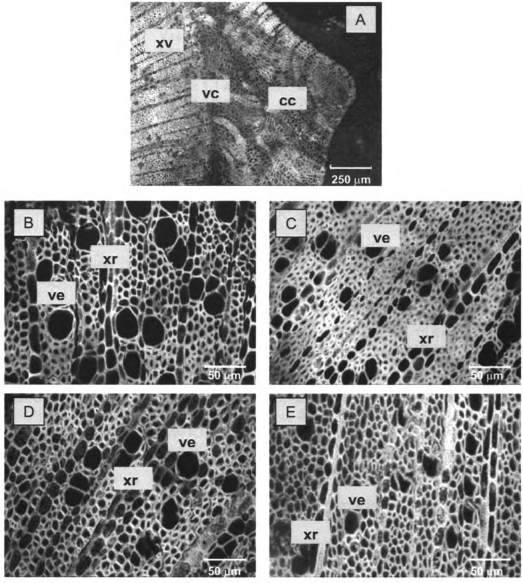


Table 2. Vessel diameter, vessel hydraulic diameter, maximum vessel diameter, and total number of vessels measured (n) six months after bud-grafting sweet cherry scion/rootstock combinations in 2001. Maximum vessel diameter indicates the largest measured vessel in each treatment. Ungrafted rootstocks were used as controls, and sampling locations were harvested at approximate locations in grafted combinations. Although graft union sections were initially examined in three locations (acropetal, middle, and basipetal), the data were combined as one section because there was no significance between the three segments of the graft union. There were a total of three replicate samples analyzed for each treatment, thus n = the number of vessel cells that were measured.

Treatment		Vessel Diameter[1] (µm)	Maximum Vessel Diameter[1] (µm)	Vessel Hydraulic Diameter (µm)	n
Ungrafted	Gisela 5	16.7 ± 0.6[2]cde[3]	24.5	16.6 cde[2]	29
	Gisela 6	21.5 ± 1.1 a	27.3	20.7 a	16
	Colt	19.8 ± 1.0 abc	26.6	19.1 ab	25
Homografts					
Gi 5/Gi 5	*Scion*	14.0 ± 0.5 fg	18.4	13.6 f	20
	Graft Union	15.8 ± 0.6 defg	26.1	15.8 def	46
	Rootstock	15.9 ± 1.0 defg	21.8	15.1 ef	10
Gi 6/Gi 6	*Scion*	15.9 ± 1.0 defg	23.6	15.8 cdef	20
	Graft Union	17.3 ± 0.6 cd	26.2	17.2 bcde	54
	Rootstock	19.0 ± 0.7 abc	24.4	18.9 ab	28
Colt/Colt	*Scion*	16.5 ± 1.2 cdef	24.9	15.8 cdef	16
	Graft Union	15.3 ± 0.6 efg	29.7	15.3 ef	54
	Rootstock	19.5 ± 1.3 abc	28.5	18.5 abc	12
Heterografts					
Rainier/Gi 5	*Scion*	16.8 ± 0.8 cde	23.5	16.6 cde	24
	Graft Union	13.7 ± 0.4 g	22.4	13.9 f	50
	Rootstock	18.0 ± 1.0 bcd	25.1	17.2 bcde	16
Rainier/Gi 6	*Scion*	20.2 ± 1.1 ab	28.3	19.2 ab	18
	Graft Union	17.4 ± 0.6 cd	30.8	17.4 bcd	54
	Rootstock	19.6 ± 1.3 abc	26.0	18.9 abc	12
Rainier/Colt	*Scion*	17.2 ± 1.3 cde	28.5	16.4 cdef	16
	Graft Union	17.0 ± 1.0 cde	31.4	16.5 cde	40
	Rootstock	16.4 ± 0.8 cdefg	20.3	15.6 def	10

[1] Mean values of total vessel cells measured
[2] Represents one SE of the mean
[3] Letters denote differences in mean separation within a column by LSD, $P \leq 0.05$.

Table 3. Vessel frequency per mm^2 in 2001, at six months after bud-grafting. Ungrafted rootstocks were used as controls, and sampling locations were harvested at approximately the same locations in grafted combinations. Heterografts represent commercially available combinations. Although graft union sections were initially examined in three locations (acropetal, middle, and basipetal), the data were combined as one section because there was no significance between the three segments of the graft union. Statistical differences are shown for overall treatments, as there were no significant differences detected between sections. Mean separation calculated using LSD, $P \leq 0.05$.

Treatment		Vessel Frequency per mm^2 ± SE	
Ungrafted	Gisela 5	43.4 ± 4.2	b
	Gisela 6	52.1 ± 11.3	a
	Colt	35.6 ± 4.4	c
Homografts			
Gi 5/Gi 5	*Scion*	32.5 ± 3.4	
	Graft Union	30.9 ± 5.3	cd
	Rootstock	24.0 ± 2.9	
Gi 6/Gi 6	*Scion*	30.9 ± 2.4	
	Graft Union	34.0 ± 6.6	c
	Rootstock	38.3 ± 6.7	
Colt/Colt	*Scion*	25.0 ± 3.5	
	Graft Union	27.5 ± 4.2	d
	Rootstock	22.5 ± 1.4	
Heterografts			
Rainier/Gi 5	*Scion*	33.7 ± 4.0	
	Graft Union	31.9 ± 5.3	cd
	Rootstock	32.4 ± 4.5	
Rainier/Gi 6	*Scion*	37.4 ± 4.5	
	Graft Union	27.2 ± 3.8	cd
	Rootstock	31.5 ± 5.2	
Rainier/Colt	*Scion*	28.2 ± 4.5	d
	Graft Union	24.3 ± 2.9	
	Rootstock	26.4 ± 4.5	

Figure 4. 2002 vessel lumen area (VLA) (A) as determined in mature vascular tissue at six months after bud-grafting, and vessel number per mm^2 (B) as determined by measurement of the entire diameter from pith to outer bark. Ungrafted rootstocks were used as controls, and sampling locations were harvested as to approximate locations in grafted combinations. Error bars indicate one SE of the mean (n≥ 3). The asterisk (*) indicates unsuccessful growth of the scion in all replicates of Gi 6.

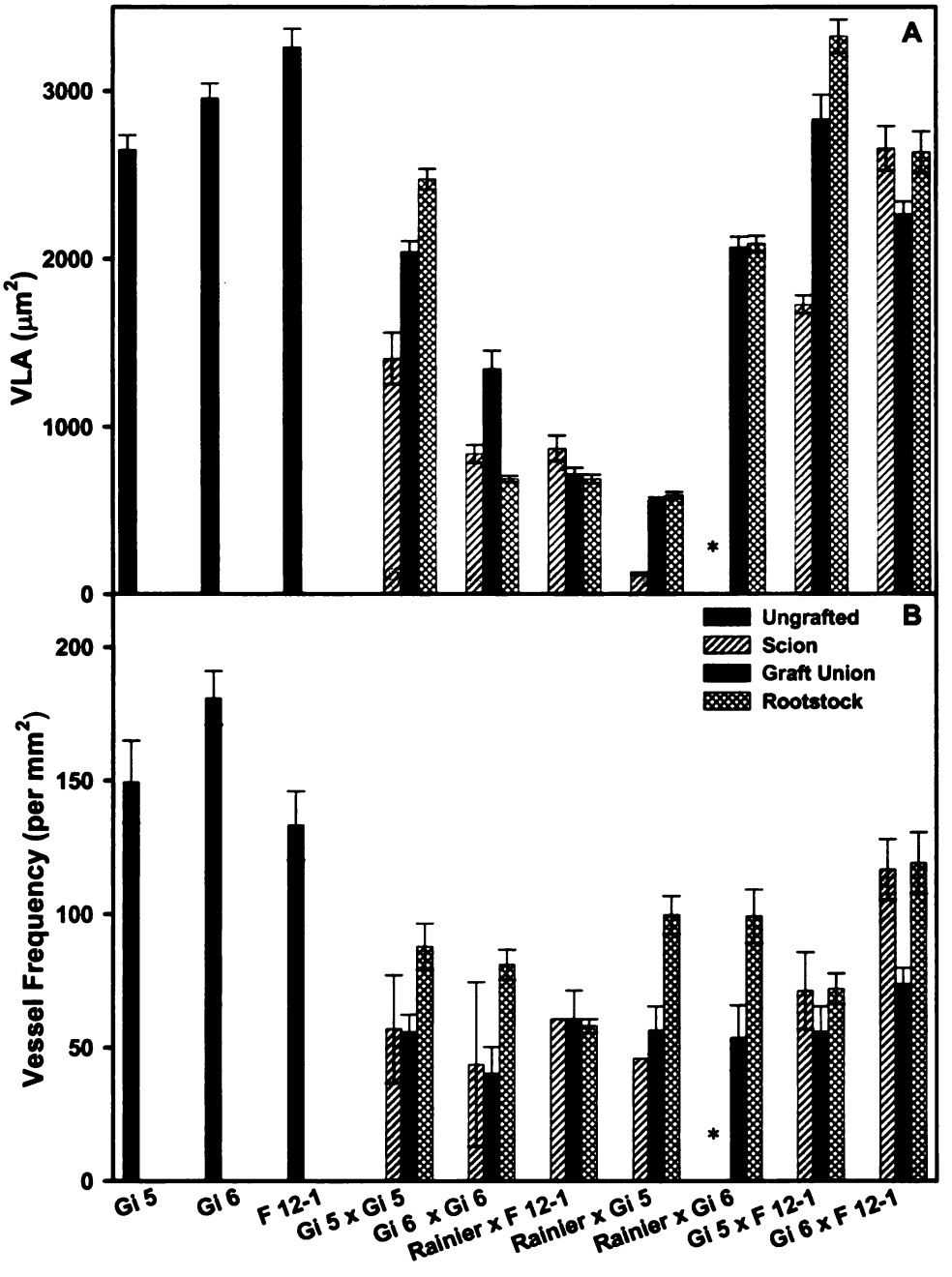


Figure 5. SEM micrographs of vessels located in mature vascular tissue that was used in measurements of vessel frequency and vessel lumen area (100x). Ungrafted rootstocks were used as controls, and sampling locations were harvested as to approximate locations in grafted combinations. Ungrafted Gisela 5 (A), ungrafted F 12/1 (B), grafted scion and rootstock tissue, Rainier/Gi 5 (C) and Rainier/F 12/1 (D). There appeared to be more vessel elements in Rainier/Gi 5 than Rainier/F 12/1 in graft union tissue (C, D). The pith is located to the right of each picture, while immature vessels are located near the vascular cambium (left; not pictured). ve: vessel element.

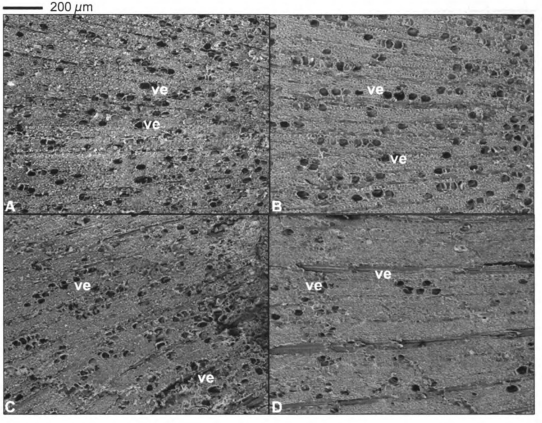


Table 4. Vessel diameter (VD), vessel hydraulic diameter (VHD), maximum vessel diameter, total number of vessels measured (n), and number of replicate samples measured for each treatment (N) in 2002. Maximum vessel diameter indicates the largest measured vessel in each treatment. Vessels were measured from the pith to the vascular cambium. Ungrafted rootstocks were used as controls, and sampling locations were harvested at approximate locations in grafted combinations.

Treatment		Vessel Diameter (µm)	Maximum Vessel Diameter (µm)	Vessel Hydraulic Diameter (µm)	n	N
Ungrafted	Gisela 5	27.9 ± 0.5[1]g[2]	51.6	27.6 j	288	3
	Gisela 6	27.0 ± 0.5 h	79.8	26.9 k	242	3
	F 12/1	33.3 ± 0.5 a	56.2	33.3 a	240	3
Homografts						
Gi 5/Gi 5	*Scion*	22.7 ± 0.9 j	47.5	24.3 l	144	3
	Graft Union	29.1 ± 0.4 g	46.6	28.1 ij	286	5
	Rootstock	30.8 ± 0.4 bc	55.3	30.9 c	494	5
Gi 6/Gi 6	*Scion*	15.5 ± 0.5 k	31.0	17.6 m	128	2
	Graft Union	29.8 ± 0.6 e	54.7	29.7 def	240	6
	Rootstock	30.7 ± 0.2cd	37.5	30.3 cd	450	6
Rainier/F 12/1[3]	*Scion*	13.4 ± 0.7 k	42.9	16.0 m	72	2
	Graft Union	28.9 ± 0.4 efg	27.2	28.2 hij	142	3
	Rootstock	32.1 ± 0.3 ab	28.0	32.9 ab	234	3
Heterografts						
Rainier/Gi 5	*Scion*	4.0 ± 0.2 l	14.0	6.6 n	166	2
	Graft Union	29.6 ± 0.2 ef	24.0	29.5 efg	320	4
	Rootstock	29.8 ± 0.2 e	26.4	29.7 de	280	4
Rainier/Gi 6	*Graft Union*	29.9 ± 0.5 de	50.3	30.1 cde	364	6
	Rootstock	29.2 ± 0.3 ef	53.5	29.0 fgh	546	6
Reciprocal Heterografts						
Gi 5/F 12/1	*Scion*	26.8 ± 0.4 h	45.3	27.5 jk	206	3
	Graft Union	28.7 ± 0.7 fg	59.9	28.7 ghi	200	4
	Rootstock	31.9 ± 0.5 b	66.9	32.1 b	358	4
Gi 6/F 12/1	*Scion*	22.8 ± 0.7 i	53.8	23.3 l	156	3
	Graft Union	26.9 ± 0.5 h	55.9	26.9 jk	282	4
	Rootstock	31.7 ± 0.6 b	53.3	31.9 b	236	4

[1] Represents one SE of the mean

[2] Letters denote differences using mean separation calculated within column by LSD, $P \leq 0.05$.

[3] Rainier/F 12/1 was used as both a homograft and heterograft due to the unavailability of own-rooted Rainier

Figure 6. Composite SEM images of ungrafted rootstocks and grafted treatments indicating differences in diameter (100x). The presence of vascular anomalies increases in grafted combinations as dwarfing capability increases (Gi 5; dwarfing, F 12/1; vigorous). Arrows indicate the vascular cambium in each image. In all images:
p: pith; np: non-functioning phloem; ve: vessel elements.

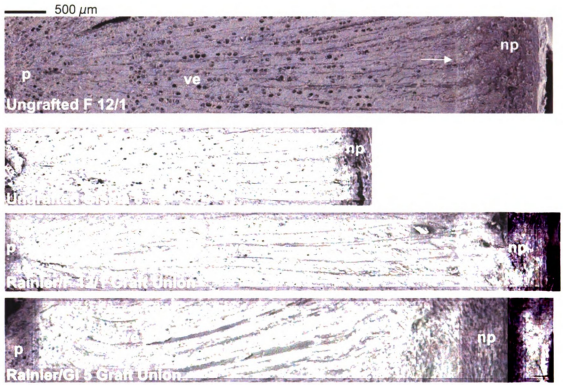
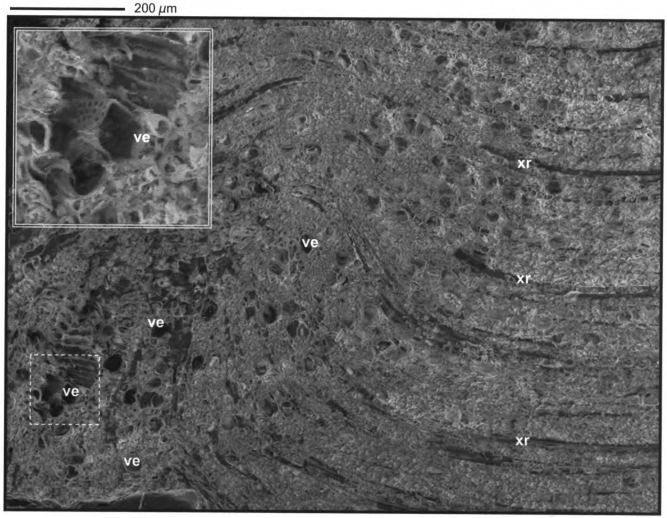


Figure 7. Vascular anomalies perpendicular to the tree axis were observed in graft union tissue of heterografts with the dwarfing rootstock, Gisela 5. Pictured below is Rainier x Gi 5 (100x) showing whorls of vascular tissue and vessels developed on a bias to the longitudinal axis of the tree (inset, 416 x). In image; ve: vessel element; xr: xylem ray.


200 μm
ve
ve
ve
ve
ve
xr
xr
xr

CHAPTER THREE

Examination of the Vascular System in Sweet Cherries (*Prunus avium* L.) Grafted onto Dwarfing and Non-Dwarfing Rootstocks

Abstract

Dye transport through vascular pathways were examined in the tissues surrounding the graft union of second-leaf, field-grown trees of Lapins/Gi 5 (dwarfing) and Lapins/Colt (non-dwarfing). Trees were dug and placed into containers with xylem-mobile dye, then allowed to transpire for six hours before sectioning the tree into 3 to 5 cm long segments. Tissues were separated into scion, graft union, and rootstock. Samples were further sectioned into 120 μm thick segments for examination under a laser confocal microscope.

Lapins/Gi 5 had a significantly larger stem cross-sectional area in the central graft union than did Lapins/Colt. Dye transport in the graft union of both Lapins/Gi 5 and Lapins/Colt was significantly less than in rootstock sections on a cross-sectional basis, and in Lapins/Gi 5 dye transport diminished acropetally. Dye was distributed more uniformly radially across the graft union in Lapins/Colt than in Lapins/Gi 5, with an apparent accumulation of dye in areas of maximum transport. Xylem vessel diameter did not differ between Lapins/Gi 5 and Lapins/Colt; however, both graft union sections displayed lower calculated mean hydraulic diameter than rootstock sections. These observations suggest that the efficiency of xylem vessels in Lapins/Gi 5 was reduced across the graft union. This is likely due to ongoing differentiation of tissues and increased vascular abnormalities.

Introduction

Dwarfing rootstocks have been used in a number of pomological systems to increase productivity and precocity, and to reduce both labor costs and chemical inputs. Dwarfing rootstocks for sweet cherries are relatively recent (Gruppe, 1985) compared to apples (e.g.; Beakbane and Thompson, 1939; Beakbane, 1953). Research on the horticultural and physiological effects of dwarfing cherry rootstocks is relatively limited (Blaunsa et al., 2000; Edin et al., 1996) and the scientific community has definitively not agreed upon the mechanisms of dwarfing in any perennial system (Atkinson et al., 2003; Jones, 1974; Kamboj et al., 1999; Sommelidou et al., 1994a, b).

A number of hypotheses have been proposed to describe the dwarfing effect of rootstocks on scion growth. These include physical limitations caused by whorls of vascular tissue (Simons, 1986, 1989); decreases in plant hormone concentrations and/or hormone ratios between scion and rootstock (Kamboj et al., 1999; Sommelidou et al, 1994b); increases in carbohydrate concentrations in scion tissue because of vascular incompatibility or physical limitations (Bieleski, 2000; Breen, 1975; Tabuenca, 1962); and decreases in water movement through the graft union (Atkinson et al., 2003, Gur and Blum, 1975).

Water transport may decrease through the graft union due to anomalies in vascular differentiation between the tissues of dwarfing combinations. The degree of reduced transpiration may be related to the degree of dwarfing imparted by a particular rootstock. In apples, roots of dwarfing rootstocks (e.g., M.27) had lower hydraulic conductances than roots of semi-dwarfing rootstocks

(e.g., MM.106; Atkinson et al., 2003). This may be associated with factors like lower xylem: phloem ratios and abnormalities in xylem anatomy of dwarfing rootstock systems (Atkinson et al., 2003; Beakbane and Thompson, 1947).

The development of vascular abnormalities, such as the production of excess callus cells in the graft union may affect xylem transport. For example, whorls of xylem vessels are associated with increased dwarfing in apples (Mosse, 1962; Simons, 1986). Vascular tissue can develop at angles contrary to the longitudinal axis of the tree, potentially hindering water transport. The incidence of xylem ray angles has been correlated with the rootstock's degree of dwarfing (Simons, 1986; 1989). Gisela 5, a dwarfing rootstock for sweet cherry, developed xylem vessels with acute angles to the longitudinal axis of the tree (Chapter 2). These occur as callus cells differentiate after the grafting of scion and rootstock.

In grafted cherries, Colt and F 12/1 rootstocks differed in nutrient uptake. Colt, considered a semi-dwarfing rootstock in this study, accumulated higher concentrations of Mg^{2+} ions in the scion than did F 12/1, a non-dwarfing rootstock (Troyanos et al., 1997), while in ungrafted rootstocks, Mg^{2+} and Ca^{2+} uptake in Colt was at least 2-fold higher than in F 12/1 (Blanusa et al., 2000). It was suggested that these differences in uptake were due to differences in specific root length and transpiration rates because ungrafted Colt rootstocks had longer roots and higher transpiration rates than ungrafted F 12/1 (Troyanos et al., 1997).

However, recent reports using ungrafted Gi 5, Gi 6, and Mazzard, in addition to grafted cherry combinations of Rainier/Gi 5, Rainier/Gi 6, and Rainier/Mazzard, showed no difference in water and nitrogen use efficiency (Zavalloni, 2004).

Numerous macroscopic and microscopic techniques have been developed to examine vascular elements (Czymmek et al., 1994; Simons, 1972). The uptake or injection of dye can be used to visualize the vascular pathway (Atkinson et al., 2003; Oparka and Santa Cruz, 2000; Thompson and Schulz, 1999). Safranin staining has been used to examine xylem transport in trees (Atkinson et al., 2003; Ellmore and Ewers, 1986).

The objective of this research was to compare the vascular pathway of water movement in sweet cherries grafted onto dwarfing and non-dwarfing rootstocks. It was hypothesized that water movement into the scion tissues is lower for dwarfing vs. non-dwarfing combinations, and that vessel diameters in the graft union are smaller in the dwarfing combination. This will provide anatomical information on water transport in dwarfing cherry systems.

Materials and Methods

In April 2003, two-year-old nursery grafted trees were field-planted at the MSU Horticulture Research and Teaching Center, East Lansing, Mich. on a well-drained Marlette fine sandy loam, with a minor slope (2-6 %). Two rootstocks were included: Gisela 5 (dwarfing) and Colt (non-dwarfing), with 'Lapins' as the scion. Spacing was 1.8 m between trees and 3.0 m between rows. The orchard

floor consisted of grass alleys with herbicide strips. Trees were fertilized as necessary and sprinkler irrigated at a rate of ~2.5 cm per week.

At the peak rate of shoot expansion (0.7 to 1.0 $mm \cdot day^{-1}$), 10 whole-tree replicates of each scion-rootstock combination were excavated, including the intact root system to minimize disruption to the water column. Trees were transported in sealed polyethylene bags to buckets containing fresh water and cut underwater to minimize the introduction of embolisms and cavitation of vessels. Trees were severed above the rootshank and below the graft union to maximize the amount of rootstock stem available for transporting water. The tree was then transferred into a filtered solution of aqueous 0.1% safranin O dye (w/v) (Sperry et al., 1988). Trees were allowed to transpire in full sunlight (max. ~1250 $W \cdot m^{-2}$) for six hours, then were cut into three transverse and three longitudinal sections centered on the graft union (3 to 5 cm long), and categorized as: 1) scion tissue (above the graft union), 2) central graft union tissue, and 3) rootstock (below the graft union) tissue. These were further sectioned into 120 μm thick transverse sections using a sliding microtome. Individual tissue replicates were examined by fluorescence microscopy (Zeiss LSM Pascal, Jena, Germany; argon 488 nm laser line, 73 μm pinhole aperture, and a 560 nm long pass filter) to quantify functional xylem across the stem and graft union. Safranin O has a peak excitation wavelength of 488 nm and a peak emission wavelength of 530 nm. Sections without dye revealed little autofluorescence using the same fluorescence parameters as stained sections (data not shown).

