IRRIGATION AND FERTILIZATION PRACTICES FOR YOUNG PEACH TREES IN THE SOUTHEASTERN UNITED STATES

by

BRUNO CASAMALI

(Under the Direction of Dario Chavez)

ABSTRACT

Traditionally, in the southeastern United States, new peach orchards do not receive supplemental irrigation until three or four years after field planting. Thus, the only source of water comes from rainfall. However, droughts are becoming more common in the region, making supplemental irrigation more important. Fertilizer recommendations for peaches in the southeastern United States were likely created decades ago and may not reflect the peach trees' requirements under modern cultivation practices. The objectives of this research were to investigate the effects of irrigated vs. non-irrigated trees, drip- vs. micro-sprinkler-irrigated trees, and four different fertilizer levels (25%, 50%, 100%, and 200%; with 100% = current fertilizer recommendations) on young 'Julyprince' trees grafted onto 'Guardian™' rootstock. In 2016, below average rainfall (severe drought) was recorded throughout the year. Drought negatively affected non-irrigated trees, reducing canopy volume (56%), trunk cross-sectional area (39%), photosynthetic activity (40%), and leaf and stem water potential (39%) when compared to irrigated trees. Further, non-irrigated trees had increased expression of genes related to ABA biosynthesis (*ChlH* and *PpCYP707A3*),

osmoregulation (*SIP1* and *P5SC)*, and reactive oxygen species scavenger (*POD*); and decreased relative expression of genes related to dehydration and aquaporins (*PpDhn3* and *Pp-δTIP1*). In 2017 and 2018, drought stress was not observed. However, the negative effects in tree growth and physiological responses of the 2016 season carried over to 2017 and 2018. Irrigated trees had 23% greater fruit yield than non-irrigated trees in 2017. Treatment effects on fruit quality were minimal and without representative trends across years. Differences between irrigation systems were not consistent; however, drip is more efficient than micro-sprinkler irrigation, with ~38% of water savings. Different fertilizer levels had no major effects on young trees' growth and yield, fruit quality, nitrogen partitioning, and relative gene expression. Overall, irrigation since planting increased tree growth and commercial yield, especially under drought conditions. Similarly, reductions in fertilizer recommendations can be made without negative effects to plants, but with large potential economic savings.

INDEX WORDS: *Prunus persica*, drought, supplemental irrigation, tree growth, photosynthetic assimilation, water potential, fruit yield, fruit quality, allocation, nitrogen concentration, dry weight, gene expression

IRRIGATION AND FERTILIZATION PRACTICES FOR YOUNG PEACH TREES IN THE SOUTHEASTERN UNITED STATES

by

BRUNO CASAMALI

B.S., Federal University of Rio Grande do Sul, Brazil, 2010

M.S., University of Florida, 2015

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2019

IRRIGATION AND FERTILIZATION PRACTICES FOR YOUNG PEACH TREES IN THE SOUTHEASTERN UNITED STATES

by

BRUNO CASAMALI

Major Professors:	Dario Chavez
	Marc van Iersel
Committee:	Anish Malladi
	Wesley Porter
	Juan Carlos Melgar

Electronic Version Approved:

Ron Walcott
Interim Dean of the Graduate School
The University of Georgia
December 2019

DEDICATION

I would like to dedicate this work to my parents and my brother, who always supported me during my endeavors. To those that believed me and gave emotional support during the difficult times - their wise words pushed me through the final line. To the peach growers and researchers.

ACKNOWLEDGEMENTS

I would like to thank my family for, one more time, sponsoring my dreams. They are present in all aspects of my life, being the “rock” and safe place where I can go to find energy and resources to continue life. They have supported my education their whole life, and I greatly appreciate.

My advisor, Dr. Dario Chavez. Thank you for opening the doors of your lab group, for helping me think out of the box, for supporting my research with no restrictions, and above all, thank you for being a great human being and friend.

My co-advisor, Dr. Marc van Iersel. Thank you for the countless advices and help during my research, providing insightful knowledge, and allowing me to use your lab equipment and personnel expertise when needed. Thank you for being a great Grad Student Coordinator, making the students’ life a little easier.

I would like to thank my committee members, Dr. Malladi, Dr. Melgar, and Dr. Porter, for their support, suggestions, advices, and feedbacks on my research. Dr. Malladi, thank you for walking me through all the steps from RNA extraction to gene expression analysis.

I would like to thank our lab technicians Malgosia and Will, and the several grad students and interns for their help collecting data and processing samples, and for their friendship. Their help was fundamental for this research.

I would like to thank the UGA Department of Horticulture, faculty, staff, and fellow grad students for their support, assistance, and friendship.

I would like to thank my girlfriend Lauren, for keeping up with me, for being my partner to talk about everything and enjoy life, and for providing emotional support. Thank you for allowing your cat Arwen to live with me; she has been a real emotional support pet.

My friends from Brazil, USA, and around the world. Thank you for the countless moments to enjoy life, relieve the stress, and supportive words and knowledge during my PhD.

I would like to thank my former undergraduate and Master's advisors, Dr. Koller, Dr. de Souza, Dr. Schwarz, Dr. Bergamaschi, Dr. Darnell, and Dr. Williamson, for their guidance, advice, and incentive to continue my studies, being the foundational stones of my academic achievements.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Accurate and precise irrigation and fertilization management for agricultural crops has become a subject of great interest. This is largely because of current problems with drought and fertilizer runoff affecting several regions of the world. In the southeastern United States, young peach trees are not irrigated until three or four years after field planting. During that period, trees rely only on natural precipitation as a water source. This puts orchards at risk of being affected by droughts. In addition, fertilization recommendations are not well suited for the southeastern United States. Fertilizer recommendations currently used in Georgia (Horton et al., 2015) are believed to be based on studies performed 50-60 years ago in different regions. The study of irrigation and fertilization for young peach trees in Georgia will generate information about irrigation practices from orchard establishment to production and will allow the tailoring of fertilizer recommendations. This will create opportunities for improvement of yield and overall production efficiency. The improved irrigation and fertilizer recommendations could induce earlier and greater fruit production and improve the sustainability and life of an orchard.

Peaches

Peaches are considered a symbol of life longevity in China. Peaches have been observed since ancient times, with reports of wild peach pits in China from 6000-7000

BC. Evidences of peach domestication date back to 3000 BC. *Prunus persica* is a diploid species (2n=16); with lanceolate, glabrous, and serrate leaves, and a glandular petiole; with flowers that are generally pink; with pubescent or glabrous fleshy fruits; and with a stony endocarp (Layne and Bassi, 2008). The genus *Prunus* is comprised of approximately 63 species and 100 accepted taxa overall (species, subspecies, or varieties) (USDA Natural Resources Conservation Service, 2015). Most species do not produce fruit with desired commercial characteristics (Bassi and Monet, 2008). Commercially important fruits in this genus are peaches, plums, cherries, almonds, nectarines, and apricots (USDA Natural Resources Conservation Service, 2015).

The history of peaches in Georgia dates back to the mid-1500s. Peaches were first introduced to Georgia's coast by Franciscan monks. After the initial introduction during the colonial period, peaches were grown by Cherokee Indians. By the mid-1800s, the first successful peach variety named 'Elberta' was released and grown commercially. 'Elberta' boosted Georgia's peach production due to its superior fruit quality and shipping ability compared to what was available at the time. Fruit from this variety could be commercialized in the northeast United States. Because of that, Georgia was named the "Peach State". Currently, around 50-60 varieties are grown in Georgia, white or yellow flesh, freestone or clingstone, and early to late ripening from mid-May to mid-August (UGA Extension, n.d). Although peach trees thrive under Georgia weather and soil conditions, several diseases can significantly reduce tree longevity and production (Beckman et al., 1998; Ritchie and Clayton, 1981). Furthermore, climate variability (Ray et al., 2015) and change (Rosenzweig et al., 2014) are causing negative environmental impacts such as lack of chill, late winter and early

spring freezes (Crouch, 2017), and drought during spring/summer months (Conrad II and Knox, 2016).

Irrigation

Peach tree irrigation has been the subject of much research over the past decades. However, current research in irrigation has been mostly done in Mediterranean regions. Peaches evolved in Asia in humid and subtropical conditions (Proebsting Jr. and Middleton, 1980). Chances are that peaches naturally have high water requirements and are sensitive to water stress (Berman and DeJong, 1996). However, peaches do not tolerate waterlogging (Iacona et al., 2013). Appropriate and precise irrigation management is required for optimal growth and tree health. Possible outcomes of this research will be the development of irrigation guidelines for peach production. Irrigated trees may display greater tree growth and higher yield.

Research studies on irrigation of peach trees are numerous and have explored different aspects such as fruit development, fruit yield, and tree growth. For instance, Williamson and Coston (1990) evaluated the effect of two irrigation rates, replacement of 12.5% or 100% of the daily evapotranspiration (ET) during all stages of fruit development (stages based on Connors, 1919), in 2.5-year-old own-rooted ‘Redheaven’ peach trees in South Carolina. No significant differences were found in fruit yield and size, and in most of the vegetative growth parameters evaluated. Layne et al. (2002) investigated the combination of three irrigation and fertilization treatments on 1-year-old ‘Redglobe’ peach trees in South Carolina. Trunk cross-sectional area, tree height, and fruit yield were greater in treatments with supplemental irrigation, whereas the rainfall

only treatment reduced fruit yield and marketable fruit. Goldhamer et al. (2002) imposed regulated deficit irrigation (RDI) or partial root zone drying on 3-year-old ‘September Snow’ peach trees grown in California. No differences between treatments were found for fruit yield, fruit load, and fruit growth rate. Control treatment [crop evapotranspiration (ETc)+10%] yielded heavier fruit, with larger diameter at stage 3 of development. Trees had greater trunk diameter and stem water potential than drought-stressed trees. Mercier et al. (2009) evaluated photosynthetic activity and physiological responses of 2-year-old ‘Alexandra’ peach trees cultivated under well-watered condition (control), light water restriction, and high-water restriction in France. Photosynthetic activity, stem water potential, stomatal conductance, and transpiration rates were lower in trees under high water restriction when compared with control or light water restriction treatments. Leaf temperature was lower for the control treatment. Lopez et al. (2011) applied four irrigation treatments on 11-year-old ‘Ryan’s Sun’ peach trees in Spain. No irrigation treatment induced smaller fruit maturing later in the season, and with greater dry matter concentration when compared to fruit from the full irrigation treatment. Total soluble solids (TSS), total titratable acidity (TTA), fruit firmness, crispness, and sourness increased with no irrigation; however, sweetness, juiciness, and intensity of peach flavor decreased. Marsal et al. (2016) investigated fruit-related parameters of 6-year-old ‘Baby Gold 6’ peach trees grown under four irrigation treatments based on different ETc and stem water potential levels in Spain. No significant differences were found in fruit yield between the treatments. Inconclusive results were found for fruit weight.

Drought stress in plants can also affect several mechanisms at the cellular level, such as adjustment of chlorophyll antenna size, thermal dissipation of light energy,

changes in the xanthophyll cycle, altered source-sink relations and carbon partitioning, alternative oxidative pathway and uncoupling of proteins in the light reaction centers, bypass in the Krebs cycle preventing the formation of reductants, formation of antioxidant enzymes and substrates to scavenge reactive oxygen species, synthesis of solutes promoting osmotic adjustment, abscisic acid biosynthesis promoting stomatal closure, regulation of aquaporin activity, and inhibition of ethylene accumulation (Bhargava and Sawant, 2013).

Irrigation and deficit irrigation affect peach trees in several ways as described above, with most of the research focused on the effects of fruit yield and quality. The lack of information about irrigation management for young and adult peach trees in the southeastern United States presents the opportunity for the development of research aiming to identify the best irrigation practices for peach production in the region. This may result in an increase of the growers' profits in the first years after field planting and improvement in tree growth.

Fertilization

Fertilization management is a basic practice in agriculture across different crops. Accurate information and recommendations for young peach production in the region are needed. Current fertilizer recommendations (Horton et al., 2015) are not well suited for the southeastern United States and are believed to be based on studies performed in the 1950-60's (Gammon Jr. and Shoemaker, 1963; Smith and Taylor, 1952). Adequate fertilization management is important for an optimal equilibrium between vegetative and reproductive tree development, maximizing fruit production. Other

possible outcomes of this research will be the reduction of fertilizer runoff and reduction in production costs.

