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EVALUATION OF NITROGEN-FERTILIZER UPTAKE, NITROGEN-USE AND WATER-USE EFFICIENCY IN SWEET CHERRY (*Prunus avium* L.) ON DWARFING AND STANDARD ROOTSTOCKS

presented by

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has been accepted towards fulfillment
of the requirements for the

Ph.D degree in Horticulture

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March 18, 2004

Date

MSU is an Affirmative Action/Equal Opportunity Institution

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By

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Horticulture

2004

ABSTRACT

EVALUATION OF NITROGEN-FERTILIZER UPTAKE, NITROGEN-USE AND WATER-USE EFFICIENCY IN SWEET CHERRY (*Prunus avium* L.) ON DWARFING AND STANDARD ROOTSTOCKS

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Optimum management of nitrogen (N) and water is of critical importance in order to maintain growth and high production of fruit trees in modern orchards. The objectives of this dissertation were to evaluate in sweet cherry on dwarfing and standard rootstocks: 1) the N-fertilizer uptake efficiency, and nitrogen-use efficiency (NUE), at different phenological stages; 2) the water-use efficiency (WUE) under non-limiting water availability and water deficit condition, and 3) the effect of water deficit on growth and physiological parameters. N-fertilizer uptake efficiency, NUE, and WUE were evaluated five times during the growing season on one-year-old potted sweet cherry cv. 'Rainier', grafted on the dwarfing rootstock 'Gisela 5', the semi-dwarfing rootstock 'Gisela 6', and the standard rootstock 'Mazzard'. Also the same rootstocks without scion were compared. N-fertilizer uptake was influenced by the accumulation of dry matter and was higher from rapid shoot growth until the beginning of leaf senescence. Overall, there were no differences in N-fertilizer uptake between dwarfing and standard rootstocks. NUE was significantly higher in 'Mazzard' compared to either the dwarfing or the semi-dwarfing rootstocks without scion. Values of NUE were similar for 'Mazzard', and cv. 'Rainier' grafted on dwarfing, semi-dwarfing, and standard rootstocks, in all the periods considered. WUE was higher in the standard rootstock without scion, compared to both dwarfing rootstocks without scion. N-fertilizer uptake and NUE were also evaluated in

field-grown, five-year-old sweet cherry cv. 'Sam' grafted on 'Mazzard' and 'Gisela 5'. $K^{15}NO_3$ was applied at full bloom, rapid shoot growth, and at the beginning of leaf senescence. N-fertilizer was absorbed in greater amounts when applied at bloom or at rapid shoot growth than at the beginning of leaf senescence. When N-fertilizer was applied at bloom, the percent of N-fertilizer was higher in leaves of sweet cherry on dwarfing than standard rootstocks indicating that N-fertilizer contribute more to the total N of dwarfing trees than standard trees. NUE, as well as N retranslocation from senescent leaves, did not differ between the rootstocks. Plant growth and gas exchange parameters, water-use efficiency and leaf carbon isotope composition were evaluated on one-year-old potted sweet cherry cv. 'Rainier' grafted on 'Mazzard' and 'Gisela 5' under two different water treatments: a) well-watered (control), which received 100% of the amount of water lost by ET, and b) water deficit treatment, which received 50% of the water applied to the control. Gas exchange parameters were affected earlier than growth parameters. Growth parameters measured in sweet cherry on standard and dwarfing rootstocks were affected similarly. Cumulative leaf area was the first growth parameter to be affected by water deficit. WUE was not significantly different between rootstocks, and did not appear to increase under water deficit condition, indicating that irrigation should be considered as an important practice in sweet cherry orchards, especially when dwarfing rootstocks are selected.

DEDICATION

This thesis is dedicated to my husband Daniele and my mother Piera

for their continuous support and encouragement.

ACKNOWLEDGMENTS

I would like to thank my major professor and mentor Dr. Jim Flore for his guidance, support, and for believing in me over these years. I am especially thankful for his friendship, his enthusiasms and creativity that have been an incredible source of encouragement during my graduate experience at MSU.

I would like to express my gratitude to the members of my graduate committee Dr. Phil Robertson, Dr. Ken Poff, Dr. Ron Perry, and Dr. Eric Hanson. In particular, I would like to thank Dr. Phil Robertson for the valuable discussions, for his advises, and for giving me confidence throughout the project; Dr. Ken Poff for his friendship and his constant reminders to 'think outside the box'; Dr. Ron Perry for his friendship, insight and support throughout the years, and Dr. Eric Hanson for his help during the experiments and for keeping his door always open for advises. I'm also very grateful to Dr. Martin J. Bukovac for the numerous valuable discussions and Prof. Bruno Marangoni for encouraging me to pursue a Ph.D. at MSU.

Special thanks must be extended to Adriana Nikoloudi for her friendship and her tireless help throughout the years – thank you.

I would like to thank Dario Stefanelli, Roberto Zoppolo, Marlene Ayala, Randall Vos, Judith Nyiraneza, John Sorochan, and Royal Fader for their friendship and their help during my graduate studies at MSU.

My sincere gratitude goes also to my family and my husband Daniele for their understanding and support.

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KEY TO SYMBOLS OR ABBREVIATIONS

Symbol	Parameter	Unit
A	net carbon dioxide assimilation rate	$\mu mol\ CO_2\ m^{-2}s^{-1}$
ABA	abscissic acid	
ATP	adenosine tri-phosphate	
C_3	three-atom carbon cycle	
C_4	four-atom carbon cycle	
C_a	ambient air CO_2 concentration	$\mu L\ L^{-1}$
CAM	crassulacean acid metabolism	
C_i	leaf internal CO_2 concentration	$\mu L\ L^{-1}$
$\delta\ ^{13}C$	plant carbon isotope composition	‰
$\Delta\ ^{13}C$	plant carbon isotope discrimination	‰
DABB	days after bud break	
DAFB	days after full bloom	
DW	dry weight	
E	transpiration rate	$mol\ H_2O\ m^{-2}\ s^{-1}$
ET	evapotranspiration	$L\ plant^{-1}\ day^{-1}$
Gi5	Gisela 5	
Gi6	Gisela 6	
GOGAT	glutamate synthase	
GS	glutamine synthetase	
g_s	stomatal conductance	$mol\ H_2O\ m^{-2}\ s^{-1}$

LSMEANS...	Least-Squares means	
M.....	Mazzard	
NDFF.....	nitrogen derived from fertilizer.....	g
NFF.....	nitrogen from fertilizer.....	%
NUE.....	nitrogen-use efficiency.....	
p_a.....	leaf external partial pressure.....	MPa
p_i.....	leaf internal partial pressure.....	MPa
PDB.....	PeeDee belemnite limestone	
PEP.....	phosphoenolpyruvate	
R_a.....	$^{13}C/^{12}C$ atmospheric ratio	
R_p.....	$^{13}C/^{12}C$ plant ratio	
R/Gi5.....	Rainier/Gisela 5	
R/Gi6.....	Rainier/Gisela 6	
RLER.....	relative leaf emergence rate.....	leaves day^{-1}
R/M.....	Rainier/Mazzard	
R_s.....	$^{13}C/^{12}C$ PeeDee belemnite ratio	
RSGR.....	relative shoot growth rate.....	cm day^{-1}
RuBP	ribulose-1,5-bisphosphate	
Rubisco.....	ribulose-1,5-bisphosphate Carboxilase/Oxigenase	
ψ_l.....	leaf water potential.....	MPa
SE.....	standard error	
S/Gi5.....	Sam/Gisela 5	
S/M.....	Sam/Mazzard	

STD.....	standard deviation	
SWC.....	soil water content.....	% (v/v)
TCSA.....	trunk cross sectional area.....	cm^2
WUE.....	water-use efficiency	

LITERATURE REVIEW

LITERATURE REVIEW

Introduction

Sweet cherries (*Prunus avium* L.) are one of the commercial fruit trees with the highest economical returns per acre (Lang 2000; USDA, 2002, www.usda.gov). Together with traditional standard rootstocks, new selections of sweet cherry dwarfing rootstocks are commercially available. Selection of the appropriate rootstock is important for maximizing the profitability of the orchard, since the vigor of the rootstock has a major impact on orchard costs such as harvest, pruning and spraying. Little is known on how rootstocks cause a dwarfing growth habit and on the effects of dwarfing rootstocks on nitrogen (N) uptake and use efficiency, and on water use efficiency. Nitrogen is a major component of amino acids and proteins and represents 2 to 5% of the plants dry weight (Marschner, 1995). Understanding the seasonal pattern of N uptake in fruit trees can be of great value for optimizing the timing of N fertilization. Physiological base process that influence plant N utilization such us the nitrogen use efficiency (NUE) could also be affected by the rootstock since NUE is based on the relation between plant N content and growth rate (Small, 1972). Optimization of N-fertilizer uptake efficiency, as well as the utilization of plants with high NUE, could reduce fertilizer costs and amount of nutrient loss, lowering the negative effects of N application on soil, water and air quality. Understanding how rootstocks respond to water deficit is essential for selecting the proper rootstock, or the proper management practices when drought stress is likely to occur. Plant parameters such as growth, gas exchange and water relations are affected by water deficit conditions and among them, leaf formation and expansion were one of the most sensitive plant parameters to water stress, in peach trees (Olien and Flore,

1990). Leaf carbon isotope composition is also influenced by water stress and in particular is correlated with water use efficiency as shown in several crops (Farquhar et al., 1982; Knight et al., 1994). The main objective of this research is to compare standard and dwarfing sweet cherry rootstocks in terms of N-fertilizer uptake efficiency, NUE and WUE.

Importance of cherry production in United States and the World

Among fruit trees and nut, sweet cherry fruit production is ranked as the eighth most valuable production in US (USDA, 2002). The largest producer of sweet cherries is Iran (13% of the world total production), followed by United States (12%), Turkey (12 %), Italy (8%), and Germany (7%) (USDA, 2002). During 1997 to 2001, US sweet cherry production averaged 210,000 tons. Sweet cherries are primarily grown in Washington State, Oregon and California, followed by Michigan, which produces about 10% of the yearly total US production (NASS, USDA 2002, www.nass.usda.gov/wa/swtchery.pdf). The average value of sweet cherry production in Michigan was $17 million between 1996 and 1999 (NASS, USDA 2002). Many of the fruit crops in US are becoming less profitable resulting from increasing labor costs and foreign competition. Using vigorous trees with long establishment period before significant fruiting occurs can reduce the profitability of orchards (Lang, 2000). Together with traditional standard rootstocks, several selections of dwarfing or semi-dwarfing sweet cherry rootstocks are now available and can be used to increase the profitability of sweet cherry orchards.

Cherry rootstocks and physiology of dwarfing process

Cherry rootstocks

The primary commercial sweet cherry rootstocks are seedling or clonal selections of *P. avium* L., known as 'Mazzard', or *P. Mahaleb* L., known as 'Mahaleb' (Perry, 1987). 'Mazzard' and 'Mahaleb' are vigorous trees and not precocious (Webster and Schmidt, 1996). The main advantages of these rootstocks are their graft compatibility with both sour and sweet cherry, and the large availability of plant material (Webster and Schmidt, 1996).

Reduction of tree size on sweet cherry has been recently achieved with the use of intra- and interspecific hybrid clones. A successful program for breeding dwarfing rootstock was initiated by W. Gruppe in 1965, at Giessen University, Germany (Franken-Bembenek, 1996). Approximately 6000 hybrids were obtained from the cross of more than 10 different *Prunus* species (Franken-Bembenek, 1996). After several rootstock trials at different locations, 25 GI-clones were selected and among them were the clones GI 148/2, later introduced in the market as 'Gisela 5' (Gi5), and GI 148/1, introduced in the market as 'Gisela 6' (Gi6). Both Gi5 and Gi6 are hybrids between *P. cerasus* (cv. 'Schattenmorelle') × *P. canescens* (Franken-Bembenek, 1996). Franken-Bembenek (1996) reported that trunk cross sectional area of four to five-year-old Gi5 was 40 to 65 % of 'Mazzard', while the one of Gi6 was 65 to 150% of 'Mazzard'. The high variability in vigor of rootstocks tested was determined by the different climatic conditions of the locations of the trial (Franken-Bembenek, 1996). In another field trial Gi5, grafted with 15 sweet cherry cultivars, was compared in terms of vigor to 'Mazzard F12/1', grafted with five cultivars (Franken-Bembenek, 1998). After nine years, trunk cross sectional

area of Gi5 was always lower than 'Mazzard F12/1', but the intensity of the reduction depended on the cultivar considered (Franken-Bembenek, 1998). Franken-Bembenek (1998) concluded that, beside trunk cross sectional area, cultivar had a high influence on cumulative yield and yield efficiency. An extensive rootstock trial in East Malling, UK, included Gi6 as one of the genotypes tested with sweet cherry cultivars 'Van' and 'Merton Glory' (Webster and Lucas, 1997). Trees on Gi6 were precocious with high yield efficiency and, only with cv. 'Van', smaller than the standard 'Colt' (Webster and Lucas, 1997). Webster and Lucas (1997) concluded that Gi6, although very promising in terms of precocity and total yield, may exhibit different dwarfing potential depending on the scion cultivar used. Gi6 and Gi5 have been reported to have low suckering intensity, medium frost hardiness, and high waterlogging tolerance for Gi6, while only medium waterlogging tolerance for Gi5 (Franken-Bembenek, 1996). Besides Gi5 and Gi6, other promising dwarfing or semi-dwarfing rootstocks include Edabriz, and the series Maxma and Weiroot (Webster and Schmidt, 1996; Lang, 2000)

Characteristics of dwarfing rootstock

Tree size can be controlled by dwarfing rootstocks or compact scion cultivars (Bargioni, 1996). Dwarfing rootstocks offer flexibility in growth control with various scion cultivars (Bargioni, 1996), and have several advantages: precocity, the possibility of intensive plantings (1000 to 1700 tree ha^{-1}), and reduced costs associated with pruning, spraying, and harvest. Dwarfing trees have a limiting number of growing points and short duration of shoot extension, but can maintain high fruit yields as the tree mature. Reducing the tree size may improve the distribution of light throughout the canopy and

therefore minimize the internal shading in the tree; better interception of light is usually correlated with higher dry matter productions (Jackson, 1980). The capability of roots to acquire nutrients and water affect scion performance in terms of total production and fruit quality. The root system of dwarfing rootstocks explores a more restricted soil volume compared to standard rootstocks, and therefore dwarf trees have higher probability to be limited in water, nutrients and oxygen than standard ones. Dwarfing rootstocks may determine early senescence, reduced shoot elongation and declining number of flowering spurs on older wood with tree aging (Webster and Schimidt, 1996).

Hypotheses on dwarfing mechanism

Several hypotheses have been proposed to explain how rootstocks cause a dwarfing growth habit, but none has been fully able to explain the dwarfing mechanism. The majority of studies have been carried out on dwarfing apple rootstocks. There is a strong interdependence between rootstock and scion. Rootstock and scion before combination as one plant may have different growth rates. After grafting, the two genotypes develop a uniform growth rate, and the low growth rate of one of the two genotypes can result in a dwarf tree (Lockard and Schneider, 1981). The interdependence between rootstock and scion growth has been shown in peach seedlings by physically restricting the root volume (Richards and Rowe, 1977). Root dry weight was reduced of 39% when roots were physically restricted and at the same time top dry weight was reduced of 34% (Richards and Rowe, 1977).

Phenols have also been proposed as an important factor in the dwarfing mechanism in apple, although results have been contradictory. The amount of phenols

required to reduce growth varies with the phenol type and the plant tissue. Scholz (1957) found that the inhibiting effect of phenols extracts from interstock bark of apple trees with different vigor was correlated with the vigor of the plant sampled and with the interstock dwarfing capability. On the other side, Martin and Williams (1967) found that certain phenols like phloridzin, were higher in the bark of the vigorous M16 than the dwarfing M9 indicating that phenols in general did not have a direct effects on the reduced growth rate of M9.

The proposed mechanisms for dwarfing dwarfing growth habit induced by rootstock in apple tree by Lockard and Schneider (1981) is based on the communication between shoot and root through plant hormones. Auxins produced in the shoots move to the roots, and promote root growth (Goodwin et al., 1978). Part of auxins flowing basipetally through the phloem are degraded in the bark by indoleacetic acid oxidase, peroxidase, and phenols present in the phloem and cambial cells. An inadequate supply of auxin to the roots decreases root growth (Beever and Woolhouse, 1975), reducing the amount of cytokinins synthesized by the root system. Cytokinins produced in the roots, are translocated to the shoots, where they influence shoot growth (Goodwin et al., 1978) and auxins production, in proportion to their amount. Since bark of different plants contain different amounts of indoleacetic acid oxidase, peroxidase, and phenols, different quantities of auxins will reach the root system, causing different tree size. Cytokinins exert some controls over gibberellins metabolism in the shoots, which also influence shoot growth (Lockard and Schneider, 1981). In general, no consistent relationship has been found between gibberellin content (Graebe and Ropers, 1978) and abscisic acid (ABA) content in different plant tissues and the dwarfing mechanism in plants, therefore

Lockard and Schneider (1981) considered the involvement of gibberellins and ABA to be less important than the one played by auxins and cytokinins in the dwarfing mechanism.

In general, besides the use of dwarfing rootstocks to control tree growth, dwarf plants can be obtained by using dwarfing rootstocks as interstocks between scions and rootstocks, or by increasing the height of budding and grafting (Parry, 1986). Dwarfing interstocks are used when soil conditions are not optimal for the dwarfing rootstock itself or to allow a better tree anchorage.

The use of dwarfing cherry rootstock clones as interstock was less successful and effective than for apple (Webster, 1998, Webster and Schmidt, 1996). Inconsistency on the effect of height of budding on obtaining dwarf plants also suggests that dwarfing effect on scion in sweet cherry could have a different mechanism of growth control compared to the one operating in apple trees (Webster, 1998). Webster (1998) suggested that rootstock control on scion vigor in sweet cherry is perhaps more related to the physiology of the root system than to the stem of the rootstock.

Effect of cultivar and rootstock on leaf nutrient concentration

Foliar mineral concentrations can be affected by scion and rootstock (Ferree, 1998; Kruczyńska et al., 1990; Ponchia et al., 1997; Rom et al., 1995; Tagliavini et al., 1992). Comparison of plant nutritional status, yield efficiency (kg of fruit cm^{-2} of trunk cross sectional area), and graft compatibility of 'Bartlett' pears grafted on rootstocks with different vigor evidenced little differences on leaf nutrient content, including N (Chaplin and Westwood, 1980).

Year-to-year variations of leaf mineral concentration were more important than different rootstock and interstem combinations in determining final leaf mineral concentration of 'Golden Delicious Smoothee' (Ebel et al., 2000). Little differences in N, P, Fe, Zn, and B were found in leaves of dwarfing and semi-dwarfing apple rootstocks in the different years, while K, Ca, and Mn varied during the five-years experiment, but not consistently (Ebel et al., 2000). Lord et al. (1985) evaluated leaf nutrient level of different apple rootstocks and interstock/rootstock combinations, with different vigor. Although nutrient levels differed in the combinations tested, it was impossible to draw any conclusion regarding a specific combination effect on leaf mineral concentration due to the inconsistency of results between years (Lord et al., 1985).

Leaf nutrient content of 'Montmorency' sour cherry grafted on standard rootstocks 'Mazzard' and 'Mahaleb' was compared in a four-year study, in two locations, in Michigan (Hanson and Perry, 1989). Leaf N, K, Ca, Mg, B, and Mn concentrations were affected by rootstocks and locations (Hanson and Perry, 1989). Leaf nutrient levels did not appear to be related to crop load or tree vigor (Hanson and Perry, 1989). Hanson and Perry (1989) speculated that although root distribution pattern played a role in nutrient uptake, it could not fully explain the differences. Differently, crop load seemed to be the cause of different leaf mineral concentrations obtained in sweet cherry cv.'Bing' on rootstocks with different vigor, in a four-year study, in two locations (Nielsen and Kappel, 1996). Yield efficiency was higher in dwarfing trees GM 9, GM 61/1, Gi6, Gi 195/1 and Gi196/4 than standard trees (Neilsen and Kappel, 1996). Rootstocks with higher yield efficiency than 'Mazzard' had lower K and Mg than 'Mazzard', while the effect of higher yield efficiency on leaf N concentration was less evident than for K and

Mg (Nielsen and Kappel, 1996). Among the genotypes with lower N concentration than 'Mazzard', only GM 61/1 and GM 9 had also a lower trunk cross sectional area than 'Mazzard' (Nielsen and Kappel, 1996). Nielsen and Kappel (1996) speculated that the inadequate nutrition could have been the reason, in part, for lower tree size.

Nitrogen in the plant

Forms of N absorbed by plants

The three major forms of inorganic N present in soils are nitrate (NO_3^-), ammonium (NH_4^+), and organic nitrogen. Fruit trees satisfy their N requirement mostly by taking up NO_3^- and NH_4^+. The reliance of plants on one form or another varies with the relative availability of the two forms and transformation of N in the soil, with environmental conditions, and plant species characteristics (Titus and Kang, 1982). Nitrogen absorption by trees is affected by light (Frith and Nichols, 1975), soil pH, temperature and mineral composition, as well as carbohydrate supply to the roots (Marschner, 1995). Attempts to compare the importance of NO_3^- and NH_4^+ nutrition under field and controlled environment conditions have not been conclusive, and results are often contradictory. Uptake and assimilation of NO_3^- and NH_4^+ require metabolic energy in the form of ATP. However, there is a different demand of ATP and carbon skeletons for the uptake of the two N forms: two ATP are required per NH_4^+ absorbed, while assimilation of NO_3^- requires 12 ATP (Bloom, 1997). Uptake of NO_3^- by roots depends on the concentration of NO_3^- in soil solution, on the volume of soil exploited by roots, and on the efficiency of roots to absorb NO_3^- (Engels and Marschner, 1995). At

low NO_3^- concentrations, high affinity NO_3^- transporters are required, together with more transporters per unit root surface, and with greater root density (Lawlor, 2002).

The form of N absorbed by the plants has a great influence on the cation/anion uptake ratio and thus on the rhizosphere pH. Nitrate uptake is correlated with a higher rate of HCO_3^- or OH^- net release (or H^+ consumption) that causes an increase in the pH of the rhizosphere while NH_4^+ uptake is correlated with a higher rates of H^+ release, which causes a decrease in the pH of the rhizosphere (Marschner, 1995). As a result, in neutral or alkaline soils, rhizoshere acidification in plants fed with NH_4^+ may enhance mobilization of sparingly soluble calcium phosphate, and favor the uptake of P, Fe, Mn, and Zn. On the other side, in acidic soils the increase of pH induced by NO_3^- uptake may enhance P uptake. Rhizosphere pH may differ from the bulk soil pH up to two units, depending on root-induced changes and soil factors like pH buffering capacity (Marschner, 1995).

There are several organic N forms taken up by plants, such as urea and several amino acids. Shim et al. (1973a) demonstrated that young apple trees can absorb ^{14}C-urea after only two hours from its application. Urease is the enzyme responsible for the hydrolysis of urea in ammonia and carbon dioxide. Shim et al. (1973b) found urease in apple leaves, roots, and bark, and roots showed the highest urease activity. In general, the uptake of amino acids is considered an adaptation to ecosystems with limited availability of N. Spencer and Titus (1971) showed that glutamate and aspartate were readily taken and metabolized by one-year-old apple trees. Recent studies on non-mycorrhizal *Pinus sylvestris* have confirmed that the amino acid uptake rate was comparable to the uptake rates of NO_3^- and NH_4^+ (Persson and Näsholm, 2001). Little is

known about the concentration of amino acids in the soil solution therefore it is difficult to estimate its contribution to the total N in plants.

Assimilation and translocation of nitrate and ammonium

In order to be assimilated, NO_3^- needs to be reduced to NO_2^- via nitrate reductase, which is localized in the cytoplasm (Marschner, 1995). Furthermore, NO_2^- needs to be reduced to NH_4^+ by the enzyme nitrite reductase, localized in chloroplasts in leaves, or in proplastids in roots (Marschner, 1995). Nitrate can be assimilated in the root or translocated via xylem into the shoot. The amount of NO_3^- translocated from the root to the shoot depends on the site of its reduction. Nitrate reduction in apple trees occurs preferentially in roots (Frith, 1972), and more specifically in fine roots (Eckerson, 1931). Nitrate has been detected in apple leaves under high availability of NO_3^- in the soil or medium, or under high concentrations of NH_4^+ in the medium. Under high availability of NO_3^- nitrate reductase in the roots is saturated and NO_3^- is translocated to the aerial part of the plant (Titus and Kang, 1982). Under high availability of NH_4^+ there is a feedback inhibition of the activity of root enzymes but not of the leaf nitrate reductase activity (Frith, 1972). Nitrate reductase has also been detected in leaves of apricot, sour and sweet cherry, plum, pear, grapevine, and walnut (Leece et al., 1972; Pérez and Kliewer, 1978). When pear, sweet cherry, plum, walnut and grapevine were compared in terms of leaves nitrate reductase activity, walnut resulted to have the greatest activity while sweet cherry the least (Pérez and Kliewer, 1978).

Unlike NO_3^-, NH_4^+ is assimilated only in the roots since in plant cells even low concentrations are toxic. Ammonium absorption is a function of its assimilation into

complex compounds (Barker and Mills, 1980; Marschner, 1995). Ammonium assimilation in roots has a large requirement for carbon skeletons; therefore NH_4^+ absorption is often carbon limited. These carbon skeletons are provided by the tricarboxylic acid cycle and the removed intermediates are replenished by increased activity of PEP carboxylase (Lawlor, 2002). The NH_4^+ is converted into amino acids by the GS/GOGAT enzyme reaction (Marschner, 1995). The first amino acid product of NH_4^+ assimilation is glutamine, synthesized by the enzyme glutamine synthetase, GS (Titus and Kang, 1982). Synthesis of glutamate proceeds from glutamine in the presence of glutamate synthase, GOGAT (Titus and Kang, 1982).

Movement and accumulation of nitrogenous compounds

Upward movement of nitrogenous compounds in fruit trees occurs through the xylem (Tromp and Ovaa, 1976). Movement of N compounds in plants occur also from the xylem to the phloem tissue, in the radial direction, through ray cells and cambium (Shim et al., 1973b). The major amino acids mobilized from the roots to the shoots are aspartate and glutamate, their amide, and arginine (Titus and Kang, 1982).

Nitrate can be mobilized from the roots to the shoots via xylem and can accumulate in the leaves. Nitrate does not appear to be phloem mobile (Pate, 1980), therefore tissues with low transpiration rate will not contain high quantity of NO_3^- (Millard, 1988). Most of the NO_3^- in leaves will be accumulated in the vacuole (Smirnoff and Stewart, 1985).

Internal cycling of N in trees

Important factors that regulate year-to-year tree N status are the tree storage and remobilization capabilities. Millard (1988) considers N to be stored if it can be remobilized from one tissue and used for growth of another tissue. In deciduous trees, N is stored over the winter in roots and bark as amino acids and proteins (Millard and Proe, 1991; Tromp, 1983; Titus and Kang, 1982) while over the summer N is stored in leaves as Rubisco (Titus and Kang, 1982; Millard, 1996). During senescence, N is withdrawn from leaves and stored in woody tissue over the winter and subsequently it is remobilized and used for spring growth (Millard, 1996). RuBP carboxylase and other enzymes related to photosynthesis are the major proteins broken down during leaf senescence (Titus and Kang, 1982). This decline in enzyme leads to the general decrease in plant leaf protein that occurs during senescence (Titus and Kang, 1982).

Early season growth and bloom of deciduous trees, like grape (Conradie, 1991), apple (Nielsen et al., 1997 and 2001 a and b; Titus and Kang, 1982) and peach (Tagliavini et al., 1999; Muñoz et al., 1993) are supported by N remobilized from plant storage organs (Weinbaum et al., 1984; Millard and Nielsen, 1989). Millard (1996) reported that the ability of trees to remobilize N in spring is related to the previous year N supply, and is independent of the current season N supply. Root N uptake becomes increasingly important as the season progresses. Weinbaum et al. (1978) found that in non-bearing prune (*P. domestica* L.) trees N-fertilizer was absorbed in greater amount when supplied after the beginning of rapid shoot growth. In grape, N absorption is most rapid between bloom and veraison (Williams, 1987 a and b) while between veraison and harvest, N uptake tends to decline (Conradie, 1991).

Nitrogen fertilization in fruit trees

Application of nitrogen (ground or foliar) influences plant growth and development during the current and subsequent season (Weinbaum, 1987). Several factors affect fertilizer use efficiency such as plant nutrient demand (Weinbaum et al., 1992), form of nutrient applied (Barker and Mills, 1980), application method (Sanchez et al, 1995), and the rate and timing of application (Taylor et al., 1975; Conradie, 1991).

Fertilizer use efficiency is defined as the fraction of applied fertilizer that is absorbed and used by a specific plant (Weinbaum et al., 1978). Fertilizer not absorbed by the target plant can be taken up by weeds, lost in the atmosphere as gases, incorporated into stable organic fractions in soil, or leached below the rooting zone (Robertson, 1997).

The synchrony between plant N demand and N soil availability is of critical importance for increasing fertilizer use efficiency. The seasonal pattern of uptake and N accumulation in trees reflects N demand and can be used for timing N application in order to maximize N-fertilizer uptake (Weinbaum et al., 1992). N uptake during the season, and partitioning of N to different plant organs, has been investigated in several fruit tree species (Weinbaum et al., 1978, 1984; Muñoz et al., 1993; Policarpo et al., 2002; Sanchez et al., 1992). During spring, bud break takes place when conditions for root uptake are not always optimal and there is a lack of new carbon skeletons necessary for the synthesis of amino acids. At this time, N remobilization is of critical importance in supplying N to the developing tissues. In a study on 9-year old walnut (*Juglans regia* L.) trees, Weinbaum and van Kessel (1998) determined that approximately 60% of the annual N demand derived from N redistribution from internal pools, and the remaining part was met by N from fertilizer-soil pool. Nitrogen applied after bloom or during rapid

shoot growth satisfied the N demand of new growth in grapes (Conradie , 1991), prune (Weinbaum et al., 1978), peach (Policarpo et al., 2002), pears (Sanchez et al., 1990) and apple (Neilsen et al., 2001 a and b). Kinetic of N uptake was assessed in late and early maturity peach (*P. persica*) cultivars under a Mediterranean climate (Policarpo et al., 2002). Uptake was relatively low for the first month after bud burst (approximately 30% of the N applied), and then it increased to 80-90% of the N applied during the rest of the season, until leaf senescence when it slightly decreased (Policarpo et al., 2002). Leaves were the major N sink during the majority of the season but after summer N was partitioned mainly to tree permanent organs, and specifically to the coarse roots (Policarpo et al., 2002). Even if the two cultivars were characterized by different phenology, they absorbed a similar amount of N over the season and they had similar N partitioning (Policarpo et al., 2002). It has been reported in many fruit crops that after shoot growth termination, leaves switch from being a strong sink, to be a source of N for reproductive organs (Millard, 1988). Crop load of the tree determine the potential size of the fruit sink. Late season applications increase the N storage pool that will be remobilized in the following year (Millard and Thomson, 1989; Sanchez et al., 1991). Nitrogen fertilization during summer or post-harvest was more efficient than N application at bud break for 'Thompson Seedless' grape (Peacock et al., 1989).

Carbohydrate and N metabolism

Carbohydrate and N metabolism are closely related in all phases of plant growth and development. Neilsen et al. (2001a) in young apple trees on M9 dwarfing rootstock indicate that crop load effects on N partitioning to fruit and shoot leaves were related to

dry matter partitioning and therefore N uptake was considered closely coupled with carbon supply.

The major leaf protein is Rubisco, which catalyses the reaction between CO_2 and RuBP giving rise to triose phosphate, and also oxygenate RuBP through photorespiration in C_3 plants (Lawlor, 2002). Rubisco has low catalytic rate therefore C_3 plants required it in large amount (Lawlor, 2002). Lawlor et al. (1989) evaluated that the amount of Rubisco in wheat flag leaves was approximately 7 g m^{-2} of leaf, which constituted 30% of the total N and up to 50% of the soluble leaf protein.

The pentose phosphate pathway, glycolysis, and the tricarboxylic acid cycle are closely coupled to amino acids biosynthesis with regards to the supply of carbon skeletons and energy. Because carbon and N are acquired separately by leaves and roots respectively, the study of how their metabolism is coupled provide information about physiological integration at the whole plant level (Garnier and Roy, 1994).

Environmental problems related to the utilization of mineral N

Of all the essential elements, N is the most commonly applied in orchards, and at the greatest rate. Excess of N stimulates vigorous growth, and therefore shading within the tree, which negatively affects flower bud development, fruit set, fruit quality, and sometimes delays fruit maturity (Weinbaum et al., 1992). Excess N causes late season growth, resulting in higher winter injury for the tree. Overfertilization is associated with high levels of residual nitrate in the soil, which potentially contribute to groundwater and atmospheric pollution, as a result of leaching and denitrification (Weinbaum et al., 1992;

Merwin et al., 1996). Nitrogen is widely regarded as responsible for the hypoxia (low oxygen) zone in the Gulf of Mexico (Keeney and Hatfield, 2001).

Efficient use of N fertilizer in crop production can reduce costs and minimize the detrimental effects of N movement into surface or ground water (Throop and Hanson, 1997). High leaching losses of nitrate occur when the N fertilizer rate is not adjusted to the crop N demand (Weinbaum et al., 1992) and does not consider the level of available nitrogen in soil.

Nitrogen-use efficiency: definition and importance

Nitrogen-use efficiency (NUE) is defined as the amount of dry matter produced per unit of N taken up (Robertson, 1997). Several indicators have been used to evaluate NUE in plants and among them are the plant C:N ratio (Maranville and Madhavan, 2002), the inverse of N concentration in plant biomass (Chapin, 1980; Nakamura et al., 2002; Tateno and Kawaguchi, 2002), or the amount of utilizable plant material (seed, grain, fruits, forage) per amount of absorbed N (Baligar et al., 2001; Maranville et al., 1980). High plant C:N ratio indicates high plant NUE. In non-cultivated ecosystems NUE appears to be higher where soil N availability is low (Robertson, 1997). Nitrogen-use efficiency has been evaluated in different plants such as barley (Gonzales Ponce et al., 1993), rice (Cassman et al., 1993), and wheat (van den Boogaard et al., 1995). Nitrogen fertilization in general leads to lower plant C:N ratios as shown by the failure to increase the crop yield with addition of N above a plant-specific saturation level (Robertson, 1997).