Images in this dissertation are presented in color. Images were analyzed using the 'trace measurement', color and intensity thresholds in Sigma Scan Pro 5.0 (Systat, Richmond, Calif.; Olmstead et al., 2004). The image was calibrated to a known dimension, and the desired anatomical feature measured by defining two points on an image. Images of transverse sections were analyzed using the color threshold tool, in which a range of color is selected for measurement. The entire diameter of the section was analyzed, including the bark which translocated a small percentage of safranin O. Extended focus images of vessels within regions of translocation were converted to a grayscale image and analyzed using an intensity threshold to determine percent uptake per unit cross-sectional area (n=10). Differences between sections in the percent area stained in the rootstock were considered as the maximum values for that specimen. Subsequent sections were compared with the rootstock to determine whether dye was accumulating or decreasing acropetal direction (from below to above the graft union) through the sections.

Randomly selected vessels within the fluorescing area (i.e., area of maximum water transport) were chosen for measurement (n=25). Vessel diameter (VD) was determined from the mean of two perpendicular measurements across the widest part of the vessel lumen, averaged for all vessels within the area of maximum water transport by Σ[(horizontal + vertical)/2]/n (n=25). Maximum vessel diameter was taken as a measurement of the largest single VD measured.

Hydraulic vessel diameters were calculated to determine the hydraulic efficiency of xylem vessels in the area of dye uptake in scion, graft union, and rootstock tissue. Narrow vessels can reduce mean VD and often contribute little to overall hydraulic efficiency. Because wider vessels are more important to flow, a hydraulically weighted mean accounts for the variation and gives a better estimate of VD. Hydraulic vessel diameter is computed from $\Sigma d^5 / \Sigma d^4$ where d is vessel diameter (μm), and yields a hydraulically weighted mean of vessel diameter (Pockman and Sperry, 2000).

The experiments were in a completely randomized design with rootstock type as the main treatment. General linear models (GLM) were used as appropriate, with alpha levels set at 0.05 *a priori*. Data were assumed to be normal with homogeneous variances and continuous. When normality was not satisfied, data were transformed logarithmically. Mean separation was by Tukey's HSD and Fisher's LSD as appropriate (SAS; Cary, N.C.).

Results

In both dwarfing and non-dwarfing plant systems, dye was transported in the two outermost growth rings (Figure 1). However, dye uptake in Lapins/Colt was distributed more uniformly radially across graft union tissue and longitudinally throughout the sections than in Lapins/Gi 5; thus, a greater amount of water was transported through all sections in Lapins/Colt (Figure 2A). This effect was especially visible in the longitudinal sections (Figure 1A-F). Dye is visible throughout rootstock, graft union, and scion tissue in Lapins/Colt (Figure

1D-F); however, much less intense staining occurred in the longitudinal sections of scion and graft union tissues of Lapins/Gi 5 (Figure 1A-C). Graft union tissue in Lapins/Gi 5 also had significantly less stem area stained than either the scion or the rootstock tissue ($p < 0.01$) (Figure 2A). Dye uptake was significantly greater in the respective tissues of Lapins/Colt than in Lapins/Gi 5 (Figure 2A).

In both Lapins/Gi 5 and Lapins/Colt, scion sections had the smallest stem cross-sectional area ($p \leq 0.05$) (Figure 1A, 1D). Furthermore, scion sections of Lapins/Gi 5 had significantly smaller stem cross-sectional area than all other tissues examined (Figure 2B). However, graft unions of Lapins/Gi5 consistently had larger stem diameters than all other sections, including the graft unions of Lapins/Colt (Figure 1A-C, 2B). In Lapins/Colt, rootstock sections were the largest, followed by graft union and scion sections (Figure 1D-F, 2B).

Despite its large cross-sectional area, water transport in the graft union of Lapins/Gi 5 was less (1.9%; $p = 0.05$) than any other tissue section (Figure 2A). In addition, the graft union of Lapins/Gi 5 had the greatest decline (59%) in area stained compared to the rootstock. Lapins/Colt exhibited a gradual decrease in water uptake from rootstock to scion, with a more moderate decline (29%) between rootstock and graft union sections.

Within areas of maximum water transport, the dye intensity reflected in upper sections of Lapins/Gi 5 was reduced (Figure 3). In scion tissue of Lapins/Gi 5, only a small number of vessels were functional, compared to Lapins/Colt scion tissue (Figure 3A, 3D). However, there were no differences in vessel diameter among of sections of Lapins/Gi 5 (Table 1). There was a

significant decrease in vessel diameter (VD) within the graft union in Lapins/Colt; however, this did not significantly affect water transport to the scions as graft union tissue did in Lapins/Gi 5 (Figure 2A). In addition, graft union tissue of Lapins/Gi 5 appeared to accumulate dye compared to either scion or rootstock tissues (Figure 3A-C, 4). In contrast, it appears that functional vascular elements in Lapins/Colt are dispersed laterally across the stem, allowing radial diffusion of the dye (Figure 3D-F). However, there were progressively less hydraulically active vessels as one moved acropetally throughout the sections (Figure 4).

Values of VD in areas of maximum water transport were significantly smaller in graft union tissue (31.2 μm) of Lapins/Gi 5 compared to rootstock tissue (33.2 μm) ($p < 0.05$; Table 1). VD in scion and graft union tissues of Lapins/Gi 5 were not significantly different ($p > 0.05$), suggesting that VD reduction occurs between the rootstock and graft union sections (Table 1). Lapins/Colt also had significantly smaller vessels in the graft union (32.0 μm) ($p < 0.001$, Table 1). There was no statistical difference between scion and rootstock tissue. Maximum vessel diameter in areas of maximum water transport exhibited a similar trend, with the lowest value in graft union sections for both treatments. In Lapins/Gi 5 and Lapins/Colt, scion and rootstock VD for the respective treatments were not statistically different, although VD was smaller in Lapins/Gi 5 than in Lapins/Colt in both tissues.

Hydraulic vessel diameter, a measure of theoretical efficiency of water transport, was least in the graft union tissues of both treatments, but was only significantly different from either scion or rootstock tissues in Lapins/Colt

(Table 1). There were no significant differences among tissues surrounding the graft union of Lapins/Gi 5, suggesting that transport is not hindered between scion, graft union and rootstock tissue due to vessel size (Table 1). Overall, hydraulic vessel diameter of Lapins/Gi 5 was significantly lower than scion and rootstock tissues of Lapins/Colt.

Discussion

One would expect the smallest stem diameter in a young grafted tree to be in the scion (Hartmann et al., 1997), and this was the case for both Lapins/Gi 5 and Lapins/Colt. An apparent swelling in the graft union of dwarfing tree fruit combinations has been attributed to callus and parenchymatous tissue in apples (Simons and Chu, 1983) and cherries (Deloire and Hebant, 1983; Chapter 2).

Prunus avium L. is classified as a semi-ring porous species, which are characterized by greatest water transport in mature xylem vessels of the outer growth rings that contain secondary xylem vessels, or in the larger vessels of inner rings (Ellmore and Ewers, 1986; Zimmerman and Jeje, 1981). It is apparent in Lapins/Gi 5 tissues that there is a reduction of dye transport throughout the tree, beginning in the graft union and diminishing as transport moves acropetally, suggesting that there is a reduction in the velocity of water transport. Longitudinal sections revealed that water transport is limited in the graft union region.

Lapins/Gi 5 graft union sections hindered water transport from the rootstock but not between the graft union and scion sections. The translocation of safranin O indicated that water transport in Lapins/Gi 5 is reduced compared to Lapins/Colt, a more vigorous system. This suggested that the functional capacity of the vascular system of Lapins/Gi 5 was less than for Lapins/Colt. This agrees with previous water transport results in apple dwarfing systems (Atkinson et al., 2003).

Smaller VDs were differentiated in Lapins/Gi 5 scion and rootstock tissues compared to Lapins/Colt, indicating that there are inherently narrower vessels in this combination. This may be due to the genetic nature of the rootstock as is has been suggested that dwarfing rootstocks inherently produce smaller vessels (Beakbane and Thompson, 1947). In addition, a wounding response was observed, as VD in both Lapins/Gi 5 and Lapins/Colt graft union was significantly less than in either scion or rootstock tissue.

Hydraulic vessel diameters revealed that there were no differences among sections of Lapins/Gi 5; however, there was a significant reduction in hydraulic vessel diameter in graft unions of Lapins/Colt. Overall, this reduction of vessel diameter in the graft union appears to be overcome only in Lapins/Colt, as determined by hydraulic vessel diameter measurements of scion and rootstock tissue. This can account for the differences in dye uptake and diffusion in Lapins/Colt xylem tissues.

An alternative hypothesis (to the reduction of lateral dye movement and overall dye uptake in Lapins/Gi 5), is a reduction in the transpiration and

photosynthesis rate, which could reduce dye uptake. Differences in transpiration, water use efficiency (WUE), or photosynthesis rate of scions on dwarfing rootstocks have been contradictory, with no reductions observed either transpiration or photosynthetic rate of apples (cv. Starking Delicious) on dwarfing rootstocks (Barden and Ferree, 1979), while reductions in both transpiration and photosynthetic rates of scions (cv. Delicious and Golden Delicious) have been observed on similar rootstocks (Looney, 1968; Schechter et al., 1991). Water use efficiency has also not been correlated with vigor. In a study of Rainier/Gi 5, Rainier/Gi 6 and Rainier/Mazzard, well-watered pot plants did not show a difference in WUE, but Rainier/Gi 5 did have higher evapotranspiration rates. (Zavalloni, 2004). A preliminary study on photosynthetic rate of fully expanded source leaves in Lapins/Colt and Lapins/Gi 5 indicated a higher photosynthetic rate in Lapins/Gi 5 is significantly higher than that of Lapins/Colt ($P \leq 0.05$; Chapter 4). In this study, acropetal dye movement in Lapins/Colt translocated farther than in Lapins/Gi 5 (data not shown).

However, vessel diameter impact on transpiration is not the only factor that can influence xylem water transport. In dwarfing apple rootstocks, a large number of vascular components may develop on a bias to the longitudinal axis of the tree, which concomitantly occurs with reduced scion growth (Simons, 1986; 1989; Sommelidou et al., 1994a). It is possible that this phenomenon occurs in dwarfing combinations of sweet cherry systems. Macroscopic examination of longitudinal sections of the graft union region of Lapins/Gi 5 confirms this, with development of xylem wood on a distinct bias (Figure 1B). Additionally, it is

possible that the vascular pathway in dwarfing combinations is extended as a result of these vascular anomalies (e.g., xylem rays on a bias), rather than physical limitations causing transport restrictions (i.e. due to reduced vessel diameter). These pathways might act to 'trap' water and allow it to diffuse into surrounding tissues. The increased pathway length may reduce water transport and solutes in the graft union (Figure 1A-F), leading to the exhibited reduction of dye uptake in the graft union of Lapins/Gi 5 (Figure 1A-C, 2B).

Conclusions

It appears that water movement in dwarfing cherry scion-rootstock combinations is reduced, as indicated visually by safranin O uptake. Although both plant systems transported the safranin O solution through the graft union, there were a number of differences. Overall, graft union stem cross-sectional area was highest in Lapins/Gi 5 compared to a gradual decrease in stem cross-sectional area of Lapins/Colt from rootstock to scion. However, because vascular components developed contrary to the perpendicular orientation of stem, the increased graft union stem cross-sectional area did not result in an increase in the stained area of functional transport. The percent of total area stained in the graft union of Lapins/Gi 5 was less than all other sections and treatment combinations.

Microscopic examination within areas of maximum transport suggested that the graft union of Lapins/Gi 5 accumulated dye, perhaps due to a reduction in water transport through this region. Lapins/Colt had a more uniform

distribution of dye throughout the scion, graft union, and rootstock tissues, hence more functional vessel elements. Vessel diameters in these regions were smallest for graft union tissues in both treatments; however, a significant decrease occurred in the graft union of Lapins/Colt. Hydraulic vessel diameter was reduced in the graft union of Lapins/Colt, while there were non-significant differences throughout the graft union of Lapins/Gi 5.

Reductions in dye uptake of dwarfing combinations may be caused by the increased amount of parenchymatous tissue located in the graft union region (Chapter 2), leading to increased avenues for xylem content dispersal. Non-functioning phloem and abnormal vascular elements in the graft union may provide a longer pathway for water to travel, thus slowing water transport in the graft union region.

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Figure 1. Transverse and longitudinal stem sections of a dwarfing combination (Lapins/Gi 5 [A-C]) and a non-dwarfing combination (Lapins/Colt [D-F]), after transport of an aqueous 0.1% safranin solution for six hours in full sunlight to determine translocation through the graft union region. Samples are representative of all replicates (n=10). A, D: scion. B, E: graft union. C, F: rootstock. Scale bar = 1 cm.

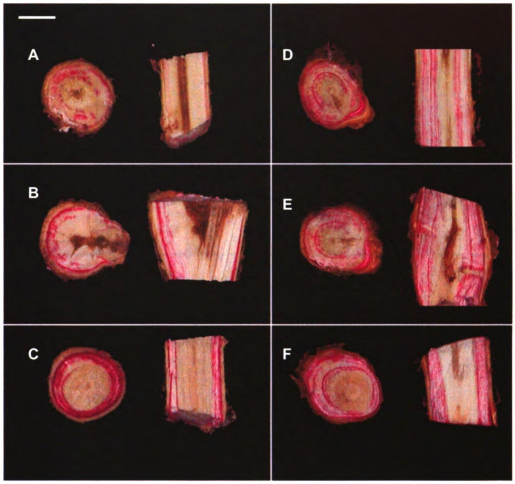


Figure 2. Stem cross-sectional area stained (A) and stem cross-sectional area (B) from transverse sections of Lapins/Gi 5 and Lapins/Colt. Error bars represent ±SE of the mean (n=10).

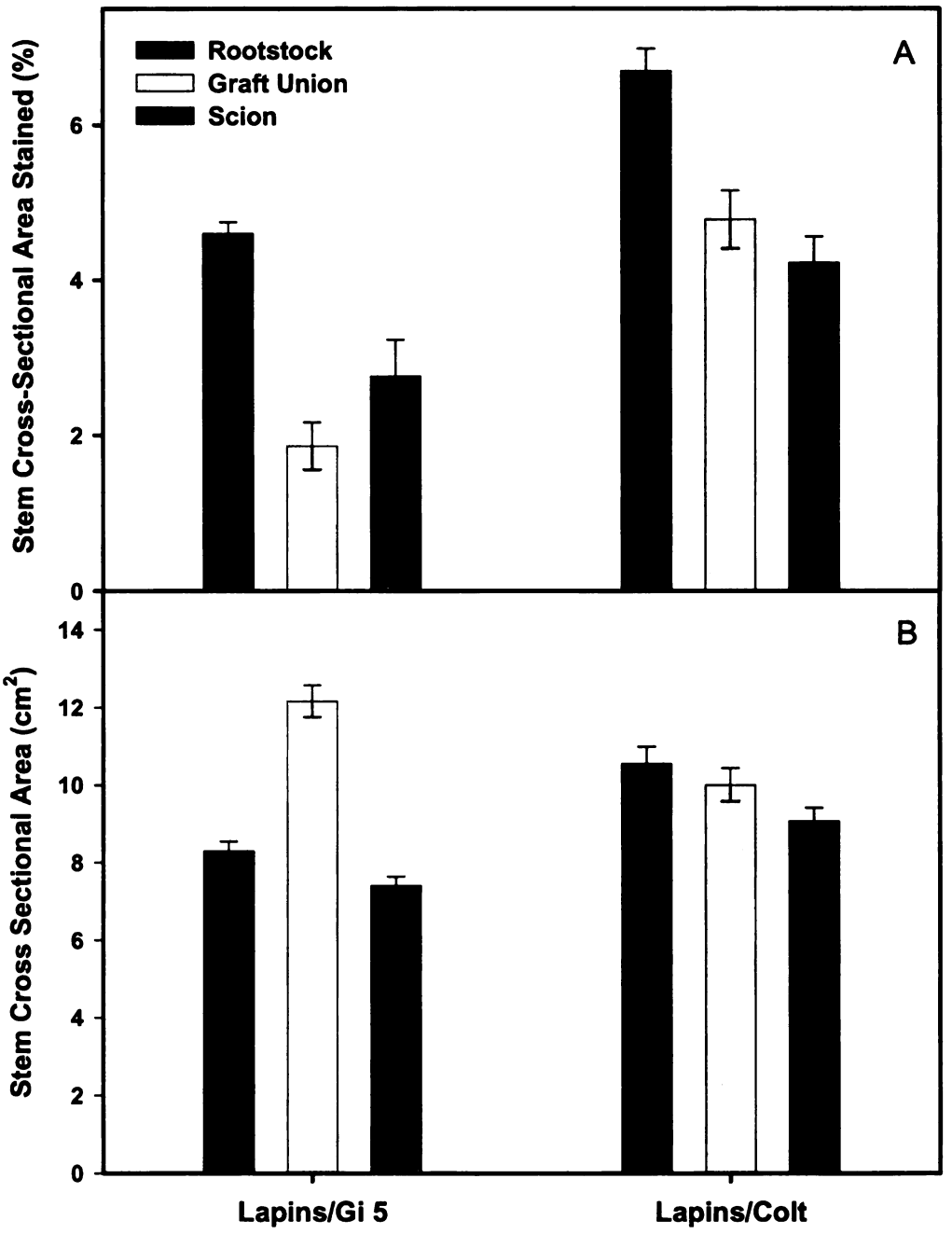
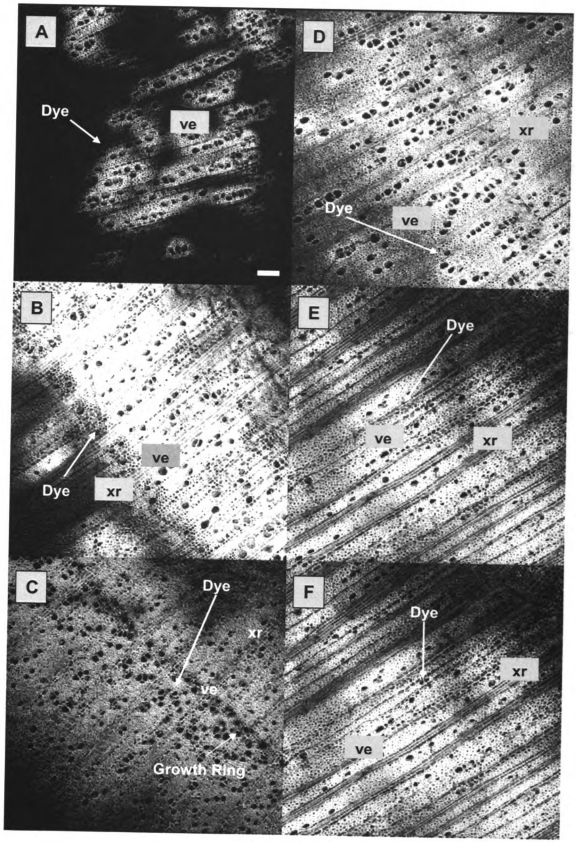


Figure 3. Extended focus fluorescence images comparing dye uptake in xylem tissue of Lapins/Gi 5 (A-C) and Lapins/Colt (D-F) [excitation with argon laser line at 488 nm and emission using a 560 nm long pass filter]. Sections were stained with safranin O. A growth ring is visible in the area of dye transport in the rootstock of Lapins/Gi 5 (C). A, D: scion; B, E: graft union; C, F: rootstock. ve: vessel element, xr: xylem rays. Scale bar = 100 μm.


A
Dye
ve
D
xr
Dye
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B
ve
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E
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ve
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C
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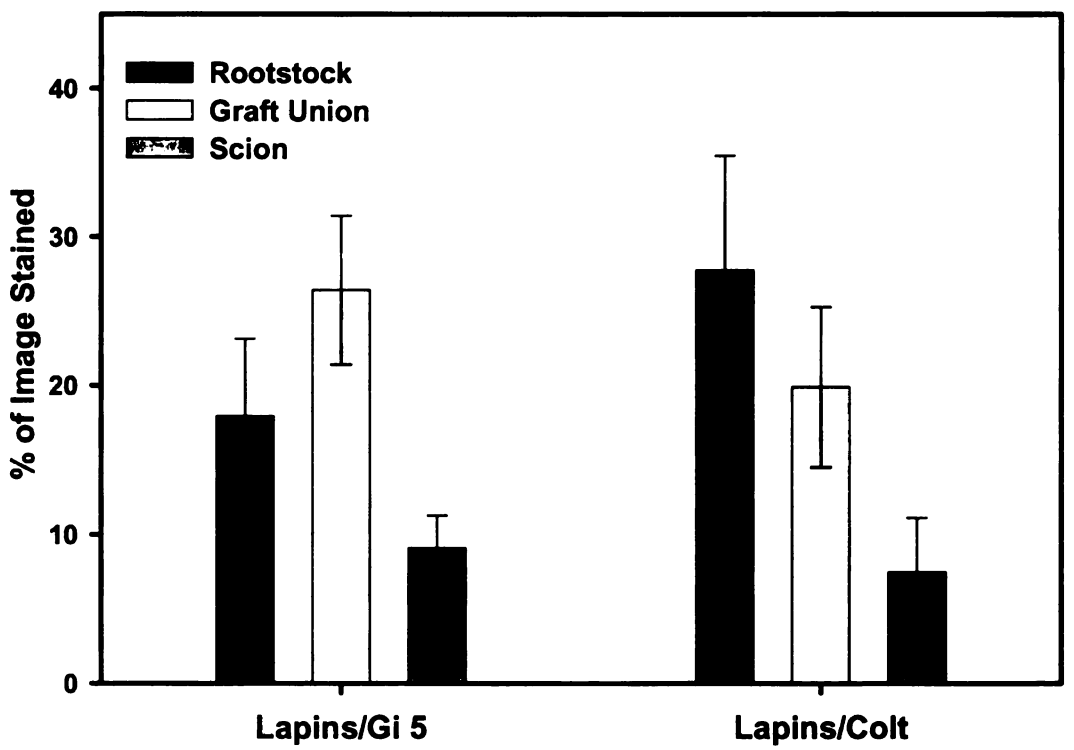
ve
Growth Ring
F
Dye
xr
ve

Table 1. Summary of xylem vessel characteristics for Lapins/Gi 5 (dwarfing) and Lapins/Colt (non-dwarfing). Total number of replicates per treatment equaled 10 and vessels per replicate measured equaled 25. Mean values of all replicates are given in the table for vessel diameter and hydraulic vessel diameter.

Treatment	Section	Vessel Diameter (µm)	Hydraulic Vessel Diameter (µm)	Maximum Vessel Diameter (µm)
Lapins/Gi 5	*Scion*	32.2 ± 0.5 bc[1,2]	37.2 ± 0.5 b	49.4
	Graft Union	31.2 ± 0.5 c	35.8 ± 0.5 b	46.3
	Rootstock	33.2 ± 0.4 b	37.8 ± 0.4 b	52.0
Lapins/Colt	*Scion*	37.2 ± 0.5 a	42.7 ± 0.5 a	57.3
	Graft Union	32.0 ± 0.5 c	38.8 ± 0.5 b	51.6
	Rootstock	39.0 ± 0.6 a	45.6 ± 0.6 a	65.3

[1] Diameter reported ± SE of the mean.
[2] Letters denote mean separation within columns by LSD at $P \leq 0.05$.

Figure 4. Percent of image stained in the area of maximum water transport by the uptake of an aqueous 0.1% safranin solution in a dwarfing Lapins/Gi 5 system and in a non-dwarfing system, Lapins/Colt. Error bars represent ±SE of the mean (n=10).



CHAPTER FOUR

Is Carbohydrate Transport Limited in Dwarfing Rootstocks?: An Examination of Established Sweet Cherry (*Prunus avium* L.) Scion on Dwarfing and Non-dwarfing Rootstocks

Abstract

To determine whether alterations in carbohydrate translocation through the graft union region is a potential factor in dwarfing, two-year-old cherry trees grafted with 'Rainier' on Gisela 5 (Gi 5; dwarfing), Gisela 6 (Gi 6; semi-dwarfing), and Colt (non-dwarfing) rootstocks were sampled for starch and soluble sugar analysis. Wood tissue and leaves from one-year-old Lapins/Gi 5 (dwarfing) and Lapins/Colt (non-dwarfing) also were examined. Scion shoot length and duration of shoot growth was reduced in combinations with Gi 5 or Gi 6. Lapins/Gi 5 tended to have more growing points than Lapins/Colt. Trunk cross sectional area in the graft union of Lapins/Gi 5 was relatively larger compared to scion or rootstock tissues, but not with Lapins/Colt. Starch concentrations were not significantly different between wood tissues of the 'Rainier' combinations; however the pattern of accumulation and reallocation varied. In 'Lapins' combinations, starch began accumulating during the period of maximum shoot elongation in rootshank tissues of Lapins/Colt, but not for Lapins/Gi 5. Soluble sugars in Rainier/Gi 5 were variable and higher overall in scion tissues than Rainier/Gi 6 or Rainier/Colt. The major soluble sugar in 'Rainier' combinations was sorbitol, while in 'Lapins' combinations, the major soluble sugar detected was sucrose. Total non-structural carbohydrates (TNC) were influenced positively by increased soluble sugar concentrations in scion tissues of Rainier/Gi 5 and scion and graft union tissues of Lapins/Gi 5, leading to an accumulation of TNC above the graft union. Thus, the accumulation of TNC in scion and graft union tissues of Rainier/Gi 5 and Lapins/Gi 5 negatively influences carbohydrate

storage to rootshank tissues, reducing available carbohydrates for subsequent season growth in these particular dwarfing combinations. Images in this dissertation are presented in color.