Nitrogen fertilization can affect a peach tree's physiology in different ways, depending on the form (Lobit et al., 2001) and amount (Jordan et al., 2014) of the nutrient applied, as well as the timing of the applications (Niederholzer et al., 2001). In Florida, Vashisth et al. (2017) conducted research to find the best N level for peaches (planting density of 272 trees/ha). They reported that the treatment receiving 90 $kg \cdot ha^{-1}$ N had the greatest yield, followed by the 0 and 269 $kg \cdot ha^{-1}$ N treatments. No differences in TTA and TSS/TTA ratio were reported, with inconsistent results on TSS for the two peach cultivars tested. No differences in peach fruit TSS, TTA, and TSS/TTA ratio among a wide range of N levels were also reported by Dolinski et al. (2018). In Brazil, four fertilizer levels (0, 30, 60, and 120 $kg \cdot ha^{-1}$ N) were tested by Ferreira et al. (2018) in a high-density orchard (1333 trees/ha) of young peach trees. For 1- and 2-year-old trees, the highest fertilizer level, which is above the recommended fertilization level for 2-year-old trees (60 $kg \cdot ha^{-1}$ N), did not increase trunk diameter, tree height, and fruit yield. However, for 3-year-old trees, the highest fertilizer treatment increased those variables. In California, Crisosto et al. (1997) reported that high levels of N resulted in poor red fruit color and delayed ripening in 'Fantasia' nectarine.

The effect of different fertilizer levels on differential gene expression of fruit trees has been investigated by some researchers. Zhang et al. (2016) reported that glutamine synthase (*GS*) and nitrite reductase (*NiR*) had reduced expression after foliar application of 0.5% (w/v) urea in peach trees. However, glutamate dehydrogenase (*GDH*), asparagine synthetase (*AS*), and nitrate reductase (*NR*) had increased

expression after foliar urea application. Liao et al. (2019) tested the relative expression of the same five genes (*GS*, *NiR*, *GDH*, *AS*, and *NR*) in citrus trees across different fertilizer levels, reporting that the lowest and highest N levels had lower gene expression in comparison with the three middle N levels.

The diverse tree responses in tree growth and fruit quality because of different rates of fertilization creates a unique and important research opportunity to improve fertilization management. Adequate fertilization may reduce production costs and improve the sustainability of peach production in the southeastern United States.

Research objectives

The overall objective of this research was to quantify the effects of different irrigation levels (irrigated vs. non-irrigated), irrigation systems (drip vs. micro-sprinkler), and fertilization levels (25%, 50%, 100%, and 200% - being the 100% the current recommended rate) on young peach trees' physiological parameters, fruit quality, nitrogen partitioning, and differential gene expression.

Specific objectives of the different chapters are:

Chapter 2) To investigate the physiological and growth responses (bud-break progression, trunk cross-sectional area, canopy volume, fruit yield, stem water potential, photosynthetic assimilation, and water use efficiency) of young peach trees under different irrigation and fertilization treatments.

Chapter 3) To evaluate the effects of irrigation and fertilization treatments on fruit weight, size, maturation index, and physicochemical quality of peach fruits.

Chapter 4) To measure the effects of irrigation and fertilization treatments on total dry weight and % N in the tissues removed from the trees during pruning, fruit thinning, harvesting, and fall defoliation.

Chapter 5) To test the expression of genes associated with drought stress and nitrogen metabolism in field grown peach trees under different irrigation and fertilization treatments.

Literature cited

Bassi, D. and R. Monet. 2008. Botany and Taxonomy, p. 1-36. In: D.R. Layne and D. Bassi (eds.). The Peach: Botany, production and uses. CAB International. Oxfordshire, UK.

Beckman, T.G., W.R. Okie, A.P. Nyczepir, P.L. Puse, and C.C. Reilly. 1998. Relative susceptibility of peach and plum germplasm to Armillaria root rot. HortScience 33:1062–1065.

Berman, M.E. and T.M. DeJong. 1996. Water stress and crop load effects on fruit fresh and dry weights in peach (*Prunus persica*). Tree Physiol. 16:859–864.

Bhargava, S. and K. Sawant. 2013. Drought stress adaptation: Metabolic adjustment and regulation of gene expression. Plant Breeding 132:21–32.

Connors, C.H. 1919. Growth of fruits of peach. N.J. Agr. Expt. Sta. Annu. Rpt. 40:82–88.

Conrad II, C.E. and P. Knox. 2016. The southeastern drought and wildfires of 2016, National Oceanic and Atmospheric Association Southeast Regional Climate Center. Chapel Hill, NC.

Crisosto, C.H., R.S. Johnson, T. DeJong, and K.R. Day. 1997. Orchard factors affecting postharvest stone fruit quality. HortScience 32:820–823.

Crouch, J. 2017. Bad news for Southeast peaches: Something freezing this way came. 7 July 2019. <https://www.climate.gov/news-features/blogs/beyond-data/bad-news-southeast-peaches-something-freezing-way-came>.

Dolinski, M.A., J.W. de O. Dangelo, F.L. Cuquel, A.C.V. Motta, and L.L.M. de Mio. 2018. Quality peach produced in fertilizer doses of nitrogen and green pruning. Bragantia 77:134–140.

Ferreira, L.V., L. Picolotto, I. dos S. Pereira, J.D. Schmitz, and L.E.C. Antunes. 2018. Nitrogen fertilization in consecutive cycles and its impact on high-density peach crops. Pesquisa Agropecuaria Brasileira 53:172–181.

Gammon Jr., N. and J.S. Shoemaker. 1963. Effect of fertilizers on mineral levels in the leaves of Flordawon peach. Florida State Hort. Soc. 1746:380–384.

Goldhamer, D.A., M. Salinas, C. Crisosto, K.R. Day, M. Soler, and A. Moriana. 2002. Effects of regulated deficit irrigation and partial root zone drying on late harvest peach tree performance. Acta Hort. 592:343–350.

Horton, D., P. Brannen, B. Bellinger, D. Lockwood, and D. Ritchie. 2015. 2015 Southeastern peach, nectarine and plum pest management and culture guide. Uni. Georgia Coop. Ext. Bul. 1171. Athens, GA.

Iacona, C., M. Cirilli, A. Zega, E. Frioni, C. Silvestri, and R. Muleo. 2013. A somaclonal myrobalan rootstock increases waterlogging tolerance to peach cultivar in controlled conditions. Sci. Hort. 156:1–8.

Jordan, M.-O., G. Vercambre, L. Gomez, and L. Pages. 2014. The early spring N uptake of young peach trees (*Prunus persica*) is affected by past and current fertilizations and levels of C and N stores. Tree Physiol. 34:61–72.

Layne, D.R. and D. Bassi, 2008. The peach: botany, production, and uses. CAB International, Oxfordshire, UK.

Layne, D.R., D.B. Cox, and E.J. Hitzler. 2002. Peach systems trial: The influence of training system, tree density, rootstock, irrigation and fertility on growth and yield of young trees in South Carolina. Acta Hort. 592:367–375.

Liao, L., T. Dong, X. Liu, Z. Dong, X. Qiu, Y. Rong, G. Sun, and Z. Wang. 2019. Effect of nitrogen supply on nitrogen metabolism in the citrus cultivar ‘Huangguogan’. PLoS One 14:e0213874.

Lobit, P., P. Soing, M. Génard, and R. Habib. 2001. Effects of timing of nitrogen fertilization on shoot development in peach (*Prunus persica*) trees. Tree Physiol. 20:35–42.

Lopez, G., M. Hossein Behboudian, G. Echeverria, J. Girona, and J. Marsal. 2011. Instrumental and sensory evaluation of fruit quality for 'Ryan’s sun' peach grown under deficit irrigation. HortTechnology 21:712–719.

Marsal, J., J. Casadesus, G. Lopez, M. Mata, J. Bellvert, and J. Girona. 2016. Sustainability of regulated deficit irrigation in a mid-maturing peach cultivar. Irr. Sci. 34:201–208.

Mercier, V., C. Bussi, F. Lescourret, and M. Génard. 2009. Effects of different irrigation regimes applied during the final stage of rapid growth on an early maturing peach cultivar. Irr. Sci. 27:297–306.

Niederholzer, F.J.A., T.M. DeJong, J.-L. Saenz, T.T. Muraoka, and S.A. Weinbaum. 2001. Effectiveness of fall versus spring soil fertilization of field-grown peach trees. J. Amer. Soc. Hort. Sci. 125:644–648.

Proebsting Jr. E.L. and J.E. Middleton. 1980. The behavior of peach and pear trees under extreme drought stress. J. Amer. Soc. Hort. Sci. 105:380–385.

Ray, D.K., J.S. Gerber, G.K. Macdonald, P.C. West. 2015. Climate variation explains a third of global crop yield variability. Nature Commun. 6:5989.

Ritchie, D.F. and C.N. Clayton. 1981. Peach tree short life: A complex of interacting factors. Plant Dis. 65:462–469.

Rosenzweig, C., J. Elliott, D. Deryng, A.C. Ruane, C. Müller, A. Arneth, K.J. Boote, C. Folberth, M. Glotter, N. Khabarov, K. Neumann, F. Piontek, T.A.M. Pugh, E. Schmid, E. Stehfest, H. Yang, and J.W. Jones. 2014. Assessing agricultural risks of climate change in the 21st century in a global gridded crop model intercomparison. Proc. Natl. Acad. Sci. 111:3268–3273.

Smith., C.B. and G.A. Taylor. 1952. Tentative optimum leaf concentration of several elements for Elberta peach and Stayman apple in Pennsylvania orchards. Proc. Amer. Soc. Hort. Sci. 60:33–41.

UGA Extension. n.d. Peaches. Cultivars. University of Georgia College of Agricultural and Environmental Sciences, Athens. 4 Mar. 2016. <https://peaches.caes.uga.edu/cultivars.html#maturity-charts>.

U.S. Department of Agriculture Natural Resources Conservation Service. 2015. Plants Database. Washington D.C. 22 Oct. 2015. <http://plants.usda.gov/java/ClassificationServlet?source=profile&symbol=PRUNU&display=31>.

Vashisth, T., M.A. Olmstead, J. Olmstead, and T.A. Colquhoun. 2017. Effects of nitrogen fertilization on subtropical peach fruit quality: Organic acids, phytochemical content, and total antioxidant capacity. J. Amer. Soc. Hort. Sci. 142:393–404.

Williamson, J.G. and D.C. Coston. 1990. Planting method and irrigation rate influence vegetative and reproductive growth of peach planted at high density. J. Amer. Soc. Hort. Sci. 115:207–212.

Zhang, C.H., B.B. Zhang, M.L. Yu, R.J. Ma, Z.Z. Song, and N.K. Korir. 2016. Isolation, cloning, and expression of five genes related to nitrogen metabolism in peach (*Prunus persica* L. Batsch). J. Hort. Sci. Biotechnol. 91:448–455.

CHAPTER 2

PLANT GROWTH AND PHYSIOLOGICAL RESPONSES TO IMPROVED IRRIGATION AND FERTILIZATION MANAGEMENT FOR YOUNG PEACH TREES IN THE SOUTHEASTERN UNITED STATES [1]

[1] Casamali, B, M.W. van Iersel, and D.J. Chavez. To be submitted to *Journal of the American Society for Horticultural Science.*

Abstract

Traditionally, new peach orchards in the southeastern United States are often not irrigated until three or four years after field establishment. During those three or four years, the only source of water comes from rainfall. Droughts in the region are becoming more common, making irrigation more important. At the same time, fertilization practices follow recommendations developed decades ago and may not be optimal for current production practices. The objective of this research was to investigate the effect of different irrigation and fertilization practices on young 'Julyprince' trees grafted onto 'Guardian™' rootstock. The treatments consisted of irrigated vs. non-irrigated trees, drip- vs. micro-sprinkler-irrigated trees, and four different fertilizer levels (25%, 50%, 100%, and 200%; with 100% = current fertilizer recommendations). Responses to the treatments varied by year. In 2016, below average rainfall (severe drought as classified by the U.S. Drought Monitor) was recorded throughout the year. This severe drought reduced the growth of non-irrigated trees in comparison with irrigated trees (average reductions of 56% in canopy volume, 39% in trunk cross-sectional area, 39% in leaf and stem water potential, and 40% in leaf photosynthesis). The negative effects in tree growth and physiological responses of the 2016 season carried over to 2017, which was characterized by a short period of below average rainfall in early spring. Non-irrigated trees displayed advanced bud break progression, reduced commercial yield (10.9 vs. 13.4 kg/tree for non-irrigated vs. irrigated trees), and smaller trunk cross-sectional area (54.0 vs. 70.1 cm^2) and canopy volume (8.9 vs. 10.9 m^3) compared to irrigated trees. In 2018, rainfall was similar to the historical average throughout the year. Major differences continued to be trunk cross-

sectional area (103.4 vs. 126.7 cm^2) and canopy volume (15.8 vs. 17.8 m^3), with non-irrigated trees being smaller than irrigated trees. No major or consistent differences were found between drip vs. micro-sprinkler irrigation or among fertilizer levels during the three years of the experiment. Irrigation increased growth, commercial yield, and resulted in superior water status than non-irrigation, especially when rainfall was below the historical average. Although no major differences were found between the irrigation systems, drip irrigation used 38% less water than micro-sprinkler irrigation. While different fertilizer levels did not induce major differences in young trees' growth and yield, potential economic savings and long-term effects of reduced fertilizer applications are being monitored as the trees mature.