In deciduous trees NUE could also be defined as the amount of dry matter loss per unit of N lost in the litterfall. The withdrawal of N from senescent leaves allows the use of the same unit of N used during the current year, to be reused for new plant organs successively (Clark, 1977; Turner, 1977). Nitrogen-use efficiency calculated in litterfall of forested ecosystem appears to be correlated with the availability of N in the ecosystem (Vitousek, 1982). Sites where symbiotic nitrogen fixers are dominant, have a relatively low NUE of litterfall since N-fixers have potentially unlimited availability of N (Vitousek, 1982). Sites relatively poor in N have relatively low N concentration in the litterfall and therefore high NUE; in such sites, NUE normally decrease when N is supplied by fertilization (Turner, 1977; Vitousek, 1982).

Several hypotheses have been formulated to explain the higher NUE in litterfall of trees on low-nutrient sites. Trees may be able to fix more carbon per unit of N and this could be achieved by using the same unit of N to fix carbon over time like in case of evergreen (Shaver, 1981). However, trees with higher NUE may have a higher retranslocation of N from leaves prior to abscission, which would determine a higher litterfall mass:nitrogen ratio (Vitousek, 1982). Another important aspect of the litterfall C:N ratio regards its effect on availability of N in the ecosystem: with high C:N ratio (higher than 20:1), decomposers are N limited and retain N in their biomass while with low C:N ratio (between 12-20:1), decomposers tend to release N into the soil solution (Vitousek, 1982). This mechanism is considered the cause of the reduced N availability in low-nitrogen site, where litterfall has the tendency to have a high C:N ratio (Vitousek, 1982).

Photosynthetic NUE is calculated as the ratio between net photosynthesis (CO_2 assimilated) and leaf nitrogen content (Field et al., 1983). In general C_4 plants have a higher photosynthetic NUE than C_3 plants (Monson, 1989). There are substantial differences between C_3 and C_4 plants in the photosynthetic components content of leaves. In C_4, Rubisco functions only as a carboxylase and therefore these have a higher rate of CO_2 fixation and require less Rubisco compared to C_3 plants. In C_3 plants, Rubisco functions as both carboxylase and oxygenase and part of the carbon fixed is lost through photorespiration. In C_3 plants 30 to 60% of the soluble proteins in leaves is Rubisco, while in C_4 plants only 5-10% of soluble protein is Rubisco (Marschner, 1995). Consequently, the N-content per unit of leaf is smaller in C_4 than C_3 plants, and the N-requirement is less to achieve the same production.

Physiology of water deficit

Water deficit, or water stress, refers to conditions in which plant water potential and turgor are reduced and affect normal functioning of the plant (Kramer, 1983); it develops in situations where water loss by transpiration exceeds absorption by the root system. Plant water deficit can be described on a daily basis, as a midday water deficit (Kramer, 1983; Werber and Gates, 1990). The increase in transpiration rate during the day causes a decrease in leaf water potential (ψ_L) and turgor; during the afternoon, stomata begin to close, transpiration rate decreases and absorption of water continue until leaf parenchyma cells are refilled and their water potential rises again (Kramer, 1983). Long-term water deficit begins with a daily cycle, which is altered by the inability of the plant to recover the water lost during the day. Plant permanent wilting occurs when soil

water potential values decreases to –1.5 MPa (Kramer, 1983). Plants can experience drought when soil water content is limiting, when atmospheric water content declines, or when both condition are present. Water deficit have implications for plant growth, physiological process, and water relations (Hsiao, 1973; Jones at al., 1985).

Plant water relations

Plant water balance is determined by water lost during transpiration and water absorbed from the soil (Lawlor and Cornic, 2002). Two driving forces that play a role when water moves from the soil into the roots are: 1) osmotic movement, in slowly transpiring plants, and 2) mass flow, in rapidly transpiring plants caused by tension or negative pressure in the xylem sap (Kramer and Boyer, 1995). As the rate of transpiration increases, the increasing in mass flow of water through the roots dilutes the root xylem sap until the osmotic mechanism becomes ineffective and absorption is controlled by the pressure potential in the xylem sap. In rapidly transpiring plants the xylem water potential can fall as low as –1.5 to –2.0 MPa (Kramer and Boyer, 1995).

Water potential, osmotic potential and turgor potential are the major contributors to cell growth (Boyer, 1988). When transpiration rate exceeds absorption, cell turgor falls, concentration of cellular content increases causing an increase in osmotic potential and water potential falls (Lawlor and Cornic, 2002). Within a cell, the pressure potential or turgor measures differences between internal and external pressure (Jones et al., 1985). Turgor potential is important for stomatal functioning. Low turgor potential negatively effects plant growth and stomatal conductance (Lawlor and Cornic, 2002). Maintenance of turgor under water deficit is a condition that has been associated with increasing

osmotic potential (Jones et al. 1985; Lakso, 1983). Plants can adjust their osmotic potential in a passive way e.g. by changing the partitioning of water between symplast and aposplast or in an active way by increasing the number of solute molecules (Jones et al., 1985). Osmotic adjustment is found to play a bigger role in maintaining the turgor of cells when plants undergoes slowly to water stress, like in situations of field-grown plants (Jones et al., 1985; Ranney et al., 1991 a and b). Osmotic adjustments have been observed in leaves and roots of cherry (Ranney et al., 1991a), and leaves of peach (Young et al., 1982).

Effect of water deficit on plant growth parameters

Processes affected by water deficit in fruit trees include cell division and elongation, flower bud differentiation, and partitioning of carbohydrates among organs (Faust, 1989). In general, under water deficit, leaf area is reduced but specific leaf weight (weight per unit area), thickness of cutin, and amount of wax on the leaf surface sometimes increase (Kramer, 1983). Shoot growth is reduced more than root growth, therefore root to shoot ratio is usually increased (Kramer, 1983). Shoot and leaf expansion are among the most sensitive plant parameters to be affected by decreases in ψ_L. Andersen and Brodbeck (1988) found that as water deficits developed in peach trees, shoot length and leaf expansion rate are inhibited before reduction in assimilation rate (A) and stomatal conductance (g_s) takes place. In another study on peach trees, growth parameters, like leaf expansion and leaf emergence rates were more sensitive than g_s and ψ_L to water stress in peach trees (Olien and Flore, 1990). In plants with commercial

importance, the effects of water deficit have negative impact on yield and fruit quality (Webster and Looney, 1996).

Effect of water deficit on physiological and morphological parameters

Closure of stomata during water stress can be responsible for the observed reduction in photosynthetic rate (Flore and Layne, 1996). Stomatal movements are regulated by the turgor of the guard cells which are affected by environmental factors such as light intensity, CO_2 atmospheric concentration, humidity, wind, and temperature, and by endogenous factors such as plant hormones, leaf water status, and internal CO_2 (Jones et al., 1985; Kramer and Boyer, 1995; Hetherington and Woodward, 2003). Root-produced ABA transported to leaves is believed to be the signal from drying roots that causes stomatal closure under water deficit (Davies et al., 1994; Davis and Zhang, 1991). Wartinger et al. (1990) showed that in almond trees under a drying cycle, as xylem ABA increased, leaf conductance decreased. At the same time, daily courses of ABA concentration in xylem sap in almond trees did not appear related to stomata conductance, perhaps because of the narrow range of ABA concentration able to affect stomata conductance (Wartinger et al., 1990). Not all authors agree on the role of ABA in stomata closure. It has been observed that stomata stay open in leaves with high ABA or remain closed even after ABA concentration has decreased (Beardsell and Cohen, 1975). Severe drought stress in kiwifruit determined reduction in stomatal aperture and decrease in overall growth (Buwalda and Smith, 1990). At the biochemical level, water deficit results in a decrease in starch and increase in sugar content of the cells. Nitrogen

metabolism is disrupted; protein hydrolysis occurs and amino acids, especially proline, accumulate (Kramer and Boyer, 1995).

A wide range of morphological and physiological characteristics in response to water deficit is affected by rootstock, scion, and their interaction (Lockard and Schneider, 1981). Rootstocks influenced transpiration rate and WUE in peach (Bongi et al., 1994), and leaf water potential in peach (Young and Houser, 1980) and apple (Olien and Lakso, 1984). Root ability to uptake water is a function of morphological and physiological characteristics of the root system. Increasing the volume of soil explored by roots leads to higher resistance of plant against stresses such as nutrient deficiency, drought and anoxia (Buwalda and Smith, 1990). Factors that influence plant hydraulic resistance are the total length and density of the root system (Jones et al., 1985). Rootstocks can affect hydraulic resistance as observed in apple and citrus (Jones et al., 1985). Cherry trees are reported to respond to low water potential by dropping leaves and by substantial osmotic adjustments (Longstrogh and Perry, 1996). Seedling rootstocks like 'Mazzard' and 'Mahaleb' are deep-rooted and can tolerate drought conditions. In areas where drought prevails, 'Mahaleb' should be preferred to 'Mazzard' for its higher tolerance to water deficit (Longstrogh and Perry, 1996).

Water-use efficiency: definition and importance

The availability of water for irrigation will be reduced in the future because of increasing competition for water for urban use, increasing depth of water table due to over pumping aquifers, and increasing problems of salinization of soils due to irrigation.

Water-use efficiency of plants will be of critical importance in agricultural systems with limited water availability.

Tanner and Sinclair (1983) reviewed different relationships that can be used to characterize plant WUE. Most researchers describe WUE as the yield of crop produced, either the total biomass or only the marketable biomass, per unit of water evapotranspired (Kramer, 1983; Harfield et al., 2001).

Physiologists prefer to express WUE as milligram of CO_2 per gram of water transpired:

$$WUE = \frac{\text{net } CO_2 \text{ uptake in mg or g} \quad (A)}{H_2O \text{ transpired in g or kg} \quad (E)}$$

Sometimes the term 'intrinsic water-use efficiency' is used when WUE is calculated by the ratio between the instantaneous rates of CO_2 assimilation (A) and transpiration (E) (Condon at al., 2002; Field et al., 1983).

There is extensive evidence that WUE varies among species in the same environment and among climate for the same species (Tanner and Sinclair, 1983; Loomis, 1983). A genotype may have a greater WUE than another if it has: 1) higher A and similar E; 2) similar A and lower E; 3) higher A and E, but proportionally higher A than E; 4) lower A and E, but proportionally lower E than A (Patterson et al., 1997).

Water-use efficiency is also affected by leaf age as shown in a study on young grapefruit trees (Syvertsen, 1985). When young grapefruit leaf expanded and matured, net photosynthesis and mesophyll conductance declined while little changes occurred in leaf stomatal conductance, determining a decline in WUE (Syvertsen, 1985).

Leaf ^{13}C composition: effect of plant water deficit

Approximately 1.11% of the carbon in the biosphere is in the form of the stable isotope ^{13}C (Boutton, 1991). Carbon isotope composition of plant tissue ($\delta\ ^{13}C$) is a measure of the $^{13}C/^{12}C$ ratio in a plant, relative to the value of the same ratio in the international standard, the limestone PeeDee Belemnite (PDB). Thus:

$$\delta^{13}C_{PDB}\ (‰) = (R_p/R_s - 1) \times 1000$$

where R_p is the $^{13}C/^{12}C$ ratio measured in plant material and R_s is the ratio of the standard (Farquhar et al., 1982). Plants have negative values of $\delta^{13}C$ due to the fact that $^{13}C/^{12}C$ ratio in the atmosphere is less than that of PDB, and there is a net discrimination against ^{13}C by plants during CO_2 fixation (Farquhar et al., 1989). The discrimination of ^{13}C can occur during diffusion of CO_2 through the stomata and CO_2 fixation by the primary CO_2-fixing enzyme (O'Leary, 1988). Also some downstream fractionations associated with plant metabolic pathways and respiration are reported (O'Leary, 1988). The rate of diffusion of $^{13}CO_2$ across the stomatal pore is lower than that of $^{12}CO_2$ by a factor of 4.4 per mill (‰) (Farquhar et al., 1982). Higher plants with the conventional C_3 pathway of carbon assimilation reduce CO_2 to phosphoglycerate via the enzyme RuBP carboxylase. Rubisco discriminates against ^{13}C due to its intrinsically lower reactivity with ^{13}C, determining a $\delta^{13}C$ of about -28‰ on average in C_3 plants (Boutton, 1991; O'Leary, 1988). C_4 plants reduce CO_2 to aspartic or malic acid through the enzyme PEP carboxylase, which does not discriminate against $^{13}CO_2$ as much as RuBP carboxylase.

Therefore C_4 plants have relatively high value of $\delta^{13}C$ with a mean around -11‰ (Boutton, 1991). Plants that exhibited a CAM methabolism have intermediate values of $\delta^{13}C$ (Farquhar et al., 1982). Carbon isotope discrimination is modified by several environmental and physiological variables such as light intensity, humidity, water availability, and photosynthesis (Farquhar et al., 1989).

Carbon isotope discrimination ($\Delta^{13}C$) is a measure of the $^{13}C/^{12}C$ ratio in plant material relative to the value of the same ratio in the air on which plants feed (Farquhar and Richards, 1984):

$$\Delta^{13}C = R_a/R_p - 1$$

where R_a is the $^{13}C/^{12}C$ ratio measured in the atmosphere and R_p is the $^{13}C/^{12}C$ ratio measured in plant material. Carbon isotope discrimination has positive values, which reflect the fact that C_3 plants actively discriminate against ^{13}C during photosynthesis. $\Delta^{13}C$ and WUE are related to the ratio between concentration of CO_2 in the leaf intercellular space and the ambient air (C_i/C_a) (Farquhar et al., 1982). In C_3 plants, $\Delta^{13}C$ decreases as WUE increases when soil water is limited, resulting in more positive $\delta^{13}C$ values of plant tissues under drought stress (Knight et al., 1994; Stewart et al., 1995). Problems associated with the use of $\Delta^{13}C$ to measure WUE is that it provides no information on the magnitudes of either photosynthetic rate (A) or transpiration (E) or whether variation in $\Delta^{13}C$ is driven by stomatal conductance or photosynthetic capacity (Condon et al., 2002).

Rational and objectives of the research

Nitrogen fertilizer application influences tree growth and development during the current year, and in the following growing season (Weinbaum, 1987). The seasonal pattern of N uptake in trees reflects their N demand and can be used for timing N application in order to maximize fertilizer uptake (Weinbaum, 1992). N uptake during the season has been investigated in grape (Conradie, 1991), prune (Weinbaum et al., 1978), peach (Policarpo et al., 2002), pear (Sanchez et al., 1990), and apple (Neilsen et al., 2001b) but little is known on the efficiency of N uptake of sweet cherry at different times during the season, especially when grown on dwarfing rootstocks.

Genetic and physiological traits of plants determine their ability to utilize N under different environmental and ecological conditions (Baligar et al., 2001). Nitrogen use efficiency has been evaluated in several crops (Gonzales Ponce et al., 1993; Cassman et al., 1993; van den Boogaard et al., 1995) but there is a lack of information on the influence of dwarfing and standard rootstocks on NUE. The utilization of plants with high NUE, together with best management practices, would achieve a more sustainable agricultural system and reduced the detrimental effect of N application on soil, water and air quality.

Understanding how rootstocks adapt and respond to drought stress is essential in selecting the proper rootstock for situation where drought stress is likely to occur.
In sweet cherry, although deep-rooted rootstocks, like 'Mazzard', are considered to be more tolerant to drought than clonally propagated rootstocks, such as 'Gisela 5' (Webster and Schmidt, 1996), little is known on the effect of the vigor of the rootstock on growth

and physiological parameter when water deficit conditions occur. Also there is a lack of knowledge on the effect of dwarfing and standard rootstocks on plant WUE.

The overall objectives of this research are to:

1. Evaluate N-fertilizer uptake efficiency at different phenological stages in dwarfing and standard sweet cherry rootstocks, and determine if dwarfing rootstocks influence N-fertilizer uptake;
2. Compare NUE of dwarfing and standard rootstocks at different phenological stages, under non-limiting availability of nitrogen;
3. Compare WUE of dwarfing and standard rootstocks under non-limiting water availability and under water deficit conditions; and
4. Determine if growth and physiological parameters of dwarfing and standard trees are affected differently under water deficit conditions.

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CHAPTER 1

CHAPTER 1

N-fertilizer uptake, nitrogen-use and water-use efficiency of one-year-old swee cherry (*Prunus avium* L.) cv. 'Rainier' on standard and dwarfing rootstocks.

Abstract

Plant demand for water and nitrogen (N) vary during the season, and is stron dependent upon weather conditions, soil moisture, stage of plant development, and c load. This research was initiated to determine if dwarfing rootstocks have an influe on N fertilizer uptake efficiency, N use efficiency (NUE), and water use efficie (WUE) when compared to standard rootstocks used in the industry. We investigated N-fertilizer uptake efficiency, NUE and WUE on one-year-old potted sweet cherry 'Rainier' grafted on the dwarfing rootstocks 'Gisela 5', on the semi-dwarfing 'Gisela and on the standard rootstock 'Mazzard'. 'Gisela 5', 'Gisela 6', and 'Mazzard' with scion were also compared. N-fertilizer uptake efficiency and NUE were evaluated times during the growing season. ^{15}N was applied to the soil at a rate of 0.5 g of N container as $K^{15}NO_3$. Plants were harvested 10-12 days after ^{15}N applications. Tota percent of ^{15}N, and dry weight of the current season growth, trunk, and roots w evaluated. N-fertilizer uptake was influenced by the accumulation of dry matter and higher from rapid shoot growth until the beginning of leaf senescence. Overall, t were no differences in N-fertilizer uptake efficiency between dwarfing and stan rootstocks. NUE was significantly higher in 'Mazzard' without scion compared to dwarfing and semi-dwarfing rootstock without scion, and had comparable values with

all the rootstocks with cv. 'Rainier'. WUE was not affected by the vigor of the rootstocks when grafted with cv. 'Rainier', but it was higher in 'Mazzard' without scion, compared to both dwarfing and semi-dwarfing rootstock without scion.

Introduction

Optimum management of water and N is of critical importance in order to maintain growth and high production of fruit trees in modern orchards. Several factors affect N-fertilizer uptake efficiency, including plant N demand (Weinbaum et al., 1992), form of N applied (Baker and Mills, 1980), application methods (Sanchez et al., 1995) and the timing of application (Taylor et al., 1975). The seasonal pattern of uptake and N accumulation in trees reflects their N demand and can be used for timing N-fertilizer application (Weinbaum et al., 1992). Application of N-fertilizer in excess often causes high levels of nitrate in soils, which represents a risk for groundwater contamination, and can contribute to increase atmospheric pollution as a result of denitrification process (Weinbaum et al., 1992). Nitrogen uptake during the season has been evaluated in grape (Conradie, 1991), prune (Weinbaum et al., 1978), peach (Policarpo et al., 2002), pear (Sanchez et al., 1990) and apple (Neilsen et al., 2001) but little is known about the efficiency of N uptake during the season in sweet cherry, especially when grown on dwarfing rootstocks.

Sweet cherries are grown mainly on rootstocks that results in full sized or 'standard sized' trees. The most common sweet cherry rootstocks are 'Mazzard' seedlings (*P. avium* L.), the clonal 'Mazzard F.12/1', or 'Mahaleb' (*P. mahaleb* L.) (Webster and Schmidt, 1996). Reduction of tree size on sweet cherry has been recently

achieved with the use of intra- or interspecific hybrid clones, such as 'Gisela 5' and 'Gisela 6' (Webster and Schmidt, 1996). Dwarfing rootstocks allow high tree densities reduction of harvest and pruning costs, and promote precocity and yield capacity of trees (Webster and Schmidt, 1996).

Differences in plants physiological traits may determine their ability to utilize N under different environmental and ecological conditions (Baligar et al., 2001). Nitrogen use efficiency (NUE) is defined as the amount of dry matter produced per unit of N absorbed (Robertson, 1997). Several indexes have been used to evaluate NUE in plants such as the plant C:N ratio (Maranville and Madhavan, 2002), the leaf C:N ratio (Tateno and Kawaguchi, 2002) or the C:N ratio of other tissues of plants (Nakamura et al., 2002). Also, NUE be expressed as the amount of utilizable plant material (e.g. seed, grain, fruits, forage) per amount of absorbed N (Maranville et al., 1980; Baligar et al., 2001). High C:N ratios indicates high plant NUE. Nitrogen use efficiency has been evaluated in barley (Gonzales Ponce et al., 1993), rice (Cassman et al., 1993), and wheat (van der Boogaard et al., 1995) but little is known on the influence of dwarfing and standard sweet cherry rootstocks on NUE.

Tanner and Sinclair (1983) summarized different relationships that can be used to characterize plant water use efficiency (WUE). Most researchers describe WUE as the yield produced per unit of water evapotranspired (Hatfield et al., 2001). The higher the biomass produced per unit of water evapotranpired, the higher the WUE. Rootstocks can influence transpiration rate, WUE, and leaf stomatal conductance (Webster and Schmidt 1996). Besides plant physiological characteristics, morphology of the root system such as depth and the number of fine roots are important factors that influence water

absorption. Little is known on the effect of the vigor of rootstocks on WUE in cherry.

A better knowledge of sweet cherry tree N requirement during the sea needed in order to synchronize application of N with tree N demand, and overall in the N-fertilizer uptake efficiency. Also, a better understanding of the tree use effi of water and N, as well as N-fertilizer uptake efficiency, could be of great val decision on the best management practice to adopt when dwarfing rootstocks are us

The objectives of this study were to evaluate, on standard, semi-dwarfin dwarfing sweet cherry rootstocks, either grafted or ungrafted: 1) the N-fertilizer efficiency at different phenological stages, and 2) NUE and WUE at di phenological stages.

Materials and methods

One-year-old 'Rainier' sweet cherry grafted on the standard rootstock 'Maz the dwarfing rootstock 'Gisela 5' (*P. cerasus* (cv. Schattenmorelle) × *P. canescens* the semi-dwarfing rootstock 'Gisela 6' (*P. cerasus* (cv. Schattenmorelle) × *P. canes* and one-year-old 'Mazzard', 'Gisela 5', and 'Gisela 6' rootstocks without scion maintained outdoors, in 11 L containers, at the Horticulture Teaching and Re Center, Michigan State University, East Lansing, MI. Dormant trees were potted o 16^{th}, 2001 in a mixture of 10% silt and clay, and 90% coarse sand, were pruned to five buds and subsequently trained to three branches. Trees were watered as neede a drip irrigation system with two emitters per pot. Nutrient solution with all es macro- and micro-nutrients (13-2-13, N-P-K, 6% Ca, 2% Mg and micronutrient

applied weekly to supply 0.5 g N per container per week. Foliar iron was periodically applied (Iron chelate DP). Daily maximum and minimum air temperature and precipitation were recorded by an automated weather station located 300 m SW of the experimental plot (Michigan Automated Weather Network, http://www.agweather.geo.msu.edu, Figure A.1, Appendix A).

^{15}Nitrogen applications

Labeled ^{15}N fertilizer was applied five times during the season, at different phenological stages (Table 1.1). Four plants per genotype were used at each application time. ^{15}N was applied to the soil at a rate of 0.5 g of N per container ($K^{15}NO_3$, 10 atom % excess, ICON Services, Mt. Marion, N.Y.). Each of the five ^{15}N applications was applied to four plants per genotype. The fertilizer was applied in 200 mL water, followed by 100 mL water in order to ensure uniform distribution into the root zone. To reduce loss of $^{15}NO_3^-$, leachate was collected in a saucer, and re-applied to the plant. Five additional trees per rootstock with scion were maintained with standard fertilizer without ^{15}N enrichment to evaluate the ^{15}N natural abundance ($^{14}N/^{15}N$ control ratio) at every harvest.

Plant growth measurements, harvests, and samples collection

Shoot length was measured from the base of the shoot to the terminal leaf, and was recorded at 18, 25, 28, 35, 42, 52, 73, 82 and 94 Days After Bud Break (DABB). Sets of four trees per genotype were destructively harvested at six different times during the season (Table 1.1). After washing the roots to remove the potting medium, trees were divided into the following components: fine roots (≤2mm diameter), coarse roots (>2mm diameter), trunk, current season shoots, and leaves. Fine roots were carefully separated from the rest of the root system and only the white, actively growing roots were included

in the fine roots component. Plant tissues were dried to constant weight at 60°C (approximately for 72 hours), and DW was recorded. Root to shoot ratio was expressed as the ratio between the belowground and the aboveground biomass. For ^{15}N analysis, a sub-sample of the different plant tissues collected at each harvest, was freeze-dried, and DW was recorded. The sub-samples were ground to pass a 40-mesh screen in a Wiley Mill and analyzed for total N and ^{15}N enrichment with mass spectrometry analysis.

Soil sampling

Soil samples were taken at every plant harvest in order to evaluate the ^{15}N present in the soil. A total of two soil cores per pot were taken with a 2.5-cm soil probe to a depth of 25 cm. Soil samples were air-dried, ground with a mortar and pestle, sieved with 500 μm screen, and analyzed for total N and ^{15}N enrichment. Soil ^{15}N natural abundance was determined from untreated soil (0.371 atom %).

^{15}Nitrogen analysis

Between 5-12 mg of dry plant tissue, depending on the plant organ, and 50 mg of dry soil were weighed into tin capsules for mass spectrometry analysis (Automated Carbon and Nitrogen Analyzer, Roboprep, Europa Scientific, Cheshire, England). Atom enrichment values were converted to percentage of N From Fertilizer (% NFF) according to the following equation (Cabrera and Kissel, 1989):

$$\% \text{ NFF} = \frac{(\text{atom \% } ^{15}\text{N in the tissue}) - (\text{atom \% } ^{15}\text{N natural abundance})}{(\text{atom \% } ^{15}\text{N in the fertilizer}) - (\text{atom \% } ^{15}\text{N natural abundance})} \times 100$$

Natural abundance of ^{15}N of untreated trees was measured, and was equivalent to 0.367%. The amount of N Derived From Fertilizer (NDFF) in different tree organs was calculated with the following equation (Millard and Nielsen, 1989):

NDFF = %NFF × DW of organ × N concentration of organ

NDFF in the soil per each pot was calculated in a similar way, used for tree organs, and expressed on total soil dry weight bases.

Nitrogen-use efficiency

Nitrogen-use efficiency was calculated as C:N ratio of the plant, and as C:N on the leaf bases. Total plant or leaf carbon was estimated by multiplying the DW of the plant or leaf by 0.45 (Gifford, 2000). Total N in the whole plant was calculated by adding the amount of N content of the different plant organs. N concentration of different plant tissue was measured by mass spectrometry analysis. Higher values of C:N ratio indicate higher plant NUE.

Water-use efficiency

Water-use efficiency was calculated in the period between 36 and 94 DABB. Evapo-transpiration of plants was estimated gravimetrically, by weighing the potted plants (METTLER, TOLEDO, SB32001 DeltaRange) and changes in weight were calculated (Tan and Buttery, 1982). Water was manually applied to the trees, on a daily base, and the amount was recorded. Precipitation was taken into account in the calculation of the water applied to the trees.

WUE was calculated as:

$$WUE = \frac{\Delta \text{ dry matter produced (g)}}{H_2O \text{ evapotranspired (L)}}$$

(Hatfield et al., 2001)

where 'Δ dry matter produced' stands for increment in plant DW calculated as the difference in DW of plants harvested at 36 and at 94 DABB, and 'H_2O evapo-transpired' stands for the total water applied to trees between 36 and 94 DABB.

Experimental design

The experiment was a randomized complete block design with six treatments (genotypes) and four single-tree replications, per genotype, at each harvest time. Analysis of variance was performed to compare the rootstocks with scion separately from the rootstocks without scion. The N application times were analyzed separately, and differences within each application time are presented. Analysis of variance was performed using the MIXED procedure (SAS, Version 8, SAS Institute, Cary, NC, USA) to detect treatment effects. Separation of significant treatment effects was performed using Least-Squares means test (LSMEANS test) with $p \leq 0.05$.

Table 1.1. Plant phenological stages during ^{15}N application, dates of ^{15}N application, and dates of plant harvests.

Phenological stage between day of ^{15}N application and plant harvest	Day of ^{15}N application	Day of tree harvest (Days After Bud Break)
5 to 10 fully expanded leaves	___	June 7 (20 DABB)
Rapid shoot growth	June 11	June 22 (35 DABB)
Rapid shoot growth	June 23	July 9 (52 DABB)
End of shoot growth	July 16	July 30 (73 DABB)
Terminal bud set	August 6	August 20 (94 DABB)
Beginning of leaf senescence	September 12	September 21 (126 DABB)

Results

Plant growth

Total shoot length per plant increased from approximately 5 to 55 cm in 'Gisela 5' (Gi5), 'Gisela 6' (Gi6) and 'Mazzard' (M) between 20 and 73 DABB and there were no significant differences among the genotypes (Figure 1.1. A). At 82 and 94 DABB, total shoot length of Gi6 continued to increase, and was significantly higher than in Gi5 or M (Figure 1.1. A). Total shoot length increased from approximately 5 cm to 80, 100 and 120 cm for 'Rainier/Gisela 5' (R/Gi5), 'Rainier/Gisela 6' (R/Gi6), and 'Rainier/Mazzard' (R/M), respectively, between 20 and 94 DABB (Figure 1.1. B). Total shoot length was not different between R/M, R/Gi5 and R/Gi6 in any of the dates measured, except at 35 DABB, where R/Gi6 and R/M shoot length was higher than R/Gi5 (Figure 1.1. B).

Total leaf dry weight (DW) per plant of Gi5, Gi6 and M increased during the season from approximately 0.2 to 4 g for Gi5 and M, and to 7 g for Gi6 (Figure 1.2. A). Gi6 and Gi5 had lower leaf DW than M at 73 DABB, Gi6 and M had higher leaf DW than Gi5 at 94 DABB, while at 126 DABB Gi6 leaf DW was higher than both Gi5 and M (Figure 1.2. A). Total leaf DW of R/Gi5, R/Gi6, and R/M increased during the season from approximately 2 to 32 g, and differed only at 52 DABB, where R/Gi5 and R/Gi6 had a higher leaf DW than R/M (Figure 1.2. B).

Total plant DW of ungrafted rootstock increased from approximately 3 to 25g between 20 and 126 DABB (Figure 1.3. A). M had a higher DW than Gi5 and Gi6 at 20 DABB, while Gi5 and M had a higher DW than Gi6 at 35 DABB (Figure 1.3. A). At 73 DABB, M had a higher DW compared to Gi5 and Gi6 (Figure 1.3. A). Instead, at 94

DABB, M and Gi6 had a higher DW than Gi5, while at 126 DABB Gi6 had a higher DW compared to both M and Gi5 (Figure 1.3. A). Total plant DW was not significantly different at any of the harvest times for R/Gi5, R/Gi6, and R/M and it increased during the season from approximately 55 to 200 g (Figure 1.3. B).

Root to shoot ratio was not significantly different between Gi5, Gi6 and M in any of the harvest times (Figure 1.4. A). The ratios were higher than one at 20 and 35 DABB, decreased to values around or lower than one at 52, 73 and 94 DABB, and were again higher than one, at 126 DABB (Figure 1.4. A). Root to shoot ratio was significantly higher for R/M than R/Gi5, R/Gi6 at 20 and 126 DABB, and was higher or around one at 20, 35 and 126 DABB (Figure 1.4. B). R/Gi5 had ratios lower than one and similar to R/Gi6, except at 35 DABB, where R/Gi6 has a ratio higher than 1 (Figure 1.4. B).

Plant total nitrogen content

Total N content per plant progressively increased from 0.05 to 0.2 g of N for Gi5 and M, and from 0.05 to 0.4 g of N for Gi6, from 35 DABB to 126 DABB (Figure 1.5. A). At 35 DABB Gi5 had a higher plant N content than Gi6 and M, while at 126 DABB Gi6 had higher plant N content than Gi5 and M (Figure 1.5. A). Total N content per plant increased approximately from 1 to 2 g of N for R/Gi6, R/Gi5 and R/M from 35 DABB to 126 DABB (Figure 1.5. B). R/Gi5 had a higher plant N content than R/Gi6 and R/M at 94 DABB, reaching values of 2.5 g of N per plant (Figure 1.5. B).

Seasonal efficiency of N-fertilizer plant uptake

At 35 DABB, Gi5 absorbed more N-fertilizer than both Gi6 and M. The percent of N-fertilizer recovery for Gi5, Gi6 and M increased from 1 to 3% during early shoot growth, to 10 to 15 % at rapid shoot growth and end of shoot growth (Figure 1.6. A).

When N-fertilizer was applied at terminal bud set and at the beginning of leaf senescence, percent of N-fertilizer recovery in Gi6 increased to 24 and 27% of the applied, respectively, and it was significantly higher than Gi5 and M (Figure 1.6. A).

N-fertilizer percent recovery in R/Gi5, R/Gi6 and R/M was between 10 and 15% of the applied at 35 DABB, and it increased to approximately 60 to 80% at 52, 73, and 94 DABB (Figure 1.6. B). At 94 DABB, R/Gi5 and R/Gi6 absorbed significantly greater amount of N-fertilizer compared to R/M. At the beginning of leaf senescence, the recovery of N-fertilizer decreased to approximately 40% of the applied in R/Gi5, R/Gi6 and R/M (Figure 1.6. B).

Percent of recovery of applied N-fertilizer in plant and soil

Total recovery of N-fertilizer for Gi5, Gi6 and M, accounting both plants and soil, was approximately 35% at 35 DABB, and it increased to 51%, 61 %, and 80% at 52, 73, and 94 DABB, respectively, while at 126 DABB was 42% of the total N-applied (Table 1.2). Total recovery of N-fertilizer for R/Gi5, R/Gi6, and R/M in both plants and soil, was approximately 66% at 35 DABB, and reached values as high as 90 % of the total N applied, in the subsequent harvests (Table 1.3).

Partitioning of absorbed N-fertilizer

Absorbed N-fertilizer was allocated in higher percent to current season growth (leaves and shoots) during the shoot growth period, in all the genotypes tested (Figure 1.7 and 1.8). At terminal bud set (94 DABB), approximately 50% of the total N-fertilizer absorbed was partitioned to roots and trunk in Gi5, Gi6 and M, while 70% of the absorbed N-fertilizer was partitioned to roots and trunk in R/Gi5, R/Gi6, and R/M (Figure 1.7 and 1.8). At the beginning of leaf senescence, roots and trunk contained

approximately 70% of the N-fertilizer absorbed, in all genotypes tested (Figure 1.7 and 1.8).

Gi5 and Gi6 harvested at 35 DABB partitioned a significantly higher percent of N-fertilizer to current season growth, while M partitioned more N-fertilizer to roots (coarse and fine) than both Gi5 and Gi6 (Figure 1.7). At 73 DABB, Gi6 and M partitioned a higher percent of the absorbed N-fertilizer to current season growth than Gi5 (Figure 1.7). Higher percent of absorbed N-fertilizer was allocated in the trunk of M than Gi6 at 94 DABB, while at the beginning of leaf senescence M had more absorbed N-fertilizer partitioned to the trunk than Gi5 and Gi6 (Figure 1.7).