Introduction

Fruit trees are generally vigorous, in some cases growing over 10 m high, requiring substantial land for orchards, as well as making management and harvest more labor intensive. In addition, fruit trees on standard rootstocks often have low precocity, requiring seven to eight years between planting and harvesting a profitable crop, as in sweet cherries (Seavert, 1997). Growers who have planted fruit trees grafted to dwarfing rootstocks in their orchards have benefited from reductions in labor costs, chemical usage per application, as well as increased productivity (2 to 3 years to harvest a profitable crop; Seavert, 1997), and increased yield (Lang and Ophardt, 2000; Lang, 2001). Best management practices of dwarfing systems could be improved by a better understanding of the underlying mechanisms of dwarfing (Jones, 1986, Zhu et al., 1999).

A number of attempts have been made to elucidate fruit tree dwarfing mechanisms, primarily in apple rootstocks (Beakbane, 1956; Kamboj et al., 1999; Lockard and Schneider, 1981; Sommelidou et al., 1994). Previously tested hypotheses for dwarfed scion growth include limitations in water and metabolite transport through the graft union (Beakbane, 1956; Poniedzialek et al., 1979), differences in plant growth regulator concentration and/or ratios in xylem sap constituents (Blaunsa, 2000; Jones, 1986, Kamboj et al., 1999), and anatomical differences in scion tissue caused by cell differentiation in dwarfing systems (Feucht et al., 1983; McKenzie, 1961; Simons and Chu, 1983). Directly applying these hypotheses to growth in cherry dwarfing rootstock combinations may be

difficult, because multiple physiological processes occur concomitantly in cherries (Lang, 2001; Tukey, 1942). For example, leaves are photosynthetically competent in apples at the time of bloom, while in cherries bloom and leaf emergences occur simultaneously (Fujii and Kennedy, 1985; Tukey, 1942). Because this developmental period is abbreviated, cherries depend heavily upon carbohydrates that originate from storage reserves, whereas apples can draw carbon from current season photosynthates as leaves become photosynthetically competent (Fujii and Kennedy, 1985; Forshey et al., 1983; Keller and Loescher, 1989).

Graft union incompatibilities of *Prunus* species have been hypothesized as a dwarfing mechanism based on increased quantity and activity of phenolic and peroxidase compounds (Usenik and Štampar, 2000; Deloire and Hebant, 1983). As an added indicator of incompatibility, lack of starch in the rootstock has been observed with some unsuccessful *Prunus* combinations (Breen, 1975; Mosse, 1962; Tabuenca, 1960; Yano et al., 2002), suggesting that limitations in carbohydrate translocation exist.

Limitations in carbohydrate transport across the graft union, whether due to anatomical development, incompatibility, or differences in sink/source strength, have important implications for cherry fruit development, because of the abbreviated fruit developmental period (Tukey, 1942). Starch reserves are utilized for bud break and then must be replenished through the supply of current season photosynthates (Keller and Loescher, 1989; M. Ayala, personal communication). However, limitations in translocation of soluble carbohydrates

in the graft union region can reduce storage reserves in various portions of the tree, ultimately leading to tree decline in extreme situations (Yano et al., 2002). Often, swelling of graft union tissue is observed in various scion/dwarfing rootstock combinations, resulting in overgrowth at the graft union. It is not known if this local anomaly of increased girth is due to varietal characteristics, lack of vascular connections, or increased concentrations of carbohydrates, parenchymatous tissue, or interspecific graft pairings (Errea et al., 1994; Mosse, 1962; Proebsting, 1926; Tubbs, 1973b).

The hypothesis tested in this research is that carbohydrate transport and storage increases in scion and graft union tissue as a result of grafting onto dwarfing rootstocks in sweet cherry. The objectives were to determine if there were differences in starch and soluble sugar concentration between sections of the tree surrounding the graft union, and differences between these sections in different scion/rootstock combinations. Alterations in carbohydrate translocation, as a result of either vascular or sink/source limitations in the graft union region, may have an impact on ultimate scion growth and precocity that directly influences management of these dwarfing combinations in sweet cherry. Semi-dwarfing (GI 148/1; Gisela 6 [Gi 6]) and dwarfing (GI 148/2; Gisela 5 [Gi 5]) cherry rootstocks were compared to a non-dwarfing rootstock (Colt).

Materials and Methods

2002 Field Grown Trees. In May 2001, 90 one-year-old trees of 'Rainier' grafted onto three different rootstocks were field-planted in a randomized complete block design at the MSU Horticulture Teaching and Research Center (HTRC) in East Lansing, Mich. Rootstocks included Gi 5 (*Prunus cerasus* x *P. canescens*; dwarfing), Gi 6 (*Prunus cerasus* x *P. canescens*; semi-dwarfing), and Colt (*P. pseudocerasus* x *P. avium*; non-dwarfing). The soil was a Marlette fine sandy loam, well-drained with a minor slope (2-6 %). Tree spacing was 2.4 m x 3.0 m, with herbicide strips and grass alleyways. Trees were sprinkler-irrigated at a rate of 2.5 cm per week, and fertilized using a controlled release fertilizer (Osmocote, The Scotts Company, Ohio). Weather data was collected from a unit of the Michigan Agriculture Weather Network (MAWN) at the HTRC (Appendix A1, A2). Growing degree days in both years were calculated using MAWN weather data and the Baskerville-Emin method (Baskerville and Emin, 1969).

In March 2002, trees were destructively harvested and sampled from three regions (~6 mm long) surrounding the graft union, in addition to rootshank and leaf tissue from bud break (day of year [DOY]; DOY 88) to leaf senescence (DOY 304) (Figure 1) (n=3). Leaf and scion wood tissue were collected and analyzed separately. Whole leaves were randomly selected from a population of fully expanded shoots to ensure that leaves were photosynthetically competent. Flowers present in graft union regions of Rainier/Gi 5 trees were removed before sampling to reduce the influence of reproductive growth on carbohydrate status. Samples were placed immediately into liquid nitrogen until transfer to a -80°C

freezer for storage. Samples were then freeze-dried, ground in a Wiley Mill (40 mesh screen), and stored in desiccant prior to carbohydrate analysis.

2003 Field Grown Trees. In April 2003, 165 one-year-old trees of 'Lapins' grafted onto either Gi 5 or Colt were planted in a completely randomized design, in a similar location as the previous year at the HTRC (similar soil and cultural conditions). As in 2002, flowers were removed from the graft union region in Lapins/Gi 5 trees before sampling to reduce the influence of reproductive growth on carbohydrate status. Destructive samples were taken from four sections of the tree surrounding the graft union, including scion, graft union, rootstock, and rootshank tissue during the period of maximum shoot elongation (DOY 122 to DOY 213) (Figure 1; n=10). Samples were placed immediately into liquid nitrogen until transfer to a -80°C freezer for storage. Samples were then freeze-dried, ground in a Wiley Mill (40 mesh screen), and stored in desiccant prior to carbohydrate analysis.

Specific growth and physiological parameters were recorded, including terminal shoot length, current season lateral shoot length, trunk diameter at three locations (above, at, and below the graft union), number of growing points, and photosynthetic rate. Trunk diameter was used to calculate trunk cross sectional area (TCSA) using the equation $(\pi \cdot d^2)/4$. Single leaf net photosynthesis was measured at solar noon on two dates (1 July and 25 July) using a CIRAS-2 portable photosynthesis measurement system (PP Systems, Amesbury, Mass.) at ambient field temperatures and CO_2 (340 $\mu l \cdot mol^{-1}$). Measurements were conducted on the first fully expanded leaf located on a current season shoot.

Carbohydrate Extraction and Starch Determination. In both years, freeze-dried tissue (0.2 g) was extracted with 3 ml of 80% (v/v) ethanol on ice. Iced samples were ground with a Brinkmann homogenizer (Brinkmann/KINEMATICA Polytron, Westbury, N.Y.) for 30 seconds, using three 10 s pulses. The homogenate was held in a boiling water bath for five minutes, cooled and centrifuged at 9,600 *g* for 10 min to give ethanol-soluble and ethanol-insoluble fractions. The supernatant was retained for soluble sugar analysis, and the pellet was extracted an additional three times in 80% (v/v) ethanol. With each centrifugation, the supernatant was collected to analyze soluble sugars. After the third centrifugation, the remaining pellet was analyzed for starch.

The pellet remaining from the ethanol extraction was resuspended using 0.2 M potassium hydroxide and placed in a boiling water bath for 30 min. Distilled water was added as necessary to maintain pellet suspension. After cooling to room temperature, the mixture was adjusted to pH 5.5 using 1 M acetic acid. Amyloglucosidase from *Aspergillus niger* (Sigma Chemical, A-9913) was dialyzed overnight (MWCO 3500, Fisher Scientific, Hampton, N.H.) in an sodium acetate buffer (pH 4.5) to remove glucose resulting from production of the enzyme. Dialyzed amyloglucosidase (3 ml) was added to the mixture and incubated for 1 h in a 55˚C water bath. Samples were then placed in a boiling water bath, cooled to room temperature, and centrifuged for 10 min at 9,600 *g*.

Released glucose was analyzed using a glucose-6-phosphate dehydrogenase (G6PDH) enzymatic linked assay. An aliquot of the sample was incubated for 30 min in a reaction mixture containing 200 mM Hepes buffer (pH

8.0), 10 mM magnesium chloride ($MgCl_2$), 10 mM dithiothreitol (DTT), 2 mM ATP, 2 mM $NADP^+$, and 4 units of hexokinase (Sigma Chemical, type VI from Baker's Yeast) and glucose-6-phosphate dehydrogenase (Sigma Chemical, type V Baker's Yeast). The reduction of $NADP^+$ by glucose-6-P dehydrogenase was determined spectrophotometrically at 340 nm using a Unicam UV 300 Spectrophotometer (ThermoSpectra, Madison, Wisc.) (Robbins and Pharr, 1987).

Soluble Sugar Determination. Soluble carbohydrates were determined as described previously (Roper et al., 1988). Supernatant from starch determination was collected into 13 x 100 mm test tubes and placed in a dry bath to evaporate ethanol from the sample. Samples were rehydrated using 1 ml of distilled water and a fraction (200 μl) of this rehydrated sample was placed into a separate tube. This was then placed into a dry bath to evaporate water from the samples. Soluble sugars were converted to oximes and then derivatized using hexamethyldisalizane and trifluoroacetic acid (Sweeley et al., 1963, Williams and Martin, 1967). Soluble sugars were then analyzed by gas-liquid chromatography and peak heights and retention times compared to sugar standards (HP 5890 II, Agilent Technologies, Palo Alto, Calif.).

Statistical Analysis. The experiments were in a completely randomized design with rootstock type as the main treatment. General linear models (GLM) were used as appropriate, with alpha levels set at 0.05 *a priori*. Data were assumed to be normal and continuous with homogeneous variances. When normality was not satisfied, data were transformed logarithmically to gain normality before

statistical analysis. Mean separation was by Tukey's HSD and Fisher's LSD as appropriate (SAS; Cary, N.C.).

Results

Growth. In 2002, Rainier/Gi 5 and Rainier/Gi 6 ceased shoot elongation at ~DOY 178 (27 June) (Figure 2), while Rainier/Colt ceased shoot elongation at DOY 204 (23 July). Rainier/Colt shoot elongation continued to increase slightly towards the end of the measurement period; however the increase in growth was not significant ($p > 0.05$). Overall, Rainier/Colt terminal shoot length was significantly greater (72.8 cm; $p \leq 0.05$) than both Rainier/Gi 5 (52.9 cm) and Rainier/Gi 6 (55.0 cm; Figure 2). In addition, Rainier/Gi 5 and Rainier/Gi 6 exhibited similar terminal shoot elongation patterns to each other throughout the sampling period.

In 2003, two different patterns of shoot elongation emerged. Terminal shoot elongation exhibited a sigmoidal growth pattern, while current lateral growth exhibited a logarithmic pattern (Figure 3A, B). Lapins/Gi 5 terminal shoot growth was reduced significantly compared to Lapins/Colt at DOY 213 (20.2 cm and 22.8 cm, respectively, $p < 0.01$). In both Lapins/Gi 5 and Lapins/Colt, terminal shoots growth ceased around DOY 199 (Figure 3A). Lapins/Gi 5 grew more slowly than Lapins/Colt, with differences as early as DOY 143 ($p < 0.01$) (Figure 3B). Lapins/Gi 5 shoot elongation in both terminal and lateral shoots was influenced by the dwarfing Gi 5 rootstock. In addition, the number of growing

points (i.e., potential bud growth) tended to be more numerous in Lapins/Gi 5 than on Lapins/Colt (data not shown).

Trunk cross sectional area (TCSA) increased slightly throughout the measurement period (Figure 4A, B). On both rootstocks, TCSA of the graft union was larger than either scion or rootstock tissues. However, the graft union TCSA was larger than scion and rootstock TCSA in Lapins/Gi 5 than in Lapins/Colt ($p < 0.001$).

Starch. Starch concentrations in 2002 were not different between woody tissues; however there was a variation in the pattern of accumulation and reallocation of starch among the sections within a rootstock (Figure 5A-C). Rainier/Gi 5 starch concentrations decreased in all sections of the tree until DOY 121 (Figure 5A), and subsequently increased in all wood tissues until concentrations peaked at DOY 212, which coincides with terminal bud set. Starch concentrations then decreased in all wood tissues through DOY 304, which coincided with leaf senescence. Leaf tissue concentrations peaked early in the measurement period (between DOY 121 and DOY 148) and gradually decreased until leaf senescence (Figure 6A). By contrast, concentrations of starch in Rainier/Colt decreased soon after bud break (DOY 88) and continued to remain relatively stable throughout the growing season, until after leaf senescence (Figure 5C).

Unlike Rainier/Gi 5 and Rainier/Colt, Rainier/Gi 6 starch concentrations at two points during the growing season (DOY 179 and DOY 212) were significantly higher in the rootshank than all other sections ($p < 0.05$; Figure 5B). In addition,

leaf tissue of Rainier/Gi 6 had the highest concentration of starch at DOY 121 (33.5 mg/g dry weight; DW) compared to either Rainier/Gi 5 (27.5 mg/g DW) or Rainier/Colt (19.8 mg/g DW). In all treatments, there was an overall trend for recovery of starch concentrations by the end of the growing season (DOY 304) (Figure 5A-C). Due to the main differences in patterns of starch accumulation and reallocation during the period of maximum shoot elongation, sampling was isolated to this period in 2003.

In 2003, starch concentrations in all tissues of Lapins/Gi 5 peaked around DOY 160 (44.0 mg/g DW) (Figure 7A). There were no significant differences between wood sections of Lapins/Gi 5 until the last sampling date, on DOY 204, on which starch concentrations in rootshank sections were higher than in graft union, rootstock and leaf tissue ($p < 0.05$; Figure 7A).

In Lapins/Colt, there were significant differences in starch concentration among wood tissues, specifically the rootshank section, beginning at DOY 147 ($p < 0.01$) (Figure 7B). Starch concentrations in rootshank sections of Colt continued to increase, peaking at 96.9 mg/g DW at DOY 196. By the end of active shoot elongation (Figure 7A, B), there was a distinct separation of starch concentration between wood sections of Lapins/Colt, with rootshank tissue having the highest concentration of starch, followed by rootstock, graft union and scion tissue (Figure 7B). Starch concentration in Lapins/Colt leaf tissue was significantly higher than Lapins/Gi 5 leaf tissue at DOY 161, while Lapins/Gi 5 had significantly higher starch concentration in leaf tissue at DOY 139 ($p < 0.05$) (Figure 6B).

Soluble sugar concentrations. The soluble sugars detected in highest concentrations were sorbitol and sucrose in wood sections of all rootstock combinations (Figure 8, 9, 10A-D). Glucose and myo-inositol were detected at levels less than 0.5% dry weight, and did not differ significantly from other detected sugars (data not shown). In 2002, soluble sugar concentrations were most variable in scion tissues of Rainier/Gi 5 (Figure 8A). There was a decrease in sorbitol and fructose from initial concentrations, followed by a peak at DOY 179, and then a gradual decrease until DOY 304. Between DOY 179 and DOY 268, there was a shift in sorbitol to sucrose as the primary available translocatable sugar. This coincided with declines in starch concentration of wood tissues (Figure 5A).

In Rainier/Gi 6, sorbitol concentrations in all four tissues were highest at DOY 179, as observed in graft union, rootstock and rootshank tissue of Rainier/Gi 5 (Figure 9A-D). As with Rainier/Gi 5, a similar pattern of reallocation and accumulation was observed. The shift in the main translocatable sugar, sorbitol to sucrose occurred during the same time interval in Rainier/Gi 6 as that in Rainier/Gi 5 (Figure 8A-D, 9A-D). Sucrose concentrations in Rainier/Gi 6 scion tissue were as high as Rainier/Gi 5 at the end of the growing season (1.9%; DOY 304).

Sorbitol concentrations in Rainier/Colt peaked between DOY 179 and DOY 212 in all tissues (Figure 10A-D). Generally, concentration increased with the basipetal direction of translocation from scion tissue to root tissue, scion (9.8 mg/g DW), graft union (14.2 mg/g DW), rootstock (15.9 mg/g DW), and rootshank

(18.9 mg/g DW) (Figure 10A-D). As with Rainier/Gi 5 scion tissue, sorbitol concentration fluctuated in all tissues of Rainier/Colt, especially during peak shoot growth.

In 2003, the main translocatable sugar detected in both treatments was sucrose, followed by sorbitol (Figure 11A-D, 12A-D). During the period of maximum shoot elongation, sucrose concentrations decreased rapidly to a minimum at DOY 161 in Lapins/Gi 5 and Lapins/Colt. However, in Lapins/Colt, there was a second peak in sucrose concentration at DOY 189 for scion graft union and rootshank tissue (Figure 12A-D). Sucrose concentrations ranged from 15.6 mg/g DW in scion tissues to 24.8 mg/g DW in rootshank tissues, and increased basipetally from scion to rootshank tissues. In Lapins/Gi 5 (Figure 11A-D), a second peak in sucrose concentration was also present, with higher concentrations for scion and graft union tissue than for either rootstock or rootshank ($p = 0.05$; Figure 11 A-D). These sucrose concentrations in Lapins/Gi 5 overall were significantly lower than sucrose concentrations in Lapins/Colt at DOY 189 ($p \leq 0.05$; Figure 11A-D, 12A-D).

Total non-structural carbohydrates (TNC). In 2002, scion tissue of Rainier/Gi 5 tended to have higher concentrations of TNC than other wood tissues, and was significantly higher during the last two sampling periods (Figure 13). Rainier/Gi 6 scion tissues were intermediate among other wood tissues of the tree, while Rainier/Colt TNC concentrations of scion tissues were the lowest of all wood tissues (Figure 13). Scion tissues had significantly higher TNC concentrations than graft union, rootstock, or rootshank tissues after DOY 268 in

Rainier/Gi 5, while scion TNC concentrations were significantly lower after DOY 268 in Rainier/Colt compared to graft union, rootstock, or rootshank tissues ($p \leq 0.05$). In addition, a similar pattern of accumulation and reallocation of TNC was reflected when comparing Rainier/Gi 5 and Rainier/Gi 6 (Figure 13).

In 2003, a similar pattern of TNC accumulation occurred in scion tissues in Lapins/Gi 5 and Lapins/Colt (Figure 14A, 14B). There were no significant differences between scion, graft union and rootshank tissues ($p \geq 0.05$); however, TNC in rootstock tissues were significantly lower during maximum shoot elongation in Lapins/Gi 5 ($p \leq 0.05$) (Figure 14A). In contrast, Lapins/Colt exhibited the same pattern in TNC accumulation and reallocation (Figure 14B) as observed in starch concentrations (Figure 7B). TNC in scion, rootstock, and rootshank tissue of Lapins/Gi 5 was significantly lower than Lapins/Colt by terminal bud set at DOY 204 ($p = 0.05$; Figure 14A, B). Rootstock tissues below scion and graft union tissues also exhibited significantly lower TNC concentration at the end of the measurement period in Lapins/Gi 5 than Lapins/Colt. Rootstock tissues in Lapins/Gi 5 exhibited significantly lower TNC concentrations throughout most of the measurement period than all other tissues examined in this treatment (Figure 14A).

Photosynthetic Measurements. Based on initial TNC concentration measurements in 2002, differences among sections led to examination of photosynthesis rate in 2003. Single leaf photosynthesis measurements were significantly higher in Lapins/Gi 5 than Lapins/Colt during the middle (12.8 $\mu mol \cdot m^{-2}s^{-1}$ and 8.2 $\mu mol \cdot m^{-2}s^{-1}$, respectively) and later period of shoot expansion

(6.5 $\mu mol \cdot m^{-2}s^{-1}$ and 3.0 $\mu mol \cdot m^{-2}s^{-1}$, respectively) (Table 1). On 1 July, the period of maximum shoot elongation, Lapins/Gi 5 had significantly higher stomatal conductance (G_s) than Lapins/Colt ($P \leq 0.01$); however, there were no differences in internal carbon dioxide concentration (C_i) between the two treatments ($p \geq 0.05$, Table 1). Internal C_i concentrations remained stable in Lapins/Colt during the two measurement dates, while C_i decreased during late shoot expansion (25 July) in Lapins/Gi 5 (Table 1).

Discussion

In 2002, the decreased shoot length in both Rainier/Gi 5 and Rainier/Gi 6 compared to Rainier/Colt (Figure 2) corroborates previous findings that scions on dwarfing rootstocks both reduce overall shoot growth and end elongation sooner on non-dwarfing rootstocks (Atkinson and Else, 2001; Edin et al., 1996; Perry et al., 1997; Sanderson, 1999; Weibel et al., 2003). The pattern of shoot elongation was reflected in 2003 lateral shoots, as trees arrived from the nursery without a terminal bud. Because of the terminal bud elimination, lateral buds initiated shoot growth along the axis of the tree. A sigmoidal growth pattern was reflected in terminal shoot growth, while a characteristic exponential growth pattern was reflected in lateral shoot growth (Figure 5). However, Lapins/Colt had a much higher shoot elongation rate than Lapins/Gi 5, similar to that observed in the previous year with Rainier/Gi 5 and Rainier/Colt.

TNC levels found in this experiment suggest that there is a reduction in carbohydrate reserves for use the following season. Thus, scions grafted onto Gi

5 dwarfing rootstocks may begin growth in early spring at a level that is not sufficient to sustain maximum shoot growth. Interestingly, this unique pattern of accumulation and reallocation of carbohydrates is found within the first one to two years that a scion is grafted onto Gi 5 or Gi 6 rootstock and is not bearing fruit. It is likely that the low levels of in reserve carbohydrate in Gi 5 dwarfing rootstock combinations is exacerbated as reproductive sink strength increases with time. Ultimately, this inherent reduction in shoot growth may shift sink strength and direct more photosynthates to reproductive growth and bud development. As previously reported, a possible shift in carbohydrate allocation may be due to a feedback response (Gucci et al., 1991).

TCSA data indicated that there was an increase in the graft union size of both Lapins/Gi 5 and Lapins/Colt (Figure 4). However, there was a greater difference between graft union and rootstock TCSA in Lapins/Gi 5 than in Lapins/Colt. Swelling has often been observed in sweet cherry dwarfing rootstock combinations (Mosse, 1962). The combination of two genetically different systems has an impact on scion growth and development, and there are current efforts to describe differences in gene expression at the scion/rootstock interface (C. Prassinos, personal comm.).