Additional index words: *Prunus persica*, tree growth, fruit yield, drought, photosynthetic assimilation, water potential

Introduction

Peach [*Prunus persica* (L.) Batsch] is a climacteric drupe grown in temperate regions of the world. Georgia is the third largest state producer in the United States with ~4,200 ha of peaches (USDA NASS, 2017). Peaches have a great significance for the state of Georgia (Okie, 2016), with its production area stable over the past ten years (USDA National Agricultural Statistics Service, 2017, 2007). It is paramount to optimize the peach production system to maintain an economically and environmentally sustainable industry. Accurate and precise irrigation (Perea et al., 2018) and fertilization management (Zhang et al., 2015) in agricultural crops have become priority research

areas. Current problems with drought and fertilizer runoff are affecting several regions of the world, including the state of Georgia. Changes in the environment and weather patterns (Rosenzweig et al., 2014) and weather fluctuations (Ray et al., 2015) soon will bring new challenges for agriculture in the southeast region of the United States. Optimal production practices (i.e. irrigation and fertilization) may help trees bear more fruit and sooner, thus increasing profitability.

Peaches evolved in Asia in humid and subtropical conditions (Proebsting Jr. and Middleton, 1980); thus, chances are that peaches naturally have high water requirements and are sensitive to water stress (Berman and DeJong, 1996). However, peaches do not tolerate waterlogging (Iacona et al., 2013). Appropriate and precise irrigation management is required for optimal growth and tree health. Most of the irrigation studies with peaches have been performed in regions with a Mediterranean type climate (e.g., Spain and California, references cited below). This is because those regions have experienced droughts (California Department of Water Resources, 2015; Hoerling et al., 2012) more often and longer than the southeast United States. In those regions, irrigation is essential for crop productivity.

In the southeastern United States, young peach trees typically are not irrigated until three or four years after planting. Thus, natural precipitation is the sole water source. Dry spring and summer seasons are becoming more common in the southeastern United States (USDA Forest Service, 2018). Growers need information about optimal irrigation management for young trees, which is currently not available.

In the southeastern United States, Williamson and Coston (1990) evaluated the effect of two irrigation levels on fruit yield and size of 2.5-year-old ‘Redhaven’ peach

trees. They reported no differences in fruit yield and size, and on most of the vegetative growth parameters evaluated. In contrast, Layne et al. (2002) investigated the combination of three irrigation/fertilization treatments on 1-year-old ‘Redglobe’ peach trees. They reported that trunk cross-sectional area and fruit yield were greater in treatments with supplemental irrigation, whereas the rainfall-only treatment reduced fruit yield and marketable fruit.

In California, Goldhamer et al. (2002) imposed regulated deficit irrigation (RDI) or partial root zone drying on 3-year-old ‘September Snow’ peach trees, finding no differences between treatments for fruit yield, fruit load, and fruit growth rate. The control treatment [crop evapotranspiration (ETc)+10%] yielded heavier fruits, with larger diameter at stage 3 of development (cell expansion stage; Connors, 1919). Control trees had trunk diameter and stem water potential greater than the RDI treatments. Similar effects on fruit yield and weight were found by Marsal et al. (2016) in Europe.

In Europe, Girona et al. (2005) reported that RDI during stage 2 of fruit growth development (pit hardening stage; Connors, 1919) resulted in the highest fruit yield and smallest fruit size. RDI during stage 2 of fruit growth development did not affect return bloom and fruit set; however, postharvest RDI negatively affected both parameters. Lopez et al. (2011) discovered that non-irrigated trees yielded smaller fruit maturing later in the season, and fruit with greater dry matter concentration when compared to the full irrigation treatment. Mercier et al. (2009) found that net photosynthesis, stem water potential, stomatal conductance, and transpiration rates were lower for peach trees under severe drought as compared with well-watered or light water restriction treatments.

Current fertilizer recommendations for peach tree orchards in the southeastern United States are variable (Ferree and Krewer, 1996; Taylor, 2012) and it is not clear how they were developed. We believe the recommendations are based on studies from the mid-1900s and/or studies performed under environmental conditions not representative of southeastern United States environment. This leaves room for potential improvements and optimization and to tailor guidelines to modern production practices. A reduction in fertilizer use, and early and/or increased fruit production (in year two or three after field planting) may generate additional profits for peach growers.

Fertilization is a basic practice for many crops, affecting the tree's physiology in different ways depending on the form (Lobit et al., 2001) and amount of the nutrient applied (Jordan et al., 2014), as well as the timing of the applications (Niederholzer et al., 2001). Several factors can affect the plants' nutritional levels, such as rainfall, fruit load, pruning, rootstock, nutritional interactions, pesticide applications (Heckman, 2001), and soil physicochemical characteristics (Baligar et al., 2001). Early reports of fertilization research in peaches are from the mid 1900s. In an extensive survey in Pennsylvania, Smith and Taylor (1952), indicated that leaf nitrogen (N) concentrations around 3.5% represents the optimal value for peaches. Later, in an effort to develop more accurate recommendations in Florida, Gammon Jr. and Shoemaker (1963) suggested that leaf N concentration around 3.0% in late May or early June was optimal. This concentration was reached by applying 44 and 78 $kg \cdot ha^{-1}$ N in the form of NH_4NO_3 for 2- and 3-year-old trees, respectively. They also reported that lower levels of N reduced yields, and high levels of N did not have negative effects on fruit color and maturity. In contrast, Crisosto et al. (1997) reported that high levels of N resulted in poor

red fruit color and delayed ripening in ‘Fantasia’ nectarine in California. In Turkey, Başar (2006) reported that the leaf N concentration of three peach cultivars, ‘Redhaven’, ‘Glohaven’, and ‘J. H. Hale’, ranged from 2.48 to 2.85% and stated that these concentrations were deficient, which agrees with Gammon Jr. and Shoemaker (1963) findings. In research designed to find the best level of N fertilization for peaches in Florida (planting density of 272 trees/ha), Vashisth et al. (2017) reported that the treatment receiving 90 $kg \cdot ha^{-1}$ N had the greatest yield, followed by the 0 and 269 $kg \cdot ha^{-1}$ N treatments. Similarly, Ferreira et al. (2018) tested four fertilizer levels (0, 30, 60, and 120 $kg \cdot ha^{-1}$ N) in a high-density orchard (1333 trees/ha) of young peach trees in Brazil. For 1- and 2-year-old trees, they found that fertilization above the recommended level for 2-year-old trees (60 $kg \cdot ha^{-1}$ N) did not increase trunk diameter, tree height, and fruit yield. However, for 3-year-old trees, the highest fertilizer treatment increased those variables. This suggests that the existing fertilizer recommendation for 3-year-old trees (80 $kg \cdot ha^{-1}$ N) is suboptimal.

Accurate irrigation and fertilization recommendations for young peach production in the southeastern United States are needed and may create opportunities to increase production efficiency and improve the sustainability of the production system. We hypothesized that 1) irrigated young trees would have a better growth and development and would have greater fruit yield than non-irrigated trees; and 2) that the current fertilizer recommendations for the southeastern United States are not well suited for peach production. The specific objective of the research was to investigate the physiological and growth responses (bud-break progression, trunk cross-sectional area, canopy volume, fruit yield, leaf and stem water potential, photosynthetic assimilation,

and water use efficiency) of young peach trees grown under different irrigation and fertilization regimes.

Material and methods

Plant material and field characteristics

Scions of 'Julyprince' peach grafted onto 'Guardian™' rootstock in the Summer of 2014 were planted on 13 Jul. 2015 (358 trees/ha at a spacing of 4.6 m within rows and 6.1 m between rows) at the Dempsey Farm, University of Georgia, Griffin, GA (33°14' 55" N, 84°17' 57" W). 'Julyprince' has an attractive, large, very-firm fruit, and is a productive and slightly-late variety (Okie and Layne, 2008), ripening in early July in Georgia. 'Guardian™' is a vigorous rootstock (Reighard et al., 1997) tolerant to peach tree short life and resistant to several root-knot nematodes (Nyczepir and Beckman, 2000). Both 'Julyprince' and 'Guardian™' are widely utilized in Georgia. The soil was a Cecil sandy loam, slope of 2-6%, pH ~5.9, and organic matter ~1.5%. Baseline soil amendments for K, P, and lime (to reach pH 6.0) were made based on soil samples taken before planting, as recommended by the Southeastern Peach, Nectarine, and Plum Pest Management and Culture Guide (Horton et al., 2015). Similarly, pest management practices followed the same culture guide.

Experimental design and treatments

The experiment was designed with three main effects: 1) Irrigation levels (irrigated vs. non-irrigated trees); 2) irrigation systems (drip- vs. micro-sprinkler-irrigated

trees); and 3) fertilizer levels (25, 50, 100, and 200%). Hereafter, these three main effects terminology will be used throughout the manuscript.

The experimental area was divided in two areas, arranging the experiment as a Split-Plot Randomized Complete Block Design. One area had the treatment combinations of 1) drip-irrigated vs. non-irrigated trees, with 2) the fertilizer levels (25%, 50%, 100%, and 200%), totaling eight combinations. These combinations were randomized and replicated in four blocks. The other area had the treatment combinations of 1) micro-sprinkler-irrigated vs. non-irrigated trees, with 2) fertilizer levels (25%, 50%, 100%, and 200%), totaling eight combinations as well. These combinations were also randomized and replicated in four blocks.

Drip-irrigated trees had four emitters (SB-20 Bowsmith; Exeter, CA) placed in a circle at ~45 cm around the trunk, with each emitter delivering 7.6 $L \cdot h^{-1}$, for a total of 30.4 $L \cdot h^{-1}$ per tree. Micro-sprinkler-irrigated trees had one micro-sprinkler (QN-08 Rain Bird; Azusa, CA) located at ~10 cm away from the trunk, delivering 30.4 $L \cdot h^{-1}$ per tree. In 2016, the micro-sprinkler head was set to deliver the water in a ~1.7 m diameter circle around the trunk because the trees were young and small. In May of 2017, the micro-sprinkler head was inverted so the water jets reached a circle of ~3.5 m diameter around the trunk because of the trees' larger size. This same configuration was used until the end of the experiment.

The irrigation control was done individually for each fertilizer level within each irrigation system (drip or micro-sprinkler). Two trees of each fertilizer level/irrigation system combination were selected. For each tree, two soil moisture sensors (10HS, Meter Group; Pullman, WA) were installed, one at 20 cm and another at 40 cm depth at

~30 cm from the trunk. Sensors were connected to nR5-DC nodes (Meter Group) and a Sensorweb base station. The base station collected the volumetric water content (VWC) data every 20 min. Based on the average VWC readings of the four sensors within each fertilizer level/irrigation system combination, solenoid valves were turned on as needed to keep the VWC values above the irrigation thresholds set as follows: 1) Irrigation off from January to early-May; 2) VWC threshold of 25% from early-May to early-August; 3) VWC threshold of 20% from early-August to mid-September; 4) VWC threshold of 15% from mid-September to late-September; and 5) irrigation off from late-September to December. These thresholds were based on soil moisture release curves for the specific soil type at the location.