Higher percent of the absorbed N-fertilizer was allocated to the trunk of R/Gi5 and R/Gi6 than R/M at 35 DABB (Figure 1.8). N-fertilizer was partitioned in higher percent to the trunk of R/Gi5 than R/Gi6 and R/M at 52 DABB (Figure 1.8). R/M presented higher amounts of N-fertilizer in roots than R/Gi5 or R/Gi6 at 52 and 73 DABB (Figure 1.8). At 94 DABB, the absorbed N-fertilizer was allocated in greater amounts to the trunk of R/Gi5 than R/Gi6 or R/M (Figure 1.8).

Nitrogen-use efficiency

Nitrogen-use efficiency, expressed as plant C:N ratio, was significantly higher in M than Gi5 and Gi6, in all the times measured (Figure 1.9. A). At 126 DABB, Gi6 had a lower C:N ratio than Gi5 (Figure 1.9. A). There were no significant differences between R/Gi5, R/Gi6, and R/M in plant C:N ratio, except at 94 DABB, where R/M had a significantly higher C:N ratio than R/Gi5 (Figure 1.9. B). C:N ratio of leaf showed a trend similar to plant C:N ratio, at all times measured and for all the genotypes tested (Figure A.2, Appendix A).

Water-use efficiency

Between 36 and 94 DABB Gi6 and M had a greater increase in DW than Gi5 (Table 1.4). Evapo-transpiration of Gi5, Gi6 and M was approximately 12 L and was not different between genotypes (Table 1.4). WUE was higher in M than Gi5 (Table 1.4). Although R/Gi5 had higher evapo-transpiration than R/Gi6 and R/M, there were no significant differences in WUE between rootstocks with scion (Table 1.4).

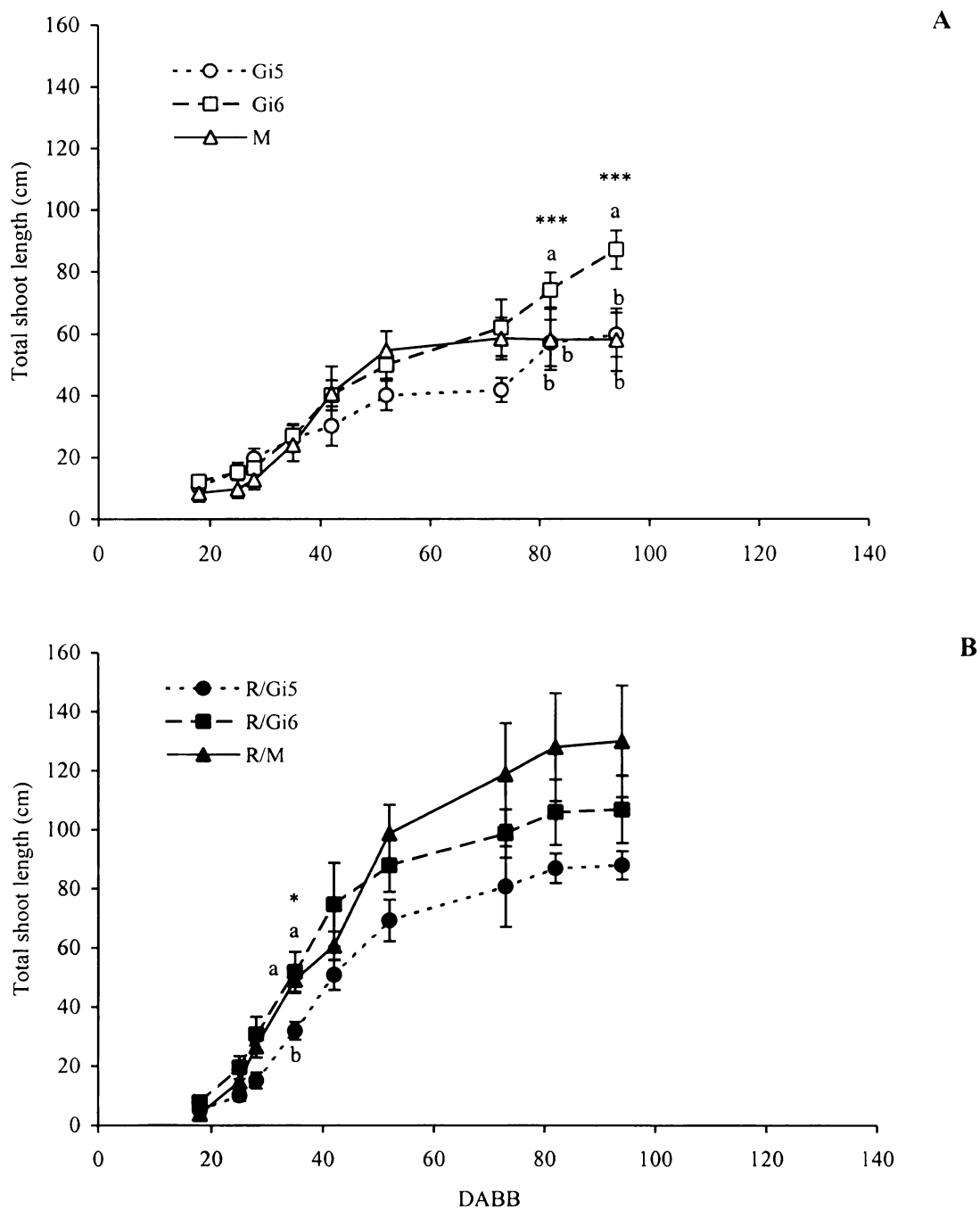


Figure 1.1. Total shoot length per plant at different Days After Bud Break (DABB). **A.** Comparison between 'Gisela 5' (Gi5), 'Gisela 6' (Gi6), and 'Mazzard' (M). **B.** Comparison between 'Rainier/Gisela 5' (R/Gi5), 'Rainier/Gisela 6' (R/Gi6) and 'Rainier/Mazzard' (R/M). Each point represents the mean (±SE) of four replications. Asterisks indicate the significance level of the plant effect; * and *** stand for significance at $p \leq 0.05$, or 0.001, respectively. Letters indicate differences between genotypes at $p \leq 0.05$ (LSMEANS test).

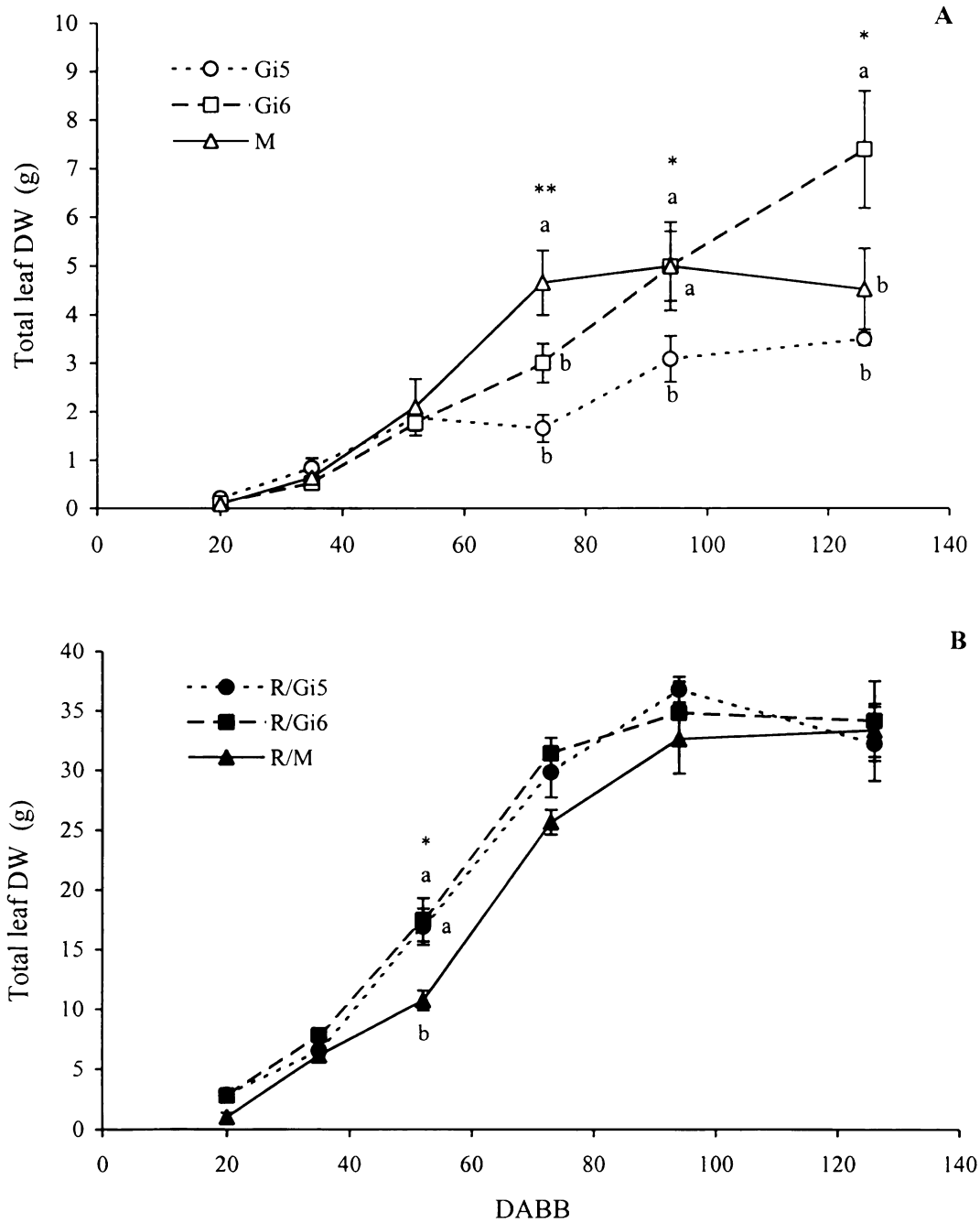


Figure 1.2. Total leaf dry weight (DW) per plant at different Days After Bud Break (DABB). **A**. Comparison between 'Gisela 5' (Gi5), 'Gisela 6' (Gi6), and 'Mazzard' (M). **B**. Comparison between 'Rainier/Gisela 5' (R/Gi5), 'Rainier/Gisela 6' (R/Gi6), and 'Rainier/Mazzard' (R/M). Analysis of variance was carried out separately for each harvest. Each point represents the mean (±SE) of four replications. Asterisks indicate the significance level of the plant effect; * and ** stand for significance at $p \leq 0.05$, or 0.01, respectively. Letters indicate differences between genotypes at $p \leq 0.05$ (LSMEANS test).

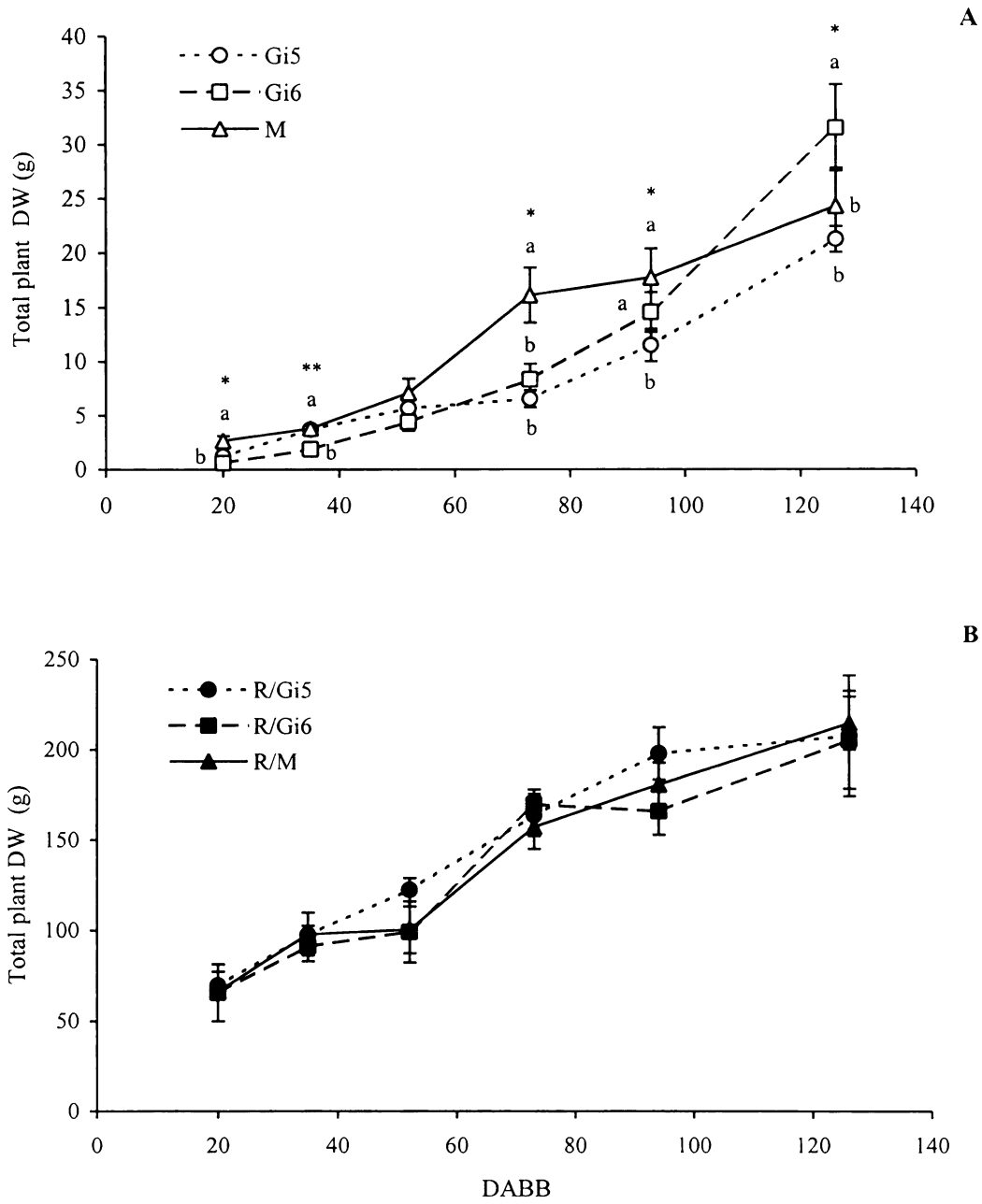


Figure 1.3. Total dry weight (DW) per plant at different Days After Bud Break (DABB). **A**. Comparison between 'Gisela 5' (Gi5), 'Gisela 6' (Gi6), and 'Mazzard' (M). **B**. Comparison between 'Rainier/Gisela 5' (R/Gi5), 'Rainier/Gisela 6' (R/Gi6), and 'Rainier/Mazzard' (R/M). Analysis of variance was carried out separately for each harvest. Each point represents the mean (± SE) of four replications. Asterisks indicate the significance level of the plant effect. * and ** stand for significance at $p \leq 0.05$, or 0.01, respectively. Letters indicate differences between genotypes at $p \leq 0.05$ (LSMEANS test).

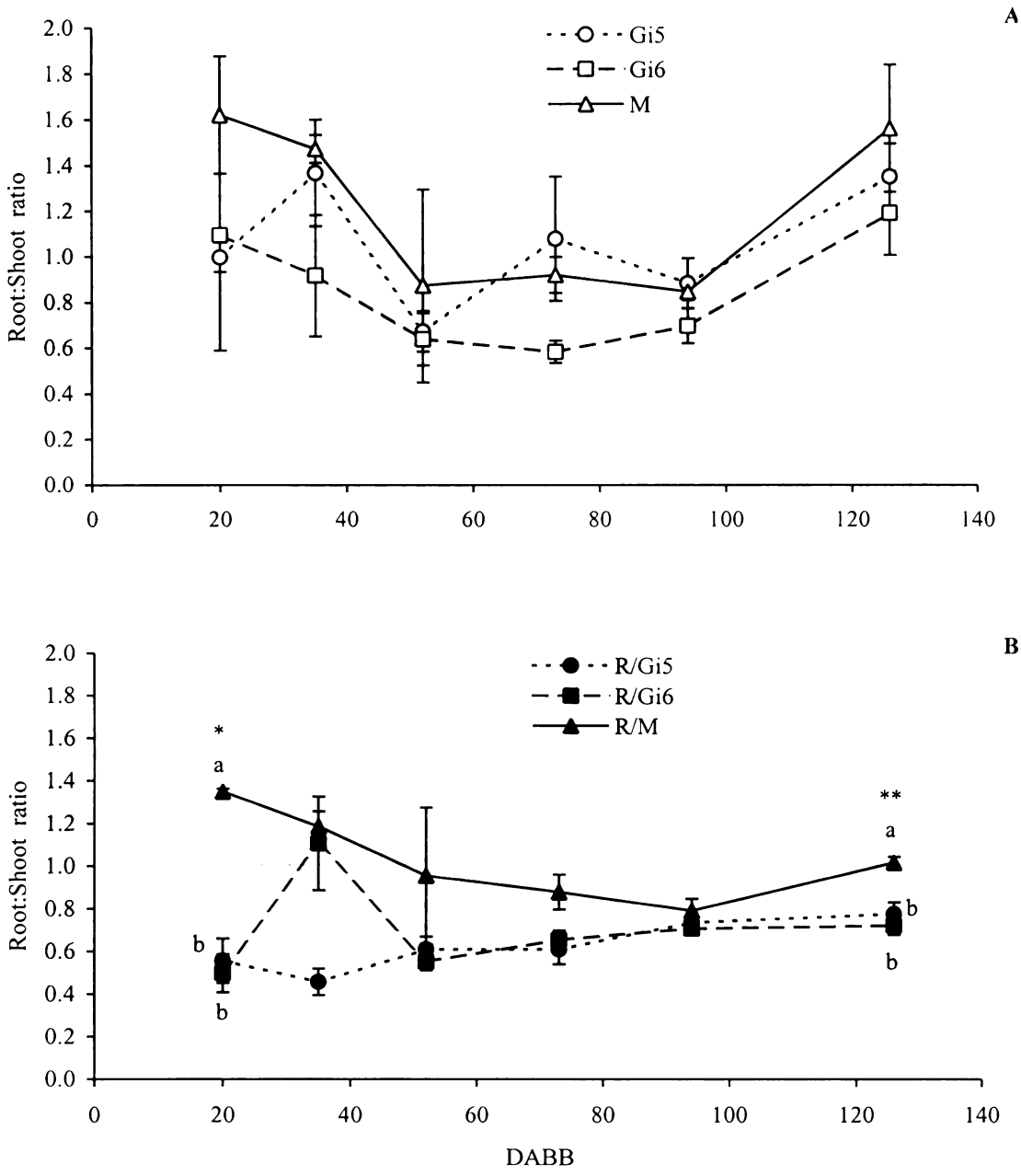


Figure 1.4. Root to shoot ratio at different Days After Bud Break (DABB). **A**. Comparison between 'Gisela 5' (Gi5), 'Gisela 6' (Gi6), and 'Mazzard' (M). **B**. Comparison between 'Rainier/Gisela 5' (R/Gi5), 'Rainier/Gisela 6' (R/Gi6), and 'Rainier/Mazzard' (R/M). Dotted lines indicate reference for root to shoot ratio equal to one. Analysis of variance was carried out separately for each harvest. Each point represents the mean (± SE) of four replications. Asterisks indicate the significance level of the plant effect; * and ** stand for significance at $p \leq 0.05$, or 0.01, respectively. Letters indicate differences between genotypes at $p \leq 0.05$ (LSMEANS test).

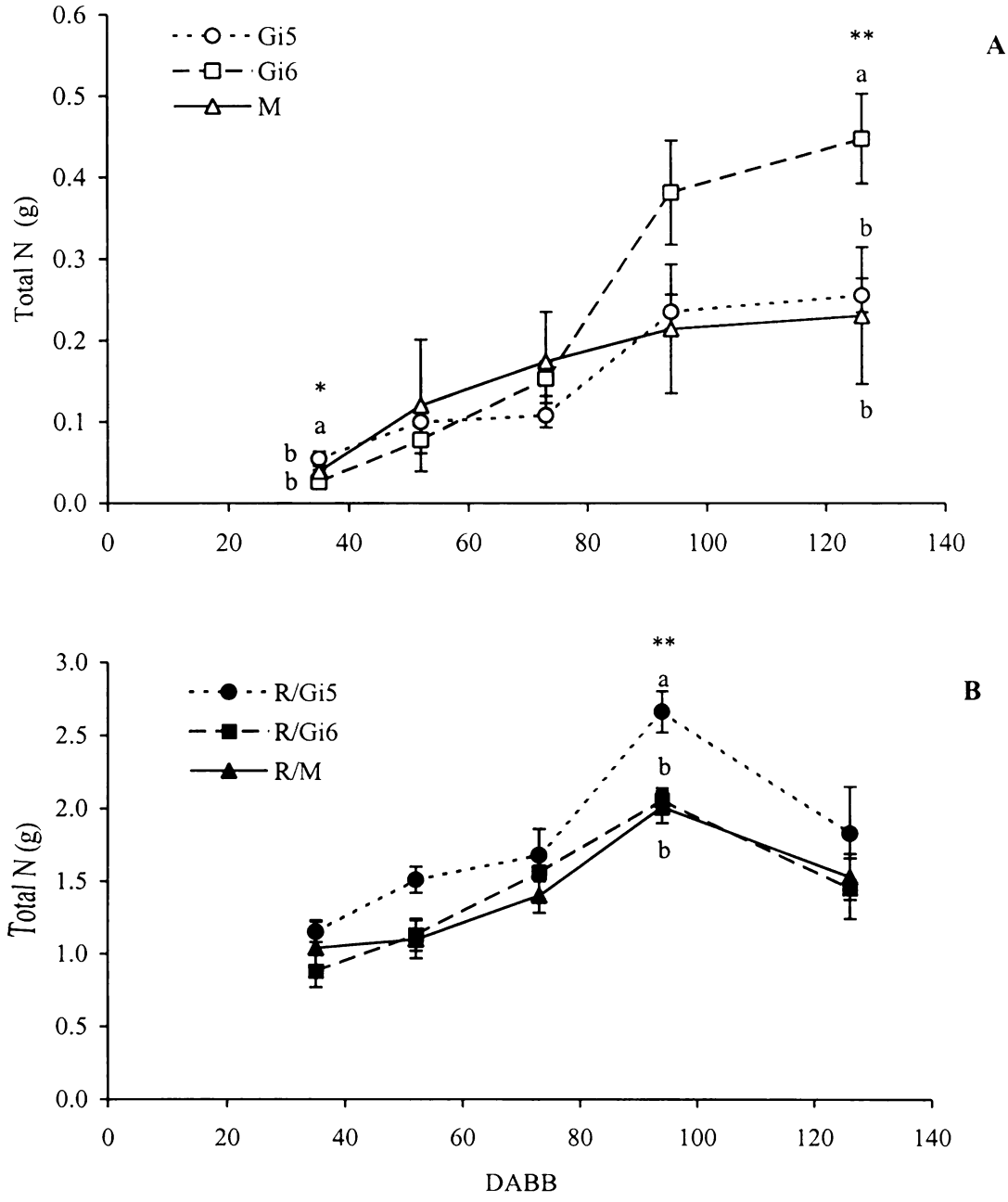


Figure 1.5. Total N content per plant at different Days After Bud Break (DABB). **A**. Comparison between 'Gisela 5' (Gi5), 'Gisela 6' (Gi6), and 'Mazzard' (M). **B**. Comparison between 'Rainier/Gisela 5' (R/Gi5), 'Rainier/Gisela 6' (R/Gi6), and 'Rainier/Mazzard' (R/M). Analysis of variance was carried out separately for each harvest. Each point represents the mean (± SE) of four replications. Asterisks indicate the significance level of the plant effect; * and ** stand for significance at $p \leq 0.05$, or 0.01, respectively. Letters indicate differences between genotypes at $p \leq 0.05$ (LSMEANS test).

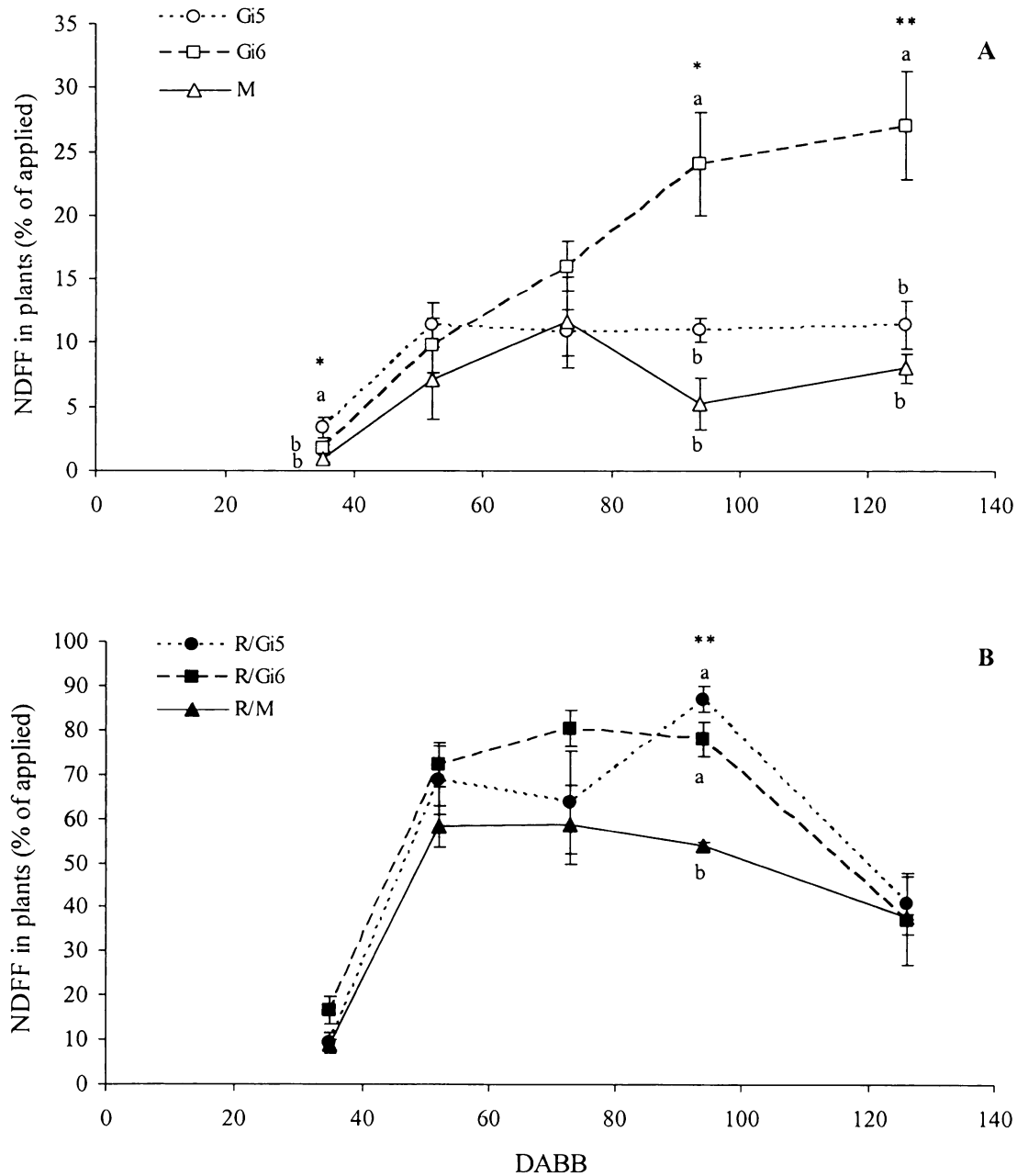


Figure 1.6. Nitrogen Derived From Fertilizer (NDFF, % of the applied) at different harvests during the growing season (Days After Bud Break, DABB). **A**. Comparison between 'Gisela 5' (Gi5), 'Gisela 6' (Gi6), and 'Mazzard' (M). **B**. Comparison between 'Rainier/Gisela 5' (R/Gi5), 'Rainier/Gisela 6' (R/Gi6), and 'Rainier/Mazzard' (R/M). Analysis of variance was carried out separately for each harvest. Each point represents the mean (± SE) of four replications. Asterisks indicate the significance level of the plant effect; * and ** stand for significance at $p \leq 0.05$, or 0.01, respectively. Letters indicate differences between genotypes at $p \leq 0.05$ (LSMEANS test).

Table 1.2. Amount of Nitrogen Derived From Fertilizer (NDFF) in plant and soil, and recovery of NDFF in plant and soil (percent of the applied N, average per harvest). Plants were fertilized with ^{15}N at different Days After Bud Break (DABB). Comparison between 'Gisela 5' (Gi5), 'Gisela 6' (Gi6) and 'Mazzard' (M).

Rootstock	N-fertilization time (DABB)	Harvest time (DABB)	Plant (mg)	Soil (mg)	Recovery of NDFF in plant and soil (% of the applied N)
Gi5	*24*	*35*	16.7 **a**[y]	167	35
Gi6	24	*35*	8.8 **b**	124	
M	24	*35*	4.6 **b**	193	
Significance[z]			*	*NS*	
Gi5	*36*	*52*	56.8	226 **a**	51
Gi6	36	52	48.9	265 **a**	
M	36	52	35.8	136 **b**	
Significance			*NS*	*	
Gi5	*59*	*73*	53.9	240	61
Gi6	59	73	80.4	268	
M	59	73	58.1	209	
Significance			*NS*	*NS*	
Gi5	*80*	*94*	54.8 **b**	398	82
Gi6	80	94	120.8 **a**	334	
M	80	94	25.8 **b**	297	
Significance			*	*NS*	
Gi5	*117*	*126*	57.3 **b**	160 **a**	42
Gi6	117	126	137.5 **a**	81 **c**	
M	117	126	39.5 **b**	147 **b**	
Significance			**	**	

[z] Analysis of variance was carried out separately for each harvest. NS, *, and **: Non Significant or significantly different at $p \leq 0.05$ or 0.01, respectively.

[y] Means within columns followed by different letters are significantly different by LSMEANS test at $p \leq 0.05$

Table 1.3. Amount of Nitrogen Derived From Fertilizer (NDFF) in plant and soil, and recovery of NDFF in plant and soil (percent of the applied N, average per harvest) Plants were fertilized with ^{15}N at different Days After Bud Break (DABB). Comparison between 'Rainier/Gisela 5' (R/Gi5), 'Rainier/Gisela 6' (R/Gi6), and 'Rainier/Mazzard' (R/M).

Rootstock	N-fertilization time (DABB)	Harvest time (DABB)	Plant (mg)	Soil (mg)	Recovery of NDFF in plant and soil (% of the applied N)
R/Gi5	*24*	*35*	47	283	66
R/Gi6	24	*35*	83	253	
R/M	24	*35*	44	276	
Significance[z]			*NS*	*NS*	
R/Gi5	*36*	*52*	345	77	80
R/Gi6	36	52	362	55	
R/M	36	52	291	72	
Significance			*NS*	*NS*	
R/Gi5	*59*	*73*	319	32	76
R/Gi6	59	73	404	42	
R/M	59	73	293	47	
Significance			*NS*	*NS*	
R/Gi5	*80*	*94*	440 **a**[y]	60.	90
R/Gi6	80	94	392 **a**	82	
R/M	80	94	272 **b**	113	
Significance			**	*NS*	
R/Gi5	*117*	*126*	204	192	78
R/Gi6	117	126	184	175	
R/M	117	126	190	222	
Significance			*NS*	*NS*	

[z] Analysis of variance was carried out separately for each harvest. NS, *, and **: Non Significant or significantly different at $p \leq 0.05$ or 0.01, respectively.

[y] Means within columns followed by different letters are significantly different by LSMEANS test at $p \leq 0.05$

Figure 1.7. Partitioning of absorbed N-fertilizer (in percent) in different plant organs, at different harvests during the growing season (Days After Bud Break, DABB). Comparison between **A**. 'Gisela 5' (Gi5), **B**. 'Gisela 6' (Gi6), and **C**. 'Mazzard' (M). Each bar represents the average of four replications. Analysis of variance was carried out separately for each harvest. Letters represent differences between organs of different genotypes, in each harvest date, at $p \leq 0.05$ (LSMEANS test).