Starch concentrations during 2002 did not show overall differences between wood sections in each treatment (Figure 5A-C). However, overall starch concentrations are lower in tissues of Rainier/Colt. Differences in starch concentration between tissue types did occur in leaves, rather than wood sections (Figure 6A). However, starch concentrations in Rainier/Gi 6 rootshank

sections began increasing on DOY 179, peaked around DOY 212, and began decreasing on DOY 268 after terminal bud set. This suggests that the Rainier/Gi 6 combination was able to store carbohydrates until sink demand increased at terminal bud set or in the root system for growth, at which point stored reserves were used. In contrast, initial starch concentrations in Rainier/Colt rootshank sections were 3.1% DW, then decreased after bud break, with stable values throughout the rest of the growing season. This suggested that Rainier/Colt trees were able to store carbohydrates, while at the same time meet sink demand in various parts of the tree.

Although there was no starch accumulation recorded among wood sections, the pattern of starch accumulation and reallocation differed between the control (Rainier/Colt) and the dwarfing combination (Rainier/Gi 5) (Figure 5A-C). The most dwarfing combination (Rainier/Gi 5) exhibited a decline in starch concentration after bud break, with a steady increase towards terminal bud set (DOY 212), before declining at leaf senescence. Alternatively, starch concentrations in Rainier/Colt remained relatively stable throughout the season (Figure 5C). In all three treatments, starch concentrations recovered to initial concentrations at the onset of growth.

In 2003 during maximum shoot elongation, starch did not accumulate in rootshank tissue of Lapins/Gi 5 to the same concentration found in Lapins/Colt. Lapins/Colt rootshank tissue accumulated more than 3X starch compared to scion tissue (Figure 7B). This has important implications for the following season growth in Lapins/Gi 5 combinations, as a significant portion of spring growth is

dependent upon stored carbohydrate reserves in the tree (Keller and Loescher, 1989; Tukey, 1942). With the obvious reduction in tree size of dwarfing combinations, there is ultimately a smaller capacity for carbohydrate storage throughout the tree (Avery, 1969, 1970). However these differences in starch storage capacities appear even in dwarfing combinations that are in their third season of growth. Thus, many of the cultural practices recommended for these dwarfing cherry systems have included intensive pruning to optimize crop load and vegetative growth (Lang, 2000, 2001).

Starch accumulation and reallocation differed between Lapins/Gi 5 and Lapins/Colt in 2003, especially in rootshank sections. Lapins/Gi 5 starch concentration peaked at terminal bud set (DOY 161) compared to the continual increase in Lapins/Colt rootshank sections. When photosynthetic rates were examined, Lapins/Gi 5 had a significantly higher rate than Lapins/Colt during the late period of shoot elongation (Table 1; Figure 3A-B). These data suggest that photosynthesis in the dwarfing combination of Lapins/Gi 5 is continuously functioning at a higher level than Lapins/Colt. This corroborates previous research in apples (Looney, 1968; Schechter et al., 1991).

Alternatively, increasing starch concentration and hence, storage in the rootshank of Lapins/Colt, probably explains lower photosynthetic rates that were recorded during shoot elongation. Excess starch concentrations can activate carbohydrate-modulated genes to signal an increase in storage synthesis (Nakamura et al., 1991). Leaf starch concentrations during this measurement period indicated that concentrations were significantly higher than in Lapins/Colt

(Figure 6B). This response was observed with non-fruiting trees and would perhaps be magnified when a crop load is present, especially on a precocious dwarfing rootstock like Gi 5 (Avery, 1969; 1970; Tubbs, 1973a). For example, when scions are grafted onto dwarfing apple rootstock M.9, there can be an 80% reduction in total tree weight when a crop load is present compared to removal of crop load (Barlow, 1971). The root system of dwarfing combinations may suffer the greatest loss in growth and carbohydrate storage, which has negative implications for subsequent growth (Avery, 1970). TNC and sucrose concentration in rootshank sections of Lapins/Gi 5 suggest that there is a reduction in the concentration of carbohydrates available throughout the season, and especially for subsequent season growth (Figure 11A-D, 14). With this smaller capacity of the dwarfing combination in general for reserve carbohydrates, the added sink strength of a crop load could exacerbate carbohydrate stress the following season.

The soluble sugar profile in all three treatments in 2002 revealed that the major pool of soluble sugar is sorbitol (Figure 8, 9, 10), as previously reported for *Rosaceae* crops (Keller and Loescher, 1989). However, during maximum shoot elongation in 2003, sucrose was detected at a higher concentration between DOY 119 and DOY 154 in Lapins/Gi 5 and Lapins/Colt (Figure 11, 12). This most likely is due to mobilization of sucrose to supply energy during a period of high sink demand, in the case of non-fruiting trees, vegetative growth. In contrast, the shift from lower sorbitol to higher sucrose concentration in 2002 between DOY 212 and 268 in Rainier/Gi 5, Rainier/Gi 6 and Rainier/Colt is

probably due to the reallocation of sorbitol for storage in reproductive buds and sucrose concentrations increased in storage organs to supply energy for the following season growth (Figure 8, 9, 10).

In 2002, accumulation of soluble sugars above the graft union led to an overall increase in TNC concentration in scion versus rootstock and rootshank tissues of Rainier/Gi 5. This was in contrast to Rainier/Gi 6, which had no differences between scion, graft union, rootstock, or rootshank tissues, and Rainier/Colt in which scion tissues exhibited the lowest overall TNC concentration (Figure 14). As one moved acropetally throughout the sampling area, there was a steady decline in TNC concentration in Rainier/Colt. These data show that scions on dwarfing Gi 5 may accumulate TNC above the graft union, with the major contributors in 2002 being soluble sugars. In addition, Rainier/Gi 5 began accumulation of TNC sooner than Rainier/Colt, which agrees with previous hypotheses that limited shoot growth is a result of earlier accumulation of carbohydrates in wood tissues (Rao and Berry, 1940).

A similar pattern was observed in Lapins/Gi 5 trees in 2003. There was an accumulation of TNC in scion and graft union tissues, compared to significantly lower TNC concentrations in rootstock tissue. The pattern of TNC accumulation in Lapins/Colt was similar to that observed in starch concentrations and it appears that the concentration of starch was a major contributor to TNC concentration in this scion/rootstock combination. This shift in starch or soluble sugar as a major contributor to TNC concentration may be due to differences in tree age and scion variety.

These data suggest that there is a limitation in carbohydrate transport between the scion and rootstock in dwarfing combinations, and that the major point of resistance is the graft union. TNC concentrations in Rainier/Gi 5 and Lapins/Gi 5 exhibited increases in tissues in and above the graft union with little accumulation in storage tissues of the rootstock and rootshank. Vessel area and number in the graft union region of this dwarfing rootstock are reduced (Chapter 2, 3), which may lead to reductions in vascular transport and the accumulation in TNC observed in this study. The effect of dwarfing rootstocks on carbohydrate storage may be magnified as tree maturity increases; thus it would be beneficial to compare carbohydrate status and photosynthetic rates on mature dwarfing and non-dwarfing combinations to further investigate differences between dwarfing and standard rootstock systems.

Conclusions

Although starch concentrations in the tree of both dwarfing and non-dwarfing combinations returned to initial concentrations by the end of the growing season, the pattern of accumulation and reallocation in a dwarfing combination, (Rainier/Gi 5 or Lapins/Gi 5) differed from that on the non-dwarfing rootstock, Colt. Scion shoot length in both years was reduced when grafted on dwarfing or semi-dwarfing rootstocks (Gi 5 or Gi 6) compared to a non-dwarfing rootstock (Colt). During maximum shoot elongation, Lapins/Colt began accumulating starch and 'refilling' its reserves in the rootshank. In contrast, starch concentrations were similar in wood tissues examined in Rainier and Lapins

grafted to Gi 5 throughout the period of shoot elongation. TNC concentrations in both Rainier/Gi 5 and Lapins/Gi 5 suggested an accumulation of TNC in tissues above the graft union in dwarfing combinations. Limited photosynthetic measurements revealed that Lapins/Gi 5 had higher midday net photosynthesis and stomatal conductance than Lapins/Colt, suggesting that photosynthesis works at a higher level in trees with a dwarfing rootstock to fulfill the sink demands of increased growing points. Based on the data collected, it appears that there is a limitation in carbohydrate transport between the scion and rootstock in dwarfing combinations.

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Figure 1. Schematic diagram of tissue sampling for carbohydrate analysis. The area surrounding the graft union was sectioned into scion, graft union, rootstock, and rootshank tissue approximately 6 mm long.

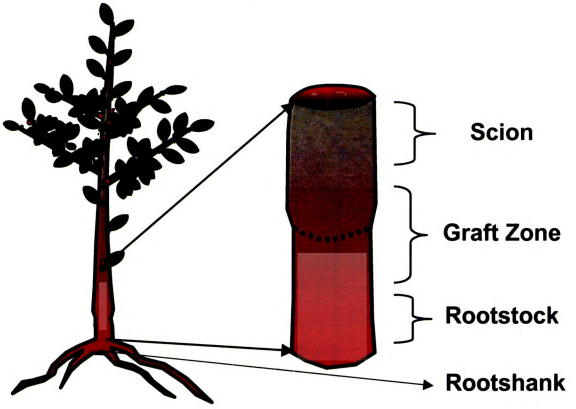

Figure 2. Shoot length and accumulated growing degree days (GDD) from budbreak to leaf senescence in 2002 for Rainier/Gi 5, Rainier/Gi 6, and Rainier/Colt, in East Lansing, Mich. Error bars indicate one SE of the mean (n=6).

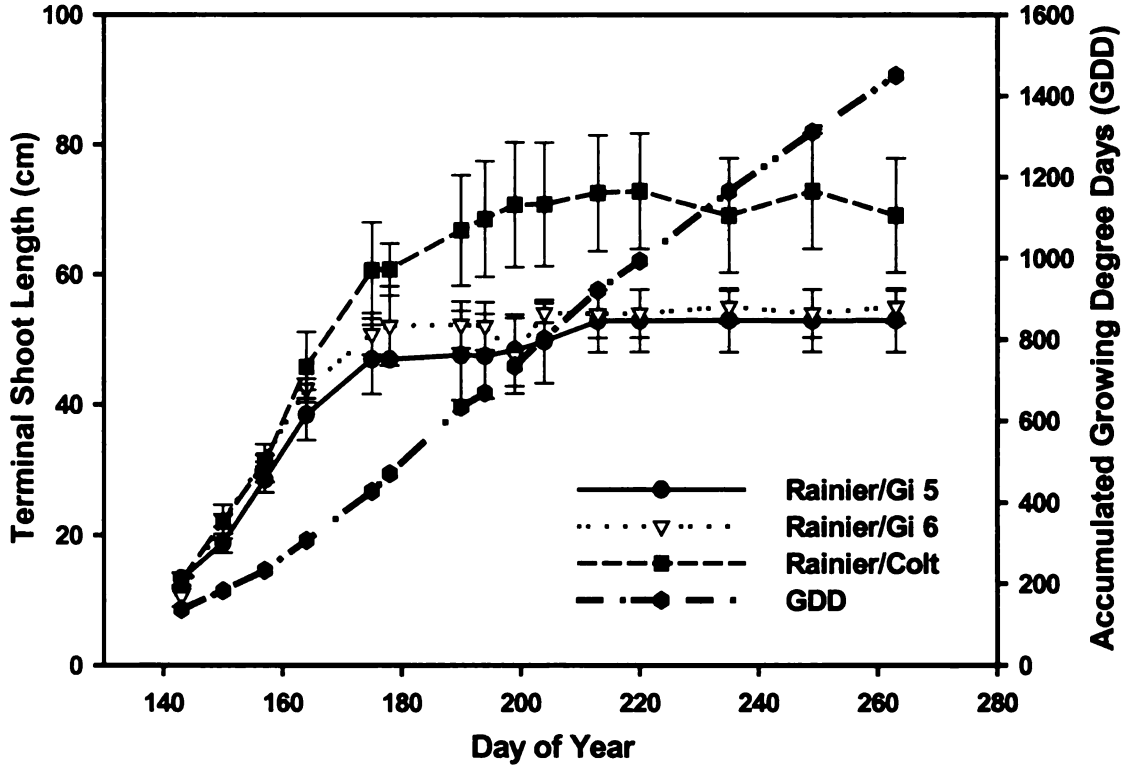

Figure 3. Terminal shoot length (A) and current year lateral growth (B) as recorded from early budbreak to leaf senescence in 2003 for Lapins/Gi 5 and Lapins/Colt in East Lansing, Mich. Error bars indicate one standard error of the mean (n=10).

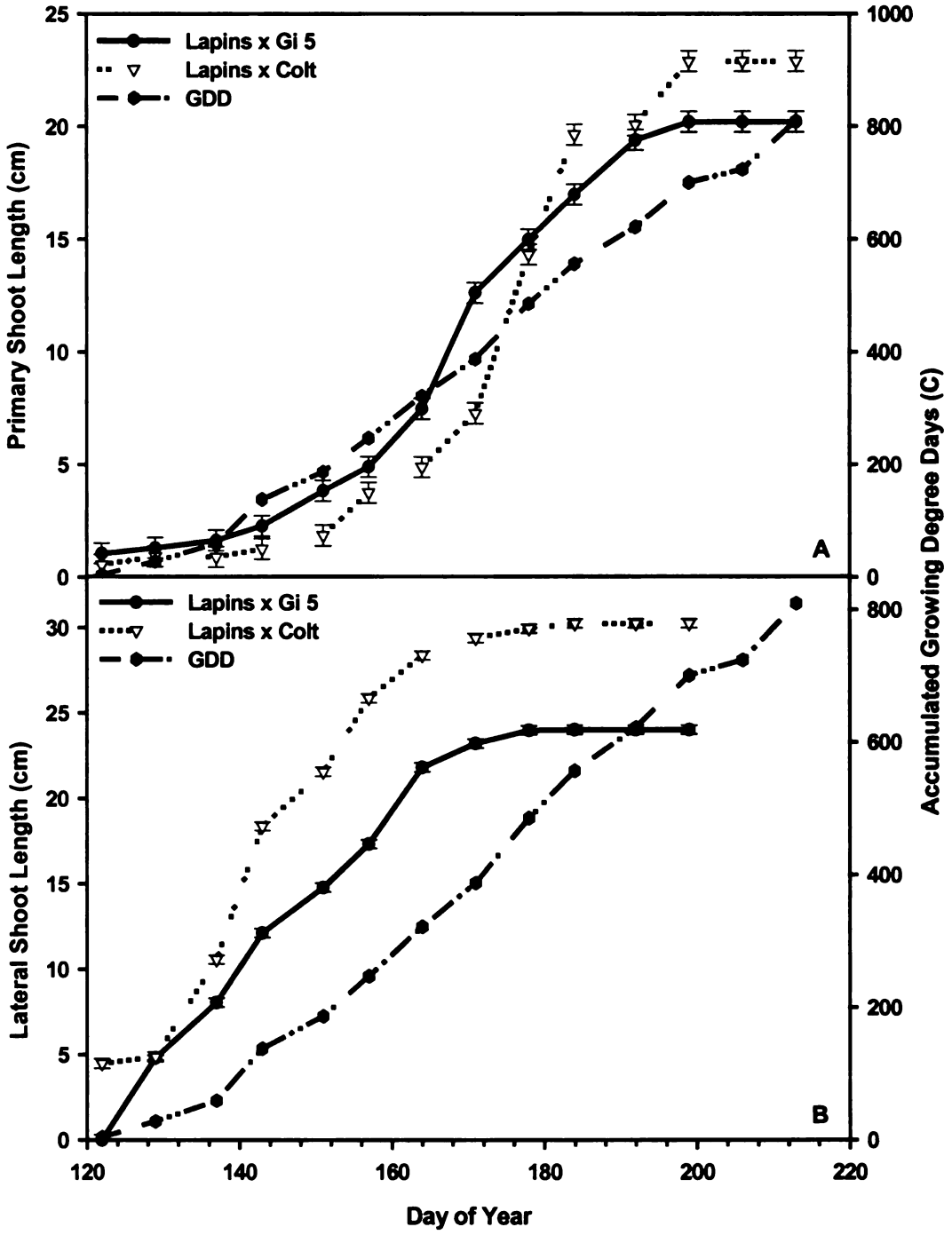

Figure 4. Trunk cross-sectional area (TCSA) of Lapins/Gi 5 (A) and Lapins/Colt (B) as determined in scion tissue above the graft union, at the graft union, and in rootstock tissue below the graft union. Measurements were made on two-year old, field planted trees in East Lansing, Mich. in 2003. Error bars indicate one standard error of the mean (n=10).

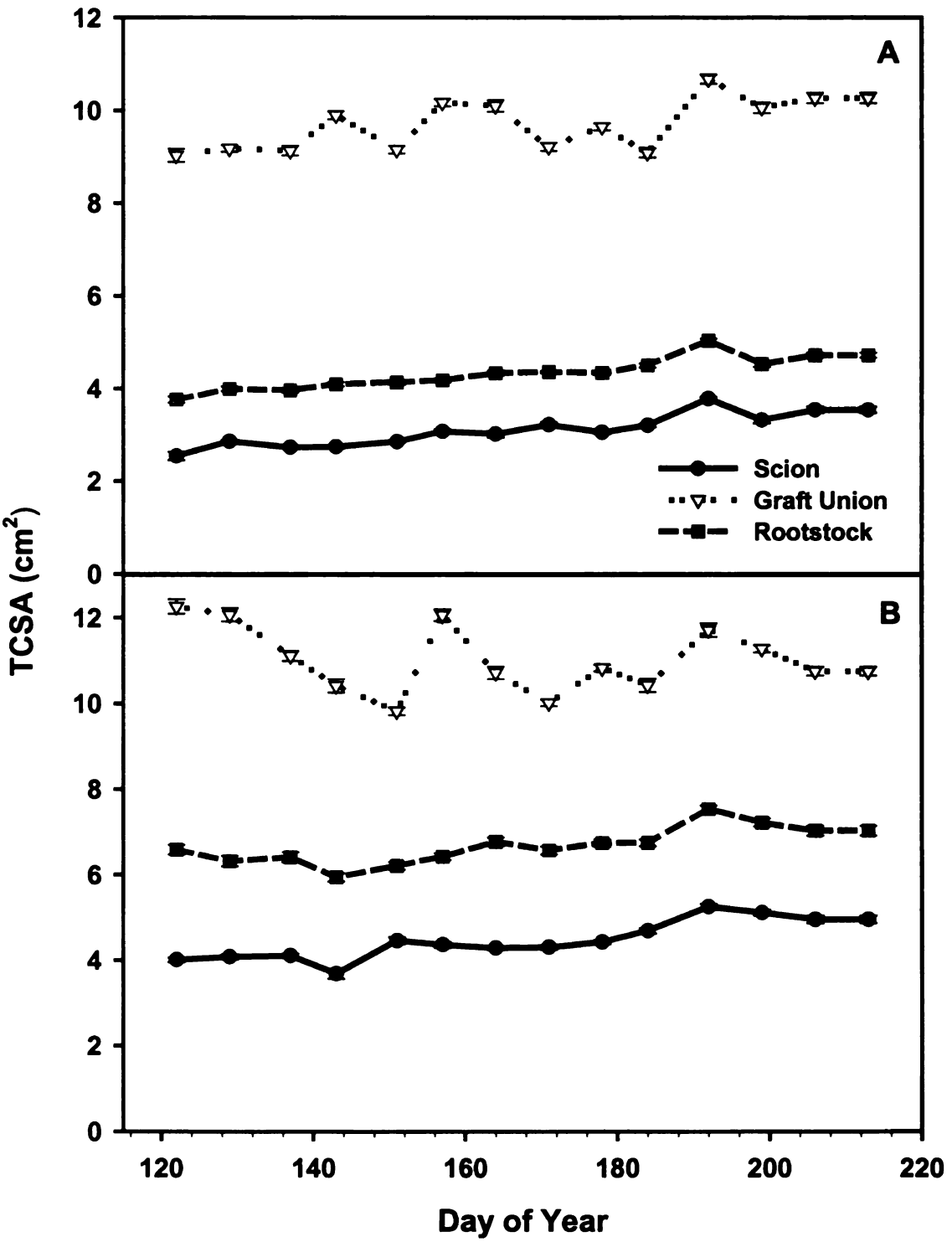

Figure 5. Starch concentrations for 2002 samples in the graft union region of dwarfing (Rainier/Gi 5) (A), semi-dwarfing (Rainier/Gi 6) (B) and non-dwarfing (Rainier/Colt) (C) trees. Three year old trees were planted in East Lansing, Mich. Error bars indicate one SE of the mean (n=3).

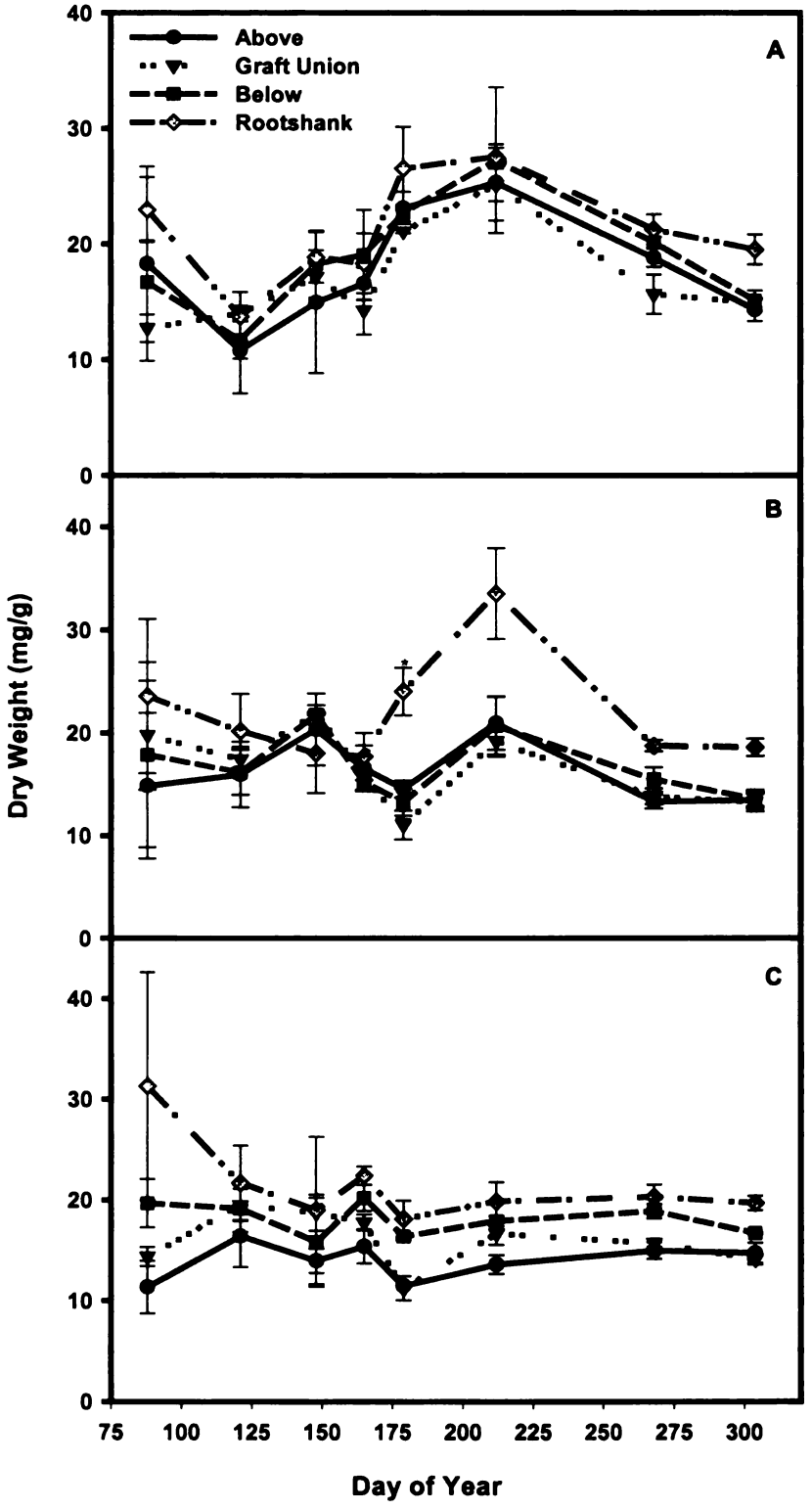

Figure 6. Starch concentrations in leaves of Rainier/Gi 5 (dwarfing), Rainier/Gi 6 (semi-dwarfing) and Rainier/Colt (non-dwarfing) trees (A) (n=3), and Lapins/Gi 5 (dwarfing) and Lapins/Colt (non-dwarfing) (B) (n=10). Two (A) and three year old trees (B) were planted in East Lansing, Mich. Error bars indicate one SE of the mean.