The experimental field was divided in four quadrants and one soil sample was taken in each quadrant center-point, at 30 cm depth. The soil moisture release curves of the four samples were determined through the evaporative method (HYPROP, Meter Group). After analyzing the four samples separately, the HYPROP-VIEW Software (Meter Group) was used to analyze all four samples together, to generate one soil moisture release curve for the whole experimental area. The results of the analysis estimated a field capacity (6 kPa) of 29.4% VWC and a permanent wilting point (PWP) (1500 kPa) of 19.1% VWC. However, the evaporative method has limitations in establishing the dry end of the moisture release curve. The real PWP is likely below the 19.1% VWC reported because of the model limitations. We aimed to have the VWC of the soil around the mid-point between the field capacity and the permanent wilting point during the most active growing period of the trees (spring and summer), and lower values of soil VWC towards the fall season. Due to lightning damage, the Sensorweb

network was inactivated from 28 July 2017 until the end of the 2017 season and from 10 Aug. 2018 until 6 Sept. 2018. Even though we had periods without the automatic irrigation by the Sensorweb network, it is important to highlight that the 2017 and 2018 seasons had rainfall similar to the 30-year historical normal (Table 2.1). If available for visualization, VWC during these periods were monitored; however no irrigation was needed because of the frequent rain linked with the reduction in VWC threshold in early-August. Starting in Mar. of 2017, VWC of non-irrigated plots was recorded every 60 min, using VWC sensors (10HS, Meter Group) placed at 20 and 40 cm depth and distant ~30 cm from the trunk, connected data loggers (Em5b, Meter Group). Irrigation volume was measured using flow meters. Drip irrigation delivered an average of 2,959 L/tree across fertilizer treatments for the three years of experiment; whereas micro-sprinkler irrigation delivered 4,743 L/tree. Non-irrigated trees received water only from rain events.

Four fertilization levels were tested (referred as 25%, 50%, 100%, and 200%). The amount of nitrogen in each level was as follows: 16, 33, 65, and 129 $kg \cdot ha^{-1}$ N for 1-year-old trees; 23, 48, 95, and 191 $kg \cdot ha^{-1}$ N for 2-year-old trees; and 24, 49, 98, and 195 $kg \cdot ha^{-1}$ N for 3-year-old trees. The current recommended levels for 1-, 2-, and 3-year-old trees are 65, 95, and 98 $kg \cdot ha^{-1}$ N, respectively (referred as 100% in this experiment). Granular fertilizer was applied by hand: one application of 10.0N-4.4P-8.3K (in March) and two applications of 15.5N-0P-0K (in May and July) for 1- and 2-year-old trees; and one application of 10.0N-4.4P-8.3K (in March) and one application of 15.5N-0P-0K (in August) for 3-year-old trees. The fertilizer applications followed the recommendations of the Southeastern Peach, Nectarine, and Plum Pest Management and Culture Guide (Horton et al., 2015).

Variables measured

Data was collected from a total of 64 trees, comprising four replications (blocks) of the 16 treatment combinations of: 1) Irrigation levels (irrigated vs. non-irrigated trees); 2) irrigation systems (drip- vs. micro-sprinkler-irrigated trees); and 3) fertilizer levels (25, 50, 100, and 200%).

Mid-day leaf water potential was assessed in July of 2016. Mid-day stem water potential was measured in Aug. of 2016; May, June, July, and Oct. of 2017; and June, July, and Oct. of 2018 using a pressure chamber (1505D-EXP PMS Instrument Company; Albany, OR). For the stem water potential measurements, a fully developed leaf for each data tree was chosen and covered with an aluminum foil bag for ~20 min before measurement to allow the water potential of the leaf to equilibrate with the water potential of the tree stem/trunk, following the equipment's manual. The leaf was then detached from the tree while inside the aluminum bag and placed inside the pressure chamber for measurements. For leaf water potential, no aluminum bag was used. All measurements were conducted around solar noon.

Flower bud development was visually assessed weekly during the bud-break period (Mar. to Apr. in 2017 and Feb. to Mar. in 2018). Each data tree received one value representative to the average flower bud stage of the tree for each week of measurement. Flower bud stages were: 1= Dormant, 2= Bud swell, 3= Green Calix, 4= Red Calix, 5= First pink, 6= First Bloom, 7= Full Bloom, and 8= Post Bloom (adapted from Washington State University, n.d.).

Trunk cross-sectional area (TCSA) was assessed in Sept. 2016, Oct. 2017, and Oct. 2018, by measuring the in-row and across-row trunk diameters at 15 cm above the

soil surface with a caliper. The TCSA was calculated as $\pi \times [(\text{in-row trunk diameter} + \text{across-row trunk diameter}) / 4]^2$.

Canopy volume (CV), as the volume of a cone, was based on tree height and in-row and across-row canopy diameters in Sept. 2016, Oct. 2017, and Oct. 2018, using the formula $CV = \pi \times [(\text{in-row canopy diameter} + \text{across-row canopy diameter}) / 4]^2 \cdot (\text{tree height} / 3)$.

Fruit yield was assessed in July 2017 and July 2018 based on three hand-harvests (July 6, 14, and 25 in 2017; and July 5, 10, and 16 in 2018). Ripe fruit was hand-harvested and separated into commercial and non-commercial yield. Non-commercial fruit comprised of small, damaged, and/or misshapen fruit. Only the results of commercial yield are reported.

Photosynthetic net assimilation (A_n) was measured in June, July, and Aug. 2016; June, July, Aug., Oct. 2017; and June, July, and Oct. 2018 using a portable photosynthesis system (LI-6400XT, LI-COR; Lincoln, NE) equipped with a Red/Blue LED light source to maintain the photosynthetic photon flux density constant at the leaf (1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$). A constant CO_2 concentration of 400 $\mu mol \cdot mol^{-1}$ was utilized in the chamber with leaf temperature and chamber humidity not kept constant. A fully developed leaf for each data tree was chosen, clamped in the equipment head, and measured around solar noon. Concomitantly, leaf transpiration (E) was measured by the system, and water use efficiency at the leaf level (WUE) was estimated by dividing A_n by E.

Statistical analysis

SAS 9.4 (SAS Institute Inc.; Cary, NC) was used to compare treatment means and interactions, using PROC GLIMMIX. Means were separated using Tukey's Honest Significant Difference test with significance level at $P \leq 0.05$.

Results

The VWC of the soil is shown in Figs. 2.1-2.3. In 2016 (Fig. 2.1), irrigation was done manually from 14 May to 22 June, period when the automated irrigation system was being tested. After that, the VWC thresholds were set in the software and the irrigation was controlled automatically until Sept. 19, when it was terminated for the season. In 2017 (Fig. 2.2), automatic irrigation started on 12 May and kept working until 28 July, when the system was damaged by a lightning strike, terminating the automatic irrigation for the season. In 2018 (Fig. 2.3), irrigation was activated on May 7 and worked until Aug. 7, when it was damaged by a storm, deactivating the system. The automatic irrigation resumed in Sept. 6 and worked for 15 more days until the system was terminated for the season in late September. During the periods when the automatic system was deactivated because of storm/lightning strike, irrigation was done manually if the soil VWC was available to be visualized and bellow the thresholds. All figures indicated that the irrigation system kept the soil VWC above the thresholds established for the sensor-based irrigation. In 2016, we did not collect soil VWC data of the non-irrigated trees; however, data from water potential, A_n, and WUE prove that non-irrigated trees were negatively affected by drought stress. In 2017 and 2018 rain

events were frequent, which associated with data from water potential, A_n, and WUE, indicate that the trees did not experience drought stress during those 2 years.

Leaf water potentials were lower in non-irrigated trees in comparison with irrigated tress in July 2016 ($P < 0.001$). Similar results were found in Aug. 2016 ($P < 0.001$) for stem water potential. During the 2017 and 2018 seasons, no differences between irrigated vs. non-irrigated trees were reported for most of the evaluation dates. The exceptions were May 2017 ($P = 0.019$) and July 2018 ($P < 0.001$), when non-irrigated trees had lower stem water potential than irrigated trees (Table 2.2).

Comparisons of water potential between drip- vs. micro-sprinkler-irrigated trees showed mixed results. No differences between these treatments were found in 2016 and early 2017. In Oct. 2017 and 2018, drip-irrigated trees had lower stem water potential than micro-sprinkler-irrigated trees ($P = 0.004$ and 0.017, respectively). However, the opposite was found in June 2018 ($P = 0.034$). Different fertilizer treatments had little effect on water potential. In Oct. 2017 and July 2018, trees receiving 200% fertilizer had the lowest stem water potential, followed by trees receiving 100 and 50% fertilizer, and trees receiving 25% fertilizer had the highest water potential ($P = 0.039$ and 0.005, respectively) (Table 2.2). Interactions between irrigation levels and fertilizer levels were seen in Aug. 2016 ($P = 0.049$) and June 2017 ($P = 0.015$), and between irrigation system and fertilizer level ($P = 0.011$). However, the interactive effects were not consistent throughout the study (Table 2.2).

Flower bud stage development followed a sigmoidal pattern in 2017 with bud swell starting in the first week of March. Bud progression for irrigated trees was delayed in comparison with non-irrigated trees on the first four evaluation days (Mar. 10 through

Mar. 31). On the fifth and sixth evaluation days (Apr. 7 and Apr. 14), no differences were found between irrigation levels (Table 2.3). Throughout the bud-break period, no differences were found between irrigation systems or among fertilization levels, with the exception of an interaction between irrigation levels and fertilizer levels on Mar. 31 (P = 0.05). Non-irrigated trees had similar flower bud stage regardless of the fertilizer treatment; however, irrigated trees receiving 100 or 200% fertilizer had more advanced flower bud stage than trees receiving 25 or 50% fertilizer. In 2018, there were no treatment effects on bud-break progression (Table 2.3).

Irrigated trees had greater TCSA in all three years (Table 2.4). However, for the fertilizer and irrigation systems treatments, different results were found in different years. In Sept. 2016, there was a significant interaction among irrigation system and fertilization treatments (P = 0.003), with trees receiving 200% of fertilizer having greater TCSA than the other treatments when micro-sprinkler-irrigated (34.3 vs. ~25.8 cm^2) and no differences were found among fertilization treatments when drip-irrigated ($\overline{x}$ = 31.5 cm^2). In Oct. 2017, no differences were found when comparing irrigation systems or fertilization treatments (Table 2.4). In Oct. 2018, drip-irrigated trees had greater TCSA than micro-sprinkler-irrigated trees (138.3 vs. 115.1 cm^2, P = 0.018), and no differences were found among fertilization treatments (Table 2.4).

Canopy volume results followed a similar trend as TCSA when comparing irrigated vs. non-irrigated trees. Irrigated trees had greater canopy volume during all three years (Table 2.4). For the comparisons among fertilization and irrigation system treatments, different years resulted in different responses. In Sept. 2016, there was an interaction among fertilization and irrigation system treatments (P < 0.001), with no

differences among fertilization treatments when drip-irrigated. However, for micro-sprinkler-irrigated trees, the highest fertilization treatment resulted in greater canopy volume. In Oct. 2017 and 2018, no differences were found among fertilization treatments or irrigation systems (Table 2.4).

The fruit yield was analyzed in 2017 and 2018, corresponding to the first and second year of fruit production for this orchard. The commercial yield of 2017 was increased by irrigation (13.4 vs. 10.9 kg/tree for non-irrigated trees, $P = 0.037$), while different irrigation systems and fertilization treatments did not induce any differences (Table 2.4). In contrast, in 2018, the commercial yield was not affected by irrigation levels or fertilization treatments. However, within the irrigated trees, micro-sprinkler-irrigated trees had a greater commercial yield than drip-irrigated trees (19.7 vs. 10.4 kg/tree, $P = 0.021$) (Table 2.4).

Photosynthetic net assimilation was measured during the same period as leaf and stem water potential, from late spring through summer. In June, July, and Aug. 2016, A_n of irrigated trees was higher than A_n of non-irrigated trees (Table 2.5). Differences between drip- and micro-sprinkler-irrigated trees were reported only in June 2017, with drip-irrigated trees having greater A_n than micro-sprinkler-irrigated trees (18.3 vs. 16.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$, $P = 0.018$). Differences among fertilizer treatments were significant only in Oct. 2018 ($P = 0.01$), when trees receiving 25% of fertilizer had the greatest A_n (13.7 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and the trees receiving 200% of fertilizer had the lowest A_n (11.6 $\mu mol \cdot m^{-2} \cdot s^{-1}$) (Table 2.5). In Aug. 2016 there was an interaction between irrigation system and fertilizer treatments ($P = 0.034$). Trees under micro-sprinkler irrigation had greater A_n when receiving 100 or 200% of fertilizer (18.7 and 19.0 $\mu mol \cdot m^{-}$

$^{2}\cdot s^{-1}$, respectively) than trees receiving 25 or 50% of fertilizer (13.3 and 13.7 $\mu mol\cdot m^{-2}\cdot s^{-1}$, respectively); however, drip-irrigated trees had similar A_n in all fertilization treatments, ~20.3 $\mu mol\cdot m^{-2}\cdot s^{-1}$ (Table 2.5).