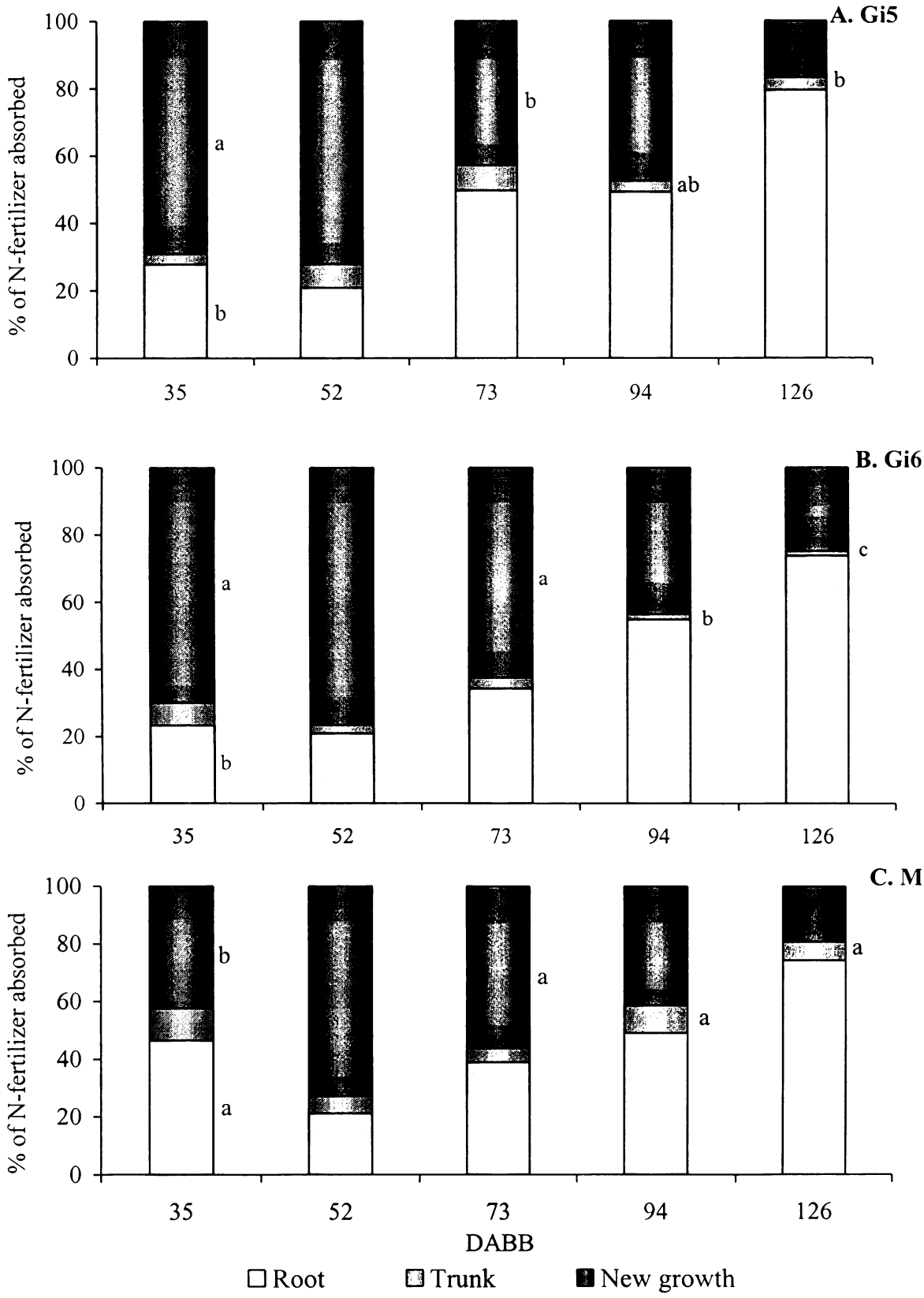
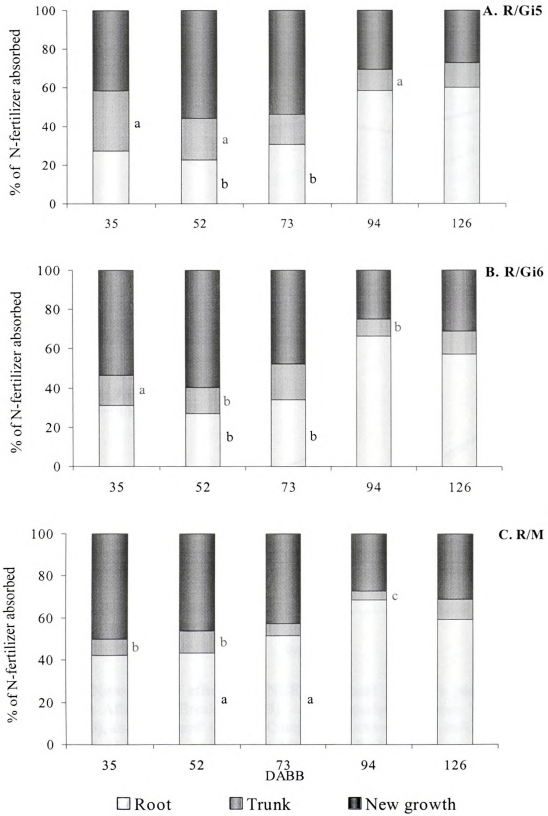

A. Gi5
B. Gi6
C. M
% of N-fertilizer absorbed
100
80
60
40
20
0
a
b
b
b
ab
b
a
b
a
b
b
c
b
a
a
a
35
52
73
94
126
DABB
Root
Trunk
New growth

Figure 1.8. Partitioning of absorbed N-fertilizer (in percent) in different plant organs, at different harvests during the growing season (Days After Bud Break, DABB). Comparison between **A**. 'Rainier/Gisela 5' (R/Gi5), **B**. 'Rainier/Gisela 6' (R/Gi6), and **C**. 'Rainier/Mazzard' (R/M). Each bar represents the average of four replications. Analysis of variance was carried out separately for each harvest. Letters represent differences between organs of different genotypes, in each harvest date at $p \leq 0.05$ (LSMEANS test).


A. R/Gi5
B. R/Gi6
C. R/M
% of N-fertilizer absorbed
100
80
60
40
20
0
a
a
b
b
a
a
b
b
b
b
c
b
b
a
a
35
52
73
94
126
DABB
Root
Trunk
New growth

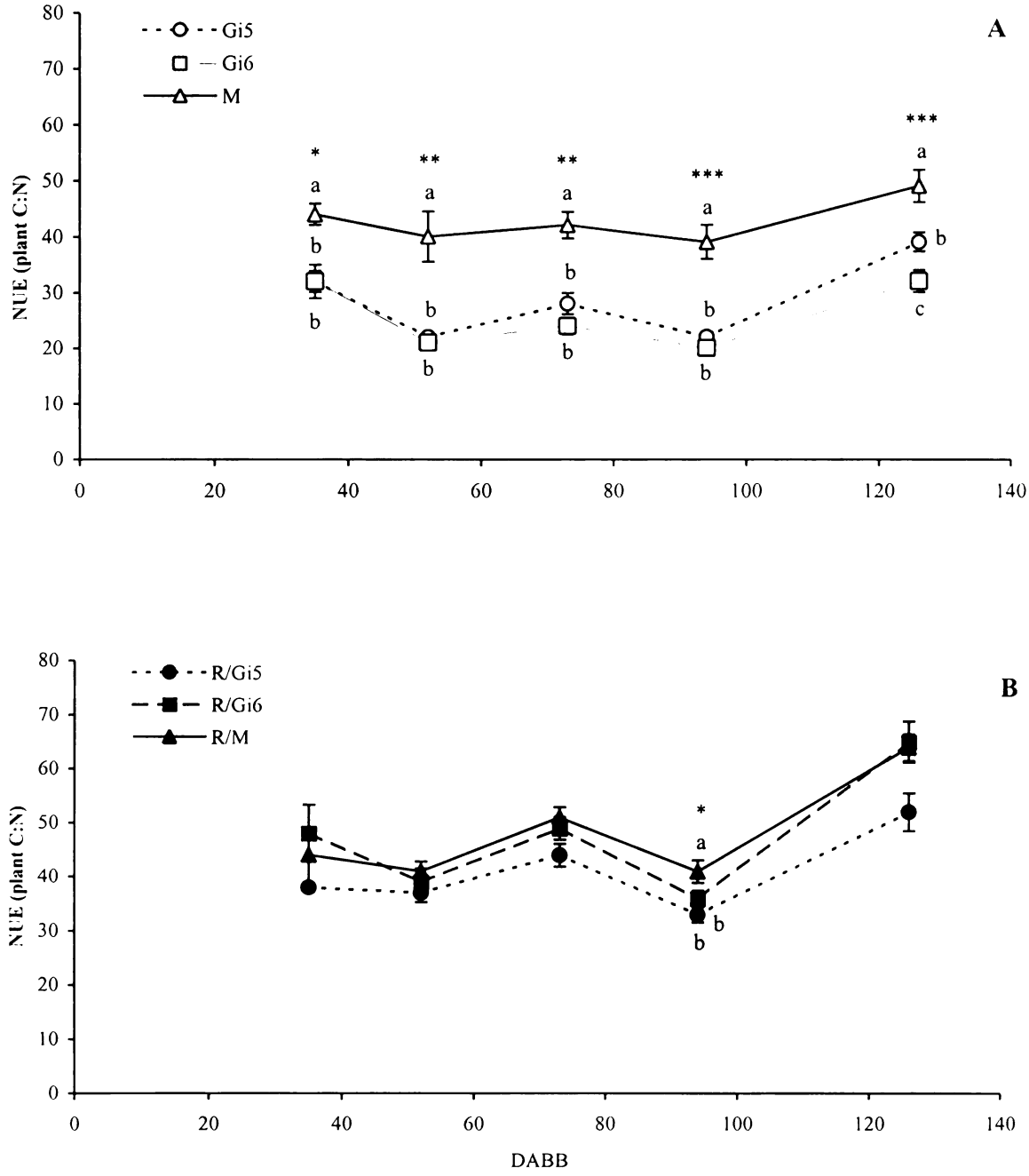


Figure 1.9. Nitrogen use efficiency (NUE) expressed as whole plant C:N ratio at different Days After Bud Break (DABB). **A**. Comparison between 'Gisela 5' (Gi5), 'Gisela 6' (Gi6), and 'Mazzard' (M). **B**. Comparison between 'Rainier/Gisela 5' (R/Gi5), 'Rainier/Gisela 6' (R/Gi6), and 'Rainier/Mazzard' (R/M). Analysis of variance was carried out separately for each harvest. Each point represents the mean (± SE) of four replications. Asterisks indicate the significance level of the plant effect; *, ** and *** stand for significance at $p \leq 0.05$, 0.01, or 0.001, respectively. Letters indicate differences between genotypes at $p \leq 0.05$ (LSMEANS test).

Table 1.4. Increase in dry weight (DW at 94 – DW at 36 DABB, g), evapo-transpiration (ET, L) and water use efficiency [ΔDW(g)/ΔET(L)] of plants, calculated between 36 and 94 Days After Bud Break (DABB). Comparison between 'Rainier/Gisela 5' (R/Gi5), 'Rainier/Gisela 6' (R/Gi6) and 'Rainier/Mazzard' (R/M) and between 'Gisela 5' (Gi5), 'Gisela 6' (Gi6) and 'Mazzard' (M).

Rootstock	ΔDW (DW at 94 – DW at 36 DABB, g)	Total ET (L)	WUE [ΔDW(g)/ET(L)]
R/Gi5	100	18.4 a[z]	5.45
R/Gi6	75	17.2 b	2.68
R/M	83	16.7 b	4.95
Significance[y]	NS	*	NS
Gi5	7.8 b	12.5	0.60 b
Gi6	12.7 a	12.2	1.04 ab
M	13.9 a	12.7	1.10 a
Significance	*	NS	*

[z] Means within columns followed by different letters are significantly different by LSMEANS test at $p \leq 0.05$

[y] NS, and *: Non Significant or significant at $p \leq 0.05$.

Discussion

Seasonal efficiency of N-fertilizer plant uptake

Understanding the seasonal efficiency of N-fertilizer uptake in sweet cherry grafted on dwarfing and standard rootstocks is an important step for optimizing the fertilization practice in modern orchards. N-fertilizer uptake efficiency was low during early shoot growth, and increased considerably when plants were fertilized during the second period of rapid shoot growth. During early shoot growth, plants had still a limited canopy, as shown by the root to shoot ratio higher than one and by the total leaf DW (Figure 1.4 and 1.1), and they were probably still in the process of developing an active root system, since they were potted approximately 24 days prior to the first ^{15}N application. Similar results have been observed in non-bearing, two-year-old prune trees, where N-fertilizer uptake significantly increased during rapid shoot growth compared to bud break or earlier N applications (Weinbaum et al., 1978). Based on the low uptake rate of N-fertilizer during the early stages of plant growth obtained in all the genotypes tested, we can speculate that early growth was sustained by the N reserves accumulated in the previous year in storage organs. Early spring growth of deciduous fruit trees is supported by remobilization of N from storage organs, such as woody tissue (Tagliavini et al., 1998; Titus and Kang, 1982; Weinbaum et al. 1978 and 1984), and is independent from the current season N availability (Millard, 1996). In apple, Neilsen et al. (1997) reported that spring remobilization of N provided the majority of N required for spur leaf growth, and half of the N used for shoot leaf growth. Fertilizer uptake followed the pattern of plant DW accumulation and more specifically of leaf DW accumulation. Plant leaf DW increased until terminal bud set (Figure 1.2, 94 DABB) and it coincided with

higher N uptake (50 to 80% of the N-fertilizer applied) while during the beginning of leaf senescence N-fertilizer uptake decreased considerably in all the genotypes tested. Absorption of N paralleled the pattern of DW accumulation in 'Sevin blanc'grapevines (Hanson and Howell, 1995), and in 'Maycrest' peach trees (Muñoz et al., 1993). The different rootstock vigor did not influence the N-fertilizer uptake efficiency. This is supported by the lack of significant differences between the rootstocks with scion, with the exception of the period of terminal bud set, where R/Gi5 and R/Gi6 were more efficient in N-fertilizer uptake than R/M (Figure 1.8).

In general, rootstocks without scion recovered less fertilizer than the same rootstocks with cv. 'Rainier'. Total plant DW was 7-fold higher in R/Gi5, R/Gi6 and R/M compared to the same rootstocks without scion, and they presented a more developed root system. In Gi5 and the standard rootstock 'Mazzard' the N uptake was between 5 and 10 % of the N-fertilizer applied from rapid shoot growth until the beginning of leaf senescence while in Gi6 N uptake increased from 10 % at rapid shoot growth to 25 % of the N-fertilizer applied at the beginning of leaf senescence. The prolonged shoot growth period of Gi6 compared to Gi5 and M caused a higher increase in plant DW for Gi6 than both M and Gi5, which drove the higher N uptake of Gi6 during the last periods of the growing season.

Partitioning of absorbed N-fertilizer

N-fertilizer partitioning in different plant organs is an important aspect to consider when N-application time is evaluated in regards to the use of N-fertilizer in the plants, e.g. for promoting growth, or increasing N reserves. At rapid shoot growth, between 40 and 60% of absorbed N was partitioned to the current season growth, indicating that

during rapid shoot growth leaves act as a major sink for N-fertilizer absorbed during the current season. Current season growth became a less important N sink at terminal bud set, where N was partitioned in higher amounts to roots than to current season growth, especially in the rootstocks with scion. At the beginning of leaf senescence the amount of N-fertilizer partitioned to the roots was approximately 60 to 80% of the absorbed N, similar to percent recovered in roots of nectarine trees (*P. persica* var. *nectarina*) (Tagliavini et al., 1999). A similar trend of N-partitioning at different phenological stages has been reported in a study with two-year-old prune trees (Weinbaum at al., 1978). During rapid shoot growth, prune trees partitioned about 70% of N-fertilizer absorbed into new shoots and leaves (Weinbaum at al., 1978), while in mid-September the major sink for N were roots, with 67% of the N-fertilizer present in the plant (Weinbaum et al., 1978). Also in young bearing peach trees, during plant growth, N was mainly partitioned to leaves, shoots and new fruits; instead, at the beginning of leaf fall, N was partitioned mainly to bark of branches, trunk and roots (Muñoz et al., 1993). N-fertilizer partitioning depends on the relative sink strength of plant organs. As previously demonstrated in other fruit trees, sink strength during the season in non-bearing sweet cherry, on dwarfing and standard rootstocks and the same rootstocks without scion switched from leaves during shoot growth, to roots and trunk, during leaf senescence.

Nitrogen-use efficiency

High NUE is a characteristic of plants adapted to low N availability, and represent plant N requirement to produce a gram of dry weight. Nitrogen use efficiency was higher in M than in both Gi5 and Gi6 while there were no differences in NUE between the rootstocks with scion. Overall, absolute values of NUE were similar for M, R/M, R/Gi5,

and R/Gi6 in all the periods considered during the season. The similar values of NUE obtained for the rootstocks with scion and the ungrafted 'Mazzard' appeared determined by the species of the aerial part of the plant rather than caused by the different rootstocks. This is supported by the fact that both the sweet cherry cv. 'Rainier' and the rootstock 'Mazzard' belong to the *P. avium* species. Leaf N composition was also affected by two apple cultivars when grown on rootstocks with different vigor (Tagliavini et al., 1992). Graft union has been indicated as one of the possible reason for alteration of leaf mineral composition (Granger and Looney, 1983), but under our experimental conditions, this was not responsible for different N leaf composition.

Water-use efficiency

Water use efficiency between 36 and 94 DABB was not different among the rootstocks with scion, while it was higher for M than Gi5 and Gi6 (Table 1.4). Considering that WUE is the result of the ratio between assimilation rate and transpiration rate, a genotype may have a greater WUE than another if it has either a higher assimilation rate and similar transpiration, or a similar assimilation rate and lower transpiration rate (Patterson et al., 1997). In the period considered, 'Mazzard' had a higher increase in DW than Gi5 and Gi6, which determined the higher WUE of M compared to Gi5 and Gi6. There were no differences in evapo-transpiration between the M, Gi5 and Gi6 therefore transpiration rate did not play a significant role in determining the higher WUE of 'Mazzard'.

Conclusions

1) Nitrogen uptake followed the accumulation of dry matter in the tree. N-fertilizer uptake was higher from rapid shoot growth, and was fairly constant until the beginning of leaf senescence, when it decreased considerably. Although the absolute values of fertilizer-N uptake obtained with potted tree are difficult to extrapolate to field-grown mature trees due to differences in magnitude of tree N demand, and availability of N from internal cycling at different tree age, the relative comparisons of genotypes N-fertilizer uptake obtained in this study, are applicable to non bearing field grown trees and can be used to optimize time of application of N fertilizer.

2) Overall, there was no difference in N uptake efficiency between dwarfing and standard trees with the exception of terminal bud set, where dwarfing and semi-dwarfing trees were more efficient in N-fertilizer uptake than standard trees. Rootstocks without scion differed in N uptake at terminal bud set and beginning of leaf senescence, and Gi6 was more efficient than both Gi5 and M due to its higher increase in plant DW.

3) NUE was higher in M than Gi5 and Gi6 while rootstocks with cv. 'Rainier' grafted on had similar NUE. Considering that 'Mazzard' had similar values of NUE of R/Gi5, R/Gi6 and R/M, the aerial part of the plant appears to have a higher incidence in determining plant NUE rather than the root system of the plant.

4) There was no difference in WUE between R/Gi5, R/Gi6 and R/M although there was a higher evapo-transpiration of R/Gi5 compared to R/Gi6 and R/M. M had a higher WUE compared to both Gi5 and Gi6 due to the higher increase in biomass in the period considered.

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CHAPTER 2

CHAPTER 2

Nitrogen uptake and nitrogen-use efficiency of field-grown sweet cherry (*Prunus avium* L. 'Sam') on dwarfing and standard rootstocks.

Abstract

This research was initiated to determine if the dwarfing rootstock 'Gisela 5' influences nitrogen (N)-fertilizer uptake and N use efficiency (NUE), when compared to the standard rootstock 'Mazzard'. The objectives were to evaluate in dwarfing and standard rootstocks: 1) efficiency of N-fertilizer uptake, at different times during the growing season; 2) the NUE, expressed as C:N ratio of leaves; and 3) retranslocation of N from senescent leaves. The experiment was conducted in 2002, on five-year-old sweet cherry trees of cv. 'Sam' grafted on the standard rootstock 'Mazzard' and the dwarfing rootstock 'Gisela 5'. The orchard was located in Copemish, Michigan on a loamy-sand soil. During the growing season, a total of 21g of N as $K^{15}NO_3$ was applied three times: at full bloom (11 May), at rapid shoot growth (40 days after full bloom, DAFB), and at the beginning of leaf senescence (126 DAFB). Shortly after bloom a severe frost eliminated all the flowers in the trees resulting in total crop loss. Leaves were sampled at one, five, 10 days following the ^{15}N applications, and then every week thereafter, until leaf senescence occurred. N content and % of ^{15}N were determined in the leaves sampled during the season. Before leaf senescence, trees were enclosed in netting bags to collect abscised leaves in order to evaluate total amount of N and N derived from fertilizer lost during leaf fall. N-fertilizer was absorbed in the greatest amount when applied at bloom

or at rapid shoot growth. When N-fertilizer was applied at bloom and rapid shoot growth, the percent of N-fertilizer was higher in leaves of sweet cherry on dwarfing than standard rootstocks. Therefore it appears that dwarfing trees rely more on N-fertilizer than standard trees. NUE was lower at bloom, and increased progressively during the season until 60 DAFB. Overall, NUE as well as N-retranslocation from senescent leaves did not differ between dwarfing and standard trees.

Introduction

Application of N influences tree growth and development during the current year and in the following growing season (Weinbaum, 1987). Several factors affect N-fertilizer use efficiency including, plant demand for N (Weinbaum et al., 1992), the form of N applied (Barker and Mills, 1980), the application method (Sanchez et al, 1995), and the rate and timing of application (Taylor et al., 1975; Conradie, 1991; Sanchez et al., 1992).

The seasonal pattern of uptake and N accumulation in trees reflects their N demand and can be used for timing N application in order to maximize fertilizer uptake (Weinbaum et al., 1992). The synchrony between plant N demand and N soil availability is an important factor to consider, in order to increase the efficiency of N-fertilizer applications. N uptake during the season, and partitioning of N to different plant organs, has been investigated in several fruit tree species (Weinbaum et al., 1978, 1984; Muñoz et al., 1993; Neilsen et al., 2001 a and b; Policarpo et al., 2002). Little is known about the pattern of N uptake in sweet cherry trees, particularly when grown on the new dwarfing rootstocks. Sweet cherries in the United State are grown mainly on standard size

rootstocks like 'Mazzard' seedling (*Prunus avium* L.), the clonal 'Mazzard F.12/1' or 'Mahaleb' (*P. mahaleb* L.) (Perry, 1987). Recently, sweet cherry on dwarfing rootstocks (i.e. 'Gisela 5', *P. cerasus* (cv. Schattenmorelle) × *P. canescens*), are being extensively planted. The main advantages of a dwarfing rootstock are the precocity of production, the possibility of intensive plantings, and lowering production costs of pruning, spraying, and harvest. When dwarfing rootstocks are used, an appropriate management of N is needed in order to maintain a proper balance between reproductive and vegetative growth in trees, and to sustain high quality fruit production (Lang, 2000).

A better knowledge of sweet cherry tree N requirements during the season is important in order to avoid problems related to overfertilization. Excess N-fertilizer in soils creates high risk for groundwater contamination as a result of nitrate leaching (Merwin et al., 1996). A large number of sweet cherry orchards in Michigan are located on sandy and sandy-loam textured soils characterized by rapid drainage and low nutrient retention. Overfertilization has been also associated with the risk of atmospheric pollution from N_2O production in soil (Weinbaum et al., 1992). Excess N in fruit trees is detrimental for fruit quality (Faust, 1989), determines vigorous growth (Weinbaum, 1992), and decreases tree cold hardiness.

Nitrogen use efficiency is defined as the amount of dry matter produced per unit of N absorbed (Robertson, 1997). Several indicators have been used to evaluate Plants NUE and among them are the plant C:N ratio (Maranville and Madhavan, 2002), the inverse of leaf N concentration (Tateno and Kawaguchi, 2002) or other plant tissue N concentration (Nakamura et al., 2002), and the amount of utilizable plant material (seed, grain, fruits, forage) per amount of absorbed N (Maranville et al., 1980; Baligar et al.,

2001). High plant C:N ratio indicates high plant NUE. In non-cultivated ecosystems NUE appears to be higher where soil N availability is low (Robertson, 1997). Nitrogen use efficiency has been evaluated in several crops (Gonzales Ponce et al., 1993; Cassman et al., 1993; van den Boogaard et al., 1995) but little is known on the effects of dwarfing and standard sweet cherry rootstocks on NUE.

Nitrogen-use efficiency can also be defined as the amount of litterfall dry matter lost per unit of N lost. The withdrawal of N from senescent leaves allows the reuse of the same unit of N to build new plant organs, the following season (Clark, 1977; Turner, 1977). Nitrogen use efficiency calculated in forest litterfall appears to be correlated with the availability of N in the ecosystem (Vitousek, 1982). Differences in N retranslocation from senescent leaves as affected by dwarfing and standard sweet cherry rootstocks has not been previously evaluated and will be important for an overall understanding of plant NUE. Plants with high NUE or high fertilizer uptake efficiency could reduce fertilizer costs, and nutrient loss. A better understanding of the trees NUE, as well as of N-fertilizer uptake efficiency, will be of great value for deciding the best management practices to adopt in terms of timing of fertilization in sweet cherries, on dwarfing or standard rootstocks.

The objectives of this study were to compare: 1) N-fertilizer uptake efficiency and partitioning to different plant organs, in standard and dwarfing sweet cherry rootstocks, at three different phenological stages: bloom, rapid shoot growth, and beginning of leaf senescence, and 2) to compare standard and dwarfing sweet cherry trees NUE and N retranslocation from senescent leaves.

Materials and methods

The experiment was carried out in 2002 in a five-year-old sweet cherry orchard (*P. avium* L. 'Sam') grafted on either the dwarfing rootstock 'Gisela 5' (*P. cerasus* (cv Schattenmorelle) × *P. canescens*) or the standard rootstock 'Mazzard' (*P. avium* L.). The orchard, located in Copemish, MI, (Lat. 44°29' N, Long. -86°08' W) was on a loamy-sand soil (84.4 % sand, 7.9% silt and 7.7% clay) with pH = 6.8, CEC = 3.1 meq/100 g, and 1.47 % of organic matter. The trees were trained as a vase, with a spacing of 4 × 4.8 m, between trees and rows for 'Sam/Mazzard' (S/M), and 3.5 × 4.8 m, between trees and rows for 'Sam/Gisela 5' (S/Gi5). Natural grass sod was maintained between the rows, and an herbicide strip of a total width of approximately 2 m was maintained under the trees. Standard production practices were used to control insects and diseases (MSU Fruit management guide, E154). In previous years the orchard was annually fertilized with urea at the rate of 85 kg ha^{-1} of N, distributed in two equal applications, at bloom and at pit hardening. Daily maximum and minimum air temperature, and precipitations during the growing season were recorded with an automated weather station of the Michigan Automated Weather Network, located at the USDA National Resources Conservation Service of Bear Lake, approximately 25 km west of the orchard (http://www.agweather.geo.msu.edu, Figure B.1, Appendix B).

^{15}Nitrogen Applications

Twelve trees for each rootstock with similar trunk cross sectional area and blossom density were selected at full bloom (10 May) in six adjacent rows, three rows with S/M and three rows with S/Gi5. Approximately seven days after full bloom (DAFB), a severe frost caused a complete loss of the cherry flowers in the orchard.

Nitrogen was applied three times during the season: at full bloom (11 May), at rapid shoot growth (20 June, 40 DAFB) and at the beginning of leaf senescence (11 September, 120 DAFB). At each application time, four trees of each rootstock received 21 g of N as $K^{15}NO_3$ (7.5 % atom, ICON Services, Mt. Marion, N.Y.) while the other eight trees for each rootstock were fertilized with an equal amount of non-enriched KNO_3. Each tree received ^{15}N only one time during the season.

To increase the efficiency of N-fertilizer applications, the fertilizer was applied in a targeted method. A strip 2 × 4 m wide below the treated trees (corresponding to the location of higher root density) was kept vegetation-free with the use of herbicide. The location of higher roots density was identified with the help of soil cores, taken at 0-30 and 30-60 cm depths, from non-treated trees. The fertilizer was dissolved in 18 L of water and applied uniformly with watering cans to the vegetation-free area under each tree. Another 18 L of water were applied after the N application, to ensure uniform distribution of the fertilizer into the root zone. Considering only the treated area (8 m^2 $tree^{-1}$) the three N-applications provided to every tree a total of 63 g of N, equivalent to approximately 80 kg N ha^{-1}. Only at bloom, to prevent leaching of the N-fertilizer due to heavy rain, the treated area was covered with black polyethylene after the distribution of ^{15}N for approximately 10 days.

Plant measurements and samples collection

Tree trunk circumference was measured on the 15 May, 2002 and on the 11 May, 2003 at 45 cm from the ground with a metal measuring tape to evaluate the yearly increase in trunk cross sectional area.

Leaf samples were collected at one, five and 10 days after each ^{15}N applicatior and then every week thereafter until leaf senescence started. Each sample consisted c eight well-exposed fully expanded leaves, collected from the middle portion of shoots c the current season growth, from every ^{15}N treated tree. For the initial ^{15}N applicatio until the end of May each leaf sample consisted of four to five shoot leaf clusters sinc leaves from shoots of the current season's growth were not present yet. All leaf sample were freeze-dried, and dry weight (DW) was recorded. All samples were ground in Wiley Mill to pass a 40-mesh screen.

Partitioning of newly absorbed N in the tree, approximately 40 days after each ^{15} application was assessed by collecting fine roots (≤ 2mm diameter), coarse roots (> 2mr diameter), 2001 wood (one-year-old wood), 2002 wood (current season shoot), buds fror shoot, buds from spur, and leaves. Buds from shoots were separated from 5 to 6 currer season shoots, while buds from spurs were separated from spurs on one-year-old wooc In samples collected 40 days after the initial ^{15}N application new buds were not full formed and could not be separated from the wood itself. Roots were sampled by takin two to three soil cores with a 5-cm soil probe to a depth of 30 cm. Samples were freeze dried, ground in a stainless steel mill to pass a 40-mesh screen, and finally analyzed fc total N and ^{15}N enrichment with mass spectrometry analysis.

At 120 DAFB tree canopies were enclosed with netting bags to collect th senescent leaves as they abscised from the trees. Abscised leaves were collected in tw times, 160 DAFB and 180 DAFB, dried at 60°C for approximately 72 hours until ther was no change in weight, and DW was recorded. A leaf sub-sample was ground in

Wiley Mill to pass a 40-mesh screen and analyzed for total N and ^{15}N enrichment with mass spectrometry analysis.

^{15}Nitrogen analysis

Between 5-12 mg of dry sample were weighted into tin capsules for mass spectrometry analysis (Automated Carbon and Nitrogen Analyzer, Roboprep, Europa Scientific, Cheshire, England). Atom ^{15}N enrichment values were converted to percentage of N From Fertilizer (% NFF) according to the following equation (Cabrera and Kissel, 1989):

$$\% \text{ NFF} = \frac{(\text{atom \% } ^{15}\text{N in the tissue}) - (\text{atom \% } ^{15}\text{N natural abundance})}{(\text{atom \% } ^{15}\text{N in the fertilizer}) - (\text{atom \% } ^{15}\text{N natural abundance})} \times 100$$

^{15}N natural abundance of plant tissues was obtained from untreated trees and was equal to O.366%. The value of ^{15}N natural abundance subtracted from the N-fertilizer was also considered equal to 0.366%. The amount of N Derived From Fertilizer (NDFF) present in the abscised leaves was calculated with the following equation (Millard and Nielsen, 1989):

$$\text{NDFF} = \text{DW of leaves} \times \text{N concentration of leaves} \times \%\text{NFF}$$

Nitrogen-use efficiency

Nitrogen-use efficiency was expressed as leaf C:N ratio. Dry weight was converted to total carbon by multiplying by 0.45 (Gifford, 2000), while total N in leaves was measured by mass spectrometry analysis.

Experimental design

The experiment was a two-way factorial randomized design, rootstock genotyp (two genotypes) × timing of ^{15}N application (three times). Four replicate trees of eac genotype were treated with ^{15}N at each N application time. Analysis of variance wa performed using MIXED procedure with SAS (Version 8, SAS Institute, Cary, NC, USA to detect treatment effects. Repeated-measure analysis was used to detect treatmen effects for NUE and percent of ^{15}N in leaves. When treatments effects were significant means were separated using Least-Squares means test (LSMEANS test) with $p \leq 0.05$.

Results

Trunk cross sectional area

Trunk cross sectional area was significantly higher in 'Sam/Mazzard' (S/M) tha 'Sam/Gisela 5' (S/Gi5) in both 2002 and 2003 (Table 2.1). The yearly increase in trun cross sectional area was approximately two-fold greater in S/M than in S/Gi5 (Table 2.1)

N-fertilizer uptake efficiency

N-Fertilizer was detected in leaves ten days after the ^{15}N application at bloom five days after the ^{15}N application at rapid shoot growth, and 12 days after the ^{15}N application at the beginning of leaf senescence (Figure 2.1.). When ^{15}N-fertilizer wa applied at bloom and at rapid shoot growth it accounted for a maximum of 12% and 6% in leaves of S/Gi5 and S/M, respectively (Figure 2.1. A and B), whereas when it wa applied at the beginning of leaf senescence accounted for only a maximum of 4 % in bot S/Gi5 and S/M (Figure 2.1. C). Leaf percent of NFF from trees treated with ^{15}N at bloom was significantly higher in S/Gi5 than S/M from 10 until the 38 DAFB, and at 52 DAF

(Figure 2.1. A). When ^{15}N was applied at rapid shoot growth similar concentrations o N-fertilizer were found in leaves of both genotypes except at 80 DAFB, when leaves o S/Gi5 had a significantly higher percent of N-fertilizer than S/M (Figure 2.1. B). Afte the ^{15}N application at the beginning of leaf senescence the percent of fertilizer-N wa higher in S/Gi5 than S/M at 136 and 143 DAFB (Figure 2.1. C).

N-fertilizer partitioning

Nitrogen concentration in fine roots of S/Gi5 and S/M was higher 40 days after N application at the beginning of leaf senescence than 40 days after N application at fu bloom and rapid shoot growth while coarse roots had higher N concentration at th beginning of leaf senescence than rapid shoot growth (Table 2.2). Nitrogen concentratio of 2002 wood and leaves was higher at 40 days after ^{15}N application at full bloom than a 40 days after ^{15}N application at rapid shoot growth and beginning of leaf senescence i both S/M and S/Gi5 (Table 2.2). S/M had higher N concentration in 2001 wood whe sampled at 40 days after full bloom than rapid shoot growth and beginning of lea senescence, while 2001 wood N concentration in S/Gi5 was higher 40 days after fu bloom and beginning of leaf senescence than rapid shoot growth (Table 2.3). While % o NFF was similar in fine roots, in either rootstock, in S/M the percent of N-fertilizer i coarse roots was higher at 40 days after the ^{15}N application at the beginning of lea senescence than rapid shoot growth and full bloom (Table 2.4). In S/Gi5, the percent o NFF in coarse roots was higher 40 days after rapid shoot growth and beginning of lea senescence ^{15}N applications than after at 40 days after full bloom (Table 2.4). N-fertilize was higher in S/Gi5 than S/M in 2001 wood, 2002 wood, and leaves at 40 days after fu

bloom and rapid shoot growth while it was similar at the beginning of leaf senescenc (Table 2.4).

Buds from shoots and spurs had a higher N concentration in trees ^{15}N fertilized a the beginning of leaf senescence than rapid shoot growth in both trees considered (Tabl 2.5). N-fertilizer contributed in higher percent to the total N in buds from shoots in tree treated at rapid shoot growth than at the beginning of leaf senescence in S/Gi5 than S/M (Table 2.6). Differently, S/M buds from shoots had higher percent of N-fertilizer tha S/Gi5 in trees fertilized at the beginning of leaf senescence (Table 2.6). N-fertilizer i buds from spurs accumulated in greater amounts in S/M at 40 days after ^{15}N applicatio at the beginning of leaf senescence than at 40 days after rapid shoot growth (Table 2.6) N-fertilizer in buds from spurs accumulated in similar amount in S/Gi5 in both period considered, and in significantly higher amount than in S/M spurs buds at 40 days afte ^{15}N application at rapid shoot growth (Table 2.6).

Nitrogen-use efficiency

Leaf N concentration during the season decreased from approximately 50 mg N g $^{-1}$DW at full bloom to approximately 20 mg N g^{-1}DW at 40 DAFB, and then remaine fairly constant until the end of the season (Figure 2B. Appendix B).

Leaf C:N ratio (Figure 2.2) was low at the beginning of the season (approximatel 8), and it increased progressively until 60 DAFB (approximately 20) and remained fairl constant until the end of the season. S/Gi5 had a significantly higher C:N ratio than S/M at 5, 38, 42, 52, and 74 DAFB (Figure 2.2). C:N ratio was higher for S/M than S/Gi toward the end of the season 136 and 150 DAFB (Figure 2.2).