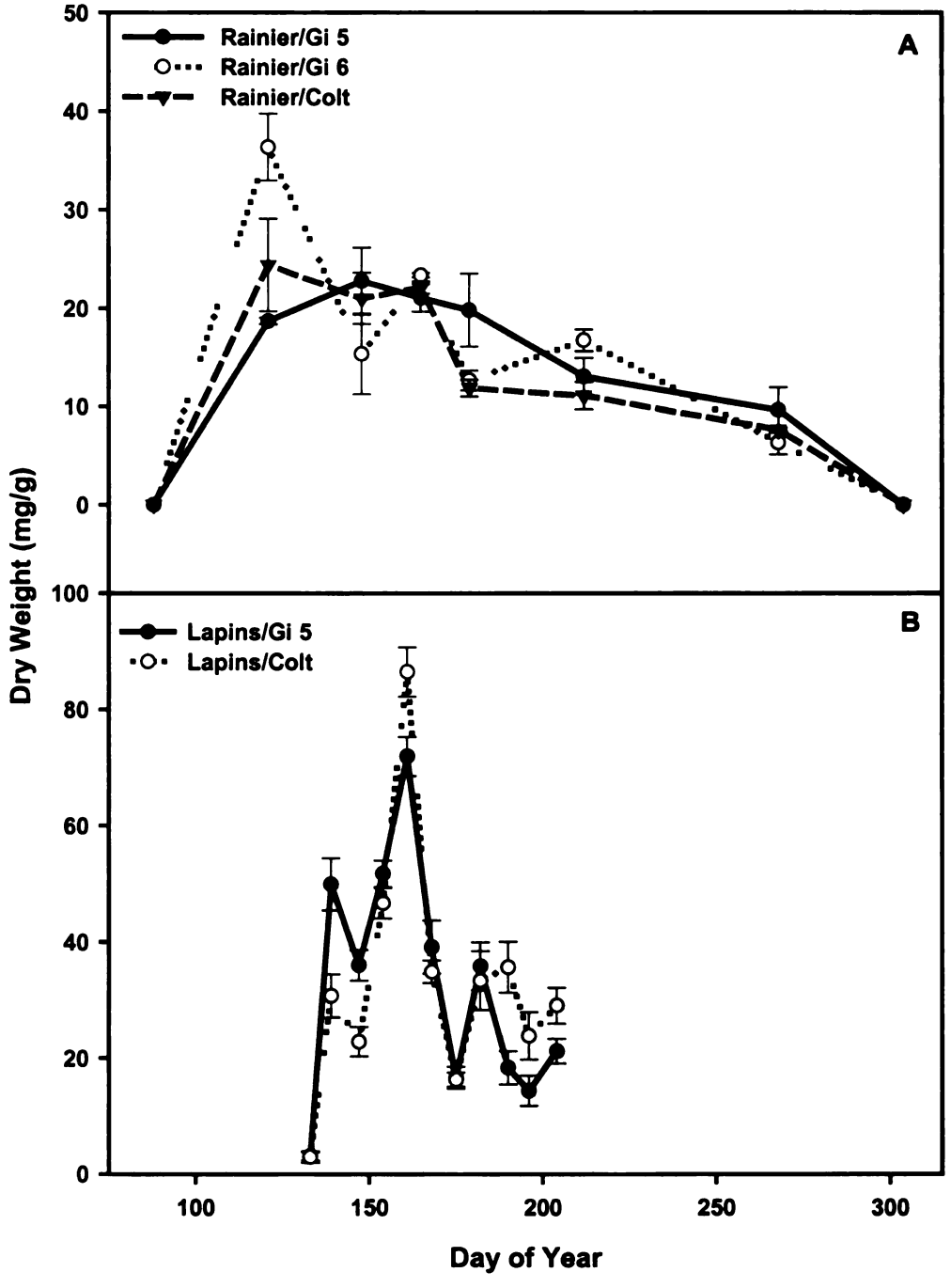

Figure 7. Starch concentrations recorded for Lapins/Gi 5 (A; dwarfing rootstock) and Lapins/Colt (B; non-dwarfing rootstock) in 2003. Samples were from four sections of the tree, including scion, graft union, rootstock, and rootshank tissue. Error bars indicate one SE of the mean (n=10).

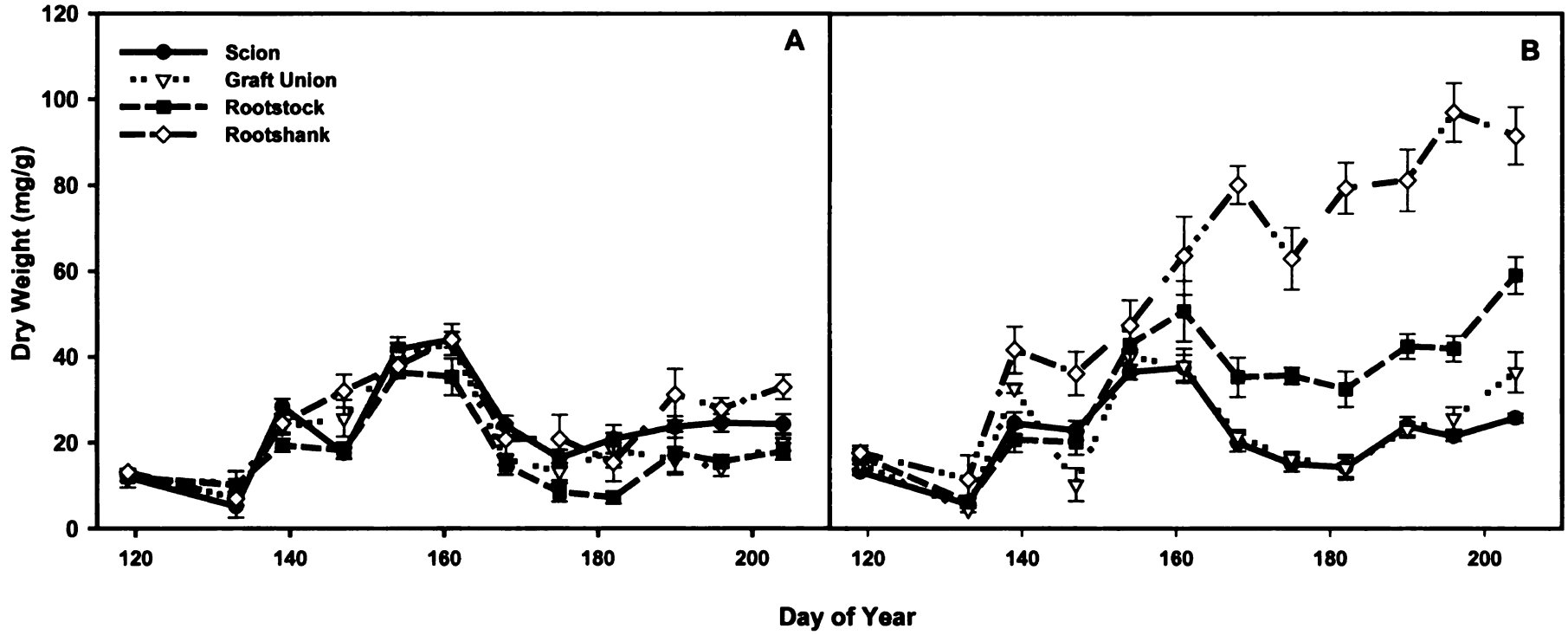

Figure 8. Soluble sugars in the scion (A), graft union (B), rootstock (C), and rootshank (D) of Rainier/Gi 5. Samples from trees planted at the Horticulture Teaching and Research Center in East Lansing, Mich., were taken throughout the 2002 growing season, from bud break until leaf senescence. Error bars indicate one SE of the mean (n=3).

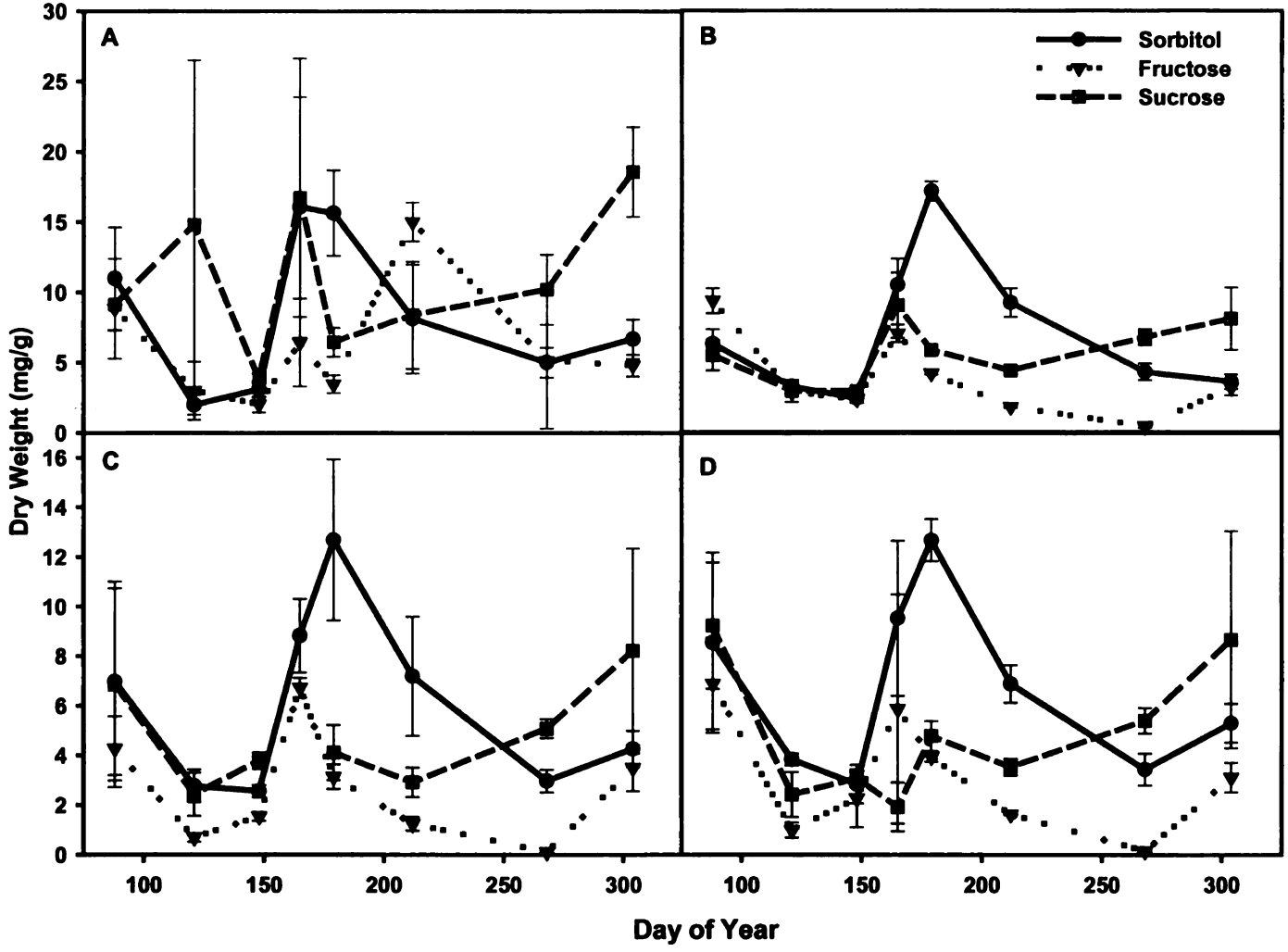

Figure 9. Soluble sugars in the scion (A), graft union (B), rootstock (C), and rootshank (D) of Rainier/Gi 6. Samples from trees planted at the Horticulture Teaching and Research Center in East Lansing, Mich., were taken throughout the 2002 growing season, from bud break until leaf senescence. Error bars indicate one SE of the mean (n=3).

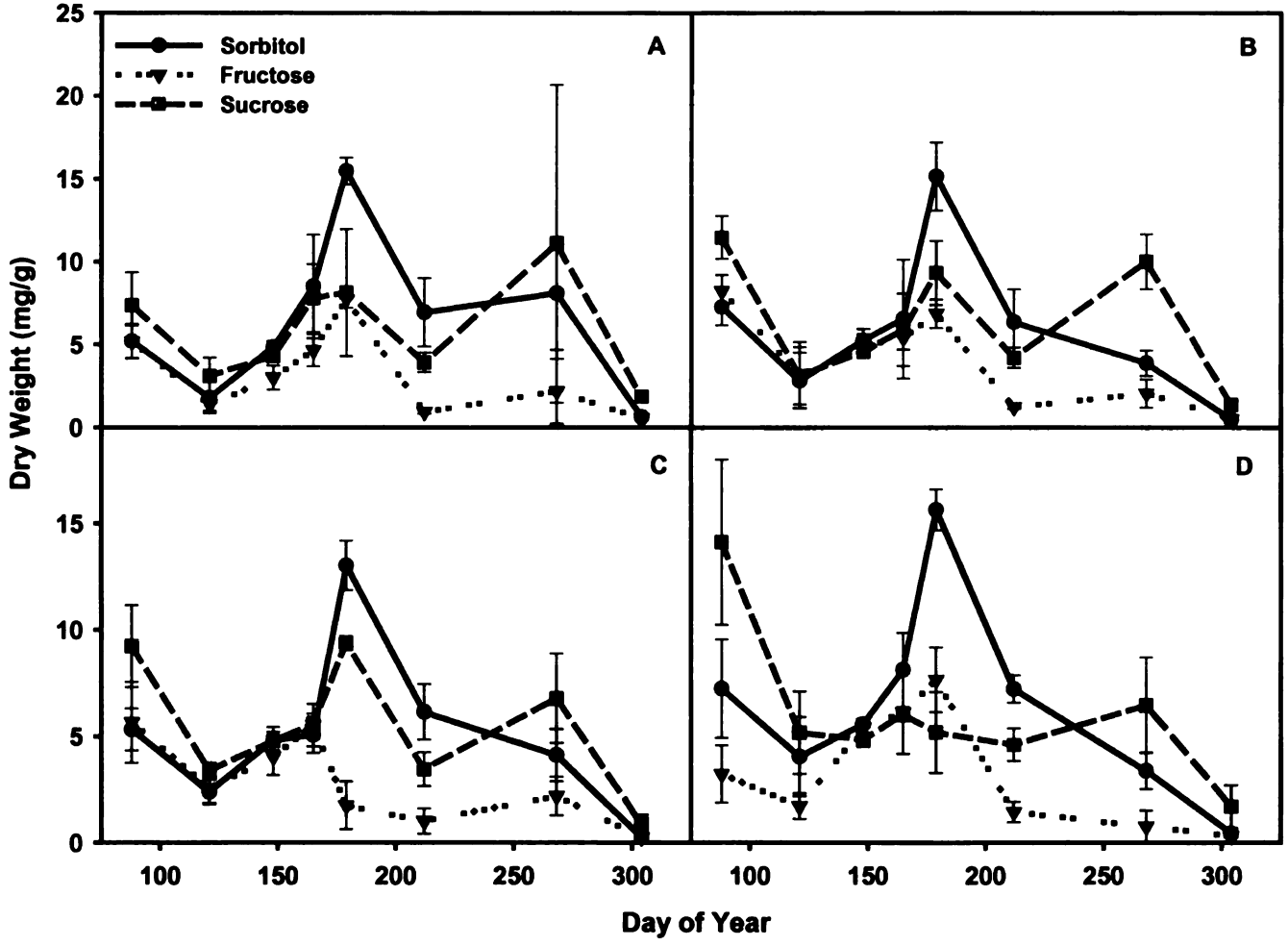

Figure 10. Soluble sugars in the scion (A), graft union (B), rootstock (C), and rootshank (D) of Rainier/Colt. Samples from trees planted at the Horticulture Teaching and Research Center in East Lansing, Mich., were taken throughout the 2002 growing season, from bud break until leaf senescence. Error bars indicate one SE of the mean (n=3).

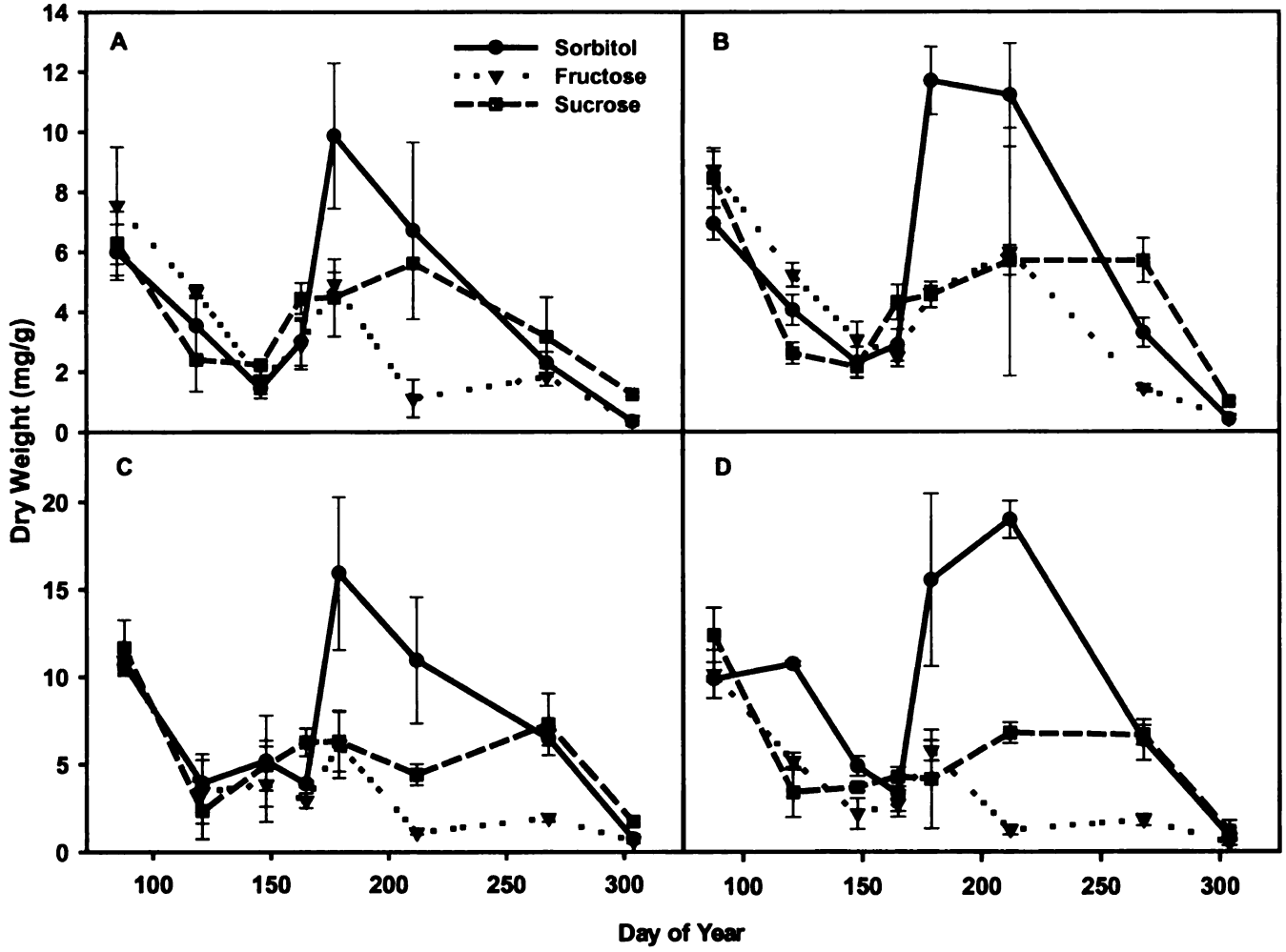

Figure 11. Soluble sugars in Lapins/Gi 5 (dwarfing) in 2003. Two year old trees were field planted at the HTRC in East Lansing, Mich. and harvested during the current growing season. Trees were sectioned into scion (A), graft union (B), rootstock (C), and rootshank (D) tissue. Error bars indicate one SE of the mean (n=10).

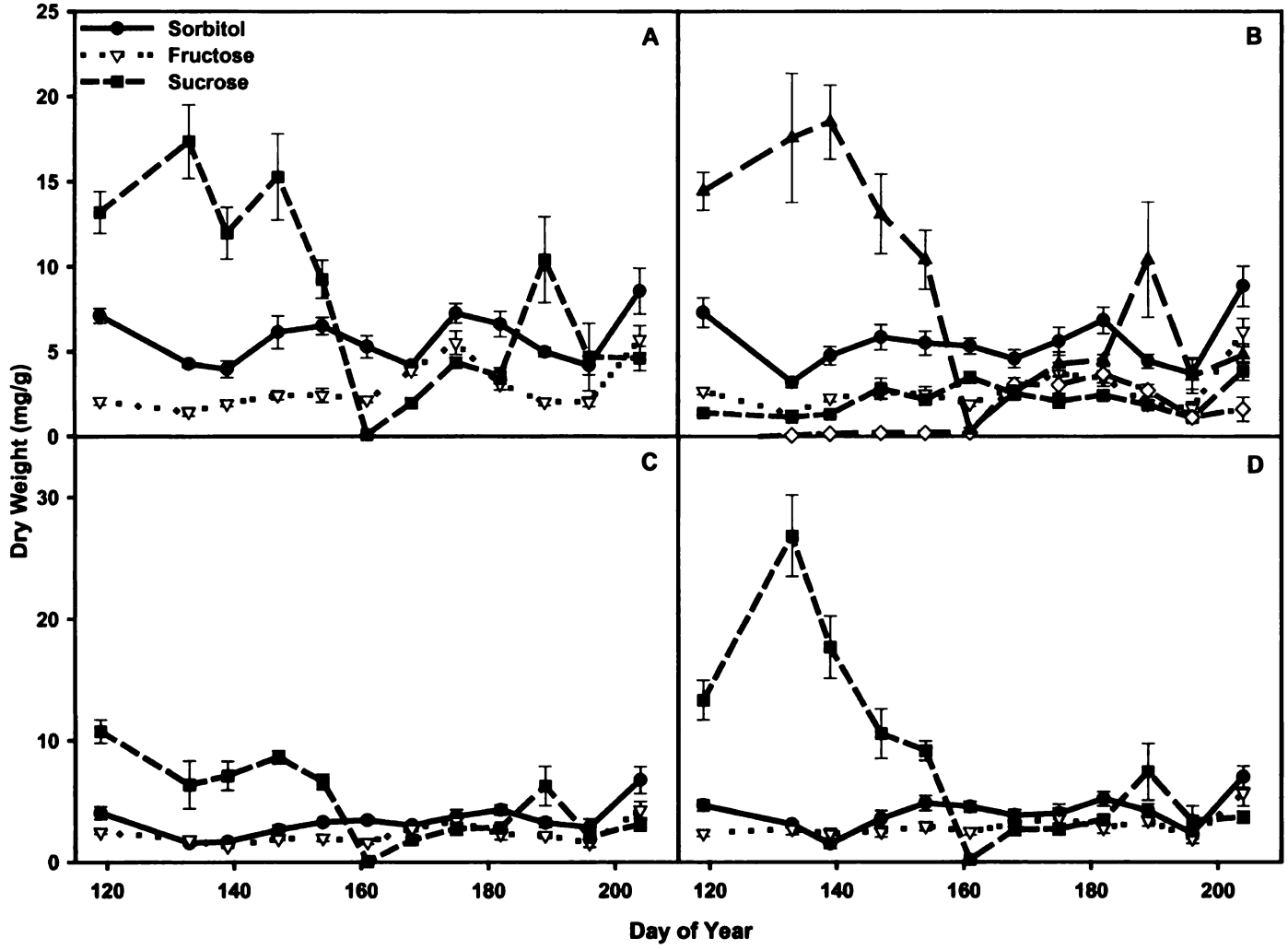

Figure 12. Soluble sugars in Lapins/Colt (non-dwarfing) in 2003. Two year old trees were field planted at the HTRC in East Lansing, Mich. and harvested during the current growing season. Trees were sectioned into scion (A), graft union (B), rootstock (C), and rootshank tissue (D). Error bars indicate one SE of the mean (n=10).