Non-irrigated trees had greater WUE than irrigated trees in June, July, and Aug. 2016, and June and July 2017. In July 2016, micro-sprinkler-irrigated trees had greater WUE than drip-irrigated trees (4.1 vs 3.3 $mmol\cdot mol^{-1}$, $P = 0.002$) (Table 2.5). No differences were found among the different fertilizer levels, except for Oct. 2018 ($P = 0.02$), when trees receiving 200% of fertilizer had the highest WUE (3.3 $mmol\cdot mol^{-1}$) and the trees receiving 50% of fertilizer had the lowest WUE (3.0 $mmol\cdot mol^{-1}$) (Table 2.5). Interactions of irrigation system and fertilizer treatments ($P < 0.001$) in Aug. 2016 and irrigation levels and fertilizer levels ($P = 0.044$) in Oct. 2017 were found, but they were not consistent throughout the experiment (Table 2.5).

Discussion

Below average rainfall resulted in low leaf and stem water potentials of non-irrigated trees in 2016, indicating that non-irrigated trees had limited soil water availability. In 2017 and 2018, because of average rain, irrigated and non-irrigated trees experienced similar soil water availability. In 2017, the VWC readings of the non-irrigated trees were slightly higher on average than those of irrigated trees, perhaps because the sensors had been recently installed. The soil near the sensors had been disturbed, possibly allowing for more water being around the sensors. This difference was not present in 2018, when the sensors were well established in the soil.

Leaf and stem water potential are related to the soil water content and weather conditions (Mahhou et al., 2005). Previous research with peach trees (Mahhou et al., 2005; Mirás-Avalos et al., 2013; Rahmati et al., 2015; Tormann, 1986) estimated that mid-day stem water potential values above -1.0 MPa are considered “no stress”, whereas values around -1.5 and -2.0 MPa correspond to “moderate stress” and “severe stress”, respectively. Our results of leaf and stem water potential varied across years, similar to results reported by Abrisqueta et al. (2015). In 2016, the low rain and soil VWC likely limited tree-available water, inducing the severe stress measured in July and August. This was more pronounced in non-irrigated trees, agreeing with results of leaf and stem water potential reported by Goldhamer et al. (1999). Despite the lack of precipitation right before the 2017 growing season (February to April), precipitation was close the historical average when the trees were actively growing (May to August). This resulted in stem water potential values not indicative of drought. Similar to 2017, 2018 had rainfall close to the historical average from January through August, not resulting in drought stress. Some statistical differences were found in 2017 and 2018, but they were not consistent over time and do not have a biological significance, since the water potential values are all within the “no stress” range.

Flower bud break progression differed in the two years of evaluation. There were differences among the treatments in the early stages of bud break progression in 2017, mainly as a result of the presence or absence of irrigation. Bud breaking progression from bud swell to full bloom advanced faster in non-irrigated than in the irrigated trees. Flower buds opening in 2017 were induced and differentiated during the Summer of 2016, when rainfall was below average (see Table 2.1), affecting the non-irrigated trees.

Drought stress can alter the hormonal and assimilate balance of trees, causing the accumulation of active substances, such as polyamines, which is linked to increase levels of floral initiation (Johnson et al., 1992). Greater flower initiation in 2016 during the drought stress likely resulted a higher number of flower buds, resulting in an advanced bud break progression. In contrast to 2017, 2018 bud development was similar in all treatments, possibly because the 2017 growing season had above average rainfall (Table 2.1). Thus, the flower buds of the 2018 season differentiated in the absence of drought in 2017.

Over the 3-year period of research, irrigated trees had larger trunk cross-sectional area and canopy volume than non-irrigated trees. The TCSA results agree with Layne et al. (2002), who also found greater TCSA in trees with irrigation, compared to rainfall-only. Similarly, Goldhamer et al. (2002) found increased trunk diameter in irrigated trees vs. regulated deficit irrigation. For canopy volume, Boland et al. (2000) reported reduced canopy volume for trees grown under deficit irrigation.

An interaction between irrigation system and fertilization treatments was found in 2016 for both TCSA and canopy volume; however, the results were not consistent during the three years of research. These results suggest that different irrigation systems and fertilizer treatments had little to no impact on TCSA and canopy volume of young peach trees in our experiment. These results agree with research done by Layne et al. (2002) who reported a small reduction in TCSA when reducing the fertilizer by 30% and with Olmstead et al. (2015) and Baldi et al. (2010), who found no differences in TCSA in response to different N levels. In our research, it appears that the highest

fertilizer level induced greater tree growth in the first year of evaluation (larger canopy volume). However, the effect did not persist into years 2 and 3.

Commercial yield differed across treatments depending on the year evaluated. In 2017, irrigated trees had a higher yield than non-irrigated trees. In 2017, the precipitation was below the historical normal from February to May and during July (Table 2.1). Therefore, non-irrigated trees likely were negatively affected by drought during the fruit growth and development period, from mid-March to mid-July. As discussed in the bud break progression section, flowers that constituted the fruits harvested in 2017 were induced and differentiated during the 2016 season, which had below average rainfall (Table 2.1). Thus, the 2016 drought may have negatively impacted the number of flower buds formed, which may have resulted in fewer harvested fruits in 2017. Further, irrigated trees were larger than their non-irrigated counterparts, which may translate to more fruiting wood, eventually resulting in higher yield.

In 2018, the yield was not affected by the irrigation level, but trees grown with micro-sprinkler irrigation had 89% higher yield than trees with drip irrigation. The effect of irrigation system on yield in 2018 was likely not a direct result of the irrigation system. An advective hard freeze affected the orchard in Mar. 2018, during bloom, and likely induced differences between the treatments. Because of the landscape topography of the orchard, the trees with drip irrigation received direct wind gusts, while the trees under micro-sprinkler irrigation were protected because of a hilltop.

Fertilizer treatments had no effect on fruit yield in either year, agreeing with Layne et al. (1996), who reported no differences in fruit yield in response to

combinations of irrigation and fertilization. Similarly, Baldi et al. (2010) did not find differences in fruit yield between fertilized vs. non-fertilized trees in the first two years of fruit production. However, fertilized trees had greater yield in the third year of fruit production, likely because the long-term lack of fertilization started to induce negative effects on the non-fertilized trees. Testing the effects of different fertilizer levels on peaches, Vashisth et al. (2017) reported inconclusive data on fruit yield, because the intermediate N treatment (90 $kg \cdot ha^{-1}$ per year) had greater yield than the 45 and 179 $kg \cdot ha^{-1}$ per year treatments, but similar yield to the 0 and 269 $kg \cdot ha^{-1}$ per year treatments.

During 2016 drought conditions, a lack of irrigation reduced A_n by ~ 40%, agreeing with earlier reports (Dichio et al., 2007; Goldhamer et al., 1999; Mahhou et al., 2005; Mercier et al., 2009). In August of 2016, drip-irrigated trees displayed greater assimilation than micro-sprinkler-irrigated trees. Both drip and micro-sprinkler treatments were set to keep the volumetric water content of the soil above the same thresholds, which would suggest similar amounts of water in the soil, regardless of the irrigation system. One possible explanation for the difference in A_n is how the systems delivered water to the soil and how the root system behaved. Drip irrigation delivers water to a smaller volume of soil, where the roots will concentrate. Micro-sprinklers create jets of water around the trunk, irrigating a greater volume of soil, where the roots will proliferate. Therefore, the root system of drip-irrigated trees likely is more concentrated in the wetted area, being more efficient absorbing water, supporting work by Tagliavini et al. (1996). In 2017 and 2018, no consistent differences among treatments were found. Fertilizer levels did not induce changes in A_n, likely because the

leaf nutritional levels were not negatively affected by the fertilizer levels (data not shown). Similarly, Pascual et al. (2016) did not find leaf nutritional differences among fertilizer treatments in two out of three evaluations.

Water use efficiency results differed among years. In 2016, and June and July of 2017, non-irrigated trees had higher water use efficiency than irrigated trees, consistent with WUE data (A_n/stomatal conductance) reported by Haider et al. (2018). Below average rainfall was present from 2016 until May 2017, increasing WUE of non-irrigated trees until July 2017. The 2018 growing season had above average rainfall, which resulted in no WUE differences for any irrigation treatments. Irrigation systems and fertilizer levels did not induce consistent WUE responses in the trees in all three seasons, which agrees with results from a prior fertility study (Haider et al. 2018).

Establishing irrigation in the first year of field planting showed benefits for young peach trees. Irrigated trees displayed greater growth with larger canopy and trunk size, which opens the possibility of having higher yields if below average rainfall is reported. Because of the drought stress that non-irrigated trees suffered in 2016, the negative effects carried over to the 2017 and 2018 seasons. Growth of non-irrigated trees did not catch up with that of irrigated trees after three years.

Conclusions

Accordingly to the United States Drought Monitor (NDMC, USDA, and NOAA, 2019), Georgia experienced drought about every other year during the past 20 years. Therefore, young peach trees are likely to be exposed to drought during their establishment, which suggests that irrigation for young trees is important. Irrigation

systems (drip or micro-sprinkler) did not induce consistent differences across variables and years of evaluation; however, drip irrigation was more efficient delivering water, resulting in ~38% of water savings in comparison with micro-sprinkler irrigation. Similar to the irrigation systems, fertilizer treatments did not induce consistent responses, having little to no effect on tree growth or physiology. The long-term effect of reductions in fertilizer levels is yet to be determined; however, we believe that a reduction in the recommended fertilizer levels for young peach trees is timely and will not be detrimental.

Irrigation from the time of establishment can serve as insurance for peach growers, as it is beneficial during periods of drought, which are becoming more common in the southeastern United States. Greater tree growth and fruit yield can be achieved by irrigating trees since establishment. In contrast, current fertilizer levels can be reduced without negative impacts on peach tree growth and yield during the initial years of field establishment. Improved irrigation and fertilization in young peach trees will increase the efficiency of peach's production system.

Literature cited

Abrisqueta, I., W. Conejero, M. Valdés-Vela, J. Vera, M.F. Ortuño, and M.C. Ruiz-Sánchez. 2015. Stem water potential estimation of drip-irrigated early-maturing peach trees under Mediterranean conditions. Comput. Electron. Agri. 114:7–13.

Baldi, E., M. Toselli, D.M. Eissenstat, and B. Marangoni. 2010. Organic fertilization leads to increased peach root production and lifespan. Tree Physiol. 30:1373–1382.

Baligar, V.C., N.K. Fageria, and Z.L. He. 2001. Nutrient use efficiency in plants. Commun. Soil Sci. Plant Analysis. 32(7&8):921–950.

Başar, H. 2006. Elemental composition of various peach cultivars. Sci. Hort. 107:259–263.

Berman, M.E. and T.M. DeJong. 1996. Water stress and crop load effects on fruit fresh and dry weights in peach (*Prunus persica*). Tree Phys. 16:859–864.

Boland, A.M., P.H. Jerie, P.D. Mitchell, I. Goodwin, and D.J. Connor. 2000. Long-term effects of restricted root volume and regulated deficit irrigation on peach: I. Growth and mineral nutrition. J. Amer. Soc. Hort. Sci. 125(1):135–142.

California Department of Water Resources, 2015. Drought in California. Sacramento, CA.

Connors, C.H. 1919. Growth of fruits of peach. N.J. Agr. Expt. Sta. Annu. Rpt. 40:82–88.

Crisosto, C.H., R.S. Johnson, T. DeJong, and K.R. Day. 1997. Orchard factors affecting postharvest stone fruit quality. HortScience 32:820–823.

Dichio, B., C. Xiloyannis, A. Sofo, and G. Montanaro. 2007. Effects of post-harvest regulated deficit irrigation on carbohydrate and nitrogen partitioning, yield quality and vegetative growth of peach trees. Plant Soil 290:127–137.

Ferree, M.E. and G.W. Krewer. 1996. Peaches and nectarines. Univ. Georgia Coop. Ext. Serv. Athens, GA.

Ferreira, L.V., L. Picolotto, I. dos S. Pereira, J.D. Schmitz, and L.E.C. Antunes. 2018. Nitrogen fertilization in consecutive cycles and its impact on high-density peach crops. Pesquisa Agropecuaria Brasileira 53:172–181.