Nitrogen in abscised leaves

Approximately 35% of the total DW of leaf abscised at senescence was collected at 160 DAFB and the other 65% at 180 DAFB. Total DW, amount of total N, and N-fertilizer present in leaves collected at 160 and 180 DAFB did not differ between rootstocks (data not shown). Dry weight, total N, total NDFF, and percent of NDFF compared to total N present in abscised leaves were significantly higher in S/M compared to S/Gi5 in all the fertilization timing (Table 2.7). Total N, NDFF, and percent of NDFF compared to total N were significantly higher in leaves abscised from S/M trees fertilized with ^{15}N at rapid shoot growth than at full bloom and at the beginning of leaf senescence (Table 2.7). Total N, NDFF, and percent of applied N-fertilizer in leaves abscised from S/Gi5 trees were not significantly different among trees fertilized in different times (Table 2.7).

Table 2.1. Trunk cross sectional area (TCSA, cm^2) of sweet cherry 'Sam/Mazzard' (S/M) and 'Sam/Gisela 5' (S/Gi5) measured 15 May, 2002, and 11 May, 2003 and percent increase of area.

Rootstock	TCSA 2002 (cm^2)	TCSA 2003 (cm^2)	Increase in area (%)
S/M	82.9 ± 12[z]	112.2 ± 17	29.3 ± 9
S/Gi5	67.6 ± 8	80.2 ± 9	12.4 ± 3
Significance[y]	**	***	**

[z] ± standard deviation of the means

[y] ** and *** stand for significant within column at $p \leq 0.01$ and 0.001, respectively

Figure 2.1. Percent of nitrogen from fertilizer (% NFF) in leaves of sweet cherry 'Sam/Gisela 5' (S/Gi5) and 'Sam/Mazzard' (S/M), collected at different Days After Full Bloom (DAFB). Arrows indicate time of ^{15}N applications. **A**. Application of ^{15}N at full bloom. **B**. Application of ^{15}N at rapid shoot growth. **C**. Application of ^{15}N at the beginning of leaf senescence. Each point represents the mean of four replications (± STD). *, **, and *** stand for significant at $p \leq 0.05$, 0.01, and 0.001, respectively.

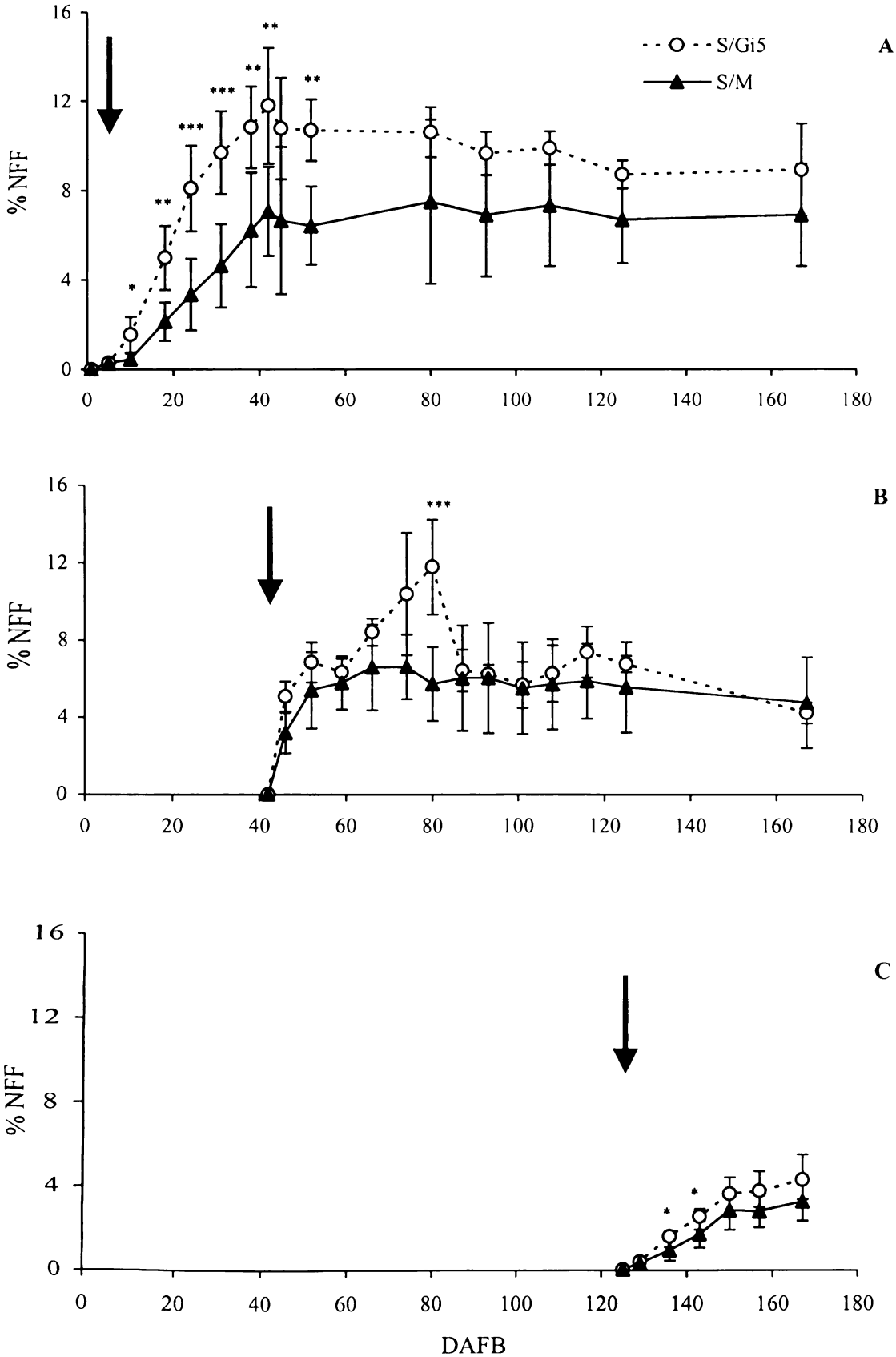
A
S/Gi5
S/M
% NFF
B
% NFF
C
% NFF
DAFB

Table 2.2. N concentration (mg N g^{-1} dry weight) in sweet cherry 'Sam/Mazzard' (S/M) and 'Sam/Gisela 5' (S/Gi5) fine roots, coarse roots, 2002 wood (current season wood), and leaves, harvested 40 days after each ^{15}N application. ^{15}N-fertilizer was applied at bloom (11 May), rapid shoot growth (20 June), and beginning of leaf senescence (11 September).

Time of N application	N concentration (mg N g^{-1} dry weight)							
	Fine roots		Coarse roots		2002 wood		Leaves	
	S/M	S/Gi5	S/M	S/Gi5	S/M	S/Gi5	S/M	S/Gi5
Full bloom	14 b[z]	15 b	12 ab	12 ab	21 a	26 a	41 a	35 a
Rapid shoot growth	15 b	17 b	8 b	11 b	8 b	11 b	20 b	18 b
Beginning of leaves senescence	18 a	20 a	11 a	17 a	11 b	11 b	19 b	20 b
Plant (P)[y]	NS		NS		NS		NS	
Ferilization time (F)	**		*		***		***	
P × F	NS		NS		NS		NS	

[z] Means within columns followed by different letters are significantly different by LSMEANS test at $p \leq 0.05$

[y] NS, *, **, and *** stand for Non Significant or significant at $p \leq 0.05$, 0.01 and 0.001, respectively

Table 2.3. N concentration (mg N g^{-1} dry weight) in sweet cherry 'Sam/Mazzard' (S/M) and 'Sam/Gisela 5' (S/Gi5) 2001 wood harvested 40 days after each ^{15}N application. ^{15}N-fertilizer was applied at bloom (11 May), rapid shoot growth (20 June), and beginning of leaf senescence (11 September).

Plant	Time of N application	N concentration (mg N g^{-1} dry weight) 2001 wood
S/M	Full bloom	14 a[z]
	Rapid shoot growth	8 b
	Beginning of leaves senescence	9 b
S/Gi5	Full bloom	12 a
	Rapid shoot growth	8 b
	Beginning of leaves senescence	12 a
Plant (P)[y]		NS
Fertilization time (F)		***
P × *F*		*

[z] Means within columns followed by different letters are significantly different by LSMEANS test at $p \leq 0.05$

[y] NS, * and *** stand for Non Significant or significant at $p \leq 0.05$ or 0.001, respectively

Table 2.4. Percent of N-fertilizer (% NFF, percent of total N) in sweet cherry 'Sam/Mazzard' (S/M) and 'Sam/Gisela 5' (S/Gi5) fine roots, coarse roots, 2001 and 2002 wood, and leaves, harvested 40 days after each ^{15}N application. ^{15}N-fertilizer was applied at bloom (11 May), rapid shoot growth (20 June) and beginning of leaf senescence (11 September).

		NFF (% of total N) (40 days after application)				
Plant	Time of N application	Fine roots	Coarse roots	2001 wood	2002 wood	Leaves
S/M	Full bloom	10.2	4.3 b[z]	4.4 b	8.2 b	7.7 b
	Rapid shoot growth	11.1	4.1 b	1.7 c	5.2 c	5.7 bc
	Beginning of leaves senescence	15.7	12.2 a	5.6 ab	7.8 b	3.3 d
S/Gi5	Full bloom	5.9	3.8 b	7.4 a	13.1 a	12.6 a
	Rapid shoot growth	14.2	12.4 a	6.1 ab	11.6 a	11.8 a
	Beginning of leaves senescence	12.9	10.0 a	5.4 b	5.7 bc	4.3 cd
Plant (P)[y]		NS	NS	**	*	*
Fertilization time (F)		NS	**	*	***	***
P × F		NS	**	*	***	**

[z] NS, *, ** and *** stand for Non Significant or significant at $p \leq 0.05$, 0.01 and 0.001, respectively

[y] Means within columns followed by different letters are significantly different by LSMEANS test at $p \leq 0.05$

Table 2.5. Nitrogen concentration (mg N g^{-1} dry weight) in sweet cherry 'Sam/Mazzard' (S/M) and 'Sam/Gisela 5' (S/Gi5) buds from current season shoots and spurs, harvested 40 days after ^{15}N application. ^{15}N-fertilizer was applied at on the 20 June (rapid shoot growth) and on the 11 September (beginning of leaf senescence).

	N concentration (mg N g^{-1} dry weight)			
Time of N application	Bud (Shoot)		Bud (Spur)	
	S/M	S/Gi5	S/M	S/Gi5
Rapid shoot growth	10 b[z]	10 b	9 b	9 b
Beginning of leaves senescence	13 a	13 a	12 a	12 a
Plant (P)[y]	NS		NS	
Ferilization time (F)	***		***	
P × *F*	NS		NS	

[z] Means within columns followed by different letters are significantly different by LSMEANS test at $p \leq 0.05$

[y] NS, and *** stand for Non Significant or significant at $p \leq 0.001$, respectively

Table 2.6. Percent of N-fertilizer (% NFF, percent of total N) in sweet cherry 'Sam/Mazzard' (S/M) and 'Sam/Gisela 5' (S/Gi5) buds from current season shoot and spur, harvested 40 days after ^{15}N application. ^{15}N-fertilizer was applied on the 20 June (rapid shoot growth) and on the 11 September (beginning of leaf senescence).

		% NFF (% of total N) (40 days after N-application)	
Plant	Time of N application	Bud (Shoot)	Bud (Spur)
S/M	Rapid shoot growth	5.0 b[z]	4.4 b
	Beginning of leaves senescence	10.3 a	10.1 a
S/Gi5	Rapid shoot growth	10.7 a	8.8 a
	Beginning of leaves senescence	7.6 b	9.5 a
Plant (P)[y]		NS[y]	*
Ferilization time (F)		NS	*
P × *F*		**	*

[z] Means within columns followed by different letters are significantly different by LSMEANS test at $p \leq 0.05$

[y] NS, * and ** stand for Non Siginficant or significant at $p \leq 0.05$, and 0.01, respectively

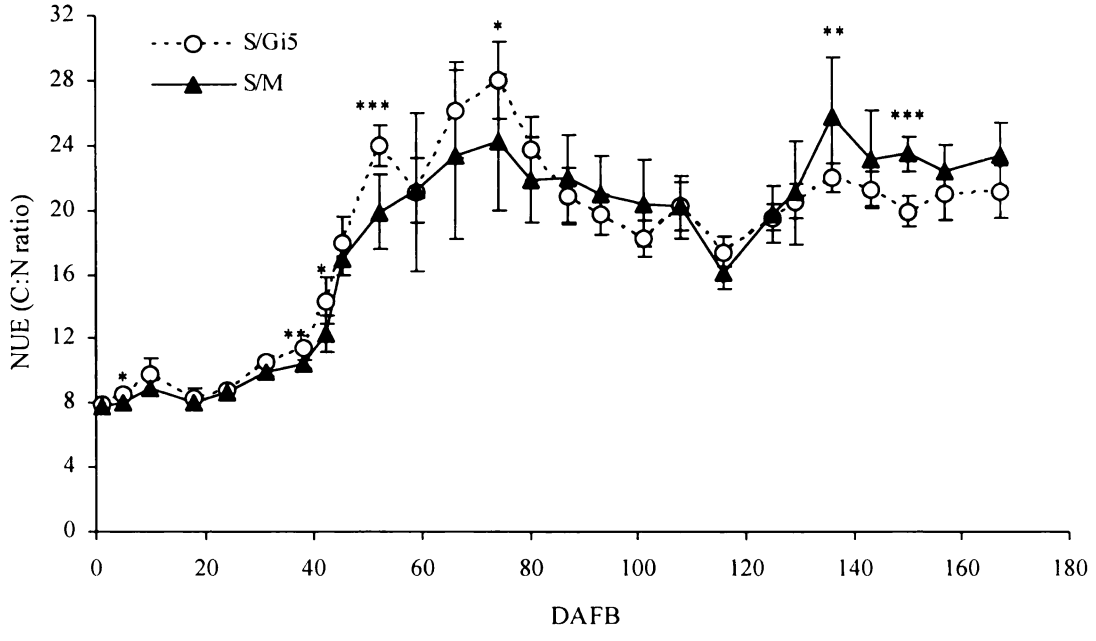

Figure 2.2. Nitrogen use efficiency (NUE) expressed as the C:N ratio of leaves in sweet cherry cv. 'Sam', measured at different Days After Full Bloom (DAFB). Comparison between 'Sam/Gisela5' (S/Gi5) and 'Sam/Mazzard' (S/M). Each point represents the mean of four replications (± STD). *, **, and *** stand for significant at $p \leq 0.05$, 0.01, and 0.001, respectively.

Table 2.7. *Total dry weight (DW), total nitrogen (N), C:N ratio, Nitrogen Derived From Fertilizer (NDFF), and percent of NDFF content compared to the total N content, measured in abscised leaves of sweet cherry 'Sam/Mazzard' (S/M) and 'Sam/Gisela 5' (S/Gi5).* ^{15}N-fertilizer was applied at bloom (11 May), rapid shoot growth (20 June) and at the beginning of leaf senescence (11 September).

Plant	Time of N application	Total DW (g)	Total N (g)	C:N ratio	NDFF (g)	% of NDFF on total N
S/M	Bloom	3289	59.6 b	25	3.2 b	5.4 b
	Rapid shoot growth	3907	68.4 a	26	5.8 a	8.4 a
	Begin of leaves senescence	3246	51.5 b	28	2.0 c	3.9 c
	Average S/M	***3481 A***[z]	***59.8***	***26***	***3.7***	***6.1***
S/Gi5	Bloom	1150	19.0 c	27	0.7 d	3.7 bc
	Rapid shoot growth	1150	19.3 c	27	0.4 d	2.1 c
	Begin of leaves senescence	1457	23.7 c	27	0.6 d	2.5 c
	Average S/Gi5	***1262 B***	***20.8***	***27***	***0.6***	***2.8***
Plant (P)[y]		**	**	NS	***	**
Fertilization time (F)		NS	NS	NS	***	*
P × F		NS	*	NS	***	**

[z] Means within columns followed by different letters are significantly different by LSMEANS test at $p \leq 0.05$. Upper case letters indicates differences between plants; lower case letters indicates between plant and treatment (P×F)

[y] NS, *, ** and *** : Non Significant or significant at $p \leq 0.05$, 0.01 and 0.001, respectively

Discussion

N-fertilizer uptake efficiency

Based on leaf ^{15}N concentration, N-fertilizer was absorbed more rapidly and i higher quantity when applied at bloom and at rapid shoot growth than at the beginning o leaf senescence, for scions on either dwarfing or standard rootstocks (Figure 2.1) Similar results were obtained in two-year-old potted prune trees (Weinbaum et al., 1978) as well as in one-year-old potted sweet cherry trees (Chapter 1, Figure 1.6) where N fertilizer was absorbed in high amounts at rapid shoot growth, and decrease consistentl in all the genotypes during leaf senescence. Nitrogen applied after bloom or during rapi shoot growth satisfied the N demand of new growth in grape (Conradie , 1991), peac (Policarpo et al., 2002), pear (Sanchez et al., 1990) and apple (Neilsen et al., 2001b) Early N applications were associated with higher bloom density and fruiting compare with summer N applications, in young 'Golden Delicious' apples on the dwarfin rootstock M9 (Neilsen et al., 2001a). Neilsen et al. (2001a) hypothesized that tree treated early in the season were able to develop a larger root system, which in genera caused greater N uptake during the season (Neilsen et al., 2001a).

N-fertilizer applied at the beginning of leaf senescence was also absorbed b sweet cherry trees, since leaf percent of N-fertilizer reached values of 4% (Figure 2.1. C) Late season N applications increase tree N storage pool, which is remobilized and used i the following year (Millard and Thomson, 1989; Sanchez et al., 1991). Deciduous plant such as grape (Conradie, 1991), apple (Titus and Kang, 1982) and peach (Tagliavini e al., 1999; Muñoz et al., 1993) are highly dependent on storage N for early spring growt and bloom. Even if the efficiency of N-fertilizer uptake at the beginning of lea

senescence was low, N-fertilizer applied in fall could significantly contribute t reserves in sweet cherry. More studies are needed to evaluate the effects of late seas applications on cold hardiness, under cold climate like the one of the Great Lakes Reg

N-fertilizer contributed more to the total N content of leaves of sweet cherrie dwarfing than standard rootstocks, particularly following the N-fertilization at blo Similar results were found in spur-type and standard 'Golden delicious' apple t under low N and high N supply (Sanchez et al., 1995). Spur-type apple trees had 3 and 72% higher percent of N-fertilizer in leaves than standard trees at low N and hig applications, respectively (Sanchez et al., 1995). Based on their higher N-ferti percent in leaves and fruit, spur-type apple trees were considered more dependent on supplies than standard trees (Sanchez et al., 1995). Several hypotheses can be formul to explain the higher percent on N-fertilizer in leaves of dwarf compared to stan trees. A possible reason for the higher percent of N-fertilizer in leaves of dwarf standard trees, could be due to the different extents of the root systems. Dwar rootstocks may explore smaller soil volumes than deep-rooted standard rootstocks, w explore larger area of soil and could uptake more naturally available N than dwar rootstocks. The root distribution pattern was also considered to play an important ro determining differences in leaf mineral contents in a four-year study on sour cherry 'Montmorency' grafted on 'Mazzard' and 'Mahaleb' (Hanson and Perry, 1989). Anc hypothesis for the higher percent of N-fertilizer in leaves of dwarf than standard tre the effect of the bloom density of trees on N-fertilizer uptake. Even if bloom density not evaluated by counting prior to frost, dwarf trees had a very high bloom density. the other side, standard trees presented very few flower clusters indicating that the

were just transitioning from the vegetative to the reproductive stage. Even if the se
frost determined the complete loss of flowers shortly after the N-fertilization at bloom
fertilizer uptake may have been higher in dwarfing than standard trees in order to sus
flowers, and subsequently the potential production of trees. Another possible specula
on the high percent of N-fertilizer in leaves of dwarfing than standard trees is that dv
trees could contain fewer N reserves in storage organs than standard trees, and dep
more on N-fertilization during the early stage of shoot growth than standard trees.

Due to the different tree size, it is difficult to speculate on the total amount o
fertilizer absorbed by the two rootstocks, since the lower percent of N-fertilizer in lea
of standard than dwarf trees may reflect, in part, a dilution of ^{15}N fertilizer due to
larger biomass of the standard trees. In general, the recommended rate for mature s
fruits in Michigan is approximately of 80 kg N ha^{-1} per year (Hanson, 1996). Lea
concentration is considered to be adequate for growth when it is approximately betwe
and 3.4 % (Nielsen and Kappel, 1996; Hanson, 1996). Under our experimental condit
application of 80 kg ha^{-1} of N supported growth in both standard and dwarfing trees s
leaf N concentration was always equal or higher than 1.9% (Figure 2.B, Appendix
The study was conducted on trees without fruits and it will be important to consider
effect of fruits as N sink. N-fertilizer may have a smaller contribution to leaf gro
when fruit competition is present, as also shown in fertilization studies on almond t
(Weinbaum et al., 1984, 1987). It will be important to evaluate both rate and timing
applications in standard and dwarfing trees at full production. In conclusion, applica
of N at bloom and rapid shoot growth in sweet cherry trees on dwarfing and stan
rootstocks was more effective than N application at the beginning of leaf senesce

When dwarf and standard trees were compared in terms of utilization of N-fertilizer, it appeared that N-fertilizer contributed more to the total N content of leaves of sweet cherries on dwarfing than standard rootstocks, especially when N was applied at bloom.

N-fertilizer partitioning

N-fertilizer absorbed at full bloom and rapid shoot growth was distributed mainly to new growth (leaves and current season shoot), in both standard and dwarf trees as a consequence of their active growth. Similar results were found in another study on two-year-old fruiting peach trees (Munoz et al., 1993). When N-fertilizer was applied at rapid shoot growth, peach trees allocated 78% of the N-fertilizer into current season growth (Munoz et al., 1993).

N-fertilizer applied at the beginning of leaf senescence was partitioned in greater amount in coarse roots than when applied at bloom, in both rootstocks (Table 2.4). Coarse roots represent the major storage organ for N as reported in peach (Bañados et al, 1997; Muñoz et al, 1993; Policarpo et al, 2002), sweet cherry (Chapter 1, Figure 1.7 and 1.8), prune (Weinbaum et al., 1978), and pear (Sanchez et al., 1992).

In standard trees, N-fertilizer in spur and shoot buds was higher when N was applied at the beginning of leaf senescence than at rapid shoot growth (Table 2.6). Differently, dwarf trees had similar percent of N-fertilizer in spur buds at rapid shoot growth and at the beginning of leaf senescence, and higher percent of N-fertilizer in shoot buds at rapid shoot growth than beginning of leaf senescence (Table 2.6). In both rootstocks, accumulation of N-fertilizer in buds followed the same pattern of N-fertilizer accumulation in current season shoot (2002 wood) and in one-year-old wood (2001 wood) (Table 2.4). Nitrogen status of developing buds is important for fruit set

(Williams, 1965). Fall N applications appear beneficial for increasing the amount of N-fertilizer in buds and therefore they may be an important practice for improving bud quality and maintaining high fruit set in trees.

Nitrogen-use efficiency

Dwarf and standard trees had similar NUE during the growing season (Figure 2.2). In the present study significant differences in NUE appeared only in a few sampling periods during the season. In a potted-tree experiment with one-year-old sweet cherry trees, similar values of NUE were obtained in 'Mazzard' rootstock, 'Rainier/Mazzard', 'Rainier/Gisela 6', and 'Rainier/Gisela 5' (Chapter 1, Figure 1.9). The values of NUE obtained with one-year-old potted trees seemed related to the presence of the same genotype, *P. avium* L., in the aerial part of the tree. Higher NUE of dwarf compared to standard trees measured during the rapid increase in leaf C:N ratio may have been caused by different growth rates of the trees. Ingestad (1979) showed a close linear relationship between relative growth rate and N concentration, in birch seedlings,. More studies are needed to evaluate the effect of growth rate of dwarfing and standard rootstocks on NUE of sweet cherry scions. Also, it will be important to compare NUE in fruiting dwarf and standard trees considering the yield produced per absorbed N.

The effect of different rootstocks or scions on leaf N concentration has been previously compared in fruit trees (Tagliavini et al., 1992; Rom et al., 1995; Ferree, 1998). Nielsen and Kappel (1996) compared leaf N concentration in several fruiting standard and dwarf sweet cherry trees. Leaf N concentration of sweet cherries on dwarfing rootstocks was occasionally lower than the ones on standard rootstock 'Mazzard'. The major difference between trees was the higher cumulative yield

efficiency of dwarf compared to standard trees. Decrease in leaf N, P, and K concentrations with increasing crop loads were also observed in apple trees (Hansen, 1980).

Retranslocation of N from senescent leaves

Nitrogen remobilization rate of sweet cherry on dwarfing and standard rootstocks did not differ as shown by the similar C:N ratio of abscised leaves. Standard trees had two times higher amounts of NDFF, calculated on the total N, in abscised leaves than dwarf trees. Since the remobilization rate of the two trees was similar, based on N-fertilizer quantity in the abscised leaves we can infer that the total amount of N-fertilizer absorbed by standard trees was higher than in dwarf trees. Based on the trunk diameter, standard trees trunk cross sectional area increased 29% while dwarfing trees trunk cross sectional area increased 12% (Table 2.1). The higher growth rate of standard trees can explain the higher N-fertilizer uptake of standard than dwarfing trees. In several studies the amount of N-fertilizer in senescent leaves was considered as part of the evaluation of plant N-fertilizer use efficiency (Weinbaum et al., 1998; Nielsen et al., 2001b). In mature walnut trees treated at the beginning of the growing season, the amount of N-fertilizer found in senescent leaves was approximately 0.2% of the N applied (Weinbaum et al., 1998). In another study on three-years-old fertigated 'Elstar' apple trees N-fertilizer in senescent leaves was 6.8% of the N applied (Nielsen et al., 2001 b). The amount of N-recovered in the abscised leaves in standard trees was comparable with the amount recovered in 'Elstar' apple trees, while in dwarf trees only 3% of the total N applied was recovered in abscised leaves.

The percent of N-fertilizer recovered in abscised leaves may be different than the one found in this study if fruiting trees are evaluated. Fruits represented a strong sink for N-fertilizer in six-year-study on walnut trees (Weinbaum et al., 1998). During the first five years, total amount of N-fertilizer recovered in leaves litter was approximately 1% of the fertilizer applied while fruits exported approximately 18% of the N-fertilizer applied (Weinbaum et al., 1998).

Conclusions

1) N-fertilizer applied during bloom and rapid shoot growth in sweet cherry on dwarfing and standard rootstocks was absorbed in greater amounts than N-fertilizer applied at the beginning of leaf senescence. Fertilization practices for sweet cherry production should be optimized considering application of fertilizer at bloom and rapid shoot growth (40 days after bloom).

2) N-fertilizer contributed in higher amount to the total N content of leaves of sweet cherries on dwarfing than standard rootstocks, especially when N was applied at bloom. N-fertilization may play an important role to sustain growth when sweet cherry are grown on dwarfing rootstocks, especially during the early stage of growth, and in soil with low organic matter. Nevertheless, N-fertilizer requirements of sweet cherry grown on dwarfing rootstocks need to be carefully evaluated to avoid risks associated to overfertilization since previous studies on apple, on the dwarfing rootstock M9, indicated low N requirements (between 8 and 44 kg N ha^{-1}) (Neilsen and Neilsen, 2002).

3) Dwarfing and standard rootstocks did not differ in terms of NUE.

4) Retranslocation of N from senescent leaves was also similar between dwarf and standard trees. Since retranslocation of N from senescent leaves is another aspect of trees NUE, it can be concluded that also based on this process, standard and dwarfing rootstocks did not affect NUE.

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CHAPTER 3

CHAPTER 3

Water-use efficiency of one-year-old sweet cherry (*Prunus avium* L. 'Rainier') on dwarfing and standard rootstocks, under well-watered and water deficit conditions

Abstract

Physiological and morphological parameters of scions may be affected by the degree of adaptation to water deficit condition of a particular rootstock. This study was conducted to determine whether standard and dwarfing sweet cherry rootstocks under water deficit conditions responded differently, relative to plant growth and gas exchange parameters, water-use efficiency (WUE), and leaf carbon isotope composition. One-year-old potted sweet cherry cv. 'Rainier' grafted on the standard rootstock 'Mazzard' and on the dwarfing rootstock 'Gisela 5' were compared under two different water treatments: 1) well-watered, which received daily 100% of the amount of water lost by ET, and 2) a water deficit treatment, which received 50% of the water applied to the control. Relative shoot growth rate, leaf emergence rate and cumulative leaf area were recorded every three to seven days during the experiment. Leaf net carbon dioxide assimilation rate, stomatal conductance, transpiration rate, internal CO_2 concentration, and WUE were measured daily for the duration of the experiment. At the end of the experiment, leaf samples were collected to determine leaf carbon isotope composition. The growth parameters measured were affected similarly in the two rootstocks, indicating a similar degree of sensitivity to water deficit in the genotypes tested. Cumulative leaf area was affected earlier by water deficit than relative shoot growth and leaf emergence rate. Gas

exchange parameters were affected earlier than growth parameters. Overall, WUE was not significantly different between dwarfing and standard rootstocks, and did not appear to increase under water deficit condition, indicating that irrigation should be considered as an important practice in sweet cherry orchards, especially when dwarfing rootstocks are selected.

Introduction

Understanding how rootstocks adapt and respond to water stress is essential for selecting the proper genotype and irrigation method for situations where drought stress is likely to occur. Unlike other stone fruits, e.g. almonds, which can often withstand drought and still produce fruit adequately, cherries require frequent supplies of water during the growing season to sustain tree growth, especially during fruit set and fruit development (Webster and Looney, 1996). In addition, availability of irrigation water will be reduced in the future because of increasing competition for urban use, therefore irrigation strategies able to increase water use, or cultivation of plants with high water-use efficiency (WUE) are becoming more important.

Water-use efficiency is defined as the amount of dry matter produced per unit of water used. It can be calculated by the instantaneous ratio of assimilation rates of CO_2 (A) and transpiration (E) (Bongi et al., 1994; Field et al., 1983) or it can be calculated on a seasonal basis (Hatfield et al., 2001). Water-use efficiency is affected by plant factors (scion and rootstock, Bongi et al., 1994) and by the environment (atmospheric CO_2 concentration, humidity, and soil water availability). Water use efficiency is an important component of drought tolerance and usually high WUE indicates the

adaptation of plants to limited water availability. Although WUE is found to increase under water deficit conditions (Flore et al., 1985), Glenn et al. (2001) reported a decline in WUE in water stressed peach.

Processes affected by water deficit in fruit trees include: cell division and expansion, flower bud differentiation, accumulation of carbohydrates (Faust, 1989), and photosynthesis (Flore and Lakso, 1989; Flore et al, 1985). Closure of stomata during water stress can be responsible for the observed reduction in photosynthetic rate (Flore and Layne, 1996). Stomatal movements are regulated by the turgor of the guard cells, which are affected by external factors such as light intensity, CO_2, humidity, wind, and temperature, as well as by endogenous factors such as plant hormones, leaf water status, and internal CO_2 (Kramer and Boyer, 1995; Hetherington and Woodward, 2003). Root-produced ABA transported to leaves is believed to regulate stomatal behavior (Davies et al., 1990). Wartinger et al. (1990) showed that in almond under a drying cycle, as xylem ABA increased, leaf conductance decreased.

Carbon isotope composition ($\delta\ ^{13}C$) is a measure of the $^{13}C/^{12}C$ ratio in plant tissues, relative to the ratio of an accepted international standard, the limestone Pee Dee belemnite (PDB). Plants have negative values of $\delta^{13}C$ because the $^{13}C/^{12}C$ ratio in the atmosphere is less than that of PDB and there is a net discrimination against ^{13}C by plants during CO_2 fixation. During water stress, the ability of plants to discriminate against the heavier isotope ^{13}C decreases, therefore the isotopic composition ($\delta\ ^{13}C$) of water stressed plants becomes more positive than the one of well-watered plants (Farquhar et al., 1982; Farquhar and Richards, 1984). Farquhar and Richards (1984) showed that carbon isotope composition is correlated with WUE in a study with different wheat genotypes. Also

carbon isotope composition was correlated with WUE in several interspecific hybrid of peach rootstock (Bongi et al., 1994).

Plants can adapt to water deficit conditions by improving water uptake or reducing water loss (Wang et al., 1998). Rootstocks have been shown to affect photosynthetic rate (Ferree and Barden, 1971), growth rate (Tubbs, 1973), and transpiration rate and WUE (Bongi et al., 1994). Besides plant physiological characteristics, morphology of the root system such as depth, and the number of fine roots are important factors for water absorption. Several reports on apple rootstocks have shown contradicting results on their ability to tolerate water stress as influenced by the vigor of the rootstock. Landsberg and Jones (1981) reported that dwarfing rootstocks, and especially M9 were more tolerant to water stress than vigorous ones. Ferree and Carlson (1987) showed that M9 was less tolerant to water stress than the vigorous MM111. Fernandez et al. (1997 a and b) showed that the dwarfing rootstock 'Mark' was the most drought sensitive, followed by the standard rootstock MM111, while the most drought resistant was the dwarfing rootstock M9 (Fernandez et al., 1997 a and b). In sweet cherry 'Mahaleb' is considered to tolerate drought better than 'Mazzard' (Lang, 2000). Little is known on the effect of dwarfing and standard sweet cherry rootstocks on growth, physiological parameters, and WUE when grown under water deficit conditions.

Periods of water shortage can occur even in regions with high annual rainfall. If water shortage coincides with a critical stage of tree and fruit growth and development, reduction in yield could take place. In order to obtain high yields, the selection of the most suitable rootstock in non-irrigated orchards is of critical importance. The objective of this study was to compare WUE of standard and dwarfing rootstocks under

well-watered and water deficit conditions. Also, we evaluated the performances of standard and dwarfing rootstocks affected by water deficit in terms of plant growth, gas exchange parameters, and leaf carbon isotope composition.

Materials and methods

One-year-old 'Rainier' sweet cherry (*P. avium* L.) grafted on Mazzard (*P. avium* L.), and Gisela 5 (*P. cerasus* (cv. Schattenmorelle) × *P. canescens*) rootstocks, were maintained outdoors, in 11 L containers at the Horticulture Teaching and Research Center, Michigan State University, East Lansing, MI. Trees were potted at dormant bud on 13 May, 2002 in a mixture of 10% silt and clay, and 90% coarse sand, they were pruned at three to five buds and subsequently trained to two to three branches. Nutrient solutions with all the essential macro- and micro-nutrients (13-2-13, N-P-K, 6% Ca, 2% Mg and micronutrients) was applied weekly in order to supply 0.5 g of N per plant, per week. Foliar iron was periodically applied (Iron chelate DP). The pots were wrapped with aluminum foil, to limit heat stress on the root system. Daily maximum and minimum air temperature and precipitation during the experiment were recorded by an automated weather station, located 300 m SW of the experimental plot, at the Horticulture Teaching and Research Center (Michigan Automated Weather Network, http://www.agweather.geo.msu.edu, Figure C.1 Appendix C).