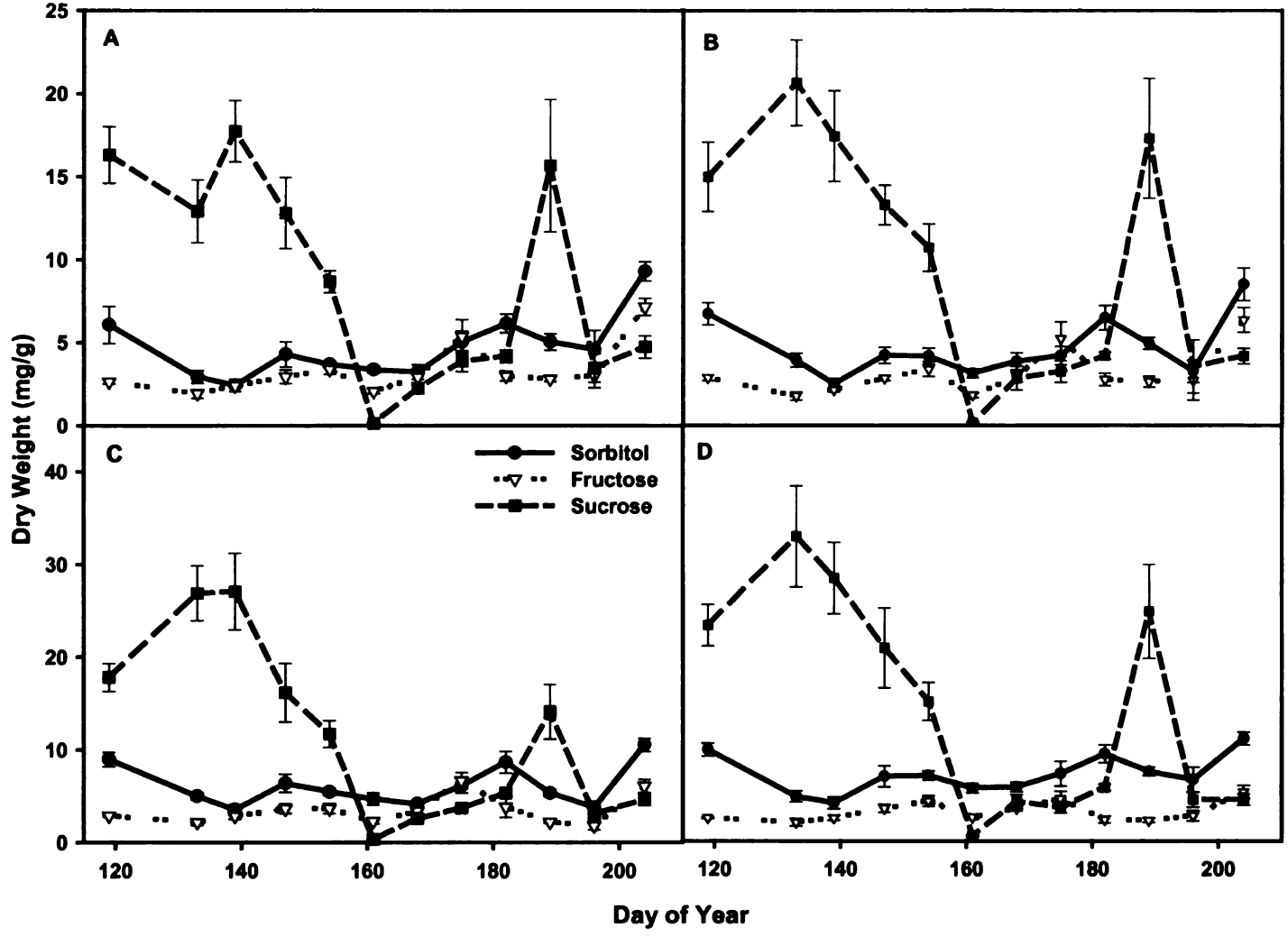

Figure 13. Total non-structural carbohydrates in woody tissues of Rainier/Gi 5 (A), Rainier/Gi 6 (B), and Rainier/Colt (C) during 29 March 2002 through 31 October 2002 from bud break (DOY 88) until leaf senescence (DOY 304). Error bars indicate one SE of the mean (n=3).

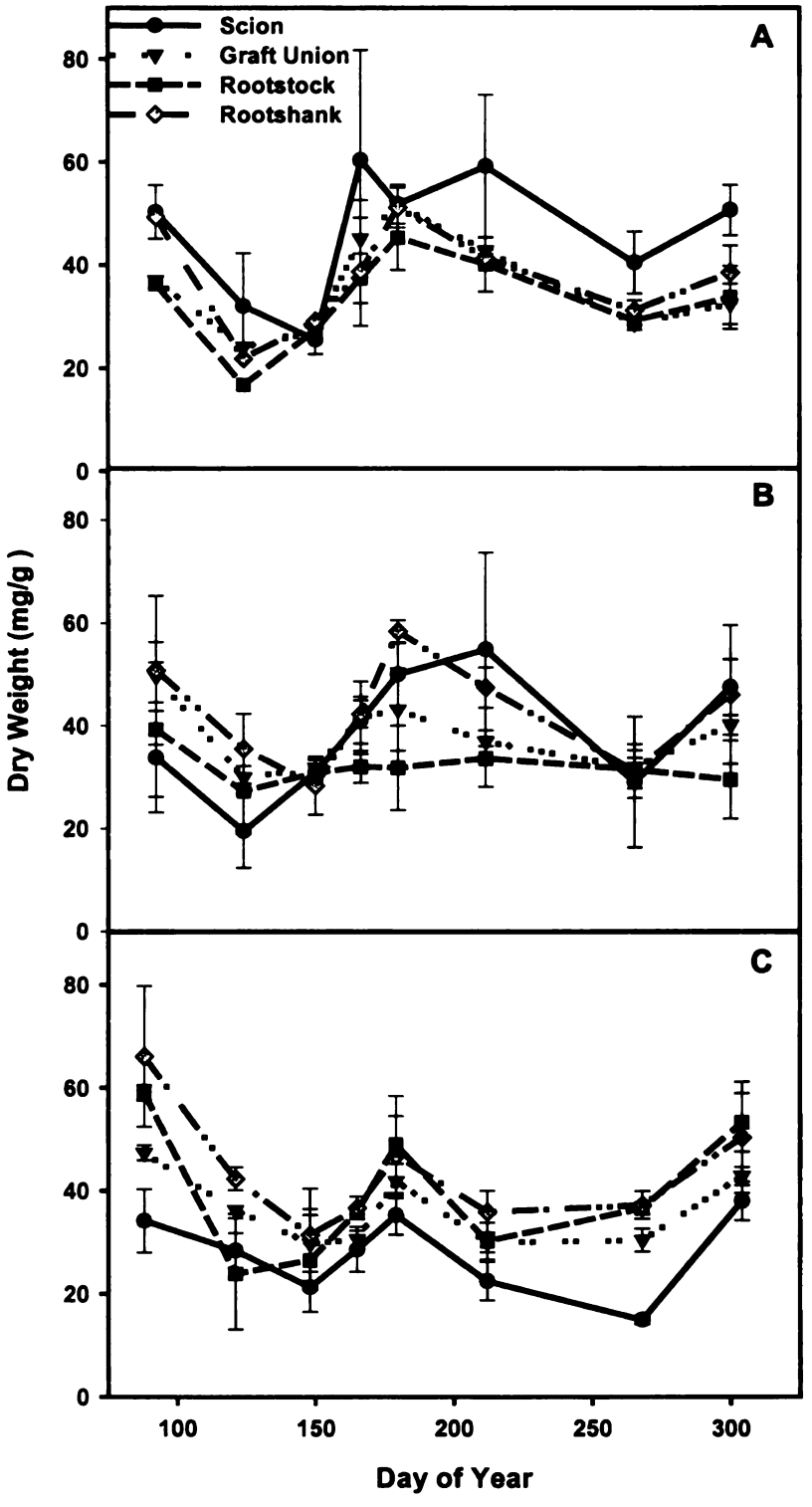

Figure 14. Total non-structural carbohydrates in woody tissues of Lapins/Gi 5 (A) and Lapins/Colt (B) during the 2003 growing season from 29 April to 1 August (maximum shoot elongation) at the Horticulture Research and Teaching Center in East Lansing, MI. Error bars indicate one standard error of the mean (n=10).

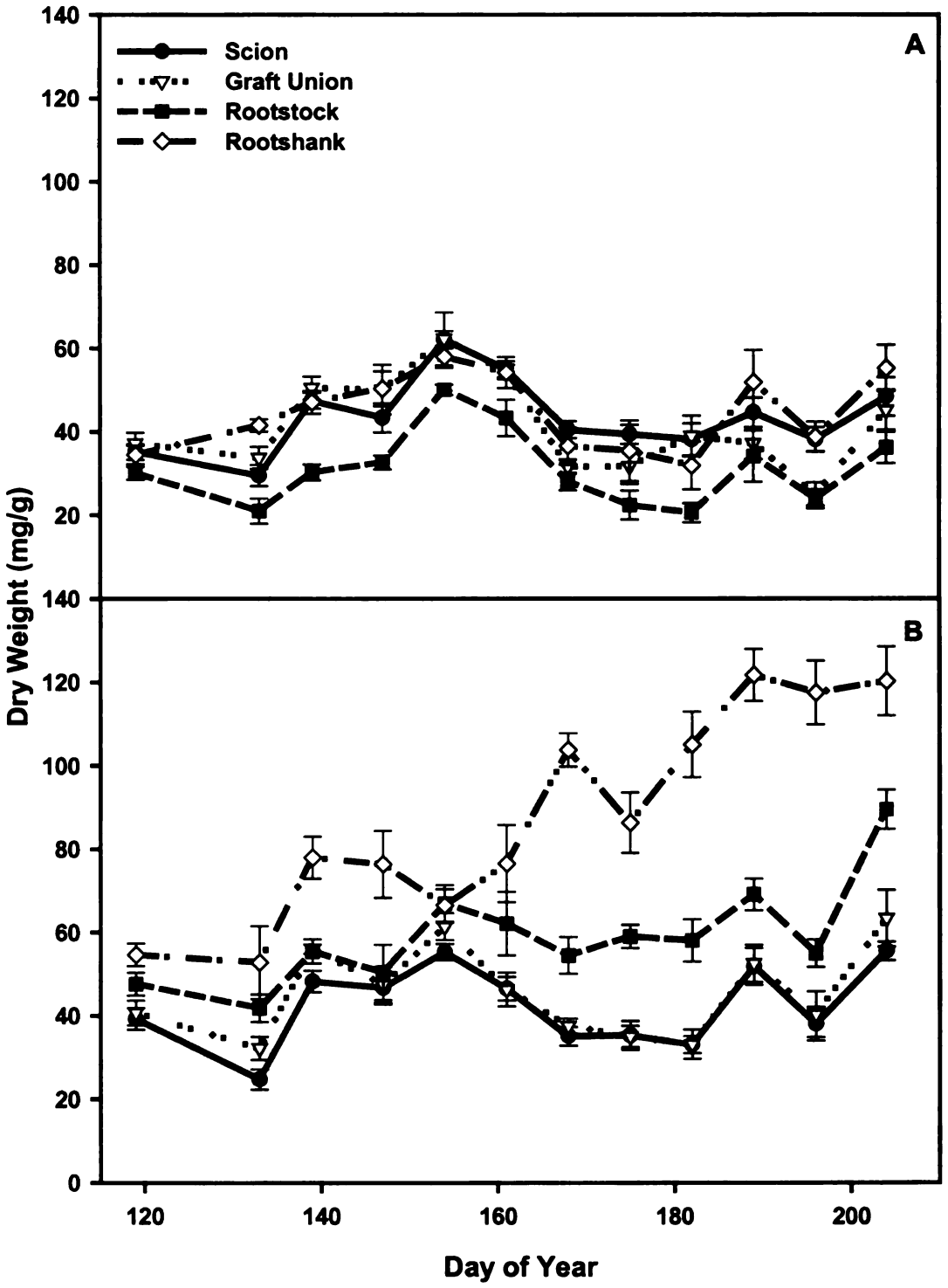

Table 1. Single-leaf photosynthesis parameters measured during the period of maximum shoot elongation in Lapins/Gi 5 and Lapins/Colt on 1 July and 25 July during the 2003 growing season at the Horticulture Research and Teaching Center in East Lansing, MI. Photosynthesis (P_n), stomatal conductance (G_s), and internal CO_2 concentration (C_i) were measured using a portable photosynthesis meter (CIRAS-2, Amesbury, MA) at solar noon (n=10).

		P_n (μmol CO_2 $m^{-2}s^{-1}$)	G_s (mmol $m^{-2}s^{-1}$)	C_i (μmol mol^{-1})
7/1/2003	Lapins/Gi 5	12.81 ± 0.91 a[1,2]	154.80 ± 10.50 a	203.40 ± 7.86 a
	Lapins/Colt	8.18 ± 0.79 b	105.20 ± 11.80 b	213.27 ± 10.10 a
7/25/2003				
	Lapins/Gi 5	6.53 ± 0.45 a	62.60 ± 4.12 a	183.53 ± 7.50 b
	Lapins/Colt	2.97 ± 0.45 b	31.07 ± 3.24 b	218.20 ± 11.20 a

[1] Parameter measurement reported ± standard error of the mean.
[2] Mean separation within columns and sampling date by LSD mean separation at $P \leq 0.05$.

CHAPTER FIVE

Storage Carbohydrates in Sweet Cherries (*Prunus avium* L.) on Dwarfing and Non-Dwarfing Rootstocks

ABSTRACT

The availability of dwarfing rootstocks in fruit tree production has led growers to increase their acreage of such systems. However, knowledge of dwarfing mechanisms is limited; especially the healing process. This research was undertaken to assess healing responses and carbohydrate status of one-year-old trees grafted onto dwarfing (Gisela 6; Gi 6, Gisela 5; Gi 5) and vigorous (F 12/1) rootstocks.

One-year-old potted rootstocks were grafted to include homografts (Gi 5/Gi 5 and Gi 6/Gi 6), heterografts (Rainier/Gi 5, Rainier/Gi 6, and Rainier/F 12/1), and reciprocal heterografts (Gi 5/F 12/1 and Gi 6/F 12/1). Scion, graft union, and rootstock tissues were harvested at one, three, and six months after budding. Starch and soluble sugar concentration were determined in these tissues. Rainier/Gi 6 combinations did not develop a successful graft union, thus results focused on Gi 5 and F 12/1 combinations.

Starch content of ungrafted rootstocks increased according to vigor (F 12/1> Gi 5). In grafted systems, reciprocal heterografts and those containing F 12/1 as a rootstock exhibited elevated starch concentrations compared to Rainier/Gi 5. Sucrose was the main soluble carbohydrate in all three tissues, and these concentrations were higher in combinations with F 12/1 as a rootstock. Rainier/Gi 5 exhibited the lowest concentration of soluble sugars in scion tissue. Overall, grafted combinations containing Gi 5 rootstock had lower concentrations of soluble sugars. Total non-structural carbohydrates (TNC) increased in

ungrafted rootstocks over the six month sampling period. In contrast, TNC concentrations decreased or were stable in grafted combinations by six months.

The early effects of dwarfing can be observed by examining carbohydrate storage in the region surrounding the graft union. Ungrafted Gi 5 exhibited lower concentrations of both starch and soluble sugars that appeared to affect scion carbohydrate storage in grafted dwarfing combinations. Initial reduced carbohydrate storage may impose a stress which may be magnified as the tree matures, negatively influencing carbohydrate storage for subsequent growth. Images in this dissertation are presented in color.

INTRODUCTION

Utilizing dwarfing rootstocks for fruit production has been beneficial for cherry production, by enhancing returns on investment due to earlier fruit production as well as lower labor costs. In addition, growers often use lower amounts of pesticides because the smaller canopy architecture lends itself to uniform chemical applications (Lang, 2000; 2001). However, dwarfing mechanisms are not well understood in cherries, or in management systems that have utilized dwarfing rootstocks for several decades (i.e., apple). In order to develop best management practices for fruit production on dwarfing rootstocks, a better understanding of the dynamic impact on growth and metabolism is necessary.

When the healing responses in grafted trees are examined, it is clear they occur along a distinct timeline, which varies depending upon the species involved. There are three distinct events that universally occur during the creation of the graft union: 1) cohesion (union) between the rootstock and scion, 2) production of callus cells, and 3) differentiation of callus and parenchyma cells into vascular elements (Hartmann et al., 1997). The timeline in which these events occur may be a function of the compatibility of the scion/rootstock combination, and the grafting method of the plant material. It is unknown if the onset of dwarfing responses occur in the early stages after grafting, with the healing response predisposing the scion to dwarf growth habits.

The method of grafting can have a direct influence on the healing responses of grafted trees. For example, in one-year-old *Malus* trees, chip-

budding produced greater numbers of successful trees than T-budding (Howard, 1977). This was important not only at the time of graft union development, but combinations that formed strong graft unions resulted in less frost damage, and greater survival during episodes of severe cold (Howard and Skene, 1974). In addition, vigorous growth was observed for combinations that were chip budded, as opposed to those that were T-budded. It was suggested that chip budding is superior to T-budding due to the decreased wounding associated with chip budding, and was particularly advantageous in climatic areas with shortened growing periods.

In addition to grafting method, compatibility between scion and rootstock must be established for a successful graft union to form. In apricots, the establishment of functional vascular connections depended upon the compatibility of the scion and rootstock (Deloire and Hebant, 1983). Histopathological tests determined that functional connections took eight to ten months to establish in a compatible union between cv. Polonais (*P. armeniaca*) and Myrobolan (*P. cerasifera*), whereas these vascular connections were disrupted in an incompatible combination, cv. Canino (*P. armeniaca*) on Myrobolan rootstock under the same conditions, during the same time period. In another study of apricot compatibility, vascular connections for both compatible and incompatible combinations did not show differences in the rate of vascular differentiation; rather the amount of parenchymatous tissue development within the graft union differed (Errea et al., 1994). In interspecific combinations of

Pyrus, an increased amount of parenchymatous or scar tissue led to structural weakness in the graft union, (Proebsting, 1926, 1928; Waugh, 1904). Growth between two genetically different systems can contribute to differences in healing responses, as with various cultivars of *P. avium* (scion) grafted onto dwarfing Gisela rootstocks (*P. cerasus* x *P. canescens*). Vessel diameters measured in vessels differentiated from callus tissue in the graft union of Rainier/Gi 5 indicate that smaller vessels developed. In addition vessels often developed at acute angles contrary to the longitudinal axis of the tree (Chapter 2). This in turn affected water transport to through the graft union of Lapins/Gi 5, a dwarfing scion/rootstock combination (Chapter 3). The combination of two genetically different systems has an impact on scion growth and development, and there are current efforts to describe differences in gene expression at the scion/rootstock interface (C. Prassinos, personal comm.).

Another theory for the dwarfing response is that a blockage in the vascular system of the graft union region may cause a reduction in water and nutrient translocation. The blockage could be due to anatomical anomalies which occur during the healing process at the junction of scion and rootstock material, like the development of phloem and xylem cells on a bias, and/or the appearance of invaginated or enclosed cambial elements (Deloire and Hebant, 1983; Simons and Chu, 1983; Poniedzialek et al., 1979). Growth of apple scion wood on dwarfing rootstocks was different in terms of the timing of cell differentiations, resulting in increased parenchymatous tissue that included the reduced rate of callus differentiation, resulting in masses of undifferentiated parenchyma cells

between stock and scion and whorls of vascular elements in the graft union region (Simons and Chu, 1984; Simons, 1986). These discontinuities may eventually be responsible for possible breakage of the tree at the graft union after a number of years (Simons and Chu, 1984; Simons, 1986; Poniedzialek et al., 1979; Proebsting, 1926).

Additionally, dwarfing rootstocks can induce anatomical changes, such as increased amounts of phloem fibers that translate to increased areas of bark and decreased areas of xylem (McKenzie, 1961). These areas of continuous phloem fibers are largely non-functional phloem that theoretically can reduce area of vascular translocation in the overall tree (Simon and Chu, 1984; Simons, 1986). The reduction in the area of vascular translocation may result in lower hydraulic conductivities in dwarfing rootstocks, and higher hydraulic conductivities in vigorous rootstocks that impacts scion growth (Atkinson et al., 2003). In fact, in dwarfing apple rootstocks (i.e., M.27, M.9, and MM.106) hydraulic resistance is increased in the graft union region, possibly due to altered vascular anatomy (Atkinson et al., 2003).

In addition to possible anatomical limitations caused by dwarfing rootstocks, variations in sink/source demands may affect the translocation of carbohydrates in the graft union region. The root systems of dwarfing systems are often smaller than their vigorous counterparts (Devyatov, 1996; Zhu et al., 1999); however, scions grafted to dwarfing cherry rootstocks may have a higher crop load and thus sink demand per total leaf canopy area (Lang, 2000). The higher sink demand to support the fruit may result in dwarfing of the vegetative

growth of the scion via various feedback processes like the increase in carbohydrate concentration or decreased nitrogen content (Gucci et al., 1991; Paul and Driscoll, 1997; Paul and Foyer, 2001). In peaches (*Prunus persica* L.) increases in carbohydrate profiles of tree components above the graft union may occur as a result of variations in sink/source demands, perhaps due to some degree of incompatibility (Gaudillere et al., 1992; Kubota et al., 1990; Salvatierra et al., 1998; Yano et al., 2002; Chapter 4). An increase of carbohydrate storage above the graft union has been thought to contribute to swelling often observed with dwarfing systems (Garner and Nicoll, 1956; Tabuenca, 1960; Yano et al., 2002).

Carbohydrate concentrations may be altered in sweet cherry dwarfing systems as previously determined in other deciduous fruit tree systems (Salvatierra et al., 1998; Yano et al., 2002; Chapter 4). In addition vessel diameter and hydraulic conductance are lower in dwarfing cherry and apple scion/rootstock combinations (Atkinson et al., 2003; Olmstead et al., 2004; Chapter 2). Alterations in total non-structural carbohydrates can be realized as early as two years after grafting; earlier changes in carbohydrate profiles have not been documented.

The objectives of this research were to determine if differences exist in non-structural carbohydrates of woody sections within and surrounding the graft union region of 'Rainier' scion wood grafted onto Gisela 5 (dwarfing rootstock; Gi 5) and Gisela 6 (semi-dwarfing rootstock; Gi 6) as compared to 'Rainier' on F 12/1 (vigorous rootstock). This experiment was designed to test the hypothesis

that carbohydrate storage within and above the graft union is altered in 'Rainier' grafted onto dwarfing cherry rootstocks from the Gisela series in comparison to 'Rainier' on non-dwarfing rootstocks (F 12/1). Further, these differences in carbohydrate allocation occur earlier in the life of the dwarfing grafted system prior to commercial levels of crop production.

Materials and Methods

Planting Material. In March 2002, ecodormant one year old ungrafted rootstocks were planted in 13.2 L pots and placed in an ambient light greenhouse with maximum temperatures of 25 °C, to initiate active growth prior to grafting. Three hundred rootstocks of Gisela 5 (*Prunus cerasus* x *P. canescens*; dwarfing), Gisela 6 (*P. cerasus* x *P. canescens*; semi-dwarfing), and two hundred twenty-five rootstocks of F 12/1 (*P. avium*) were included in this experiment. All of the rootstocks were commercially clonally propagated (Meadowlake Nursery, McMinnville, Ore.; Carlton Plants, Dayton, Ore.) to minimize genetic variation.

In April 2002, after one month of growth, trees were chip-budded to create seven different grafted combinations representing heterografts, homografts and reciprocal grafts (Table 1). Scions were budded in two locations on each rootstock stem to enhance the possible number of successful bud unions. Buds were wrapped tightly with budding tape to maintain humidity and insure maximum contact between scion and rootstock. Plants were placed outside when the threat of frost had passed, approximately the second week of May, 2002. As the budded scion grew out, rootstock stems were cut immediately

above the graft union, and the budding tape was removed to facilitate healing of the wound. Rainier/F 12/1 was utilized as both a homograft (*P. avium/P. avium*) and a heterograft due to the unavailability of both F 12/1 budwood and/or own-rooted Rainier. To illuminate specific tissue incompatibility interactions, reciprocal grafts were included to define the effect of rootstock grafted onto tissue that is commercially designated as scion material. Ungrafted rootstocks were used as controls. Samples were destructively harvested at one (DOY 150), three (DOY 210) and six months (DOY 301) after bud-grafting of the combinations had been completed (Figure 1A-C). Ungrafted rootstock samples were harvested at the same time to coincide with the grafted combinations. Based on preliminary sample analysis of 2001 tissues, samples for this experiment were divided into three sections: scion tissue, graft union, and rootstock tissue.