Gammon Jr., N. and J.S. Shoemaker. 1963. Effect of fertilizers on mineral levels in the leaves of Flordawon peach. Florida State Hort. Soc. 1746:380–384.

Girona, J., M. Gelly, M. Mata, A. Arbonès, J. Rufat, and J. Marsal. 2005. Peach tree response to single and combined deficit irrigation regimes in deep soils. Agr. Water Mgt. 72:97–108.

Goldhamer, D.A., E. Fereres, M. Mata, J. Girona, and M. Cohen. 1999. Sensitivity of continuous and discrete plant and soil water status monitoring in peach trees subjected to deficit irrigation. J. Amer. Soc. Hort. Sci. 124:437–444.

Goldhamer, D.A., M. Salinas, C. Crisosto, K.R. Day, M. Soler, and A. Moriana. 2002. Effects of regulated deficit irrigation and partial root zone drying on late harvest peach tree performance. Acta Hort. 592:343–350.

Haider, M.S., M.M. Kurjogi, M. Khalil-ur-Rehman, T. Pervez, J. Songtao, M. Fiaz, S. Jogaiah, C. Wang, and J. Fang. 2018. Drought stress revealed physiological, biochemical and gene-expressional variations in ‘Yoshihime’ peach (*Prunus Persica* L.) cultivar. J. Plant Interactions 13:83–90.

Heckman, J. 2001. Leaf analysis for fruit trees. New Jersey Agr. Expt. Sta. FS627. New Brunswick, NJ.

Hoerling, M., J. Eischeid, J. Perlwitz, X. Quan, T. Zhang, and P. Pegion. 2012. On the increased frequency of mediterranean drought. J. Climate 25:2146–2161.

Horton, D., P. Brannen, B. Bellinger, D. Lockwood, and D. Ritchie. 2015. 2015 Southeastern peach, nectarine and plum pest management and culture guide. Uni. Georgia Coop. Ext. Bul. 1171. Athens, GA.

Iacona, C., M. Cirilli, A. Zega, E. Frioni, C. Silvestri, and R. Muleo. 2013. A somaclonal myrobalan rootstock increases waterlogging tolerance to peach cultivar in controlled conditions. Sci. Hort. 156:1–8.

Johnson, R.S., D.F. Handley, and T.M. DeJong. 1992. Long-term response of early maturing peach trees to postharvest water deficits. J. Amer. Soc. Hort. Sci. 117(6):881–886.

Jordan, M.-O., G. Vercambre, L. Gomez, and L. Pages. 2014. The early spring N uptake of young peach trees (*Prunus persica*) is affected by past and current fertilizations and levels of C and N stores. Tree Phys. 34:61–72.

Layne, D.R., D.B. Cox, and E.J. Hitzler. 2002. Peach systems trial : The influence of training system, tree density, rootstock, irrigation and fertility on growth and yield of young trees in South Carolina. Acta Hort. 592:367–375.

Layne, R.E.C., C.S. Tan, and D.M. Hunter. 1996. Irrigation and fertilizer application methods affect performance of high-density peach orchards. HortScience 31:370–375.

Lobit, P., P. Soing, M. Génard, and R. Habib. 2001. Effects of timing of nitrogen fertilization on shoot development in peach (*Prunus persica*) trees. Tree Phys. 20:35–42.

Lopez, G., M. Hossein Behboudian, G. Echeverria, J. Girona, and J. Marsal. 2011. Instrumental and sensory evaluation of fruit quality for 'Ryan's sun' peach grown under deficit irrigation. HortTechnology 21:712–719.

Mahhou, A., T.M. DeJong, T. Cao, and K.S. Shackel. 2005. Water stress and crop load effects on vegetative and fruit growth of 'Elegant Lady' peach [*Prunus persica* (L.) Batch] trees. Fruits 60:55–68.

Marsal, J., J. Casadesus, G. Lopez, M. Mata, J. Bellvert, and J. Girona. 2016. Sustainability of regulated deficit irrigation in a mid-maturing peach cultivar. Irr. Sci. 34:201–208.

Mercier, V., C. Bussi, F. Lescourret, and M. Génard. 2009. Effects of different irrigation regimes applied during the final stage of rapid growth on an early maturing peach cultivar. Irr. Sci. 27:297–306.

Mirás-Avalos, J.M., R. Alcobendas, J.J. Alarcón, P. Valsesia, M. Génard, and E. Nicolás. 2013. Assessment of the water stress effects on peach fruit quality and size using a fruit tree model, QualiTree. Agric. Water Mgt. 128:1–12.

National Drought Mitigation Center, U.S. Department of Agriculture, and National Oceanic and Atmospheric Association. 2019. United States drought monitor. 11 June 2019. <https://droughtmonitor.unl.edu/Data/Timeseries.aspx>.

Niederholzer, F.J.A., T.M. DeJong, J.-L. Saenz, T.T. Muraoka, and S.A. Weinbaum. 2001. Effectiveness of fall versus spring soil fertilization of field-grown peach trees. J. Amer. Soc. Hort. Sci. 125:644–648.

Nyczepir, A.P., T.G. Beckman. 2000. Host status of guardian peach rootstock to *Meloidogyne* sp. and *M. javanica*. HortScience 35:772.

Okie, W.R. and D.R. Layne. 2008. 'Scarletprince' and 'Julyprince' peaches. HortScience 43:1603–1605.

Okie, W.T. 2016. The Georgia peach: Culture, agriculture, and environment in the American south. Cambridge University Press, New York, NY.

Olmstead, M., L. Zotarelli, J. Brecht, and M. Ross, 2015. Impact of nitrogen on vegetative growth of mature peach trees in a subtropical climate. Acta Hort. 1084:459–464.

Pascual, M., J.M. Villar, and J. Rufat. 2016. Water use efficiency in peach trees over a four-years experiment on the effects of irrigation and nitrogen application. Agr. Water Mgt. 164:253–266.

Perea, R.G., A. Daccache, J.A. Rodríguez Díaz, E. Camacho Poyato, and J.W. Knox. 2018. Modelling impacts of precision irrigation on crop yield and in-field water management. Precision Agr. 19:497–512.

Proebsting Jr., E.L. and J.E. Middleton. 1980. The behavior of peach and pear trees under extreme drought stress. J. Amer. Soc. Hort. Sci. 105:380–385.

Ray, D.K., J.S. Gerber, G.K. Macdonald, P.C. West. 2015. Climate variation explains a third of global crop yield variability. Nature Commun. 6:5989.

Rahmati, M., G.H. Davarynejad, M. Génard, M. Bannayan, M. Azizi, and G. Vercambre. 2015. Peach water relations, gas exchange, growth and shoot mortality under water deficit in Semi-Arid weather conditions. PLoS One 10:e0120246.

Reighard, G.L., W.C. Newall Jr., T.G. Beckman, W.R. Okie, E.I. Zehr, and A.P. Nyczepir. 1997. Field performance of *Prunus* rootstock cultivars and selection on replants soil in South Carolina. Acta Hort. 451 243–249.

Rosenzweig, C., J. Elliott, D. Deryng, A.C. Ruane, C. Müller, A. Arneth, K.J. Boote, C. Folberth, M. Glotter, N. Khabarov, K. Neumann, F. Piontek, T.A.M. Pugh, E. Schmid, E. Stehfest, H. Yang, and J.W. Jones. 2014. Assessing agricultural risks of climate change in the 21st century in a global gridded crop model intercomparison. Proc. Natl. Acad. Sci. 111:3268–3273.

Smith., C.B. and G.A. Taylor. 1952. Tentative optimum leaf concentration of several elements for Elberta peach and Stayman apple in Pennsylvania orchards. Proc. Amer. Soc. Hort. Sci. 60:33–41.

Tagliavini, M., D. Scudellazi, B. Marangoni, and M. Toselli. 1996. Nitrogen fertilization management in orchards to reconcile productivity and environmental aspects, p. 161–170. In: C. Rodriguez-Barrueco (ed.). Fertilizer and Environment. Developments in Plant and Soil Sciences. Springer, Dordrecht, Netherlands.

Taylor, K.C. 2012. Peach orchard establishment and young tree care. Univ. Georgia Coop. Ext. Serv. Athens, GA.

Tormann, H. 1986. Canopy temperature as a plant water stress indicator for nectarines. South African J. Plant Soil 3:110–114.

University, W.S. n.d. Peaches. Critical temperatures for blossom buds. 6 April 2019. <https://s3.wp.wsu.edu/uploads/sites/2086/2014/05/budchph.jpg>.

U.S. Department of Agriculture Forest Service. 2018. Drought impacts in the Southern region. U.S. Department of Agriculture Forest Service. Washington, D.C.

U.S. Department of Agriculture National Agricultural Statistics Service. 2007. 2007 Census of agriculture. State Profile. U.S. Department of Agriculture. Washington, D.C.

U.S. Department of Agriculture National Agricultural Statistics Service. 2017. Noncitrus Fruits and Nuts - 2016 Summary. U.S. Department of Agriculture. Washington, D.C.

Vashisth, T., M.A. Olmstead, J. Olmstead, and T.A. Colquhoun. 2017. Effects of nitrogen fertilization on subtropical peach fruit quality: Organic acids, phytochemical content, and total antioxidant capacity. J. Amer. Soc. Hort. Sci. 142:393–404.

Williamson, J.G. and D.C. Coston. 1990. Planting method and irrigation rate influence vegetative and reproductive growth of peach planted at high density. J. Amer. Soc. Hort. Sci. 115:207–212.

Zhang, X., E.A. Davidson, D.L. Mauzerall, T.D. Searchinger, P. Dumas, and Y. Shen. 2015. Managing nitrogen for sustainable development. Nature 528:51–59.

Table 2.1. Precipitation (mm) from January to December in 2016, 2017, and 2018 and the historic normal (average from 1981 to 2010) at the Dempsey Farm, Griffin, GA.

	January	February	March	April	May	June	July	August	September	October	November	December	Total
2016	110	125	66	174	67	77	66	69	159	1	69	159	754
2017	247	66	53	97	91	194	91	112	130	207	28	79	952
2018	103	98	133	118	145	134	103	106	84	132	164	267	940
1981-2010	112	104	146	113	96	96	150	131	91	83	99	99	947

Table 2.2. Effects of irrigation levels, irrigation systems, and fertilizer levels on leaf and stem water potential of 'Julyprince' peaches in 2016, 2017, and 2018.

	Leaf and stem water potential (MPa)[z]								
	2016		2017				2018		
Treatment	July	Aug.	May	June	July	Oct.	June	July	Oct.
Irrigation level									
Irrigated	-2.16 b[y]	-1.25 b	-0.47 b	-0.64	-0.69	-0.75	-0.62	-0.65 b	-0.82
Non-irrigated	-2.56 a	-1.99 a	-0.51 a	-0.63	-0.70	-0.71	-0.63	-0.74 a	-0.84
Irrigation system									
Drip	-2.28	-1.17	-0.48	-0.65	-0.68	-0.81 a	-0.59 b	-0.62	-0.87 a
Micro-sprinkler	-2.05	-1.32	-0.47	-0.62	-0.71	-0.70 b	-0.66 a	-0.68	-0.77 b
Fertilizer level									
25%	-2.34	-1.68	-0.47	-0.60	-0.68	-0.68 b	-0.60	-0.64 b	-0.78
50%	-2.32	-1.55	-0.50	-0.64	-0.73	-0.73 ab	-0.64	-0.69 ab	-0.85
100%	-2.37	-1.65	-0.49	-0.62	-0.68	-0.73 ab	-0.61	-0.68 ab	-0.83

200%	-2.41	-1.59	-0.51	-0.68	-0.69	-0.78 a	-0.67	-0.76 a	-0.85
P value									
Irrigation level	**<0.001**	**<0.001**	**0.019**	0.867	0.896	0.072	0.779	**<0.001**	0.528
Irrigation system	0.067	0.160	0.746	0.472	0.703	**0.004**	**0.034**	0.415	**0.017**
Fertilizer level	0.818	0.507	0.446	0.103	0.706	**0.039**	0.210	**0.005**	0.152
Irrigation level* Fertilizer level	0.338	**0.049**	0.908	**0.015**	0.117	0.873	0.401	0.209	0.765
Irrigation system* Fertilizer level	0.303	0.236	0.649	0.911	0.587	0.538	0.358	0.684	**0.011**

[z] Leaf water potential was measured in July 2016 only. The data for other months are stem water potential.