Water treatments

Water applied during the experiment was based on the evapotranspiration (ET) of trees, measured every two to three days. ET was estimated gravimetrically by weighting the potted trees on a scale (METTLER, TOLEDO, SB32001 DeltaRange) and recording

their weight changes (Tan and Buttery, 1982). Two different water treatments were applied: 1) well-watered (control, $W_{100\%}$), which received 100% of the amount of water lost by ET during the previous day, and 2) a water deficit treatment ($W_{50\%}$), which received 50% of the water applied to the control. All plants were initially grown for a period of six weeks under well-watered condition where water was applied as needed through a drip irrigation system, with two emitters per pot. Before starting the application of the different water regimes, all pots were irrigated to field capacity. Water treatments were imposed from 48 to 69 days after bud break (DABB). Water was applied each day, in the late afternoon. Every pot was covered with an aluminum lid to exclude rain water.

Soil water content

Soil water content (SWC, % v/v) was determined with a time-domain reflectometer (Mini Trace TDR, Soilmoisture Equipment Corp. Santa Barbara, CA), using two 15-cm steel rods per pot (pots were approximately 22 cm in height). Measurements were made 2, 5, 8, 13 and 20 days from the beginning of the treatments (Topp and Davis, 1985; Topp et al., 1982).

Plant growth measurements

Shoot length and number of leaves per plant were recorded at 1, 7, 10, 14, and 21 days after the beginning of the treatments. Shoot length was measured from the base of the shoots and relative shoot growth rate (RSGR, cm day^{-1}) was calculated from shoot length of sequential measurements, according to the following equation:

$RSGR = L_2 - L_1/\Delta t$

where L_1 and L_2 stand for plant shoot length at time 1 and 2, respectively and Δt stands for time between the two measurements, in days. The leaves were counted from the base of each shoot, and the count included every unfolded leaf.

Three days after the beginning of the experiment, the three most recently unfolded leaves per shoot were tagged, and width and length were measured. Increment in tree leaf area during the experiment was estimated by measuring width and length of the three original leaves plus every new unfolded leaf until no increment in either width or length per leaf was detected. For each genotype, a regression equation was used to estimate the cumulative leaf area when only width and length were known (Table 3.1). Measurements of leaf width (point of maximum width) and length (from the petiole base to the leaf tip) were taken at 3, 7, 11, 14, and 20 days after the beginning of the treatment.

Trunk diameter (cm) was measured at 10 cm above the graft union with a digital caliper recording NS and EW dimensions twice during the experiment, at bud break (15 May) and at tree harvest (31 July).

Gas exchange measurements

Net carbon dioxide assimilation rate (A), stomatal conductance (g_s), transpiration rate (E), and internal CO_2 concentration (C_i) were measured at 1, 3, 4, 6, 7, 9, 10, 11, 12, 13, 14, 15, 16, 19, and 21 days after the beginning of the experiment, on three leaves per plant with CIRAS-2 portable infrared gas analyzer (PP systems, Haverhill, MA). Fully expanded leaves were selected and tagged at the beginning of the experiment and photosynthetic measurements were made on the same leaves throughout the experiment. Gas exchange measurements were taken in the morning between 9:00 am to 12:00 pm. During gas exchange measurements, VPD ranged from 12 to 28 mbar and leaf

temperature ranged from 22 to 34 °C on different days, PPFD > 900 μmol $m^{-2}s^{-1}$, flow rate into the cuvette was 200 mL min^{-1}, while CO_2 concentration into the cuvette was held at 370 ppm.

On day 24 from the beginning of the treatment, gas exchange was evaluated four times during the day, in order to detect any differences in the diurnal pattern of photosynthetic rate between treatments and/or genotypes. Photosynthesis was measured between 9:00 to 11:00 am, 11:00am to 1:00 pm, 1:00 to 3:00 pm, and 3:00 to 5:00 pm.

Photosynthetic water-use efficiency

Photosynthetic water use efficiency (WUE) was calculated as the ratio between CO_2 assimilated (A, μmol CO_2 m^{-2} s^{-1}) and water loss through transpiration (E, mmol H_2O m^{-2} s^{-1}) (Wang et al., 1998; Zhang and Marshall, 1994).

Plant harvest

Trees were destructively harvested at the end of the experiment (day 30 from the beginning of the experiment) to evaluate dry weight (DW) differences between control and water deficit. After washing the roots to remove the potting medium, trees were divided into the following components: fine roots (≤ 2mm diameter), coarse roots (>2mm diameter), trunk, current growth shoots, and leaves. Plant components were dried to constant weight for approximately 72 hours at 60°C, and DW was measured.

Leaf carbon isotope determination

Leaf samples collected at plant harvest were freeze-dried, and ground in a Wiley Mill to pass a 40-mesh screen. Approximately 1 mg of DW of leaves was weighted into tin capsules for mass spectrometry analysis (Automated Carbon and Nitrogen Analyzer, Roboprep, Europa Scientific, Cheshire, England) to determine total C and $\delta^{13}C$.

Carbon isotope composition ($\delta\ ^{13}C$) is calculated as follow:

$$\delta^{13}C_{PDB}\ (‰) = [(R_s/R_{PDB}) - 1] \times 1000$$

where R_s is the $^{13}C/^{12}C$ ratio measured in plant material and R_{PDB} is the $^{13}C/^{12}C$ ratio of the PDB standard, equal to 0.0112372 (Farquhar et al., 1982).

Experimental design

The experiment was a two-way factorial complete randomized block design, analyzed as a repeated-measurement design. One factor was represented by the rootstock (two rootstocks), and the other factor was the water treatment (two water treatments). Five replications per rootstock per each water treatment were used (total of 20 trees). Analysis of variance was performed using the MIXED procedure (SAS, Version 8, SAS Institute, Cary, NC, USA) to detect treatment effects. When treatments effects were significant, means were separated using the Least-Squares means test (LSMEANS test) with $p \leq 0.05$.

Table 3.1. Leaf area regression equations, number of leaf measured (n), and R^2 for leaf area prediction. Leaf area is calculated using leaf width (cm) and length (cm). Different equations were developed for 'Rainier/Mazzard' (R/M) and 'Rainier/Gisela 5' (R/Gi5).

Plant	Number of leaves used (n)	Regression equation for area	R^2
R/M	148	-20.5067+ 5.20138 × width + 3.16405 × length	0.95
R/Gi5	168	-25.7269+ 9.17772 × width + 1.93198 × length	0.94

Results

Soil water content

Maximum soil water content (SWC) calculated at field capacity was 16% (v/v). SWC of the control trees was maintained between 10-12% (v/v) during the experiment (Figure 3.1. A). SWC of the $W_{50\%}$ trees was significantly lower than the controls starting 8 days after the beginning of the experiment, when it was approximately 80% of the SWC of the controls and continued to decrease until the end of the experiment (Figure 3.1. A and B). There were no significant differences between SWC in R/Gi5 and R/M $W_{100\%}$ and between R/Gi5 and R/M $W_{50\%}$ at any of the measurement times.

Plant growth parameters

Shoot elongation

Total shoot length increased from 10 to 90 cm per plant, between 25 and 60 Days After Bud Break (DABB) (Figure C.2 Appendix C). No differences were detected between the two rootstocks, on any of the measurement times (Figure C.2 Appendix C).

Table C.1 and C.2 , Appendix C, give a summary of occurrence over time of significant differences between $W_{100\%}$ and $W_{50\%}$ of each rootstock, and differences between controls or between water deficit plants (expressed as percent of their controls) of all growth parameters measured during the experiment.

Relative shoot growth rate (cm day^{-1}) increased steadily until 48 DABB with a maximum of 3 cm day^{-1} between 37 and 41 DABB and gradually decreased until 69 DABB (data not shown). Relative shoot growth rate started to decreased in $W_{50\%}$ plants from 7 days after the beginning of the treatments, but was not significantly lower than controls until the 14 through 21 days period. During this period of time, both R/Gi5 and

R/M $W_{50\%}$ showed a significant lower relative shoot growth rate compared to the corresponding controls (Figure 3.2). The decrease in relative shoot growth rate was similar in R/M and R/Gi5 $W_{50\%}$, when values where express as a percent of their controls (data not shown).

Leaf emergence rate

Relative leaf emergence rate (leaves day^{-1}) of R/Gi5 $W_{100\%}$ plants was four times higher than R/Gi5 $W_{50\%}$ plants, between 14 and 21 days after the beginning of the treatments (Figure 3.3. A). R/M $W_{50\%}$ had a rate equal to zero between 14 to 21 days from the beginning of the experiments and was significantly lower than the $W_{100\%}$ plants (Figure 3.3. B). Relative leaf emergence rate of R/Gi5 $W_{50\%}$ plants, expressed as a percent of their controls, was higher than that of R/M $W_{50\%}$ plants between 14 and 21 days from the beginning of the treatments (data not shown).

Cumulative leaf area

Estimated cumulative increment in leaf area of R/Gi5 $W_{50\%}$ was significantly lower than $W_{100\%}$ plants from 11 days after the beginning of the treatments (Figure 3.4. A). Estimated cumulative increment in leaf area of R/M $W_{50\%}$was significantly lower than $W_{100\%}$ plants from 14 days after the beginning of the experiment (Figure 3.4. B). Increment in leaf area was statistically higher for R/Gi5 $W_{100\%}$ plants than R/M $W_{100\%}$ plants in all the measured dates (data not shown). When expressed as a percent of their corresponding controls, no difference was detected between R/Gi5 and R/M $W_{50\%}$ plants (data not shown).

Trunk cross sectional area

Although trunk cross sectional area of $W_{100\%}$ and $W_{50\%}$ plants was not statistically different at the end of the experiment (data not shown), the percent of increase in trunk cross sectional area from budbreak until tree harvest was significantly higher in $W_{100\%}$ than $W_{50\%}$ plants, in both rootstocks (Table 3.2).

Plant dry weight

Dry weight of fine roots, coarse roots, trunk, shoot and leaves were not significantly affected by the two different water treatments, although there was a tendency for the $W_{50\%}$ plants of both genotypes to have a lower amount of total DW than the $W_{100\%}$ plants (data not shown). Root to shoot ratio was not significantly affected by the water treatments and there was no difference between the two rootstocks. The root to shoot ratio was approximately 0.5 and 0.7 for R/Gi5 and R/M, respectively.

Gas exchange parameters

Table C.1 and C.2 , Appendix C, gives a summary of occurrence over time of significant differences between $W_{100\%}$ and $W_{50\%}$ of each rootstock, and differences between controls or between water deficit plants (expressed as percent of their controls) of all gas exchange parameters measured during the experiment.

Net carbon dioxide assimilation rate

Values of net carbon dioxide assimilation rate (A) for $W_{100\%}$ plants during the experiment varied between 16.9 and 10.3 and between 14.3 and 9.05 $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$ for R/Gi5 and R/M, respectively (Figure 3.5). Statistically significant differences between $W_{100\%}$ and $W_{50\%}$ plants were detected on day 7 for R/M and on 9 day for R/Gi5 (Figure 3.5). On day 8, A of $W_{50\%}$ plants was approximately 80% of the control values, in both

genotypes, and it progressively decreased until it reached 20 % of the controls, on day 21. Control plants of R/Gi5 had a significantly higher A than R/M on days 3, 4, 9, 10, 13, 14, 15 and 21. Nevertheless, the two rootstocks under water deficit conditions did not show significant differences when A was expressed as a percent of their controls, except on day 9 of the experiment, where R/M had a higher A then R/Gi5.

Transpiration rate

Transpiration rate (E) for $W_{100\%}$ plants throughout the experiment varied between 3.7 and 2.2, and between 2.2 and 1.7 mmol $H_2O \cdot m^{-2} \cdot s^{-1}$ for R/Gi5 and R/M, respectively (Figure 3.6). Control plants of R/M and of R/Gi5 had a significantly higher E than R/M $W_{50\%}$ and R/Gi5 $W_{50\%}$ plants starting from day 7 and day 6, respectively until the end of the experiment (Figure 3.6). On day 8, E of $W_{50\%}$ plants was approximately 80% of the $W_{100\%}$ plant values, in both genotypes, and it progressively decreased until it reached 20 % of $W_{100\%}$ plant values, on day 21. Control plants of R/Gi5 had a higher E than R/M on days 3, 4, 9, 15 and 21. Transpiration of the two genotypes under water deficit conditions did not differ, when expressed as a percent of their controls, except on days 3, 10 and 11 from the beginning of the treatments, where R/M had a higher transpiration rate than R/Gi5.

Stomatal conductance

Values of stomatal conductance (g_s) for $W_{100\%}$ plants during the experiment varied between 116 and 246 and between 118 and 219 mmol $H_2O \cdot m^{-2} \cdot s^{-1}$ for R/Gi5 and R/M, respectively (Figure 3.7). Statistically significant differences between $W_{100\%}$ and $W_{50\%}$ plants were detected at day 7 for R/M and at day 6 for R/Gi5 (Figure 3.7). Control plants of R/Gi5 had higher g_s than R/M on days 3, 4, 13, and 16. The two genotypes

under water deficit conditions did not show any significant differences when g_s was expressed as a percent of their controls except on day 3 and 11, where R/M had a higher g_s of R/Gi5.

Internal carbon dioxide

Values of internal CO_2 (C_i) for $W_{100\%}$ plants during the experiment varied between 181 and 218, and between 192 and 233 $\mu L\ L^{-1}$ for R/Gi5 and R/M, respectively (Figure 3.8). Internal CO_2 was statistically higher in R/Gi5 $W_{100\%}$ than R/Gi5 $W_{50\%}$ plants on days 3, 9, 10, 11, 12, 14 and 15 (Figure 3.8. A). For R/M, it was statistically higher in $W_{50\%}$ plants than $W_{100\%}$ plants on days 3 and 4, while it was higher for $W_{100\%}$ than $W_{50\%}$ plants on days 9, 12, 14, 15, and 16 (Figure 3.8. B). The percent of C_i of $W_{50\%}$ plants during the experiment was between 80 and 110% of the $W_{100\%}$ trees, for both genotypes. Comparing the controls of the two genotypes, C_i was statistically higher for R/M than R/Gi5 on days 3, 6, 9, 12, 14, 15, and 16. When expressed as percent of the control, R/M $W_{50\%}$ plants had a higher C_i than R/Gi5 $W_{50\%}$ on days 3, 4, 10, and 12, while on days 13 and 16 R/Gi5 $W_{50\%}$ had higher values of C_i than R/M $W_{50\%}$ plants.

Water-use efficiency

Values of water-use efficiency (WUE) for $W_{100\%}$ plants during the experiment vary approximately between 3.4 and 6.5 $\mu mol\ CO_2\ mmol^{-1}\ H_2O$, for both R/Gi5 and R/M (Figure 3.9). On days 13 and 21, WUE was statistically higher in R/Gi5 $W_{100\%}$ than $W_{50\%}$ plants while on day 14 R/Gi5 $W_{50\%}$ plants had a higher WUE than $W_{100\%}$ plants (Figure 3.9. A). On days 3 and 21 of the experiment, WUE was statistically higher in $W_{100\%}$ than $W_{50\%}$ plants for R/M while on days 14 and 16 R/M $W_{50\%}$ plants had a higher WUE than $W_{100\%}$ (Figure 3.9. B). On days 9, 11, 12, 13, and 14 R/Gi5 $W_{100\%}$ plants had

a higher WUE than R/M $W_{100\%}$ plants (Figure 3.10. A). The percent of WUE of the $W_{50\%}$ plants was approximately between 110 and 90% of the controls with a few times around 70% of the controls (Figure 3.10. B). On days 3, 10, 11 R/Gi5 $W_{50\%}$ plants had a higher WUE than R/M $W_{50\%}$ plants, when expressed as a percent of their controls (Figure 3.10. B).

Daily trend of gas exchange parameters

Assimilation rate was higher in $W_{100\%}$ than $W_{50\%}$ plants, during all the intervals measured (Figure 3.11. 1). R/Gi5 $W_{100\%}$ plants had higher A than R/M $W_{100\%}$ between 9:00 and 11:00 am and 3:00 and 5:00 pm, while R/Gi5 $W_{50\%}$ plants had higher A than R/M $W_{50\%}$ between 9:00 and 11:00 am and 1 and 3 pm (Figure 3.11. 1). Transpiration rate was higher for R/Gi5 and R/M controls than the same genotypes under water deficit, in all the intervals measured (Figure 3.11. 2). WUE decreased from approximately 8 to 4 μmol CO_2 $mmol^{-1}$ H_2O from 9:00 to 11:00 am to 3:00 to 5:00 pm, respectively, in both treatments and genotypes (Figure 3.11. 3). WUE was different between $W_{100\%}$ and $W_{50\%}$ plants only between 9:00 to 11:00 am (Figure 3.11. 3). Stomatal conductance decreased from 9:00 to 11:00 am to 3:00 to 5:00 pm and was higher in $W_{100\%}$ than $W_{50\%}$ plants, in both genotypes (Figure 3.12). Internal CO_2 was higher in R/M $W_{100\%}$ plants compared to the rest, between 9:00 to 11:00 am and 11:00 am to 1:00 pm (Figure 3.13). Between 1:00 and 3:00 pm, C_i was significantly higher in R/M and R/Gi5 controls than R/Gi5 $W_{50\%}$ plants (Figure 3.13). Also, between 1:00 and 3:00 pm, C_i was significantly higher in R/M $W_{50\%}$ than R/Gi5 $W_{50\%}$ plants (Figure 3.13).

Leaf carbon isotope composition

The $\delta^{13}C$ of leaves was affected by the different water treatments (Table 3.3). Leaves of $W_{50\%}$ plants of both genotypes had a more positive $\delta^{13}C$ and a higher atom % than their corresponding controls (Table 3.3). Comparing the two genotypes, R/M had more negative values of $\delta^{13}C$ than R/Gi5 (Table 3.3).

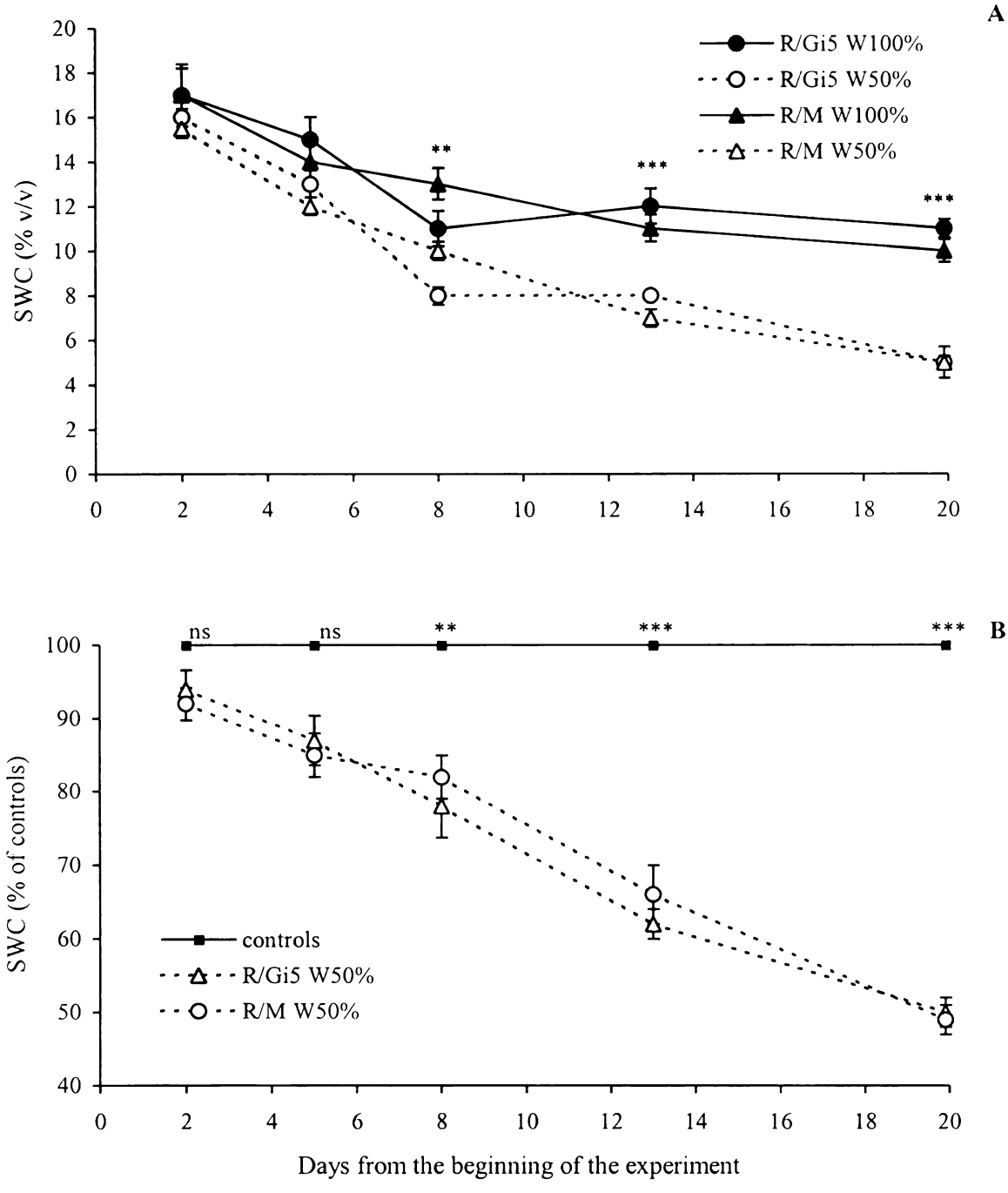


Figure 3.1. Soil Water Content (SWC) measured with Time Domain Reflectometry (TDR). **A** Comparison between 'Rainier/Gisela 5' (R/Gi5 $W_{100\%}$) under well-watered and 'Rainier/Gisela 5' (R/Gi5 $W_{50\%}$) under water deficit conditions, and between 'Rainier/Mazzard' (R/M $W_{100\%}$) under well-watered and 'Rainier/Mazzard' (R/M $W_{50\%}$) under water deficit conditions. **B** SWC of R/Gi5 $W_{50\%}$ and R/M $W_{50\%}$ expressed as % of their controls. Vertical bars indicate standard errors (SE, n=5) of means. ** and *** stand for significant at $p \leq 0.01$ and 0.001, respectively.

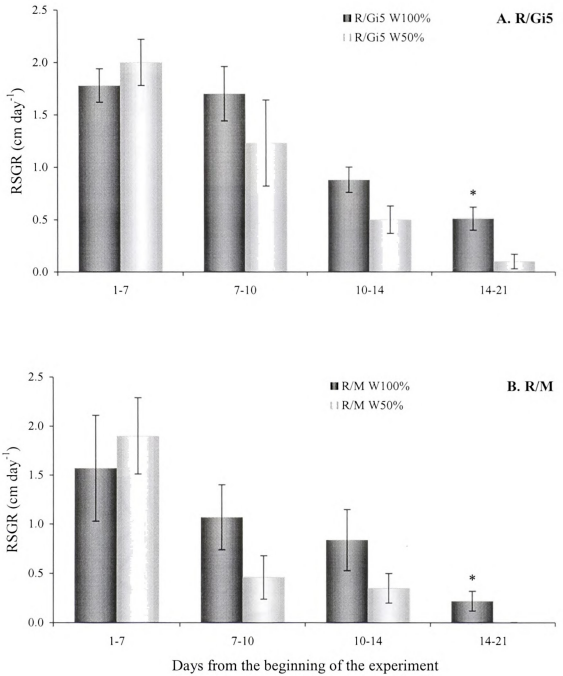


Figure 3.2. Relative Shoot Growth Rate (RSGR, cm day^{-1}) of sweet cherry cv. 'Rainier' plants. Comparison between **A** 'Rainier/Gisela 5' under well-watered (R/Gi5 $W_{100\%}$) and water deficit (R/Gi5 $W_{50\%}$) conditions, and **B** 'Rainier/Mazzard' under well-watered (R/M $W_{100\%}$) and water deficit (R/M $W_{50\%}$) conditions. Vertical bars indicate standard errors (SE, n=5) of means. * stands for significant at $p \leq 0.05$.

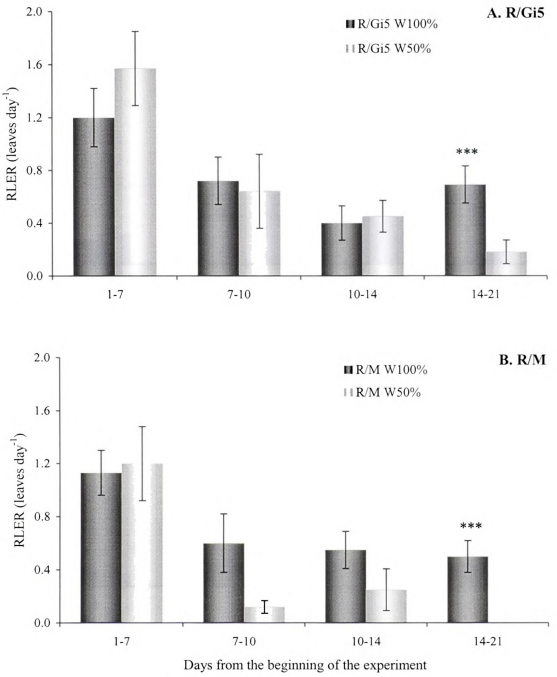


Figure 3.3. Relative Leaf Emergence Rate (RLER, leaves day^{-1}) of sweet cherry cv.'Rainier'. Comparison between **A** 'Rainier/Gisela 5' under well-watered (R/Gi5 $W_{100\%}$) and water deficit (R/Gi5 $W_{50\%}$) conditions, and **B** 'Rainier/Mazzard' under well-watered (R/M $W_{100\%}$) and water deficit (R/M $W_{50\%}$) conditions. Vertical bars indicate standard errors (SE, n=5) of means. *** stands for significant at $p \leq 0.001$.

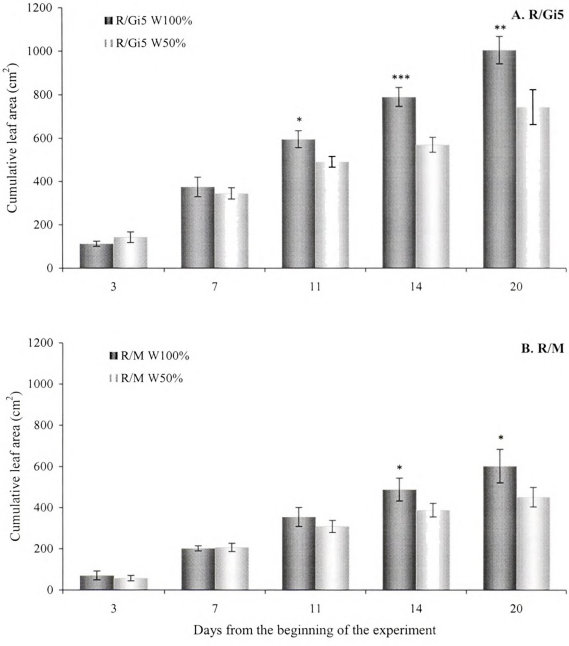


Figure 3.4. Cumulative leaf area (cm^2) of newly formed leaves of sweet cherry cv.'Rainier'. Comparison between **A** 'Rainier/Gisela 5' under well-watered (R/Gi5 $W_{100\%}$) and water deficit (R/Gi5 $W_{50\%}$) conditions, and **B** 'Rainier/Mazzard' under well-watered (R/M $W_{100\%}$) and water deficit (R/M $W_{50\%}$) conditions. Vertical bars indicate standard errors (SE, n=5) of means. *, **, and *** stand for significant at $p \leq 0.05$, 0.01 and 0.001, respectively.

Table 3.2. Percent of increase in trunk cross sectional area from bud break (15 May) until tree harvest (31 July). Comparison between R/M and R/Gi5 under well-watered (R/M and R/Gi5 $W_{100\%}$) and R/M and R/Gi5 under water deficit conditions (R/M and R/Gi5 $W_{50\%}$).

Rootstock	Increase in area (%)
R/M $W_{100\%}$	17.5 ± 1[z]
R/M $W_{50\%}$	7.1 ± 2
R/Gi5 $W_{100\%}$	11.3 ± 3
R/Gi5 $W_{50\%}$	4.7 ± 3
Significance	
Plant[y]	NS
Treatment	*
Plant × Treatment	NS

[z] Standard Error (n=4) of means

[y] NS and *: Non Significant and significant at $p \leq 0.05$, respectively

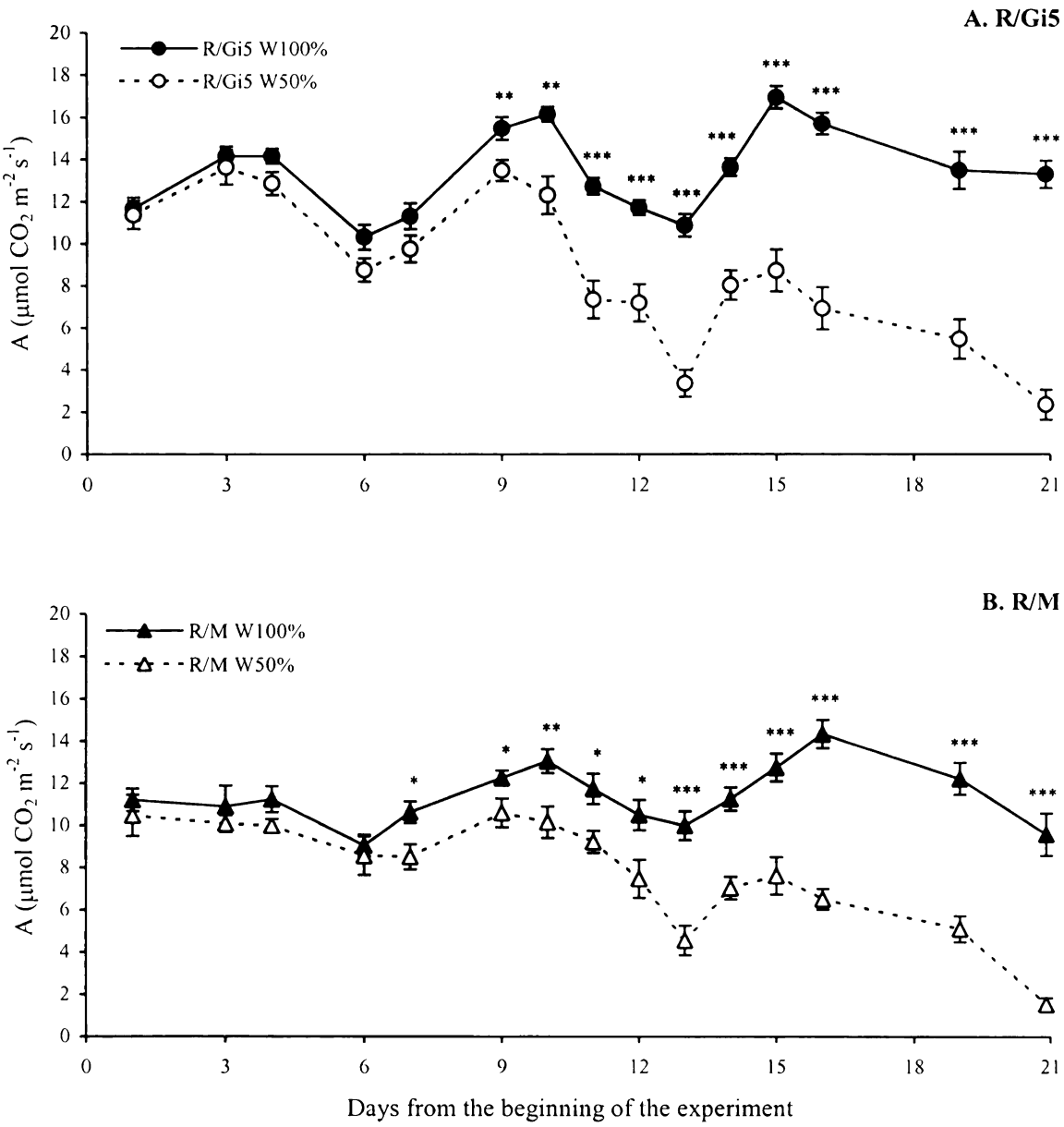


Figure 3.5. Plants net assimilation rate (A, µmol CO_2 m^{-2} s^{-1}). Comparison between **A** 'Rainier/Gisela 5' under well-watered (R/Gi5 $W_{100\%}$) and water deficit (R/Gi5 $W_{50\%}$) conditions, and **B** 'Rainier/Mazzard' under well-watered (R/M $W_{100\%}$) and water deficit (R/M $W_{50\%}$) conditions. Vertical bars indicate standard errors of means of five plants (three leaves per plant). *, **, and *** stand for significant at $p \leq 0.05$, 0.01 and 0.001, respectively.