Carbohydrate Extraction and Starch Determination. Commencing with one month after grafting, samples were harvested from three sections of each combination (Figure 1A) directly into liquid nitrogen and stored in a -80°C freezer. Samples were then freeze-dried, ground in a Wiley Mill (40 mesh screen), and stored in desiccant prior to carbohydrate analysis. Freeze-dried tissue (0.2 g) was extracted with 3 ml of 80% (v/v) ethanol on ice. Samples on ice were ground with a Brinkmann homogenizer (Brinkmann/KINEMATICA Polytron, Westbury, N.Y.) for 30 s, using three 10 s pulses. The homogenate was held in a boiling water bath for five minutes, cooled and centrifuged at 9,600 *g* for 10 min to yield ethanol-soluble and ethanol-insoluble fractions. The supernatant was

retained for soluble sugar analysis, and the pellet was extracted an additional three times in 80% (v/v) ethanol. With each centrifugation, the supernatant was collected to analyze soluble sugars. After the third centrifugation, the remaining pellet was analyzed for starch.

The pellet remaining from the ethanol extraction was resuspended using 0.2 M potassium hydroxide and placed in a boiling water bath for 30 min. Distilled water was added as necessary to maintain pellet suspension. After cooling to room temperature, the mixture was adjusted to pH 5.5 using 1 M acetic acid. Dialyzed amyloglucosidase (3 ml) was added to the mixture and incubated for 1 h in a 55°C water bath. Samples were placed in a boiling water bath, cooled to room temperature, and centrifuged for 10 min at 9,600 *g*. Amyloglucosidase from *Aspergillus niger* (Sigma Chemical, A-9913) was dialyzed overnight (MWCO 3500, Fisher Scientific, Hampton, N.H.) in a sodium acetate buffer (pH 4.5) to remove glucose resultant from production of the enzyme.

Released glucose was analyzed using a glucose-6-phosphate dehydrogenase (G6PDH) enzymatic linked assay. An aliquot of the sample was incubated for 30 min in a reaction mixture containing 200 mM Hepes buffer (pH 8.0), 10 mM magnesium chloride ($MgCl_2$), 10 mM dithiothreitol (DTT), 2 mM ATP, 2 mM $NADP^+$, and 4 units of hexokinase (Sigma Chemical, type VI from Baker's Yeast) and glucose-6-phosphate dehydrogenase (Sigma Chemical, type V Baker's Yeast). The assay mixture contained 0.5 ml of reaction mixture and 100 µl of sample, diluted with 400 µl of water as to contain less than 50 µgm of

glucose per 0.5 ml. A reagent blank was included, consisting of 0.5 ml of DD H_2O and 0.5 ml of reaction mixture, in addition to individual sample blanks, containing 0.5 ml of Hepes buffer, and sample. Optical densities for reagent and sample blanks were subtracted from sample optical densities. The reduction of $NADP^+$ by glucose-6-P dehydrogenase was determined spectrophotometrically at 340 nm using a Unicam UV 300 Spectrophotometer (ThermoSpectra, Madison, Wisc.) (Robbins and Pharr, 1987).

Soluble Sugar Determination. Soluble carbohydrates were determined as described previously (Roper et al., 1988). Pooled supernatant collected from ethanol extraction of freeze-dried samples was collected into 13 x 100 mm test tubes and placed in a dry bath to evaporate ethanol from the sample. Samples were rehydrated using 1 ml of distilled water and a fraction (200 μl) of this rehydrated sample was placed into a separate tube. This was then placed into a dry bath to evaporate water from the samples. Soluble sugars were converted to oximes and then derivatized using hexamethyldisalizane and trifluoroacetic acid (Sweeley et al., 1963, Williams and Martin, 1967). Soluble sugars were analyzed by gas-liquid chromatography and peak heights and retention times compared to sugar standards (HP 5890 II, Agilent Technologies, Palo Alto, Calif.).

Statistical Analysis. The experiments were in a completely randomized design with rootstock type as the main treatment. General linear models (GLM) were used as appropriate, with alpha levels set at 0.05 *a priori*. Data were assumed to be normal and continuous with homogeneous variances. When normality was not satisfied, data were transformed logarithmically to gain normality before

statistical analysis. Mean separation was by Tukey's HSD and Fisher's LSD as appropriate (SAS; Cary, N.C.).

Results

Starch. Grafted combinations of Rainier/Gi 6 did not develop a successful graft union after one month, thus all data concerning Gi 6 rootstocks are not reported. Concentrations of starch in ungrafted rootstocks reflect the carbohydrate storage pool of the two rootstocks without wounding (Figure 2). At one month, starch concentration in F 12/1 rootstock tissue was significantly higher than Gi 5 ($p < 0.05$). There was also an increase in the concentration of starch in F 12/1 and Gi 5 throughout the six month growth period, with highest concentration of starch observed at three months (Figure 2). Ungrafted Gi 5 trees consistently had lower starch concentrations that the F 12/1 rootstock in an unperturbed system.

In grafted systems, there were high concentrations of starch in scion tissues of Gi 5/Gi 5 and Gi 5/F 12/1 one month after grafting (MAG), compared to reduced concentrations in Rainier/Gi 5 and Rainier/F 12/1 (Figure 3A), which then fell significantly by three and six months. However, at 3 MAG, there were no differences among all tissues examined, and concentrations remained fairly stable at six MAG ($p > 0.05$) (Figure 3A). There was a general trend for systems containing Gi 5 to have lower concentrations of starch in all tissues, as compared to other grafted combinations within a category (homograft, heterograft, or

reciprocal heterograft) (Figure 3A-C). This pattern was especially evident in rootstock tissues at 1 MAG, and graft union tissues at 6 MAG.

Soluble sugar concentrations. In ungrafted rootstocks, sucrose was the predominant sugar (Figure 4), and these concentrations were higher than overall starch concentrations (Figure 2). A steady increase in sucrose concentrations was observed in ungrafted F 12/1 from month one to month six. There was no significant increase in sucrose concentration in Gi 5 between one and three month sampling dates. However, between three and six month sampling dates the sucrose concentration in Gi 5 rootstock was the same as in F 12/1 rootstock (Figure 4).

Rainier/Gi 5 scion tissue had the lowest overall concentration of all soluble carbohydrates (<2% dry weight [D.W.]) compared with graft union and rootstock tissue, as well as within the heterograft treatments (Figure 5A-F). In contrast, sucrose concentrations in Rainier/F 12/1 sharply increased 3 MAG to 26.0 % D.W., with a decrease to 5.4% D.W. 6 MAG (Figure 5D). Graft union tissue of Rainier/Gi 5 exhibited a steady increase in graft union tissue, suggesting that there was a buildup of soluble sugars in the graft union (Figure 5B). This pattern of sucrose accumulation was not observed in Rainier/F 12/1 (Figure 5E). These soluble sugars may accumulate in parenchyma tissue that develops in the graft union of dwarfing combinations (Chapter 2, 3).

In scion tissue of homografts, there was a peak in sucrose concentration at 3 MAG, which most likely coincided with maximum shoot growth. Gi 5/Gi 5 had significantly lower concentrations of all soluble carbohydrates of any

combination (Figure 6A-C). Peak concentrations reflected the rootstocks' effect on vigor, with Rainier/F 12/1 (26.0% D.W.; Figure 5D) and Gi 5/F 12/1 (30.9% D.W., Figure 6D) had the highest peak concentrations of sucrose, followed by significantly lower sucrose in Gi 5/Gi 5 (5.7% D.W.; Figure 6A). In addition, the pattern of accumulation and reallocation of sucrose in scion tissue was similar for all combinations using F 12/1 as a rootstock (Figure 5D, 6D). From the data presented here, F 12/1 when utilized as a rootstock similarly affects soluble carbohydrate accumulation and reallocation as observed in Rainier/F 12/1 and Gi 5/F 12/1 (Figure 5D-F, 6D-F).

Total non-structural carbohydrates (TNC). As with starch and soluble sugar concentrations, TNC concentrations were significantly lower for ungrafted than most grafted tissue combinations. In ungrafted rootstocks, TNCs significantly increased from a range of 3-7 % D.W. to approximately 14.5 % D.W. in six months of growth ($p < 0.001$; Figure 7). However, ungrafted Gi 5 was significantly lower than F 12/1 during the one and three month sampling periods, before increasing to 14.5% ($p < 0.01$; Figure 7). Overall, Gi 5 exhibited a different pattern of TNC accumulation than F 12/1.

In scion tissues of heterografts, bud union replications of Rainier/F 12/1 were successful throughout the six month sampling period; however, Rainier/Gi 5 had fewer successful buds (data not shown). Consequently, Rainier/F 12/1 scion tissue TNC was significantly higher than Rainier/Gi 5 ($p > 0.001$) (Figure 8A). In Gi 5/Gi 5 TNC was greater at 1 MAG than 3 or 6 MAG, while in graft union and rootstock tissues, TNC increased (Figure 8 A-C). Overall, scion tissue contained

the highest concentration of TNC throughout the sampling period in grafted combinations.

Discussion

Carbohydrate analyses confirm that carbohydrate storage is altered in ungrafted dwarfing rootstocks and their respective combinations. Dwarfing Gi 5 rootstocks consistently had lower starch concentrations than the vigorous F 12/1, suggesting that this particular dwarfing rootstock is reduced in its carbohydrate storage capacity. Reduced starch reserves in Gi 5 rootstock storage tissues appear to influence starch concentrations in scions when they are part of a Gi 5 grafted system.

In grafted combinations containing Gi 5, starch concentrations were lower in all tissues than those with F 12/1 in the grafted rootstock combination, suggesting that scion growth may be limited by the amount of carbohydrates available for growth and development. In addition, scion tissue of Rainier/Gi 5 had the lowest sucrose concentration, during the period that sucrose concentrations in graft union tissues increased in the same grafting combination. Thus, it appears that a reduction in the transport and/or accumulation of sucrose may also contribute to reduced scion growth in Rainier/Gi 5.

Reduced carbohydrate storage and translocation in dwarfing systems may affect the development of fine root hairs that are important for water and mineral uptake, thereby reducing structural components of the root system, important for carbohydrate storage. This in turn may reduce shoot growth and development.

This cascading effect would be magnified as trees produce fruit and carbohydrate sink demand increases (Avery, 1969; 1970; Tubbs, 1973). For example, apples grafted onto a dwarfing rootstock, M.9 had an 80% reduction in overall dry weight when a crop load was present (Barlow, 1971). In dwarfing cherry rootstock systems, the reduced fruit developmental period may place a high demand for carbohydrates that exist at lower concentrations than in cherry scions grafted onto vigorous rootstocks. This predisposition could induce a carbohydrate stress in the tree, consequently affecting fruit development.

Considering both starch and soluble carbohydrate concentrations, it is apparent that soluble carbohydrates have the largest impact on TNC concentration in scion tissue. In fact, soluble sugar concentrations accumulate above the graft union in two separate dwarfing systems (Chapter 4), suggesting that soluble carbohydrate pools are significantly altered in dwarfing systems. It is possible that differences in soluble sugar concentration observed in this study could persist throughout the life of the tree, further affecting shoot growth and fruit development.

Conclusions

Overall, starch concentrations of ungrafted rootstocks reflected the growth characteristics of Gi 5 and F 12/1. In grafted systems, those containing F 12/1 as a rootstock exhibited elevated starch concentrations compared to those utilizing 'Rainier' as the scion, and Gi 5 as the rootstock. Sucrose was the main soluble carbohydrate, and these concentrations were higher in combinations that utilized

F 12/1 as a rootstock. Rainier/Gi 5 exhibited the lowest concentration of soluble sugars in scion tissue. At 6 MAG, Rainier/Gi 5 had the lowest concentration of starch in scion and rootstock tissues. Total non-structural carbohydrates increased in ungrafted rootstocks over the six month sampling period; however concentrations decreased or were stable in grafted combinations.

From the data presented here, it appears that the early effects of grafting can be observed in combinations that utilize Gi 5. Ungrafted Gi 5 exhibited lower concentrations in both starch and soluble sugars that appeared to affect scion carbohydrate storage in grafted dwarfing combinations. Thus, it is possible that these effects would be magnified as the tree matures, negatively influencing carbohydrate storage for subsequent growth.

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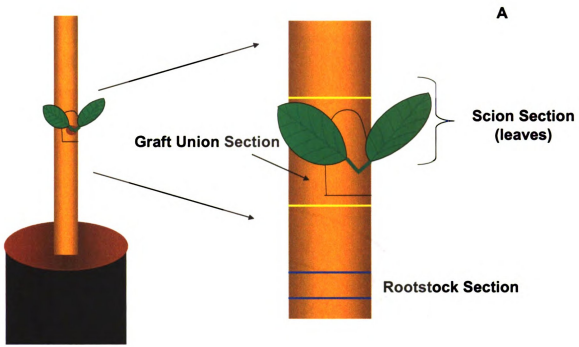
Table 1. 2002 graft combinations and resulting treatments of dwarfing (Gi 5 and Gi 6) and vigorous (F 12/1) rootstocks with `Rainier` scion. Heterografts represent commercially available combinations.

Controls	Gi 5, Gi 6, F 12/1 ungrafted trees
Homografts	Rainier/F 12/1, Gi 5/Gi 5, Gi 6/Gi 6
Heterografts	Rainier/Gi 5, Rainier/Gi 6 Rainier/F 12/1

Figure 1. Sampling parameters for one-year-old trees, showing the progression of the scion at one month after budding (A), three months after budding (B), and six months after budding (C).



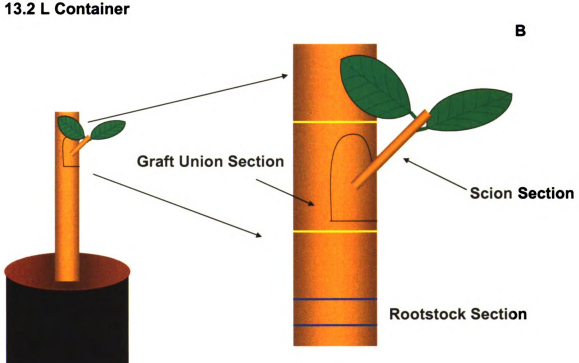
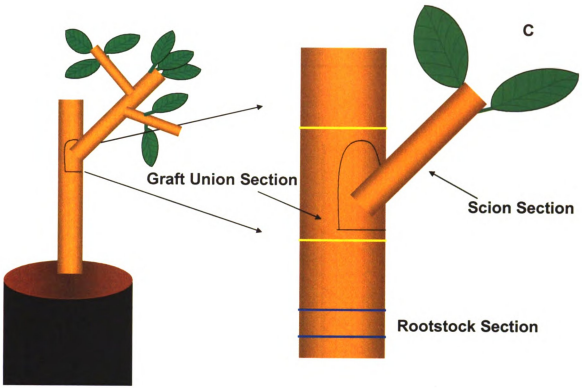


Figure 1 (continued).



13.2 L Container

Figure 2. 2002 starch concentrations in one-year-old ungrafted rootstocks. Trees were planted in 13.2 L pots and placed under ambient growing conditions in East Lansing, Mich. Gisela 5 (Gi 5; dwarfing) and F 12/1 (vigorous) were sampled at one, three, and six months to coincide with the sampling periods for grafted treatments. Error bars indicate one SE of the mean (n=5).

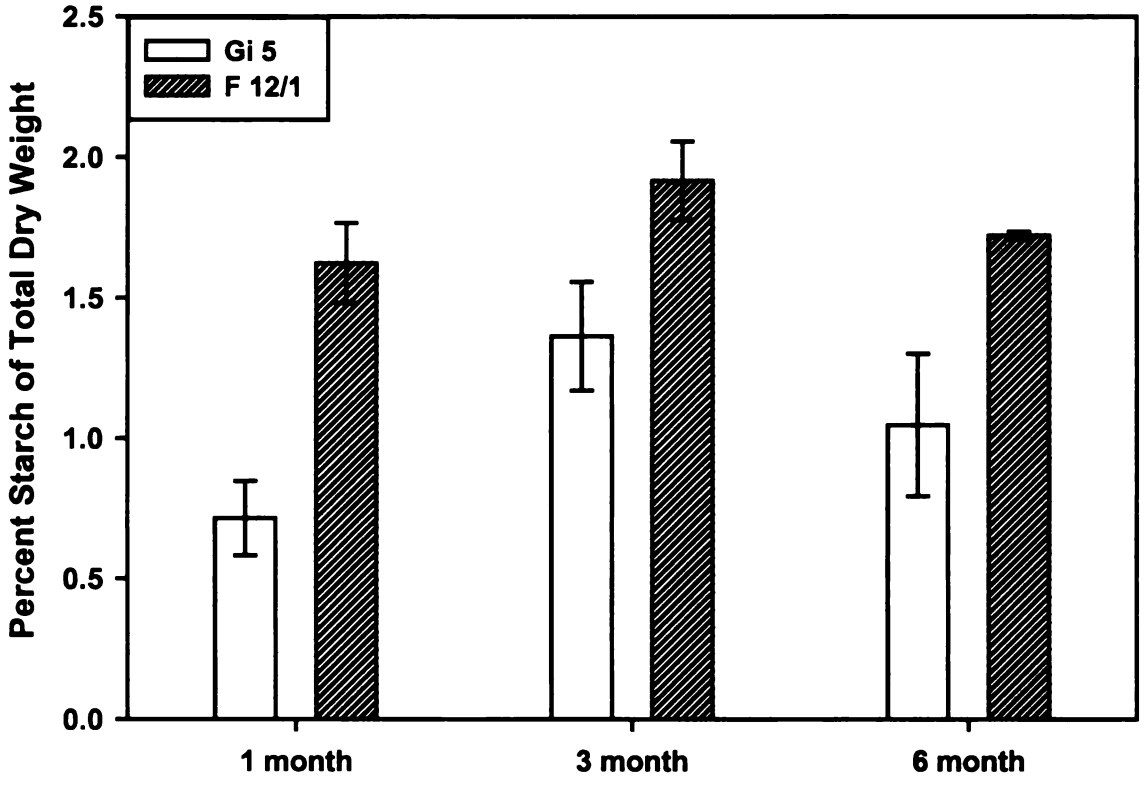


Figure 3. 2002 starch concentrations in scion (A), graft union (B), and rootstock (C) tissue of grafted one-year-old potted trees. Trees were planted in 13.2 L pots and placed under ambient growing conditions in East Lansing, Mich. Treatments included heterografts (Rainier/Gi 5 and Rainier/F 12/1), homografts (Gi 5/Gi 5 and Rainier/F 12/1), and a reciprocal graft (Gi 5/F 12/1), sampled at one, three, and six months after grafting. Error bars represent one SE of the mean ($n \geq 3$).

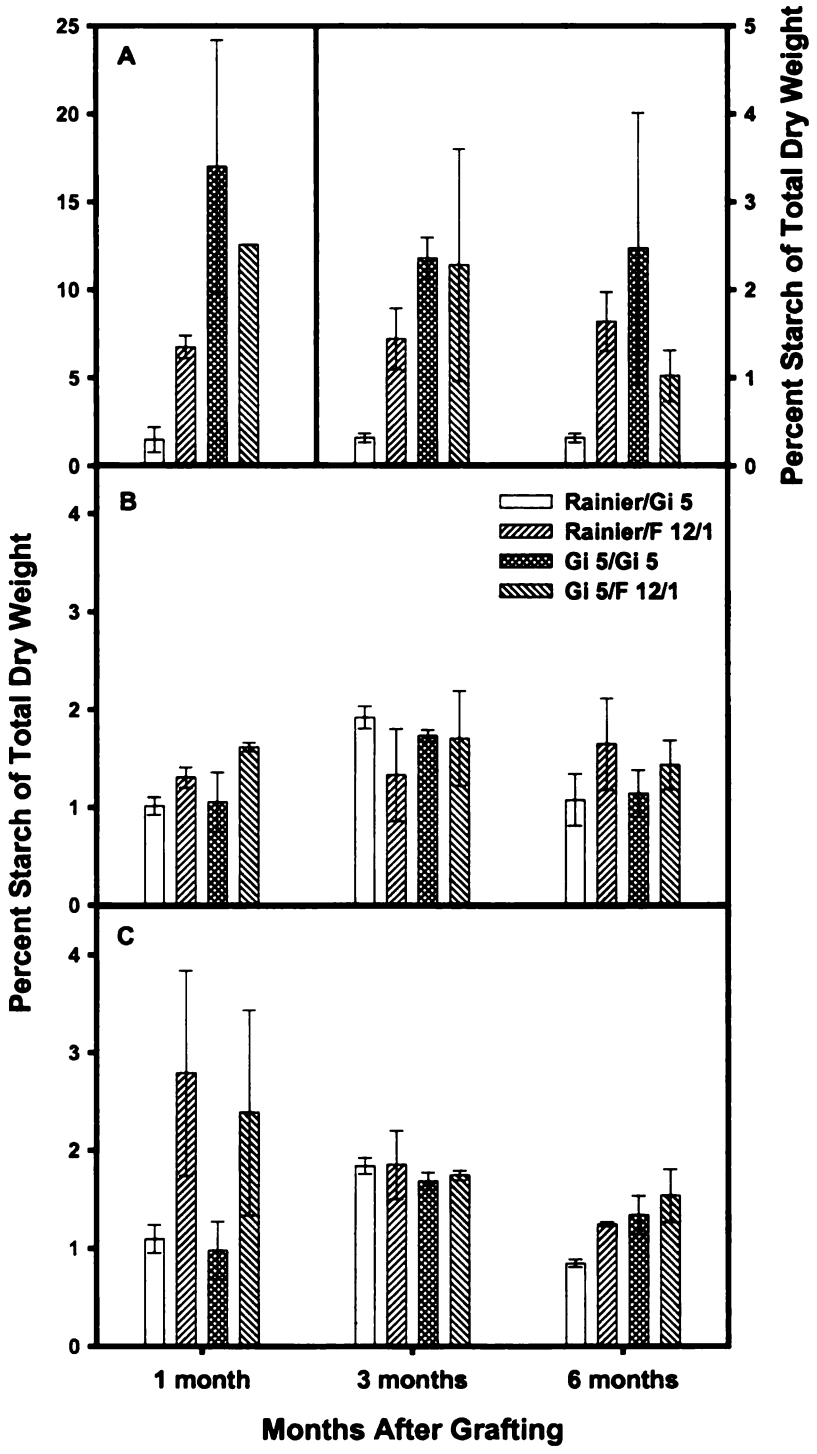


Figure 4. 2002 soluble sugar profile of one-year-old ungrafted rootstocks. Trees were planted in 13.2 L pots and placed under ambient growing conditions in East Lansing, Mich. Gisela 5 (A; dwarfing) and F 12/1 (B; vigorous) were sampled at one, three, and six months to coincide with the sampling periods for grafted treatments. Error bars indicate one SE of the mean (n=5).

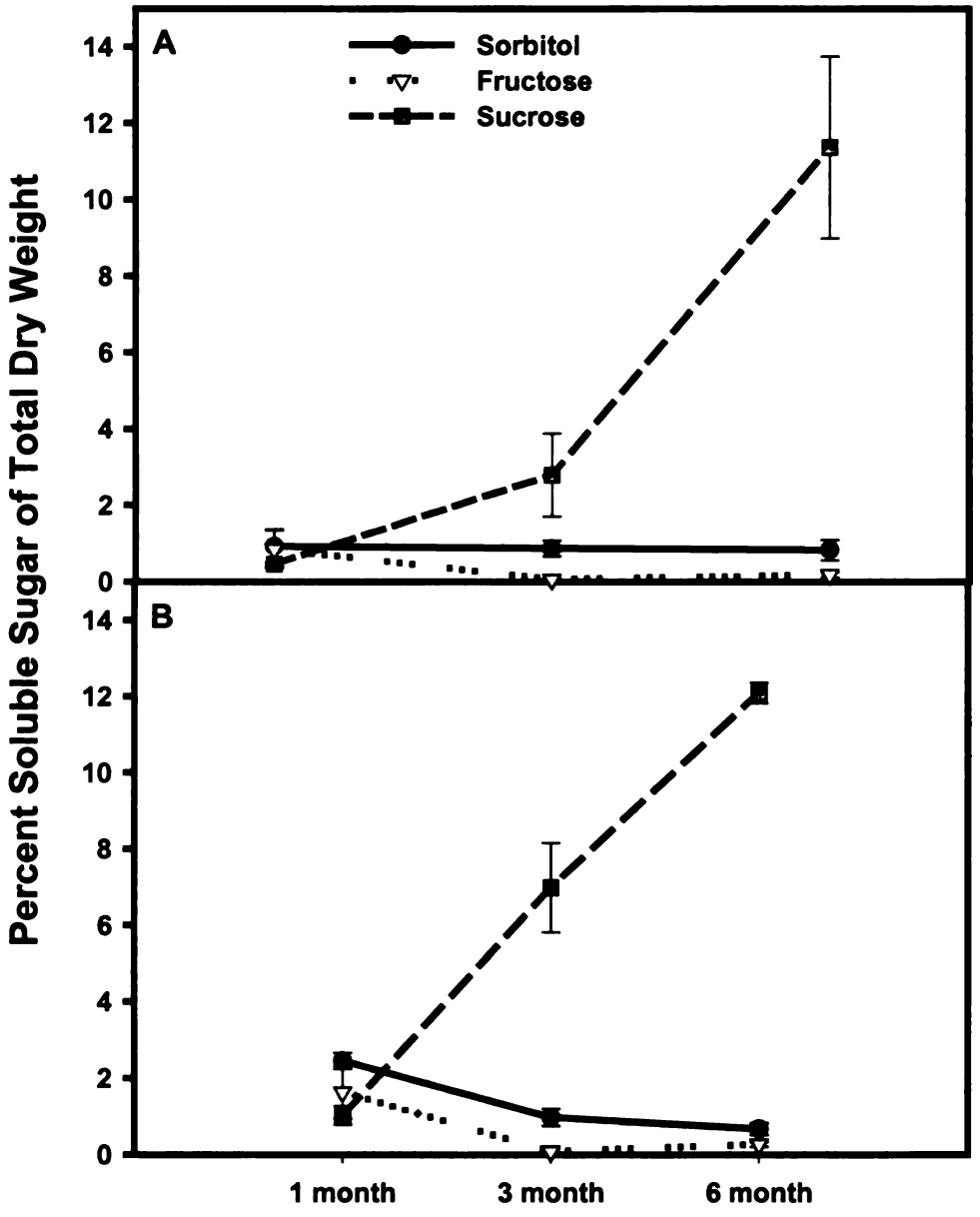


Figure 5. 2002 soluble sugar profiles of Rainier/Gi 5 scion (A), graft union (B) and rootstock tissue (C) and Rainier/F 12/1 scion (D), graft union (E) and rootstock tissue (F) Trees were planted in 13.2 L pots and placed under ambient conditions at the HTRC in East Lansing, Mich. Treatments were sampled at one, three, and six months after grafting. Error bars represent one SE of the mean (n≥3).