[y] Means followed by the same letter within a column and treatment effect are not significantly different by Tukey's honestly significant difference test, $P \leq 0.05$. Means without letters indicate non-significant differences.

Table 2.3. Effects of irrigation levels, irrigation systems, and fertilizer levels on bud-break progression of ‘Julyprince’ peaches in 2017 and 2018.

Treatment	Bud stage 2017[z]					
	10 Mar.	17 Mar.	24 Mar.	31 Mar.	7 Apr.	14 Apr.
Irrigation level						
Irrigated	2.0 b[y]	2.4 b	4.3 b	6.6 b	7.9	8.0
Non-irrigated	2.7 a	3.1 a	4.8 a	7.0 a	8.0	8.0
Irrigation system						
Drip	1.9	2.4	4.6	6.6	7.9	8.0
Micro-sprinkler	2.2	2.3	4.0	6.5	7.8	8.0
Fertilizer level						
25%	2.3	2.7	4.4	6.7	7.8	8.0
50%	2.3	2.7	4.5	6.7	7.9	8.0
100%	2.4	2.7	4.8	6.9	8.0	8.0
200%	2.4	2.8	4.6	6.8	8.0	8.0

P value

Irrigation level	**<0.001**	**<0.001**	**0.027**	**0.019**	0.271	1.000
Irrigation system	0.108	0.299	0.102	0.587	0.564	1.000
Fertilizer level	0.744	0.692	0.597	0.823	0.357	1.000
Irrigation level*Fertilizer level	0.691	0.159	0.217	**0.050**	0.680	1.000
Irrigation system*Fertilizer level	0.688	0.133	0.999	0.227	0.281	1.000

	Bud stage 2018				
	12 Feb.	19 Feb.	23 Feb.	27 Feb.	5 Mar.
Irrigation level					
Irrigated	1.0	2.9	5.0	7.1	8.0
Non-irrigated	1.0	2.9	5.0	7.1	8.0
Irrigation system					
Drip	1.0	2.8	5.0	7.1	8.0
Micro-sprinkler	1.0	2.9	5.0	7.0	8.0

Fertilizer level					
25%	1.0	3.0	5.0	7.0	8.0
50%	1.0	2.8	5.0	7.1	8.0
100%	1.0	2.8	5.0	7.1	8.0
200%	1.0	2.8	5.0	7.1	8.0
P value					
Irrigation level	1.000	0.583	0.505	0.313	1.000
Irrigation system	1.000	0.709	1.000	0.564	1.000
Fertilizer level	1.000	0.312	0.929	0.737	1.000
Irrigation level*Fertilizer level	1.000	0.821	0.929	0.538	1.000
Irrigation system*Fertilizer level	1.000	0.302	1.000	0.313	1.000

[z] Bud stages adapted from Washington State University, n.d. 1= Dormant, 2= Bud swell, 3= Green Calix, 4= Red Calix, 5= First pink, 6= First Bloom, 7= Full Bloom, and 8= Post Bloom.

[y] Means followed by the same letter within a column and treatment effect are not significantly different by Tukey's honestly significant difference test, $P \leq 0.05$. Means without letters indicate non-significant differences

Table 2.4. Effects of irrigation levels, irrigation systems, and fertilizer levels on trunk cross-sectional area (TCSA), canopy volume in 2016, 2017, and 2018, and commercial yield of 'Julyprince' peaches in 2017 and 2018.

	TCSA (cm^2)			Canopy volume (m^3)			Commercial yield (kg/tree)	
Treatment	Sept. 2016	Oct. 2017	Oct. 2018	Sept. 2016	Oct. 2017	Oct. 2018	2017	2018
Irrigation level								
Irrigated	29.7 a[z]	70.1 a	126.7 a	4.5 a	10.9 a	17.8 a	13.4 a	15.1
Non-irrigated	18.0 b	54.0 b	103.4 b	2.0 b	8.9 b	15.8 b	10.9 b	12.8
Irrigation system								
Drip	31.5	72.5	138.3 a	4.7 a	11.5	19.1	15.0	10.4 b
Micro-sprinkler	28.0	67.7	115.1 b	4.3 b	10.4	16.5	11.7	19.7 a
Fertilizer level								
25%	23.2	62.3	117.9	3.0 b	10.2	17.2	12.6	13.2
50%	23.1	59.4	108.1	3.1 b	9.3	16.0	10.9	16.3

100%	23.4	63.8	118.1	3.2 ab	10.4	17.4	11.2	13.0
200%	25.7	62.8	116.2	3.5 a	9.8	16.6	13.8	13.2
P value								
Irrigation level	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.013**	**0.037**	0.344
Irrigation system	0.056	0.306	**0.018**	**0.017**	0.112	0.071	0.097	**0.021**
Fertilizer level	0.088	0.715	0.523	**0.014**	0.341	0.621	0.264	0.725
Irrigation level* Fertilizer level	0.211	0.502	0.621	0.659	0.547	0.380	0.620	0.795
Irrigation system* Fertilizer level	**0.003**	0.400	0.941	**<0.001**	0.890	0.644	0.654	0.678

[z] Means followed by the same letter within a column and treatment effect are not significantly different by Tukey's honestly significant difference test, $P \leq 0.05$. Means without letters indicate non-significant differences.

Table 2.5. Effects of irrigation levels, irrigation systems, and fertilizer levels on photosynthetic assimilation (A_n) and water use efficiency (WUE) of 'Julyprince' peaches in 2016, 2017, and 2018.

	A_n (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$)									
	2016			2017				2018		
Treatment	June	July	Aug.	June	July	Aug.	Oct.	June	July	Oct.
Irrigation level										
Irrigated	14.9 a[z]	14.3 a	18.3 a	17.2	16.3	12.4	11.0	15.3	18.5	13.1
Non-irrigated	6.7 b	8.2 b	13.4 b	16.4	16.5	12.6	11.7	16.0	18.2	12.5
Irrigation system										
Drip	15.1	13.8	20.3 a	18.3 a	16.1	11.9	11.0	15.3	18.9	13.2
Micro-sprinkler	14.7	14.8	16.2 b	16.1 b	16.4	12.8	11.0	15.3	18.1	13.0
Fertilizer level										
25%	10.7	10.1	14.8	17.3	16.2	13.7	11.2	16.0	18.7	13.7 a
50%	10.5	11.3	15.6	16.4	15.8	12.3	10.0	16.0	18.4	13.2 ab
100%	10.1	10.9	16.3	16.9	17.4	12.1	11.8	15.2	18.4	12.8 ab

200%	11.9	12.6	16.6	16.7	16.0	11.8	12.3	15.3	17.9	11.6 b
P value										
Irrigation level	**<0.001**	**<0.001**	**<0.001**	0.151	0.677	0.718	0.316	0.213	0.531	0.211
Irrigation system	0.511	0.377	**<0.001**	**0.018**	0.771	0.500	0.993	0.983	0.154	0.785
Fertilizer level	0.099	0.173	0.306	0.689	0.060	0.086	0.094	0.627	0.551	**0.010**
Irrigation level*										
Fertilizer level	0.496	0.826	0.161	0.713	0.659	0.832	0.474	0.730	0.462	0.356
Irrigation system*										
Fertilizer level	0.175	0.063	**0.034**	0.838	0.650	0.614	0.500	0.669	0.930	0.924

WUE (mmol·mol^{-1})

	2016			2017				2018		
Treatment	June	July	Aug.	June	July	Aug.	Oct.	June	July	Oct.
Irrigation level										
Irrigated	3.5 b	3.7 b	5.8 b	4.4 b	2.9 b	3.2	5.1	2.8	3.2	3.1
Non-irrigated	5.1 a	4.9 a	7.4 a	4.6 a	3.1 a	3.1	5.0	2.9	3.1	3.1

Irrigation system										
Drip	3.6	3.3 b	5.1	4.3	3.0	3.1	5.2	2.9	3.2	3.0
Micro-sprinkler	3.3	4.1 a	6.5	4.5	2.9	3.2	5.1	2.7	3.1	3.2
Fertilizer level										
25%	4.1	4.6	6.9	4.3	3.0	3.0	5.0	2.8	3.1	3.1 ab
50%	4.4	4.1	6.9	4.5	3.1	3.2	4.9	2.9	3.2	3.0 b
100%	4.4	4.6	6.2	4.6	3.0	3.2	5.1	2.9	3.2	3.2 ab
200%	4.3	4.0	6.5	4.5	3.0	3.2	5.2	3.0	3.0	3.3 a
P value										
Irrigation level	**<0.001**	**<0.001**	**<0.001**	**0.047**	**0.028**	0.913	0.472	0.197	0.409	0.528
Irrigation system	0.123	**0.002**	0.052	0.253	0.058	0.386	0.707	0.364	0.059	0.312
Fertilizer level	0.470	0.141	0.482	0.151	0.593	0.405	0.506	0.077	0.401	**0.020**
Irrigation level*										
Fertilizer level	0.953	0.584	0.161	0.925	0.698	0.664	**0.044**	0.456	0.935	0.159
Irrigation system*	0.061	0.135	**<0.001**	0.819	0.258	0.101	0.062	0.233	0.314	0.051

Fertilizer level

---

[z]Means followed by the same letter within a column and treatment effect are not significantly different by Tukey's honestly significant difference test, $P \leq 0.05$. Means without letters indicate non-significant differences.

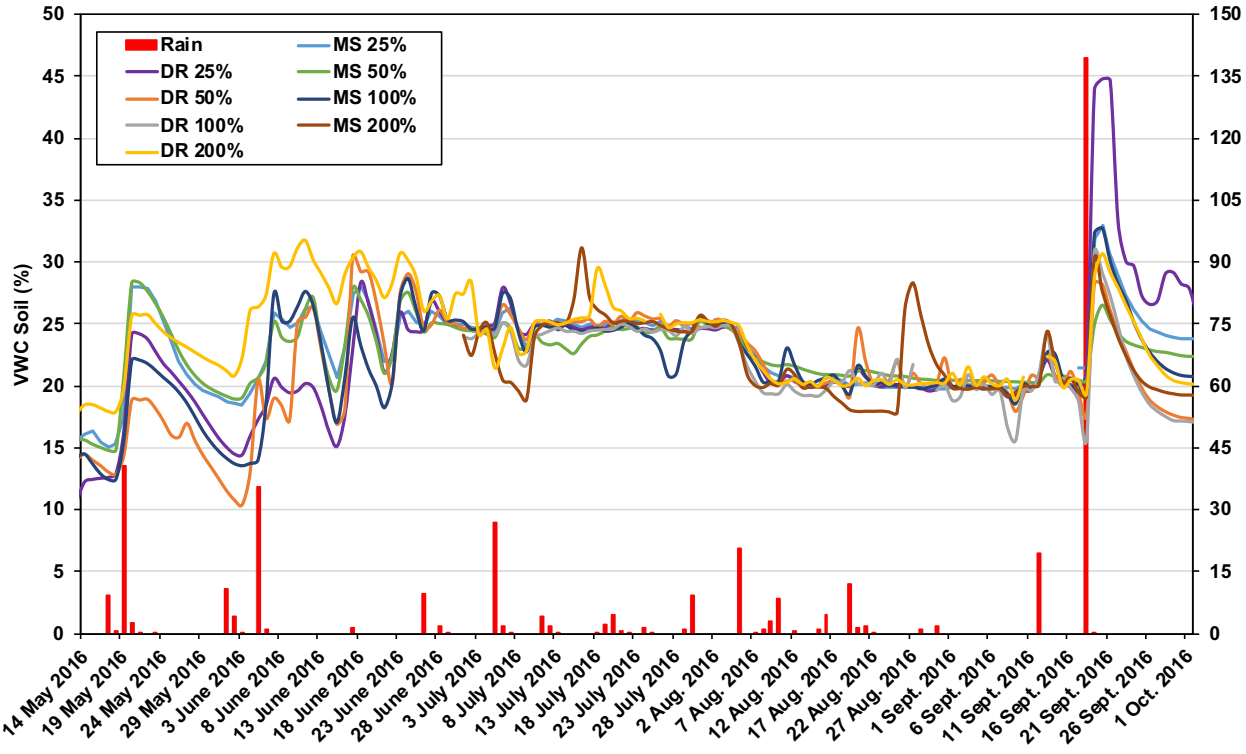

Figure 2.1. 2016 Soil volumetric water content (VWC) for the different combinations of drip (DR) and micro-sprinkler (MS) irrigation with different fertilizer levels (25, 50, 100, and 200%). Rain events are shown as vertical bars. From 14 May 2016 to 22 June 2016 the irrigation system was being tested and the irrigation was controlled by hand, trying to keep the VWC above 25%. After 23 June 2016, the automated irrigation network was turned on, following the thresholds established (early-May to early-August: VWC threshold of 25%; early-August to mid-September: VWC threshold of 20%; mid-September to late-September: VWC threshold of 15%; late-September to December: Irrigation off).