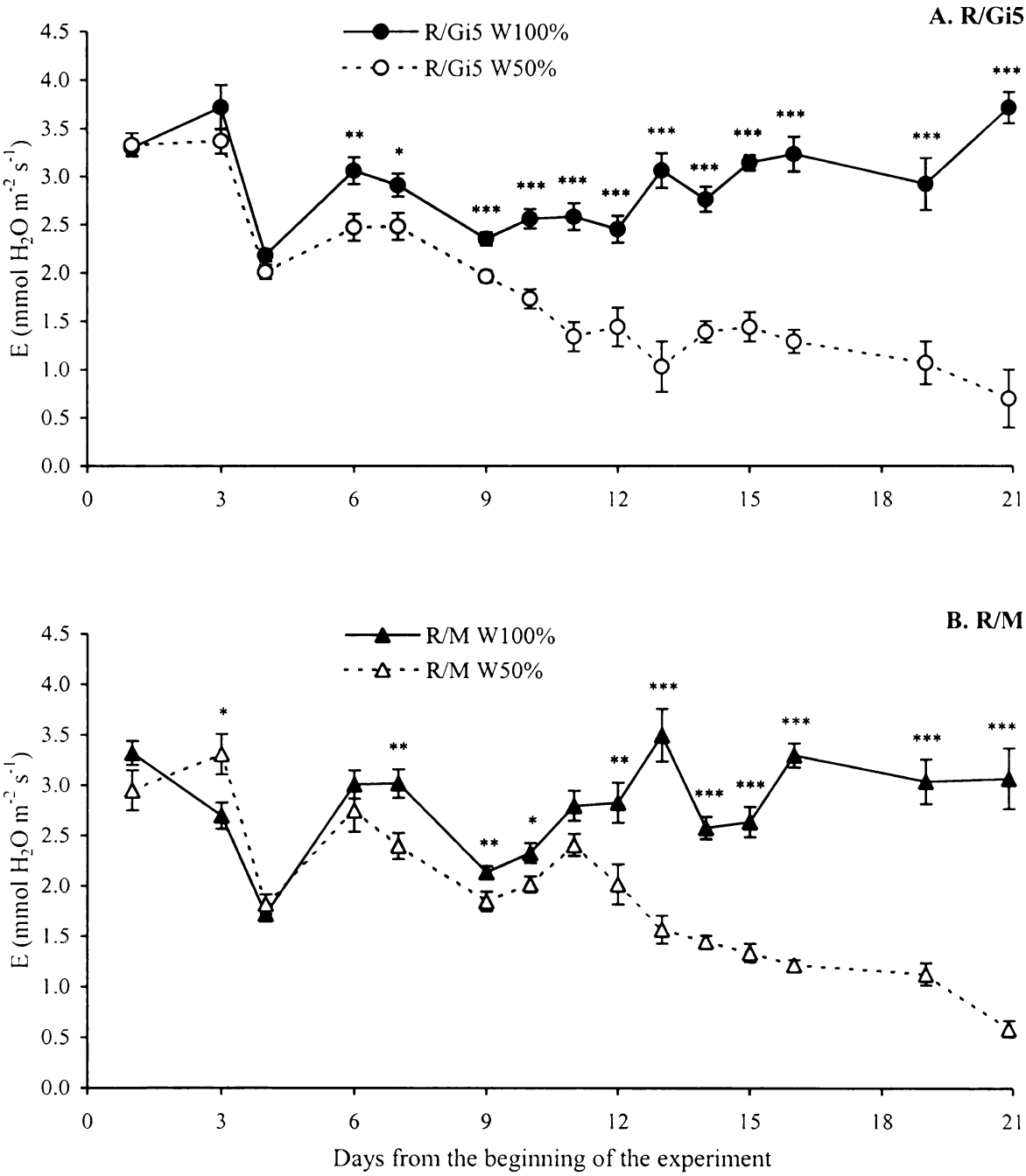


Figure 3.6. Plants transpiration rate (E, mmol H_2O m^{-2} s^{-1}). Comparison between **A** 'Rainier/Gisela 5' under well-watered (R/Gi5 $W_{100\%}$) and water deficit (R/Gi5 $W_{50\%}$) conditions, and **B** 'Rainier/Mazzard' under well-watered (R/M $W_{100\%}$) and water deficit (R/M $W_{50\%}$) conditions. Vertical bars indicate standard errors of means of five plants (three leaves per plant). *, **, and *** stand for significant at $p \leq 0.05$, 0.01 and 0.001, respectively.

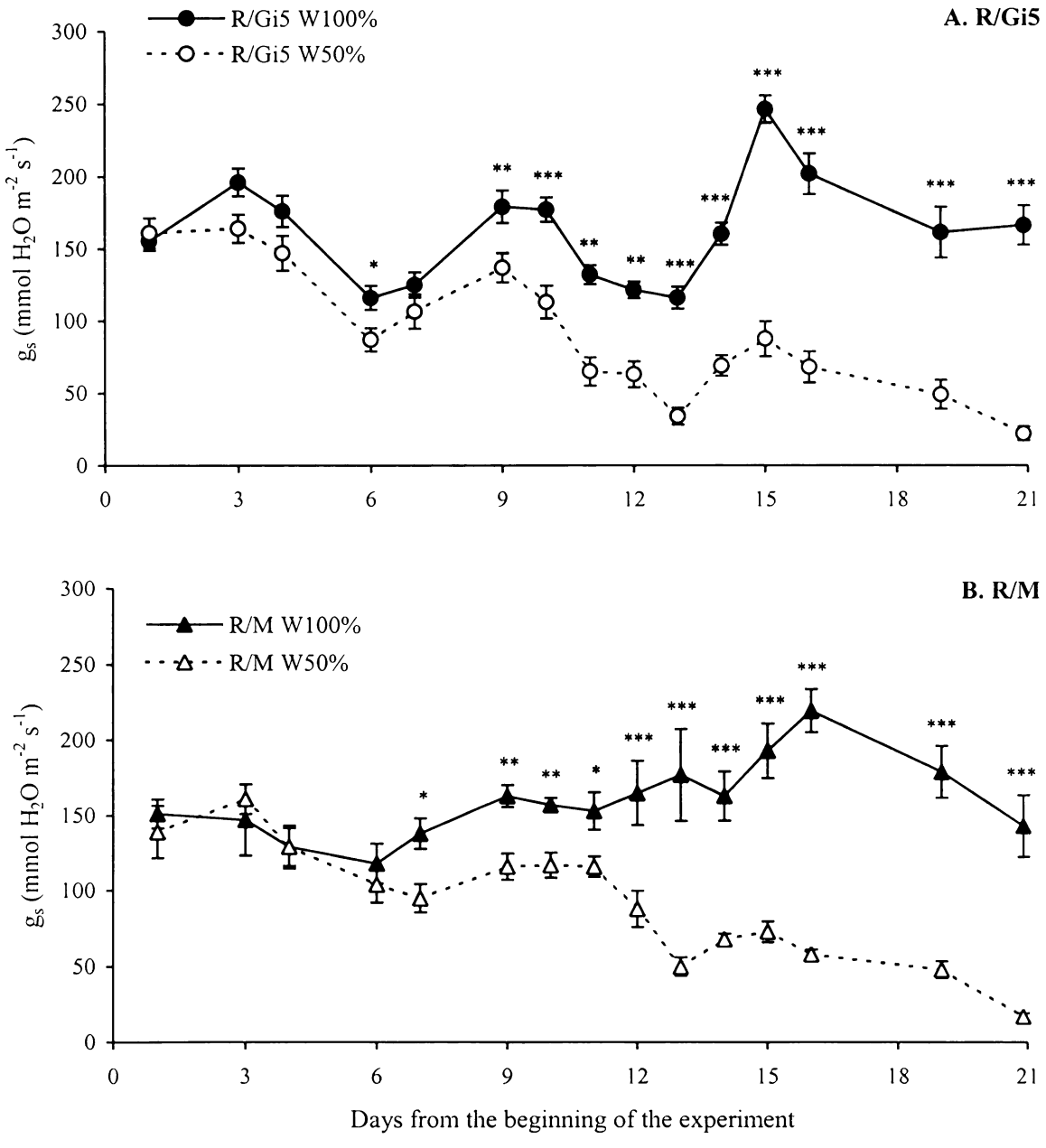


Figure 3.7. Plants stomatal conductance (g_s, mmol H_2O m^{-2} s^{-1}). Comparison between **A** 'Rainier/Gisela 5' under well-watered (R/Gi5 $W_{100\%}$) and water deficit (R/Gi5 $W_{50\%}$) conditions, and **B** 'Rainier/Mazzard' under well-watered (R/M $W_{100\%}$) and water deficit (R/M $W_{50\%}$) conditions. Vertical bars indicate standard errors of means of five plants (three leaves per plant). *, **, and *** stand for significant at $p \leq 0.05$, 0.01 and 0.001, respectively.

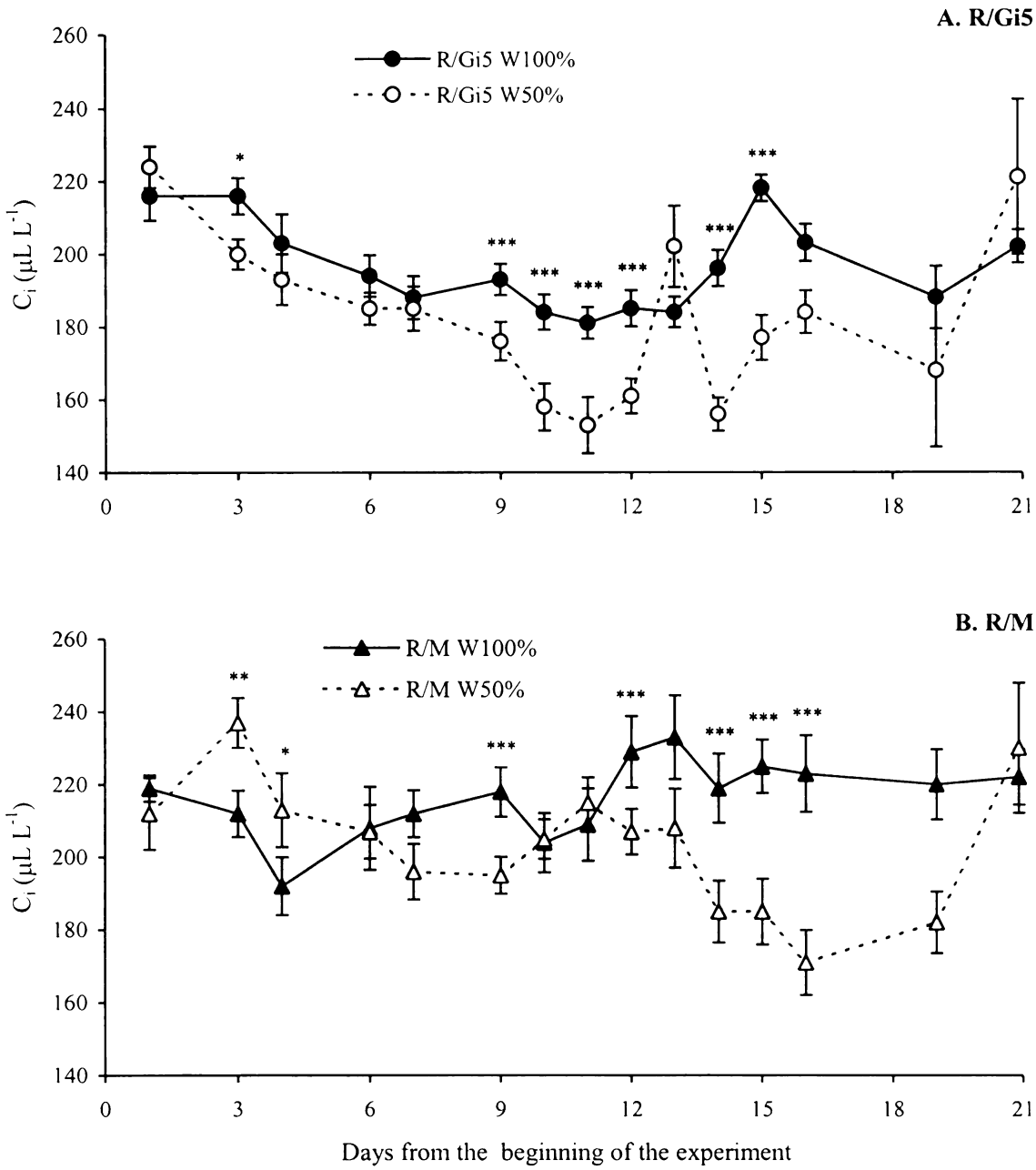


Figure 3.8. Leaf intercellular CO_2 concentration (C_i, µL L^{-1}). Comparison between **A** 'Rainier/Gisela 5' under well-watered (R/Gi5 $W_{100\%}$) and water deficit (R/Gi5 $W_{50\%}$) conditions, and **B** 'Rainier/Mazzard' under well-watered (R/M $W_{100\%}$) and water deficit (R/M $W_{50\%}$) conditions. Vertical bars indicate standard errors of means of five plants (three leaves per plant). *, **, and *** stand for significant at $p \leq 0.05$, 0.01 and 0.001, respectively.

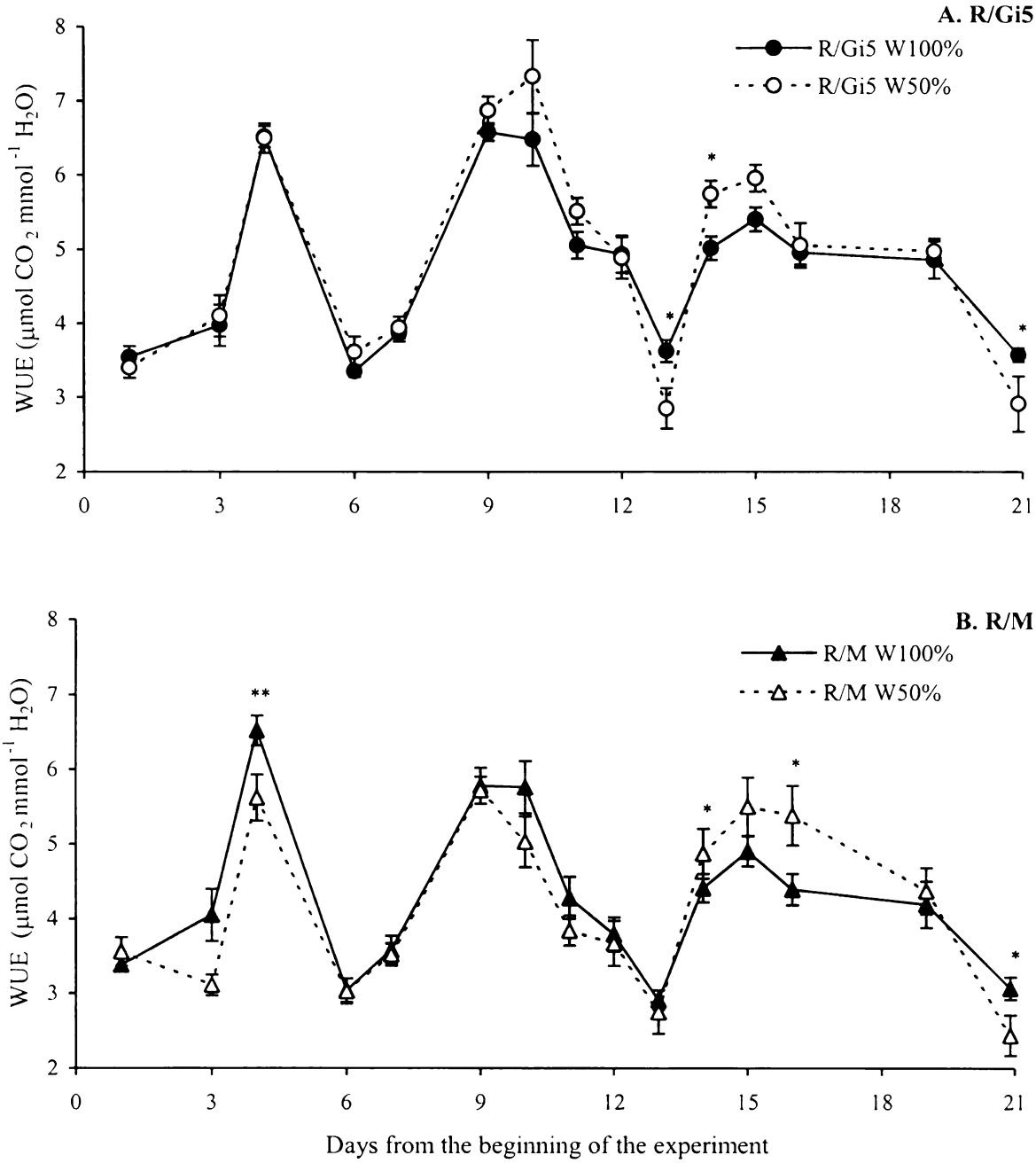


Figure 3.9. Water-use efficiency (WUE, μCO_2 $mmol^{-1}$ H_2O). Comparison between **A** 'Rainier/Gisela 5' under well-watered (R/Gi5 $W_{100\%}$) and water deficit (R/Gi5 $W_{50\%}$) conditions, and **B** 'Rainier/Mazzard' under well-watered (R/M $W_{100\%}$) and water deficit (R/M $W_{50\%}$) conditions. Vertical bars indicate standard errors of means of five plants (three leaves per plant). * and ** stand for significant at $p \leq 0.05$ and 0.01, respectively.

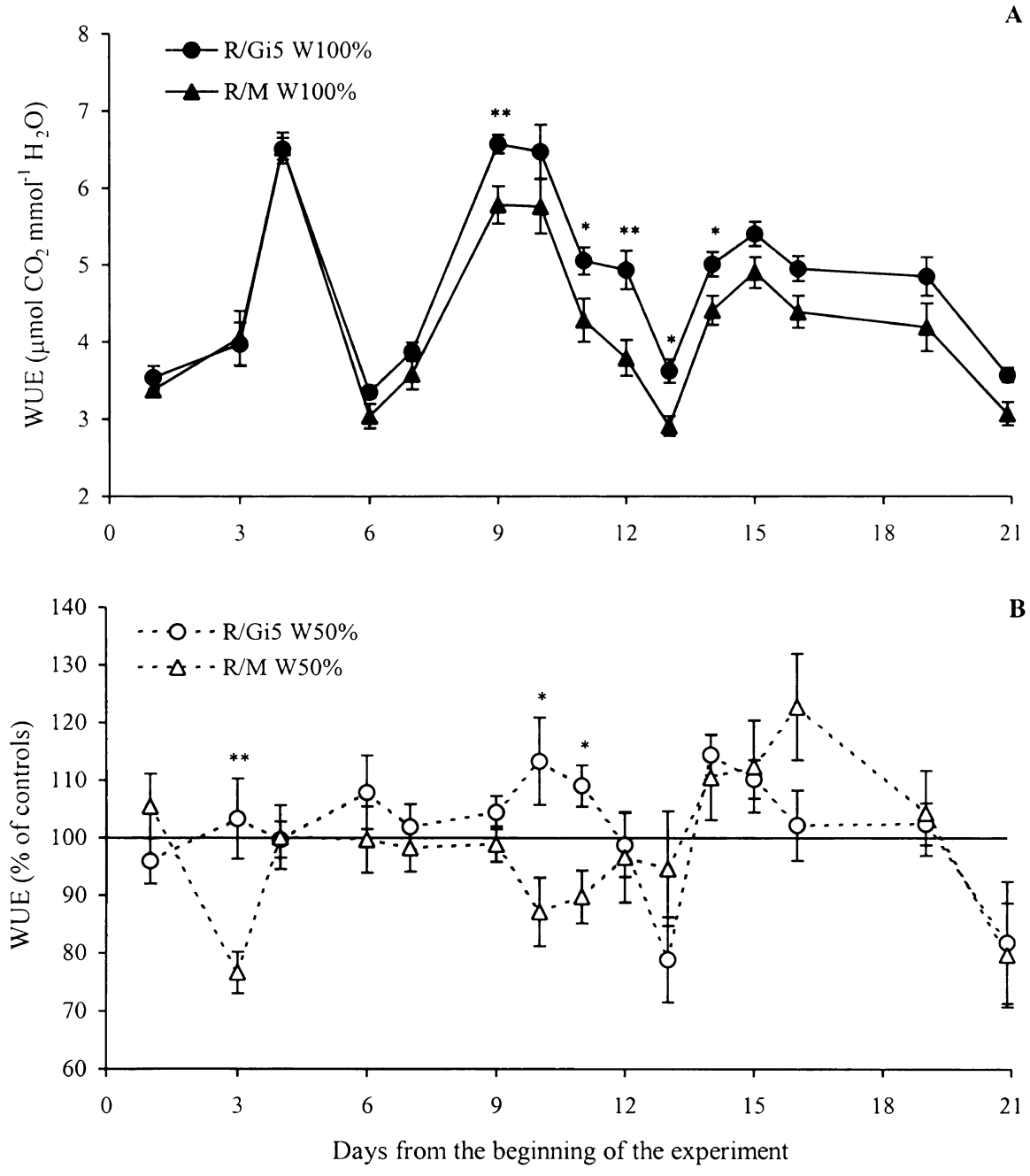
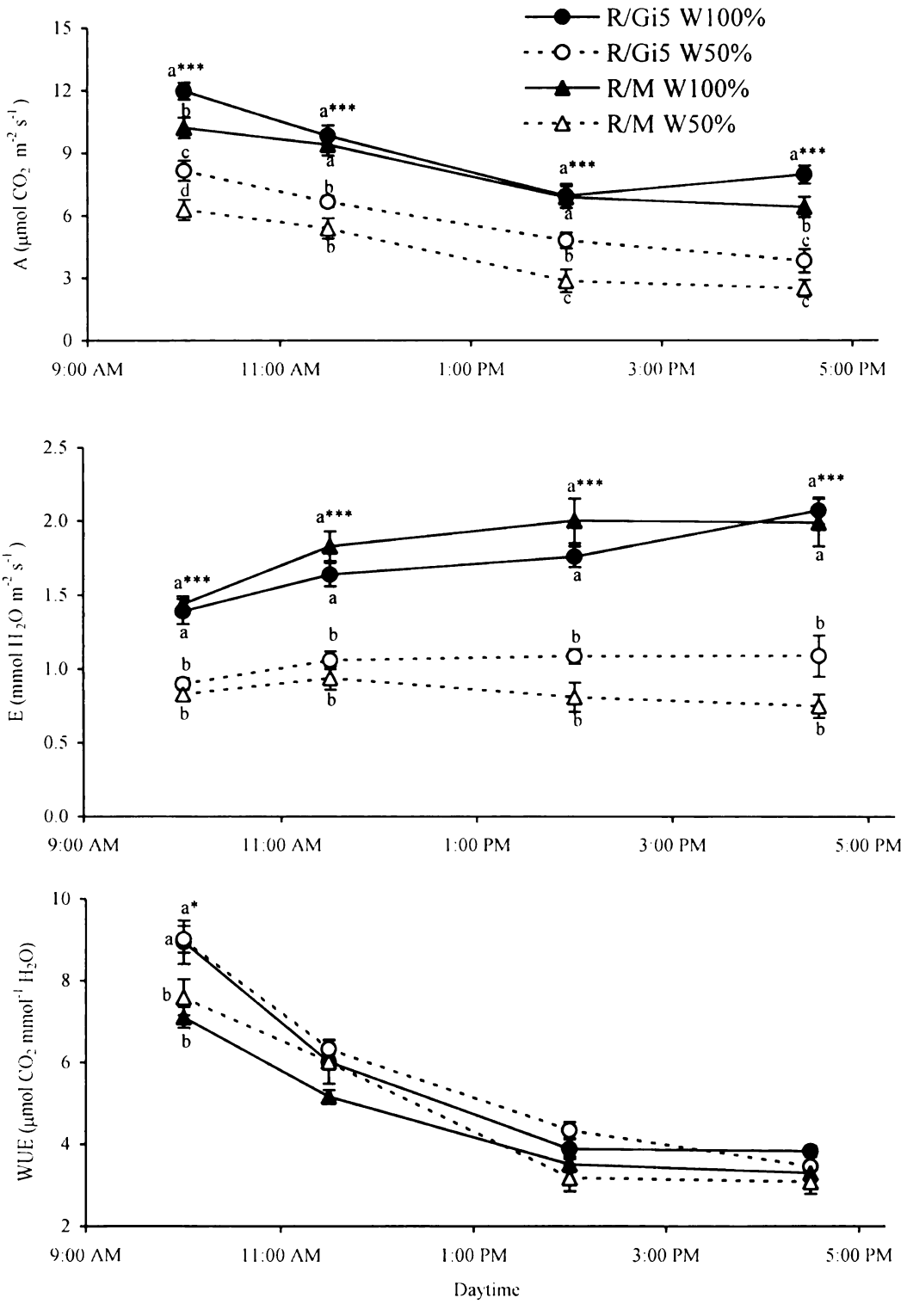


Figure 3.10. Water-use efficiency (WUE, µmol CO_2 $mmol^{-1}$ H_2O). **A** Comparison between 'Rainier/Gisela 5' (R/Gi5 $W_{100\%}$) and 'Rainier/Mazzard' (R/M $W_{100\%}$) under well-watered conditions. **B** WUE of 'Rainier/Gisela 5' (R/Gi5 $W_{50\%}$) and 'Rainier/Mazzard' (R/M $W_{50\%}$) under water deficit conditions expressed as % of their controls. Vertical bars indicate standard errors of means of five plants (three leaves per plant). * and ** stand for significant at $p \leq 0.05$ and 0.01, respectively.

Figure 3.11. Daily trend of **1.** net carbon dioxide assimilation rate (A), **2.** transpiration rate (E), and **3.** water-use efficiency (WUE) of sweet cherry cv.'Rainier'. Comparison between 'Rainier/Gisela 5' under well-watered (R/Gi5 $W_{100\%}$) and water deficit (R/Gi5 $W_{50\%}$) conditions and 'Rainier/Mazzard' under well-watered (R/M $W_{100\%}$) and water deficit (R/M $W_{50\%}$) conditions. Vertical bars indicate standard errors of the means. Treatment means with different letters are significantly different at $p \leq 0.05$ (Least-square means test). * and ***: significant at $p \leq 0.05$ and 0.001, respectively.


R/Gi5 W100%
R/Gi5 W50%
R/M W100%
R/M W50%
A (μmol CO2 m-2 s-1)
E (mmol H2O m-2 s-1)
WUE (μmol CO2 mmol-1 H2O)
9:00 AM
11:00 AM
1:00 PM
3:00 PM
5:00 PM
Daytime

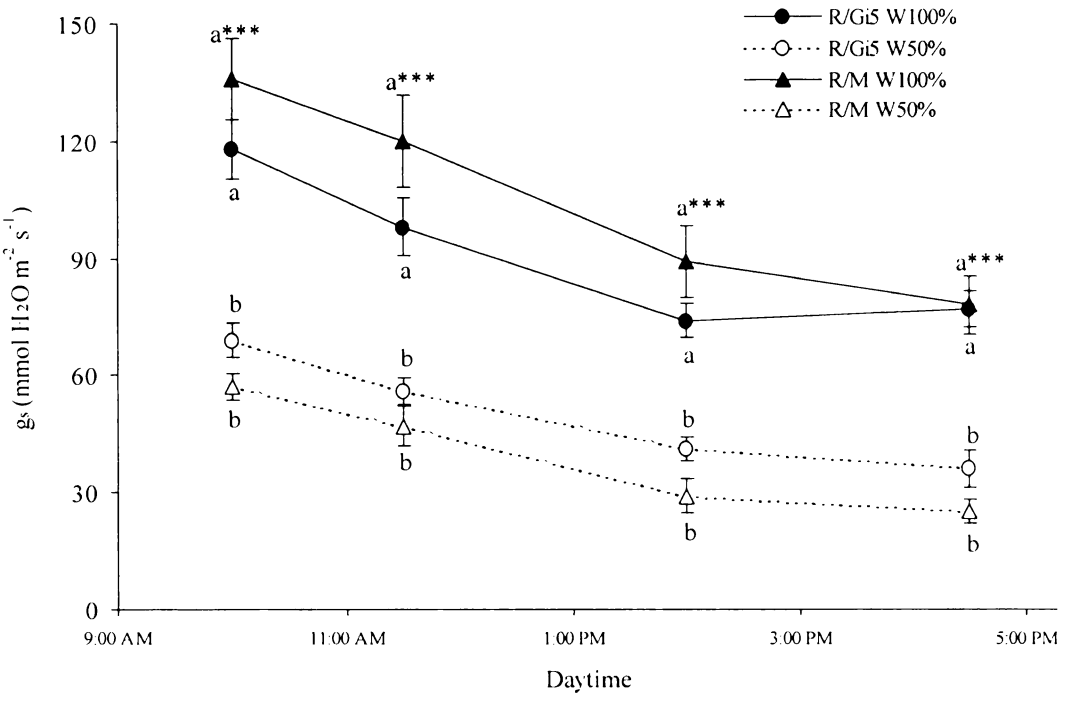


Figure 3.12. Daily trend of stomatal conductance (g_s) of sweet cherry cv. 'Rainier'. Comparison between 'Rainier/Gisela 5' under well-watered (R/Gi5 $W_{100\%}$) and water deficit (R/Gi5 $W_{50\%}$) conditions and 'Rainier/Mazzard' under well-watered (R/M $W_{100\%}$) and water deficit (R/M $W_{50\%}$) conditions. Vertical bars indicate standard errors (SE, n=5) of the means. Treatment means with different letters are significantly different at $p \leq 0.05$ (Least-square means test). * and ***: significant at $p \leq 0.05$ and 0.001, respectively.

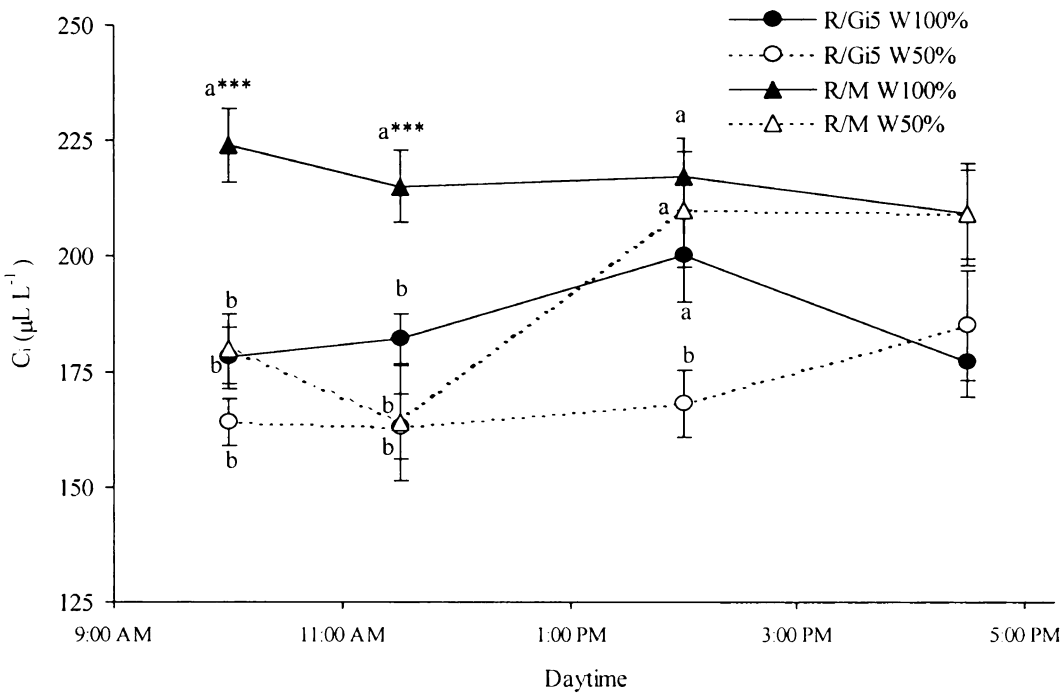


Figure 3.13. Daily trend of internal CO_2 (C_i) of sweet cherry cv. 'Rainier'. Comparison between 'Rainier/Gisela 5' under well-watered (R/Gi5 $W_{100\%}$) and water deficit (R/Gi5 $W_{50\%}$) conditions and 'Rainier/Mazzard' under well-watered (R/M $W_{100\%}$) and water deficit (R/M $W_{50\%}$) conditions. Vertical bars indicate standard errors (SE, n=5) of the means. Treatment means with different letters are significantly different at $p \leq 0.05$ (Least-square means test). * and ***: significant at $p \leq 0.05$ and 0.001, respectively.

Table 3.3. Carbon isotope composition ($\delta^{13}C$, ‰) and atom % of leaves of one-year-old sweet cherry 'Rainier/Mazzard' under well-watered (R/M $W_{100\%}$) and water deficit conditions (R/M $W_{50\%}$) and 'Rainier/Gisela 5' under well-watered (R/Gi5 $W_{100\%}$) and water deficit conditions (R/Gi5 $W_{50\%}$). Leaves were collected when trees were harvested.

Rootstock	$\delta^{13}C$ (‰)	Atom %
R/M $W_{100\%}$	-28.6 a[z]	1.79 d
R/M $W_{50\%}$	-28.0 b	1.80 c
R/Gi5 $W_{100\%}$	-27.5 c	1.81 b
R/Gi5 $W_{50\%}$	-26.7 d	1.82 a
Significance		
Plant[y]	*	*
Treatment	**	**
Plant × Treatment	NS	NS

[z] Means within columns followed by different letters are significantly different by LSMEANS test at $p \leq 0.05$.

[y] NS,*, and**: Non Significant and significant at $p \leq 0.05$, 0.01, respectively

Discussion

The first growth parameter affected by water deficit in both R/Gi5 and R/M was the cumulative leaf area. Shoot growth rate and leaf emergence rate were also affected by water deficit, but only during the last seven days of the experiment. Shoot growth rate and leaf emergence rate were not affected to the same extend of cumulative leaf area, perhaps because the experiment was done during the last period of shoot growth (Figure C.2, Appendix C). The growth parameter more affected was the length of the less vigorous shoots, in a comparison between apple rootstocks with different vigor, exposed to water stress (Fernandez et al., 1997a). In addition, trunk cross sectional area and leaf emergence rate were affected differently depending on the genotype of the rootstock, but the observed differences did not follow a certain trend based on tree vigor, as it may have been expected (Fernandez et al., 1997a). In our experiment, the growth parameters measured on the R/Gi5 and R/M under water deficit conditions were affected in a similar way and to the same extend in the two genotypes when compared to their controls. This finding indicates that the two genotypes have a similar degree of sensitivity to water deficit in terms of growth.

Leaf expansion rate and leaf emergence were more sensitive than stomatal conductance and leaf water potential to water stress in peach trees (Olien and Flore, 1990). In our experiment, gas exchange parameters were affected earlier than all growth parameters measured. Stomata closure affected A and E in a similar way in standard plants, since reduction in A and E was detected at the same time, while in dwarf plants assimilation rate was affected three days later than E, indicating that A in dwarf plants was less influenced by water deficit. This is further supported by a larger decrease in C_i

in dwarf than in standard plants. Higher internal CO_2 of standard than dwarfing controls indicated a limitation in the use of CO_2 in standards, which can be explained by their lower assimilation rate. We can speculate that stomatal conductance was not the limiting factor for assimilation rate in standard plants. In both genotypes under progressively less soil water availability, C_i generally decreased but when soil water availability decreased further, internal CO_2 showed a sharp increase. This is in accordance with observations of C_i in conifers (Bodribb, 1996), in wheat and sunflower (Lawlor, 1995), under decreasing relative water content in leaves. The sharp increase in C_i perhaps indicates also an effect on the biochemical processes of photosynthesis.

Water-use efficiency was not significantly different between the two genotypes in most of the days measured. From day 9 to day 14 from the beginning of the treatments, control plants of dwarfing rootstocks showed a higher WUE than control plants of standard rootstocks (Figure 3.10 A). The differences in WUE were mainly due to the higher photosynthetic rate of the dwarf compared to standard plants, even though transpiration was also higher in dwarf than in standards plants. WUE has been found to be higher on vigorous than dwarfing interspecific hybrid peach rootstocks but it was due to lower transpiration in vigorous than dwarf plants (Bongi et al., 1994). On the contrary, Fernandez et al. (1997 b) found similar WUE in apple rootstocks with different vigor. In our experiment, plants of both rootstocks did not show any increase in WUE compared to their controls when challenged with water deficit (Figure 3.9). Similarly, in peach trees grown at elevated CO_2, WUE was not improved in water stressed plants (Centritto et al., 2002). Since both rootstocks did not improve their WUE under water deficit conditions, other plants characteristics such as root system morphology may play an important role

under water deficit conditions. Alternatively, the values obtained in this experiment, were already high enough so any further decrease in stomatal conductance to reduce transpiration would have negatively affected the photosynthetic rate as well. In general, WUE during the experiment ranged from 3.4 to 6.4 μmol CO_2 $mmol^{-1}$ H_2O. These values are higher than the ones obtained for kiwifruit plants that are considered quite inefficient in the use of water, where WUE ranged from 2.8 to 4.7μmol CO_2 $mmol^{-1}$ H_2O from 90 to 200 days after bud break (Buwalda and Smith, 1990).