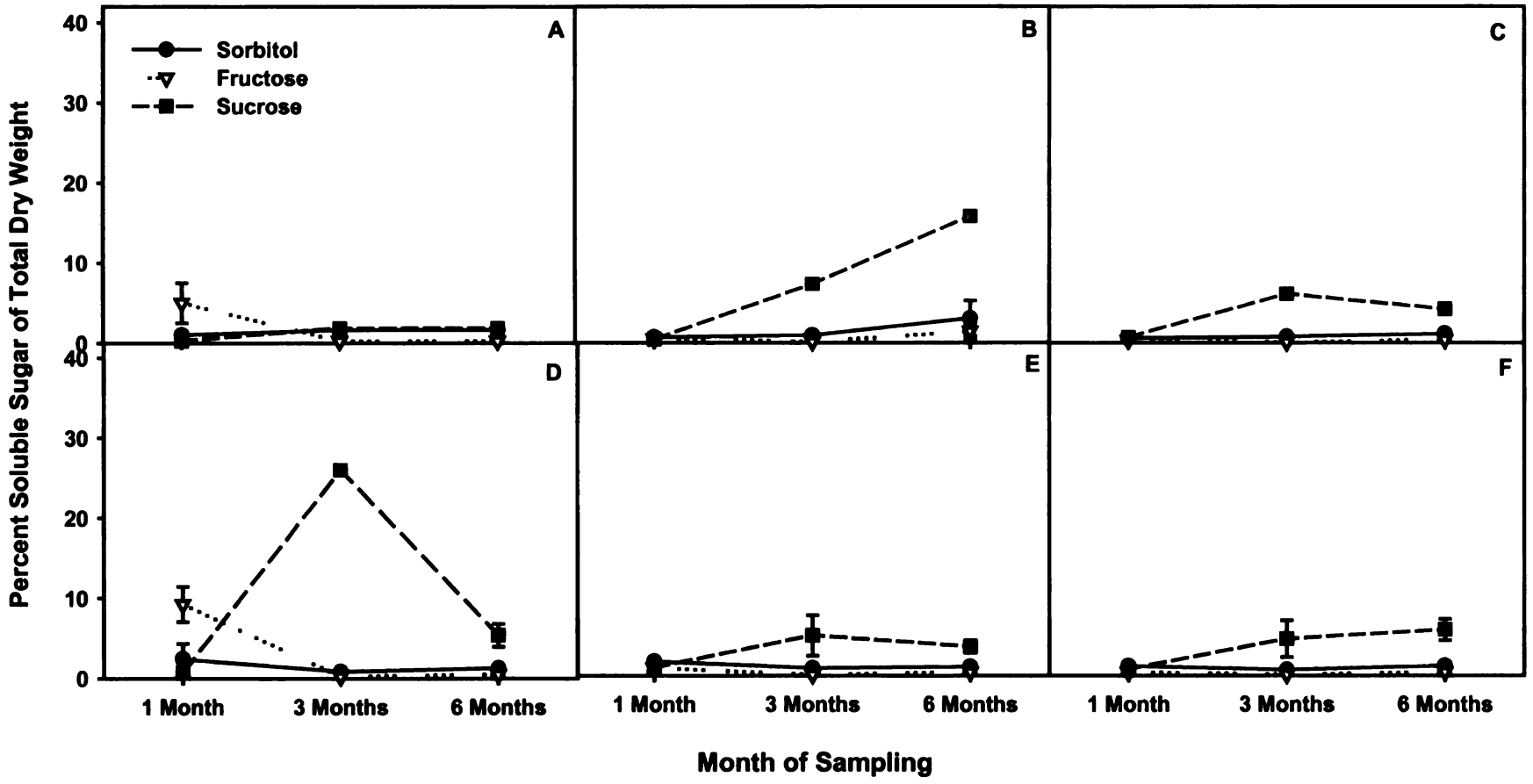


Figure 6. 2002 soluble sugar profiles of Gi 5/Gi 5 scion (A), graft union (B) and rootstock tissue (C) and Gi 5/F 12/1 scion (D), graft union (E) and rootstock tissue (F). Trees were planted in 13.2 L pots and placed under ambient conditions at the HTRC in East Lansing, Mich. Treatments were sampled at one, three, and six months after grafting. Error bars represent one SE of the mean (n=5).

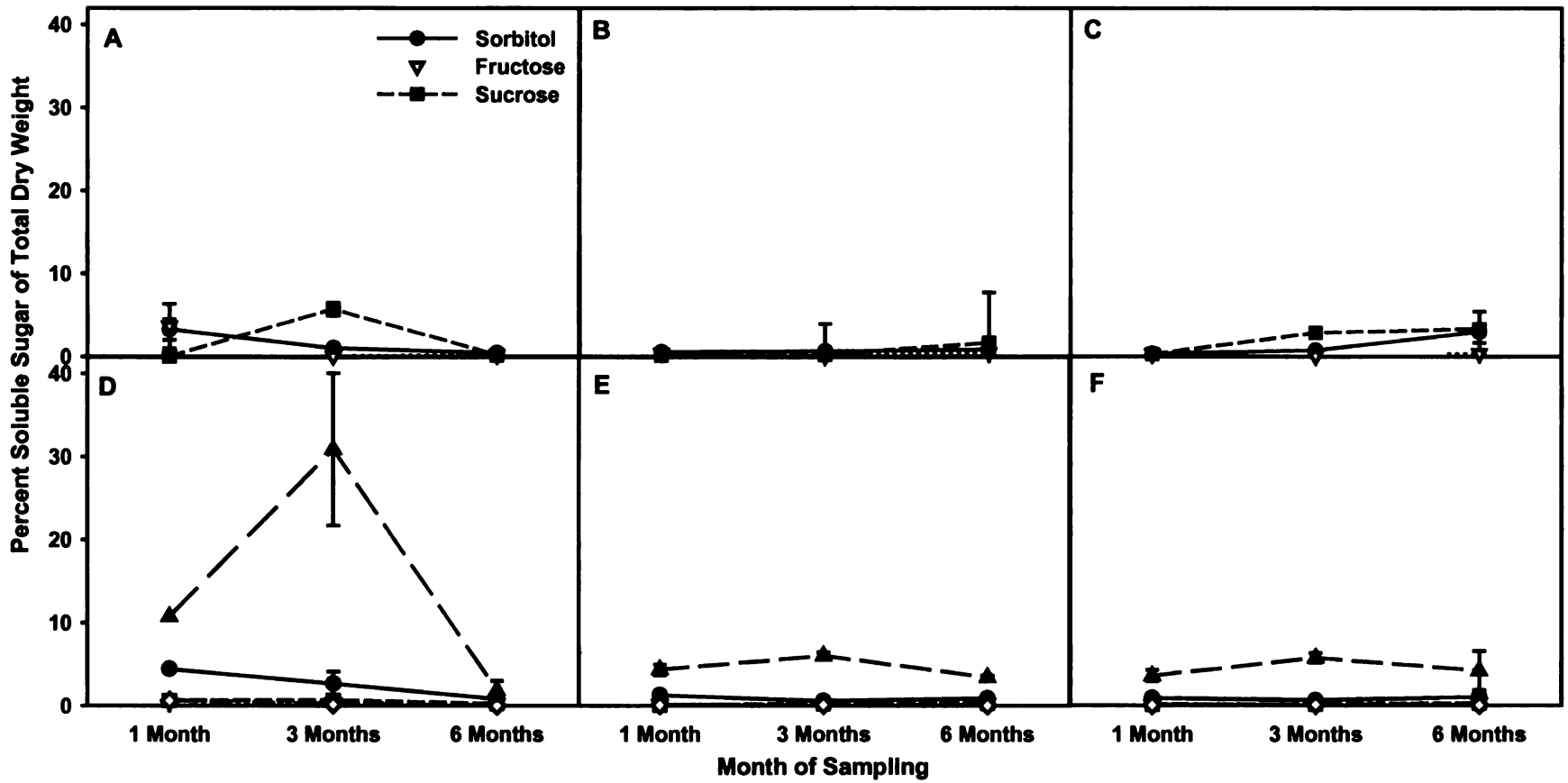


Figure 7. 2002 total non-structural carbohydrates (TNC) in woody tissues of ungrafted one-year-old Gi 5 and F 12/1. Trees were potted in 13.2 L pots and located at the HTRC in East Lansing, Mich. Error bars indicate one SE of the mean (n=5).

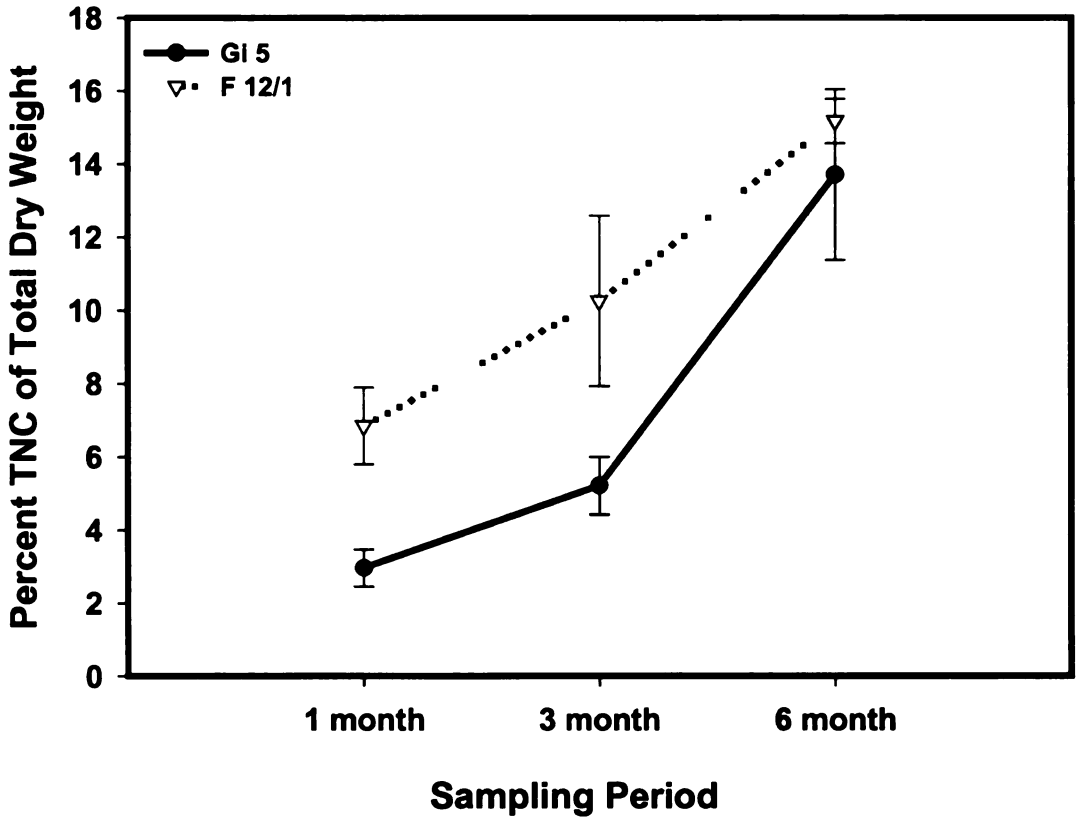
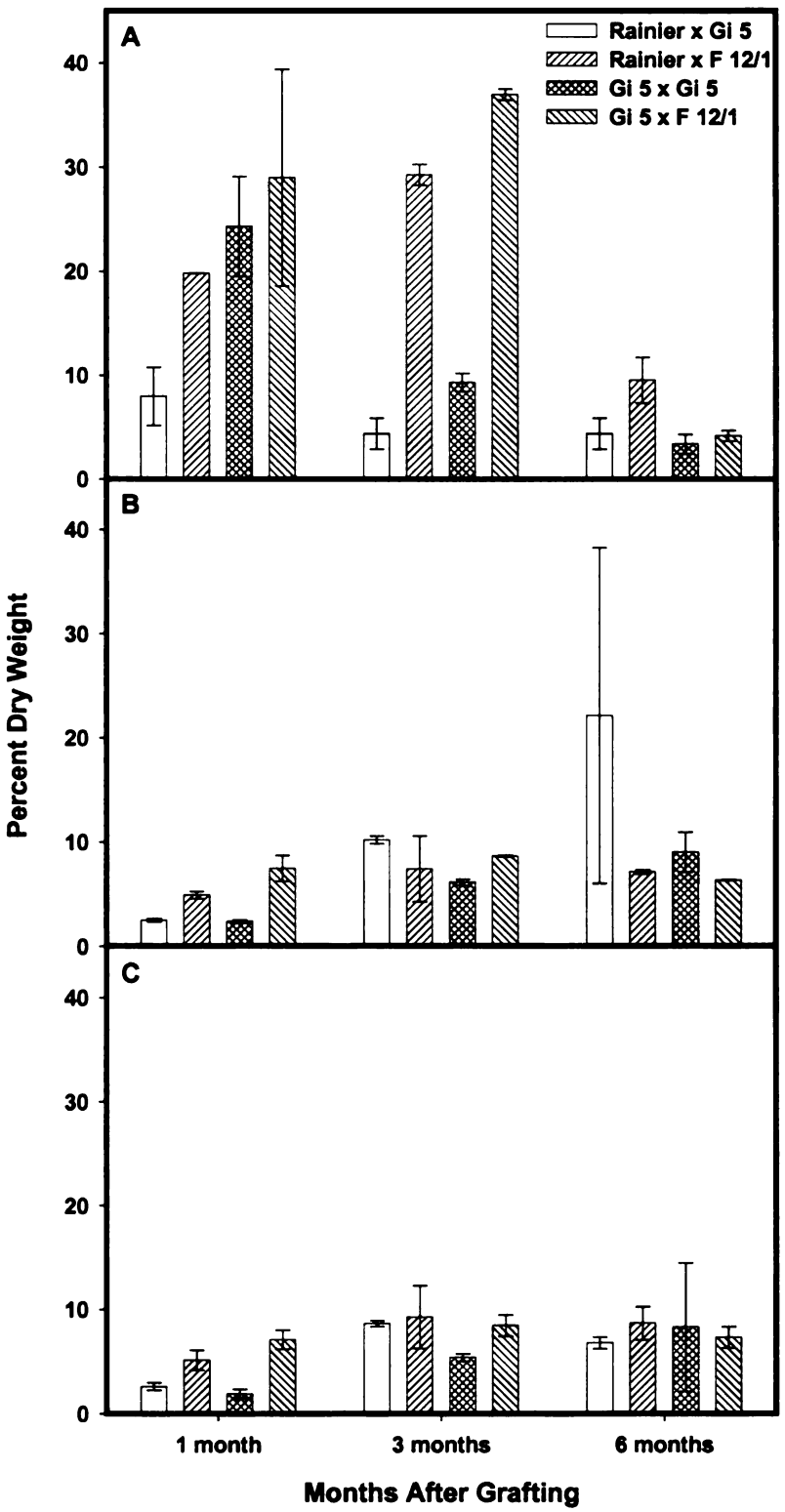


Figure 8. 2002 total non-structural carbohydrates in scion (A), graft union (B), and rootstock (C) tissues of one-year-old grafted combinations. Trees were potted in 13.2 L pots and located at the HTRC in East Lansing, Mich. Error bars indicate one SE of the mean ($n \geq 3$).



CHAPTER SIX

Summary and Conclusions

SUMMARY AND CONCLUSIONS

The research described herein examined the graft unions of three dwarfing sweet cherry scion/rootstock combinations (Rainier/Gisela 5 [R/Gi 5], Rainier/Gisela 6 [R/Gi 6], and Lapins/Gisela 5 [L/Gi 5]), and two non-dwarfing combinations (Rainier/Colt [R/C] and Lapins/Colt [L/C]). Two hypotheses were tested: 1) that vessel characteristics would be altered in dwarfing rootstock combinations, and 2) that carbohydrate concentrations would increase above the graft union due to physical limitations or alterations in sink/source strength.

Vessel area was smaller in graft union tissue of Gi 5 combinations, suggesting that a hydraulic restriction may occur in the graft union with the highest proportion of genetically mixed tissue (scion + rootstock). Vessel diameter of all ungrafted rootstocks (Gi 5, Gi 6, Colt and F 12/1) was greater compared to graft union sections of homograft (Gi 5/Gi 5, Gi 6/Gi 6, and Colt/Colt) and heterograft combinations (R/Gi 5, R/Gi 6, and R/C). Although a wounding effect was observed with decreases in estimated VHD of scion compared to graft union tissue, vessel element length was not significantly different among treatments. Estimation of vessel hydraulic diameter (VHD) supports the hypothesis that vessels in R/Gi 5 graft union tissue may have reduced conductive potential compared to scion or rootstock tissues.

Higher vessel frequency per mm^2 was found in dwarfing rootstocks compared to non-dwarfing rootstocks, suggesting that there are a greater proportion of narrow vessels produced in dwarfing rootstock combinations. Ungrafted rootstocks produced more vessels per mm^2 than in any tissue within a

grafted combination, which were larger in size than in their grafted counterparts, suggesting these differences in vessel size were due to a wounding effect. Vascular anomalies such as whorls of vascular tissue and the development of xylem vessel elements on a bias at the graft union were both prevalent in dwarfing rootstock combinations. When scions are grafted onto a dwarfing rootstock, it appears that the combination of a larger proportion of narrow vessels, with reduced hydraulic conductive potential and increased incidences of vascular anomaly greatly contributes to the dwarfed scion habit in these combinations.

Water movement in dwarfing rootstock combinations was reduced in graft union tissue compared to that of non-dwarfing rootstock combinations, as indicated by Safranin O uptake. Overall, graft union stem cross-sectional area was greatest in dwarfing rootstock combinations compared to non-dwarfing rootstock combinations which exhibited a gradual decrease in stem cross-sectional area moving acropetally, from rootstock to scion. The production of non-functioning phloem as excess tissue in the graft union of dwarfing rootstock combinations can contribute to increased graft union stem area. However, because of vascular anomalies and lack of effective transport present in dwarfing rootstock combinations, increased graft union stem cross-sectional area did not result in an increase of the stained stem area in the graft union or the scion. The percent of total area stained in the graft union of dwarfing combinations was less than all other sections (scion, graft union, or rootstock) and non-dwarfing treatment combinations (homografts, heterografts, or reciprocal heterografts). In

fact, SEM examinations within stained areas (maximum dye transport) suggest that the graft union of the dwarfing rootstock combination accumulated dye in discrete and restricted cells. This could account for the increased parenchymatous tissue located in the graft union region providing avenues for xylem content unloading. Non-functioning phloem and abnormal vascular elements in the graft union may provide a longer pathway for water to travel, thus slowing water transport in the graft union region. Non-dwarfing combinations had a more uniform distribution of dye longitudinally and radially throughout the tree, which occurred due to more functional vessel elements. Vessel diameters in graft union tissues were significantly smaller compared to scion or rootstock in the non-dwarfing rootstock combination.

Scion shoot length was reduced when grafted on dwarfing or semi-dwarfing rootstocks compared to a non-dwarfing rootstock. The pattern of accumulation and reallocation of starch in dwarfing rootstock combinations differed from non-dwarfing rootstocks. During maximum shoot elongation, the non-dwarfing rootstock combination (Lapins/Colt) began accumulating starch and sequestering reserves in the rootshank. In contrast, starch concentrations were similar in all wood tissues examined in Rainier and Lapins grafted to Gi 5 throughout the period of shoot elongation. Starch concentrations in all woody tissues of both dwarfing and non-dwarfing combinations (R/Gi 5, R/Gi 6, and R/C) returned to their respective initial concentrations by the end of the growing season.

In examination of young ungrafted rootstocks and grafted rootstock combinations, total non-structural carbohydrates (TNC) increased in ungrafted rootstocks over the six month sampling period. Ungrafted Gi 5 exhibited lower TNC concentrations than F 12/1 during the first 3 months of sampling before increasing to a similar concentration as in F 12/1. In grafted systems of <6 months, those containing a non-dwarfing rootstock exhibited elevated starch concentrations in scion tissues compared to those utilizing 'Rainier' as the scion, and a dwarfing rootstock. Sucrose concentrations were elevated in scion tissues of combinations that utilized a non-dwarfing rootstock, while R/Gi 5 exhibited the lowest concentration of soluble sugars in scion tissue. At 6 months after grafting, R/Gi 5 had the lowest concentration of starch in scion and rootstock tissues. TNC concentrations in both dwarfing rootstock combinations accumulated in tissues above the graft union.

Carbohydrate concentrations in dwarfing rootstock combinations accumulated above the graft union, reducing storage in lower portions of the tree. This suggests that there could be a carbohydrate stress associated with reduced carbohydrates available for expansion of the root system. A smaller root system can affect shoot growth, predisposing the scion to a dwarfed growth habit. These effects may be magnified as the tree matures, negatively influencing carbohydrate storage for subsequent growth and fruit production.

Appendix

Figure A1. Daily air temperatures (A), precipitation (B), maximum soil temperature (C), and total solar radiation (D) recorded by the MAWN weather unit at HTRC, East Lansing, MI during the growing season of 2002.

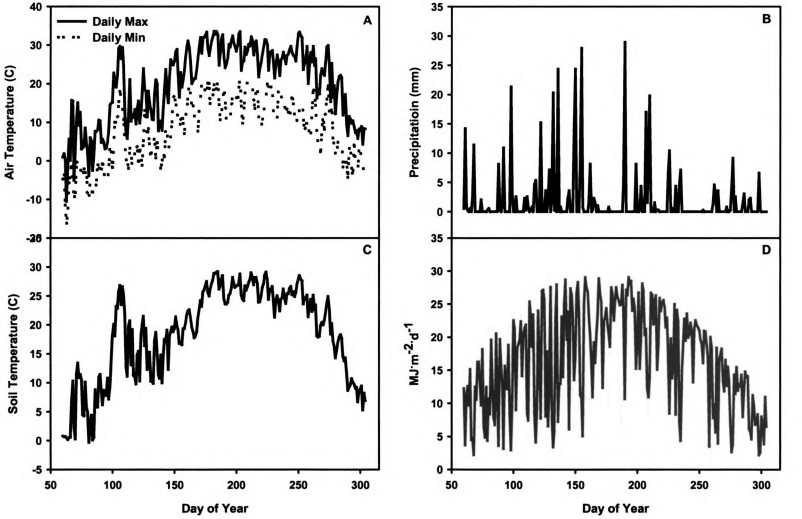


Figure A2. Daily air temperatures (A), precipitation (B), soil temperature (C), and total solar radiation (D) recorded by the MAWN weather unit at HTRC, East Lansing, MI during the period of maximum shoot elongation of 2003.