Daily measurements of gas exchange parameters indicated a general decrease in A and g_s during the day, independent from the water regimes of the plants. Werber and Gates (1990) suggested that diurnal reduction in A resulted from increased water loss and a more negative water potential, which leads to stomatal closure. Neither Gucci et al. (1991) in a study on defruited plums, nor Layne and Flore (1993) studying continuously illuminated cherry, were able to show that diurnal decline in A was caused by a change in water potential. Flore and Layne (1996) proposed that the diurnal decrease in g_s is a result of reduction in A due to feedback inhibition and not to water stress. Although leaf water status was not evaluated during this experiment, based on the observed decline of A independently from the water availability of plants, we can also speculate that reduction of g_s was due to a reduction in assimilation rate. The diurnal trend of gas exchange measurements evidenced that differences between controls and plants under water deficit were independent from the time of the day where gas exchange parameters were evaluated. Water use efficiency decreased from mid morning until late afternoon due to both a decrease in A and a slight increase in E (Figure 3.11).

In general, when soil moisture level decreases, the ratio of intercellular to atmospheric partial pressure of CO_2 (p_i/p_a) will decrease, if stomata close and photosynthesis continue to operate (Farquhar et al., 1989). This could be measured by an increased in ^{13}C isotopic composition ($\delta^{13}C$) (Farquhar et al., 1989). The higher $\delta^{13}C$ values observed in $W_{50\%}$ plants of both genotypes were caused by the increased use of intercellular CO_2. Internal CO_2 of $W_{50\%}$ plants was significantly lower than in controls from day 9 to day 16 (Figure 3.8); this was caused by a higher uptake of internal CO_2 and less discrimination against the heavier isotope ^{13}C. Duranceau et al. (1999) found that under decreasing relative water content (RWC) in leaves, ^{13}C enrichment was correlated with decreased p_i/p_a in *Phaseolus vulgaris*. When leaf RWC decreased, sucrose and starch became enriched in ^{13}C, since A was supported by the CO_2 present in the intercellular spaces (Duranceau et al., 1999).

Conclusions

1) Growth parameters measured on dwarfing and standard trees under water deficit conditions were affected in a similar way, and to the same extent, when compared to their controls. These findings indicate that the two genotypes have a similar degree of sensitivity to water deficit in terms of growth.

2) Gas exchange parameters like assimilation and transpiration rate, stomatal conductance, and internal CO_2 were affected earlier than all growth parameters measured, perhaps because the water deficit treatments were applied during the last period of shoot growth.

3) Leaves $\delta^{13}C$ values of dwarfing and standard trees under water deficit conditions increased compared to their controls. $\delta^{13}C$ values were higher in plants under water deficit due to their increasing use of intercellular CO_2.

4) Water use efficiency ranged from 3.4 to 6.4 μmol CO_2 $mmol^{-1}$ H_2O and was not significantly different between dwarfing and standard trees, in most of the days measured.

5) Dwarfing and standard trees under water deficit conditions did not show any increase in WUE compared to their controls.

These findings indicate that irrigation is an important agronomic practice to be considered in sweet cherry orchard, especially when trees are grown on dwarfing rootstocks characterized by shallow root systems, or when orchards are established on sandy, and sandy-loam textured soils, with limited water holding capacity.

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SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

This research was initiated to determine if dwarfing and standard rootstocks differed in N-fertilizer uptake efficiency, N use efficiency (NUE), and water-use efficiency (WUE). Application of N influences tree growth and development during the current year and in the following growing season (Weinbaum, 1987). The seasonal pattern of N uptake in trees reflect their N demand and can be use for timing N-fertilizer application in order to maximize fertilizer uptake (Weinbaum et al., 1992).

N uptake in one-year-old sweet cherry trees followed the accumulation of dry matter in trees. N-fertilizer uptake was high at rapid shoot growth, and was fairly constant until the beginning of leaf senescence, when it decreased considerably. Overall, there was no difference in N-fertilizer uptake efficiency between dwarfing and standard trees with the exception of terminal bud set, where dwarf and semi-dwarf trees 'Rainier/Gisela 5' and 'Rainier/Gisela 6' were more efficient in N-fertilizer uptake than standard trees 'Rainier/Mazzard'. Rootstocks without scion differed in N-fertilizer uptake at terminal bud set and at the beginning of leaf senescence, and 'Gisela 6' was more efficient than both 'Gisela 5' and 'Mazzard' due to its higher increase in plant DW. Comparisons of genotypes N-fertilizer uptake efficiency obtained in this study are applicable to non-bearing field grown trees and can be used to optimize time of application of N fertilizer.

N-fertilizer applied at bloom and rapid shoot growth in five-year-old field-grown sweet cherry trees on dwarfing and standard rootstocks was absorbed in greater amount than N-fertilizer applied at the beginning of leaf senescence. Based on these results, fertilization practices for sweet cherry should be optimized considering application of N-

fertilizer at bloom and rapid shoot growth (40 days after bloom). N-fertilizer contributed in higher amount to the total N content of leaves of sweet cherries on dwarfing than standard rootstocks, especially when N was applied at bloom. When dwarfing rootstocks are utilized in sweet cherry orchard, N-fertilization may play an important role to sustain the growth of the tree, particularly during the early stages of growth. Even if the contribution of N-fertilizer was higher in dwarf than standard trees, the overall amount of N-fertilizer absorbed by dwarf trees may be limited. Previous studies on apple grown on the dwarfing rootstock M9 indicated low N requirements, between 8 and 44 kg N ha^{-1} (Neilsen and Neilsen, 2002). Therefore N-fertilizer requirements of sweet cherry on dwarfing rootstocks need to be carefully evaluated to avoid risks associated to overfertilization.

Nitrogen-use efficiency evaluated on one-year-old potted sweet cherry was higher in the standard rootstock 'Mazzard' than in the dwarfing rootstocks 'Gisela 5' and the semi-dwarfing rootstock 'Gisela 6', while when cv. 'Rainier' was grafted as scion, the genotypes had similar NUE. Considering that 'Mazzard' had a similar value of NUE of 'Rainier/Gisela 5', 'Rainier/Gisela 6' and 'Rainier/Mazzard', the aerial part of the plant appears to have higher incidence in determining the differences in plant NUE rather than the root system. Dwarfing and standard rootstocks did not differ in terms of NUE when evaluated on the base of leaf N concentration, under field conditions. Also retranslocation of N from senescent leaves was similar between dwarfing and standard rootstocks. Since retranslocation of N from senescent leaves is another aspect of trees NUE, it can be concluded that even based on this process, standard and dwarfing trees did not differed in terms of NUE.

Understanding how rootstocks adapt and respond to water stress is essential in order to select the proper genotype and irrigation method for situations where drought stress is likely to occur. When WUE was evaluated under well-watered conditions in one-year-old potted trees over a period of 60 days, there was no difference in terms of WUE between 'Rainier/Gisela 5', 'Rainier/Gisela 6' and 'Rainier/Mazzard', although 'Rainier/Gisela 5' had a higher evapotranspiration compared to 'Rainier/Gisela 6' and 'Rainier/Mazzard'. 'Mazzard' rootstocks had a higher WUE compared to both 'Gisela 5' and 'Gisela 6' due to the higher increase in biomass during the period considered.

Growth parameters measured on dwarf trees 'Rainier/Gisela 5' and on standard tree 'Rainier/Mazzard' under water deficit conditions were affected in a similar way, and to the same extend in the two genotypes, when compared to their controls. These findings indicate that dwarf and standard trees have a similar degree of sensitivity to water deficit in terms of growth. Gas exchange parameters like assimilation and transpiration rate, stomatal conductance, and internal CO_2 were affected earlier than all growth parameters measured, perhaps because the water deficit treatments were applied during the last period of shoot growth. Leaves $\delta^{13}C$ values of dwarf and standard trees under water deficit conditions increased compared to their controls. $\delta^{13}C$ values were higher in plants under water deficit due to their increasing use of intercellular CO_2. Water use efficiency ranged from 3.4 to 6.4 μmol CO_2 $mmol^{-1}$ H_2O and was not significantly different between the two genotypes, in most of the days measured. The few differences in WUE observed during the experiment indicated a higher WUE of dwarf than standard trees, and were mainly due to the higher photosynthetic rate of the dwarf compared to standard trees. Water-use efficiency of standard and dwarf trees

under water deficit conditions was not higher compared to their controls. Overall, these findings indicate that irrigation is an important agronomic practice to be considered in sweet cherry orchard, especially when sweet cherry are grown on dwarfing rootstocks, characterized by shallow root systems or when orchards are established on sandy, and sandy-loam textured soils, with limited water holding capacity.

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APPENDIX A

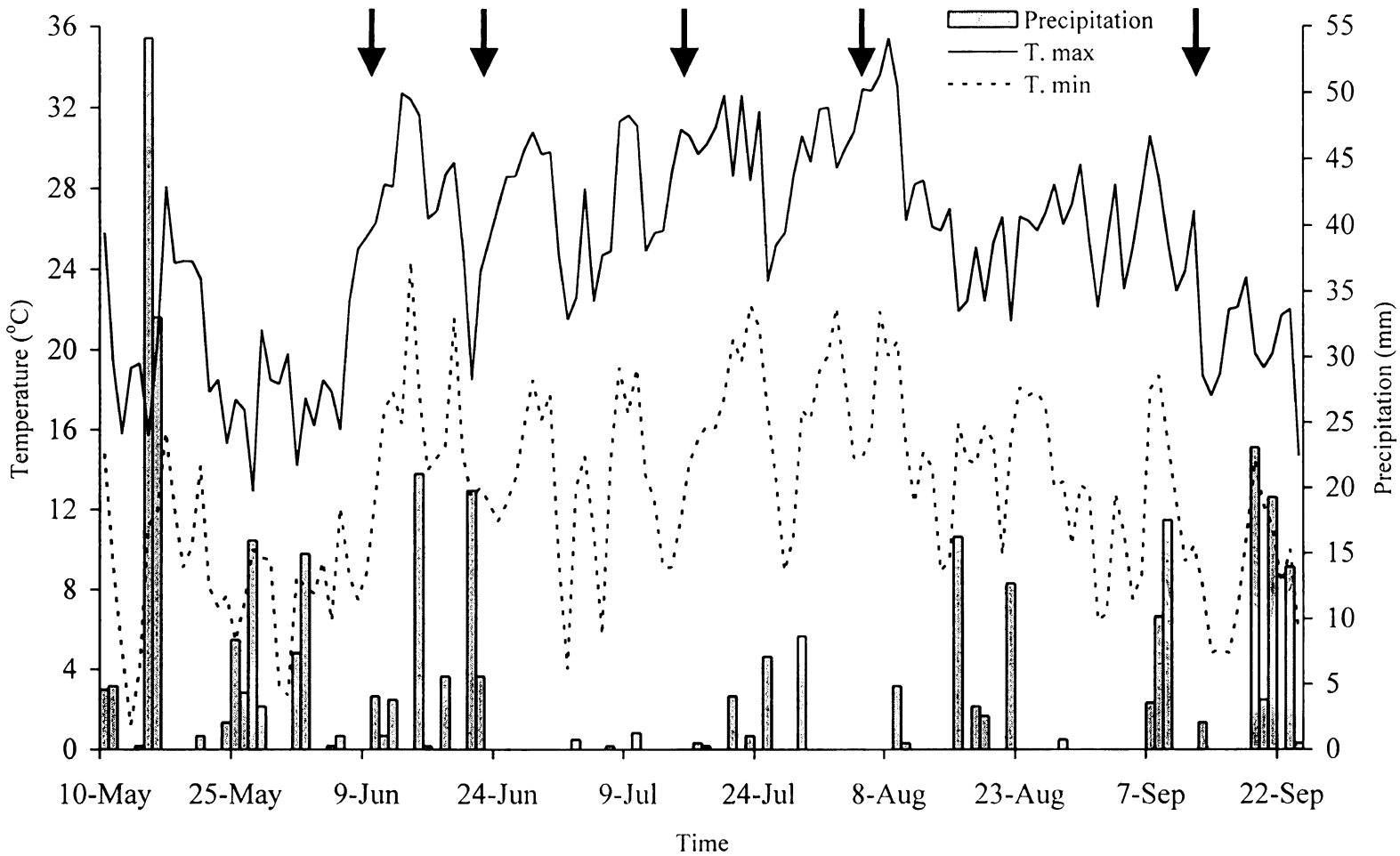

Figure A.1. Maximum and minimum temperature (°C), and precipitation (mm) recorded daily during the experiment at the MSU Horticulture Teaching and Research Center during 2001. Data were collected by an automated weather station of the Michigan Automated Weather Network. Arrows indicate ^{15}N-fertilizer application times.

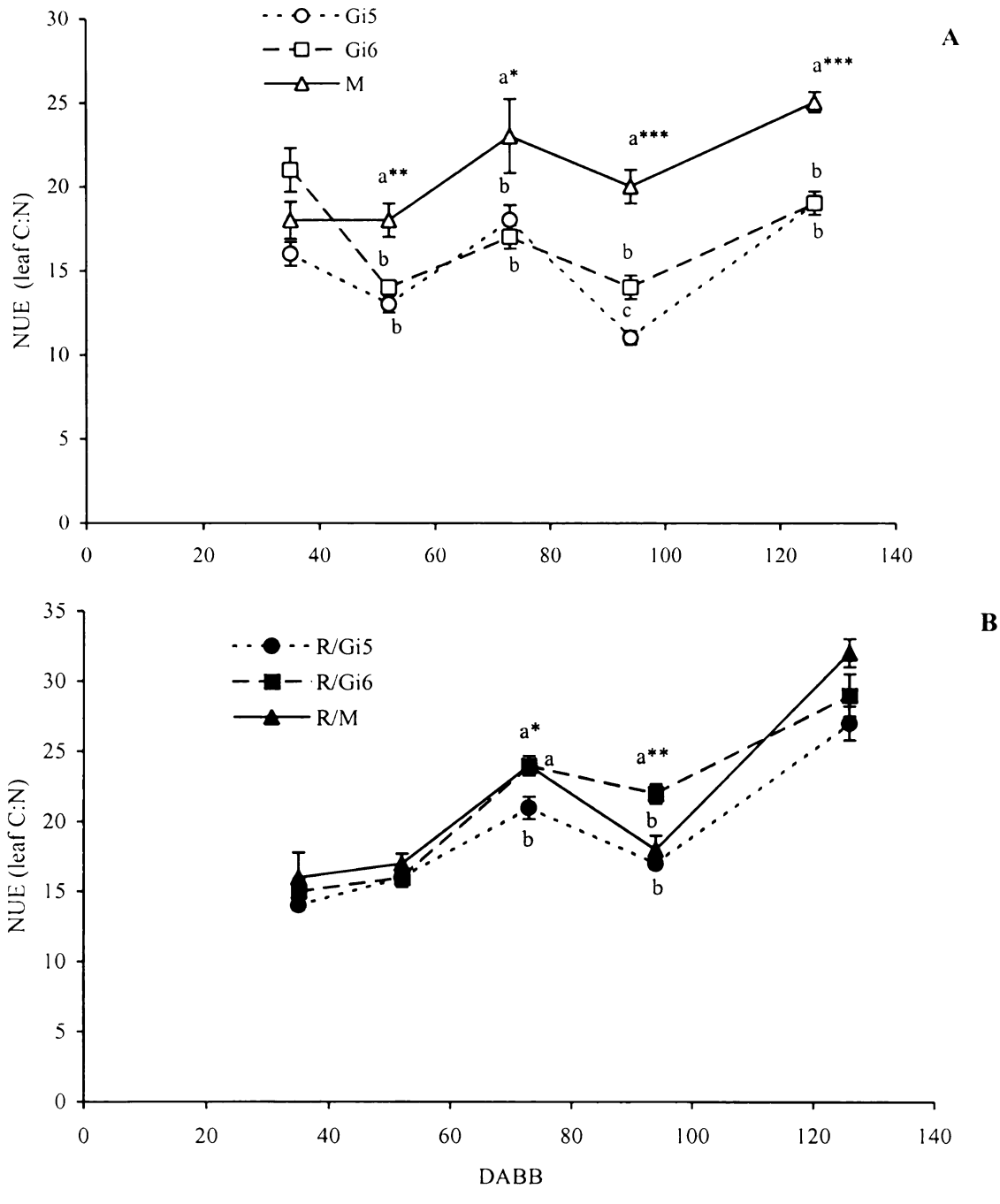

Figure A 2. Nitrogen-use efficiency (NUE) expressed as C:N ratio of leaves during the growing season (Days After Bud Break, DABB). **A**. Comparison between 'Gisela 5' (Gi5), 'Gisela 6' (Gi6), and 'Mazzard' (M). **B**. Comparison between 'Rainier/Gisela 5' (R/Gi5), 'Rainier/Gisela 6' (R/Gi6), and 'Rainier/Mazzard' (R/M). Analysis of variance was carried out separately for each harvest. Each point represents the average (± SE) of four replications. *, **, ***, stand for significance at $p \leq 0.05$, 0.01, and 0.001, respectively.

APPENDIX B

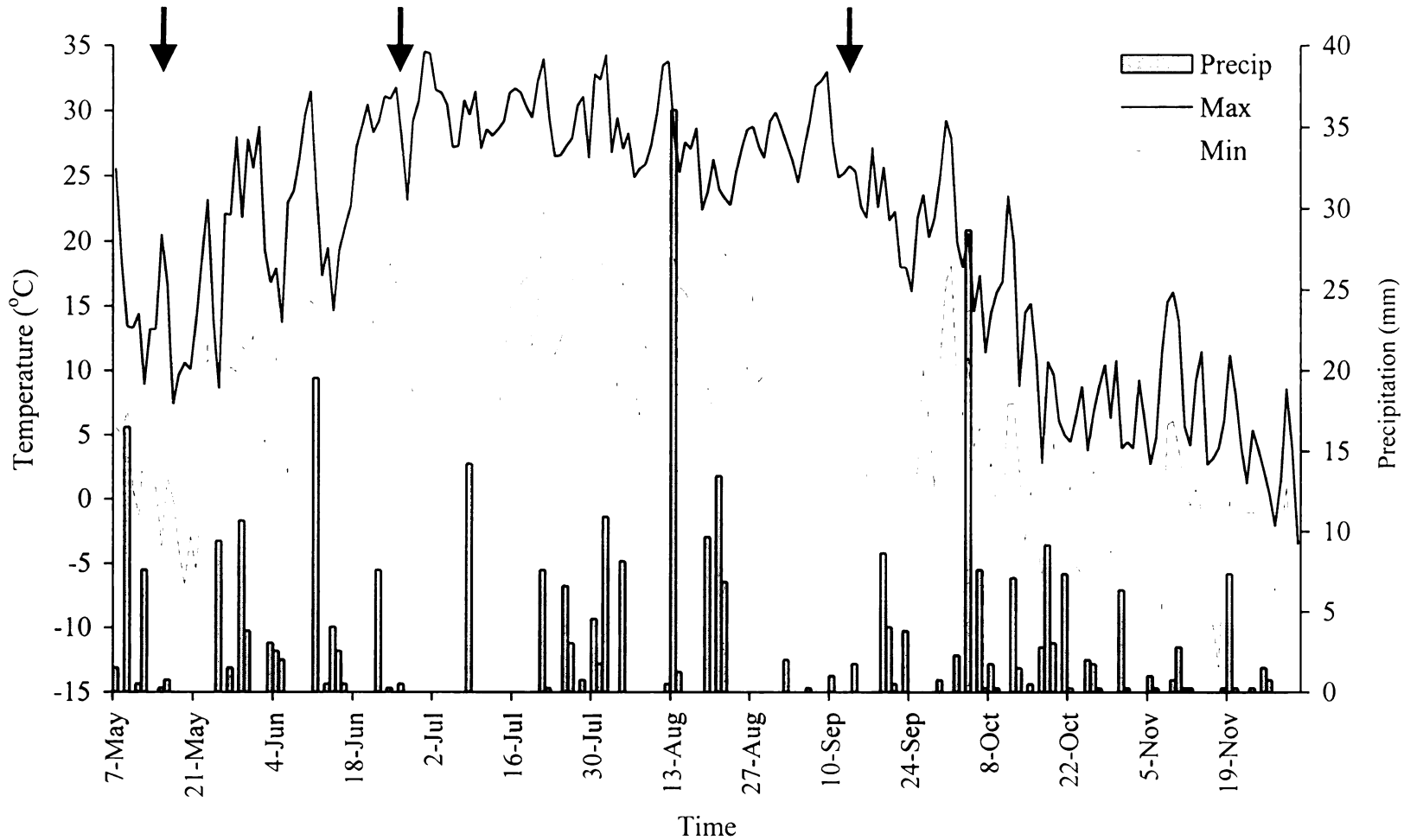

Figure B.1. Maximum and minimum temperature (°C) and precipitation (mm) daily recorded at the USDA National Resources Conservation Service of Bear Lake, during 2002. Data were collected by an automated weather station of the Michigan Automated Weather Network. Arrows indicate time of N-fertilizer applications.

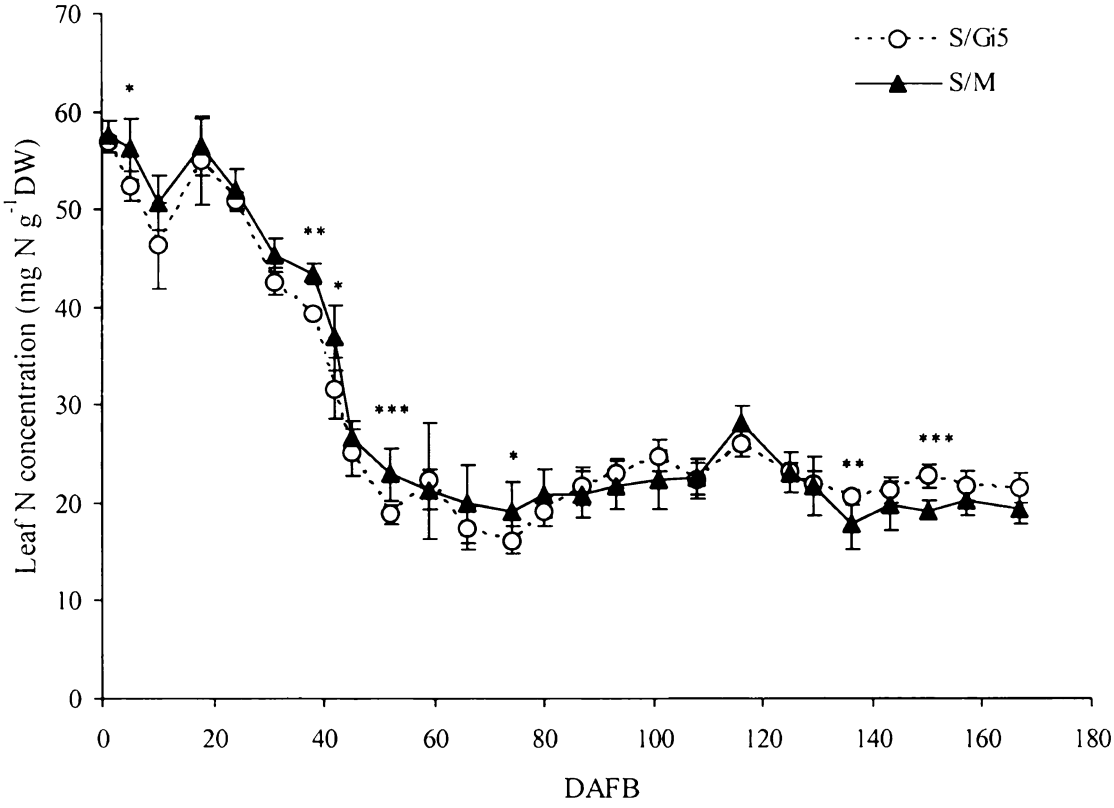

Figure B.2. Leaf nitrogen concentration (mg of N g^{-1} dry weight) in leaves of sweet cherry cv. 'Sam', measured at different Days After Full Bloom (DAFB). Comparison between 'Sam/Gisela5' (S/Gi5) and 'Sam/Mazzard' (S/M). Each point represents the means of four replications (± STD). *, **, and *** stand for significant at $p \leq 0.05$, 0.01, and 0.001, respectively.

APPENDIX C

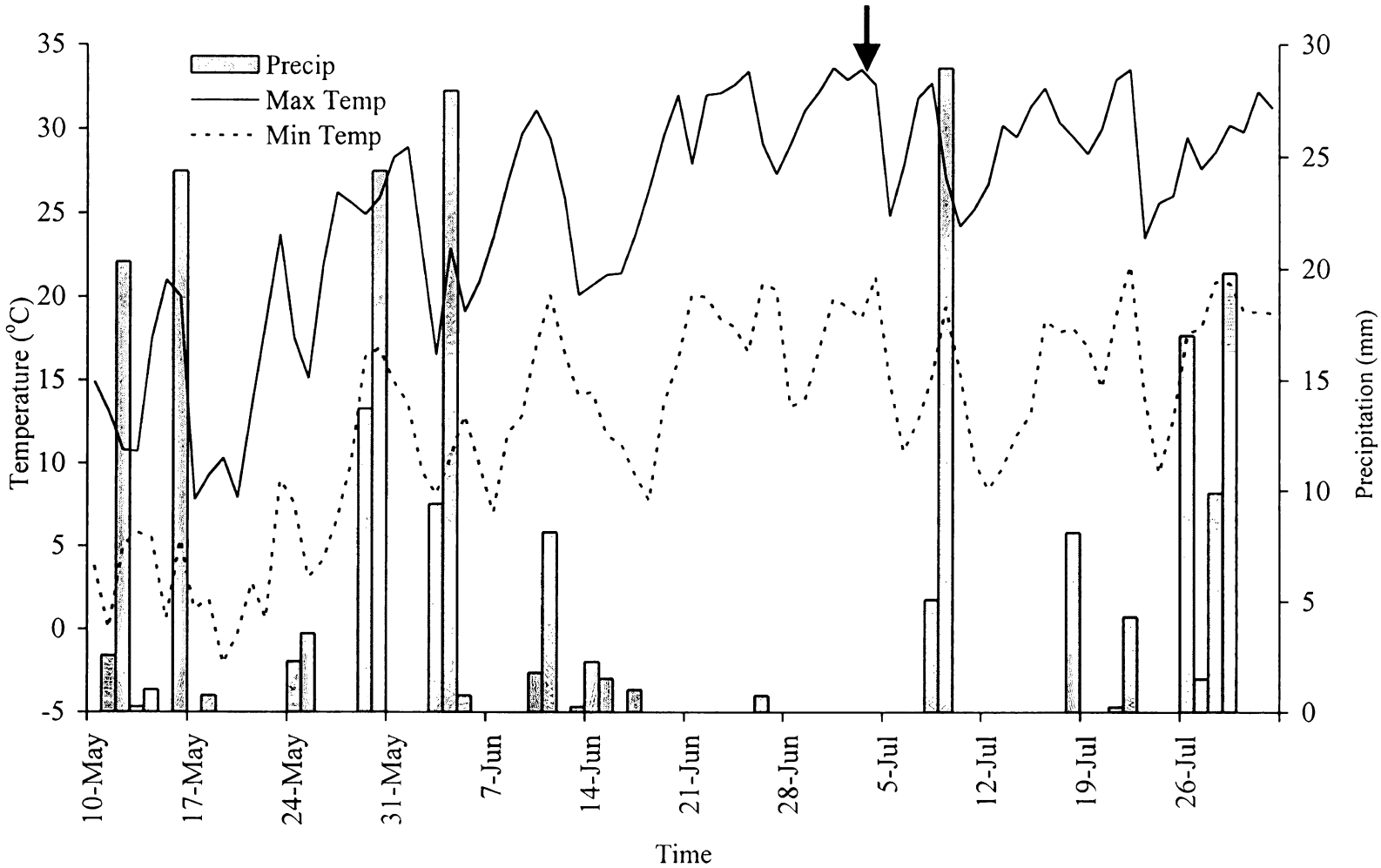

Figure C.1. Maximum and minimum temperature (°C) and precipitation (mm) recorded daily during the experiment at the MSU Horticulture Teaching and Research Center during 2002. Data were collected by an automated weather station of the Michigan Automated Weather Network. Arrow indicates beginning of water treatments.

Table C.1. Summary of statistical significant differences between control and water deficit plants of 'Rainier/Mazzard' and 'Rainier/Gisela 5' of plant growth and gas exchange parameters measured during the experiment.

Parameter	Days from the beginning of the treatments																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Rainier/Mazzard																					
Shoot RGR							ns			ns				ns							*
Leaf RER							ns			ns				ns							***
Leaf area			ns				ns				ns			*						ns	
A	ns		ns	ns		ns	*		*	**	*	*	***	***	***	***			***		***
E	ns		*	ns		ns	**		**	*	ns	**	***	***	***	***			***		***
gs	ns		ns	ns		ns	*		**	**	*	***	***	***	***	***			***		***
Ci	ns		**	*		ns	ns		***	ns	ns	***	ns	***	***	***			ns		ns
WUE	ns		ns	**		ns	ns		ns	ns	ns	ns	ns	*	ns	*			ns		*
Rainier/Gisela 5																					
Shoot RGR							ns			ns				ns							*
Leaf RER							ns			ns				ns							***
Leaf area			ns				ns				*			***						**	
A	ns		ns	ns		ns	ns		**	**	***	***	***	***	***	***			***		***
E	ns		ns	ns		**	*		***	***	***	***	***	***	***	***			***		***
gs	ns		ns	ns		*	ns		**	***	**	**	***	***	***	***			***		***
Ci	ns		*	ns		ns	ns		***	***	***	***	ns	***	***	ns			ns		ns
WUE	ns		ns	ns		ns	ns		ns	ns	ns	ns	*	*	ns	ns			ns		*

ns, *, **, *** non significant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively

Table C.2. Summary of statistical significant differences of growth and gas exchange parameters measured during the experiment. Comparison between control plants of 'Rainier/Mazzard' and 'Rainier/Gisela 5' (control) and between water deficit plants of 'Rainier/Mazzard' and 'Rainier/Gisela 5'(water deficit), calculated as percent of their controls during the experiment.

Parameter	Days from the beginning of the treatments																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Control																					
Shoot RGR							ns			ns				ns							*
Leaf RER							ns			ns				ns							ns
Leaf area			*				**				***			***						***	
A	ns		*	*		ns	ns		*	*	ns	ns	*	*	*	ns			ns		*
E	ns		*	*		ns	ns		*	ns	ns	ns	ns	ns	*	ns			ns		*
Gs	ns		*	*		ns	ns		ns	ns	ns	ns	*	ns	ns	*			ns		ns
Ci	ns		*	*		ns	ns		*	ns	ns	*	ns	*	*	*			ns		ns
WUE	ns		ns	ns		ns	ns		**	ns	*	**	*	*	ns	ns			ns		ns
Water deficit																					
Shoot RGR							ns			ns				ns							ns
Leaf RER							ns			ns				ns							*
Leaf area			ns				ns				ns			ns						ns	
A	ns		ns	ns		ns	ns		ns	ns	*	ns	ns	ns	ns	ns			ns		ns
E	ns		*	ns		ns	ns		ns	*	***	ns	ns	ns	ns	ns			ns		ns
Gs	ns		*	ns		ns	ns		ns	ns	**	ns	ns	ns	ns	ns			ns		ns
Ci	ns		***	*		ns	ns		ns	**	**	ns	*	ns	ns	*			ns		ns
WUE	ns		**	ns		ns	ns		ns	*	*	ns	ns	ns	ns	ns			ns		ns

*, **, ***: significant at $p \leq 0.05$, 0.01, and 0.001, respectively

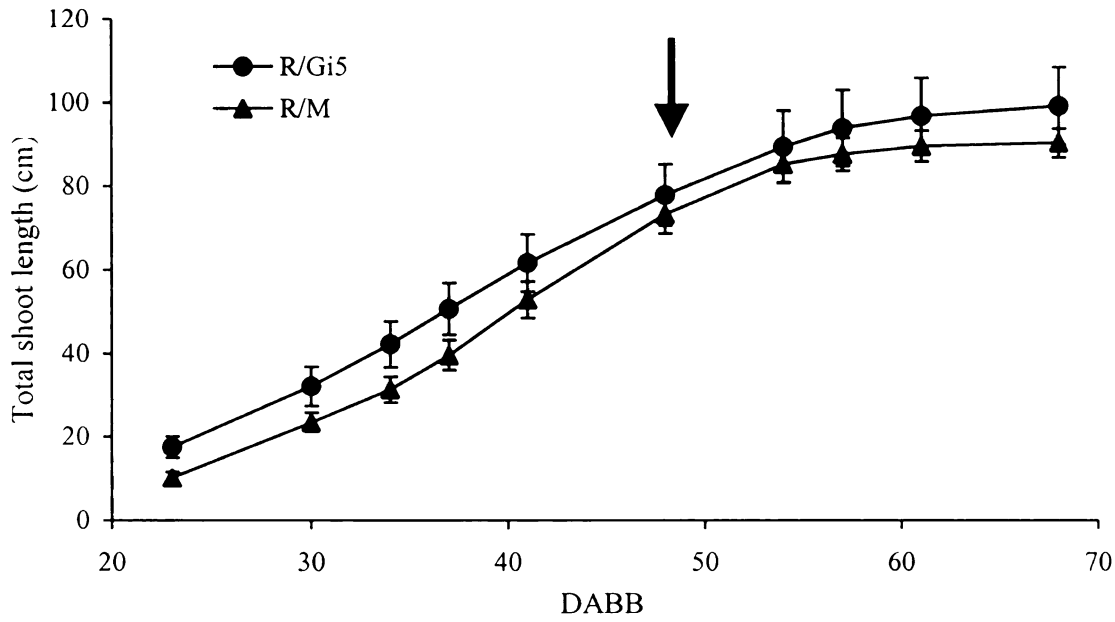

Figure C.2. Total shoot length (cm) per plant of sweet cherry cv. 'Rainier' measured at different Days After Bud Break (DABB). Comparison between 'Rainier/Gisela 5' (R/Gi5) and 'Rainier/Mazzard' (R/M). Vertical bars indicate standard errors of means (SE, n=30). Arrow indicates the beginning of different water treatments.