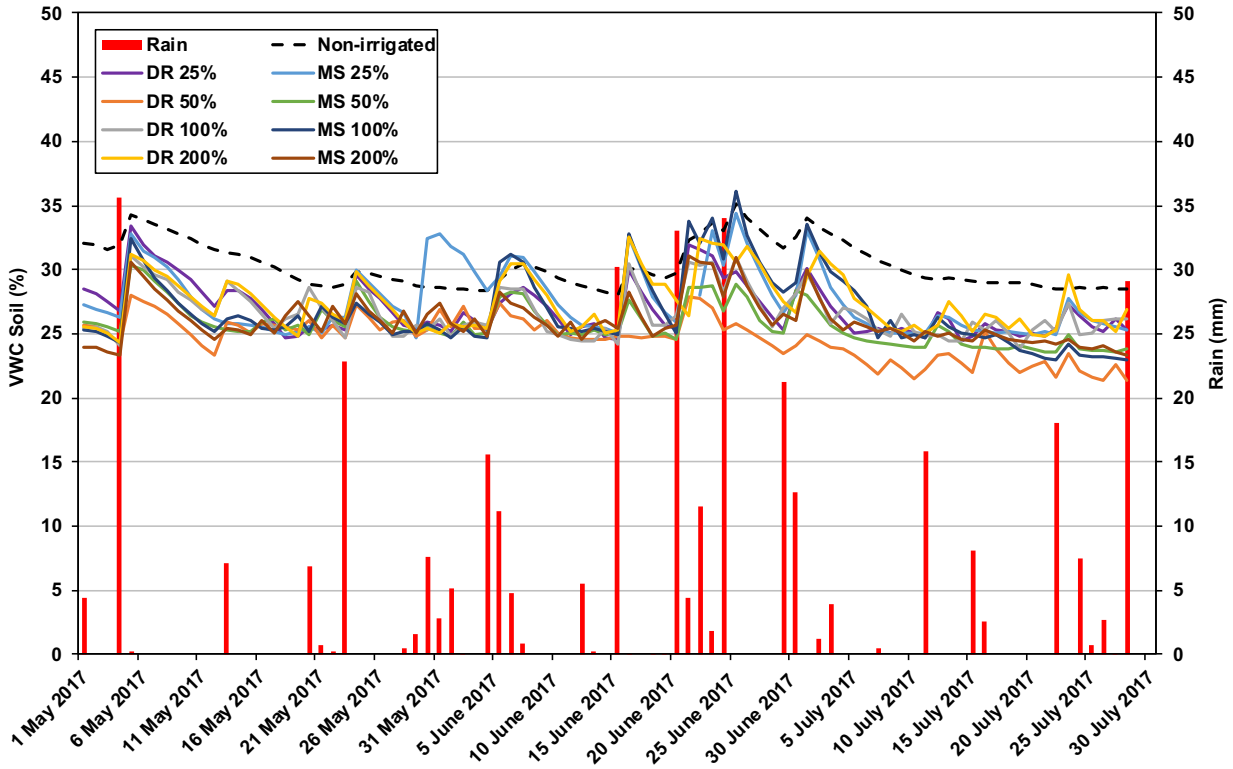

Figure 2.2. 2017 Soil volumetric water content (VWC) for the non-irrigated treatments and for the different combinations of drip (DR) and micro-sprinkler (MS) irrigation with different fertilizer levels (25, 50, 100, and 200%). Rain events are shown as vertical bars. The automated irrigation network was activated 12 May 2017, with the intent of following the thresholds established (early-May to early-August: VWC threshold of 25%; early-August to mid-September: VWC threshold of 20%; mid-September to late-September: VWC threshold of 15%; late-September to December: Irrigation off). The system was damaged and deactivated after 28 July 2017 because of a lightning strike.

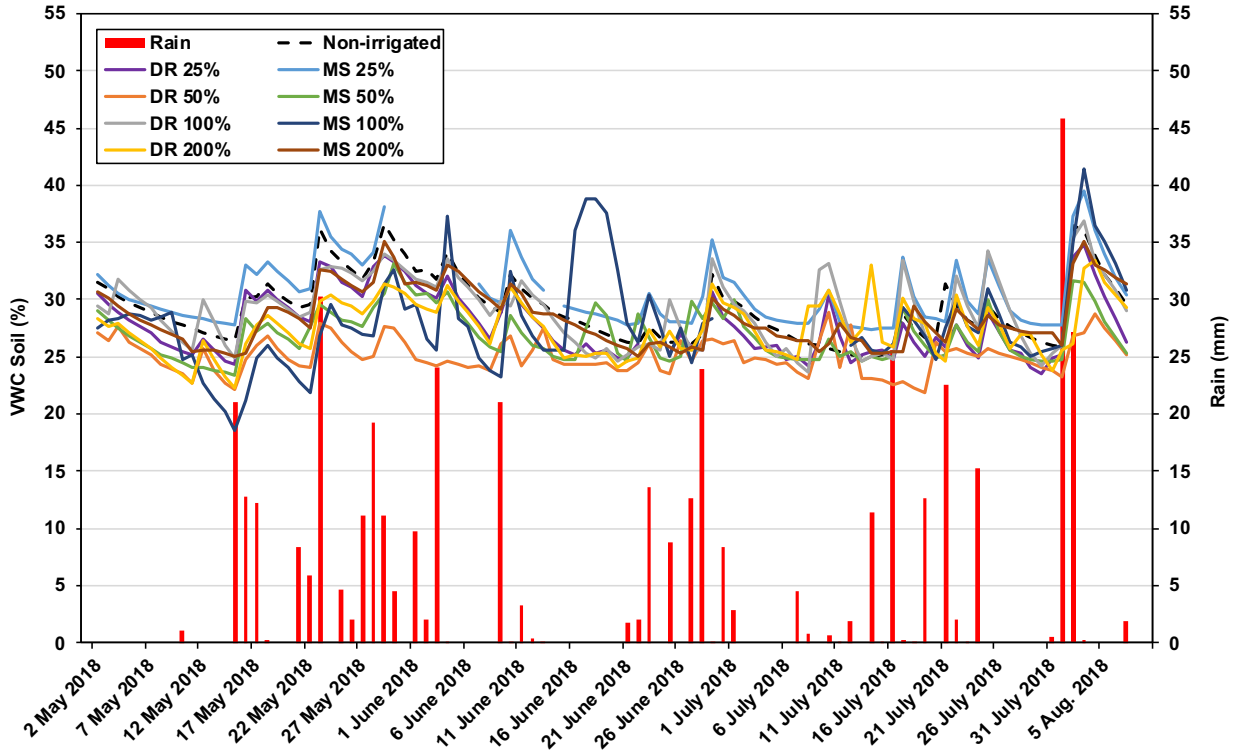

Figure 2.3. 2018 Soil volumetric water content (VWC) for the non-irrigated treatments and for the different combinations of drip (DR) and micro-sprinkler (MS) irrigation with different fertilizer levels (25, 50, 100, and 200%). Rain events are shown as vertical bars. The automated irrigation network was activated on 7 May 2018, with the intent of following the thresholds established (early-May to early-August: VWC threshold of 25%; early-August to mid-September: VWC threshold of 20%; mid-September to late-September: VWC threshold of 15%; late-September to December: Irrigation off). However, the system was damaged and the data was not recorded from 8 Aug. 2018 to 5 Sept. 2018.

CHAPTER 3

PHYSICOCHEMICAL FRUIT QUALITY ANALYSIS OF 'JULYPRINCE' PEACHES GROWN UNDER DIFFERENT IRRIGATION AND FERTILIZATION PRACTICES IN THE SOUTHEASTERN UNITED STATES [1]

[1] Casamali, B, M.W. van Iersel, and D.J. Chavez. To be submitted to *HortScience.*

Abstract

Peach fruit quality can be affected by cultivar differences, weather, and/or orchard management practices, such as fertilization, irrigation, spraying, and tree architecture. Young peach trees are not commonly irrigated until they begin producing fruit (3 years after planting). Growth of non-irrigated trees can be negatively affected by drought. In the past 20 years, the frequency and intensity of droughts have increased in the southeastern United States. In addition, current fertilizer recommendations for peach production in Georgia need an update to reflect modern production practices. The objective of this research was to investigate the effects of irrigation levels (irrigated vs. non-irrigated), irrigation systems (drip vs. micro-sprinkler), and fertilization levels (25%, 50%, 100%, and 200% of current recommendations) on the physicochemical quality characteristics of 'Julyprince' peach fruits. In 2017, no significant differences were reported among the treatments for fruit weight ($\overline{x}$ = 180.8 g), fruit diameter ($\overline{x}$ = 70.9 mm), total titratable acidity (TTA) ($\overline{x}$ = 0.72%), and total soluble solids (TSS)/TTA ratio ($\overline{x}$ = 13.9). However, irrigated trees had a slightly greater I_{ad} index than non-irrigated trees (I_{ad} index = 0.67 vs. 0.54). Micro-sprinkler-irrigated trees had greater TSS than drip-irrigated trees. In 2018, no significant differences among treatments were found for fruit diameter ($\overline{x}$ = 80.8 mm), I_{ad} index ($\overline{x}$ = 0.49), and TSS/TTA ratio ($\overline{x}$ = 13.6). Non-irrigated trees had greater TSS and TTA than irrigated trees. Fruits from micro-sprinkler-irrigated trees were ~9% heavier than those from the drip-irrigated trees. Based on these findings, irrigation may decrease the fruit quality of young peach trees. Although significant differences were found when comparing irrigated vs. non-irrigated

and drip- vs. micro-sprinkler-irrigated trees each year, they were not consistent across years. Different fertilizer levels did not impact fruit quality.

Additional index words: *Prunus persica*, fruit size, fruit weight, I_{ad} index, °Brix, total titratable acidity, supplemental irrigation

Introduction

Peach fruit quality can be affected by diverse factors (Minas et al., 2018), such as fertilization (Chatzitheodorou et al., 2004), rootstocks (Giorgi et al., 2005; Orazem et al., 2011), nutrient foliar sprays (Sotiropoulos et al., 2010), fruit position within the tree canopy (Alcobendas et al., 2013), and irrigation (Alcobendas et al., 2013; Zhou et al., 2017).

Georgia was named the peach state due to its importance within the state's history and economy (Okie, 2016). In the last few years, the southeastern United States peach production has been affected by a myriad of environmental factors such as lack of chill, late winter and early spring freezes (Crouch, 2017), and drought during spring/summer (Conrad II and Knox, 2016). To keep this important horticultural industry vigorous, efficient and economically sound agricultural practices need to be utilized. Growers should optimize their production systems to prevent or minimize negative environmental impacts. Precision irrigation (Perea et al., 2018) and optimized fertilizer management (Zhang et al., 2015) are two cultural practices that can be implemented by growers to improve their orchards' health, profitability, and sustainability.

Irrigation in the southeastern United States is traditionally installed by peach growers when an orchard becomes commercially productive (3 years after planting). Besides the lack of financial return from young trees, installing an irrigation system is costly (Fonsah et al., 2007) and growers may prefer to wait to invest in irrigation to dilute the establishment costs over time. Because of that, the early investment in irrigation is believed to be not justifiable.

Peach is a climacteric fruit with variable quality (Belisle et al., 2018) and flavor. Minas et al. (2018) compiled the results from different peach irrigation trials and their effects on fruit quality. They reported that results are variable, suggesting that specific characteristics of each experiment and environment greatly affect fruit quality. Bryla et al. (2005) studied different types of irrigation scheduling and systems (furrow, microspray, and drip) for peach trees. They reported minor effects on TTA and fruit color distribution, and no effects on flesh firmness and TSS. In another study, regulated deficit irrigation (RDI) during stage 2 (pit hardening, Connors, 1919) of peach fruit development did not change TSS and TTA in comparison with full irrigation (Lopez et al., 2008). In contrast, Alcobendas et al. (2013) showed that RDI during stage 2 of peach fruit development increased TSS and TTA in comparison with full irrigation. Similarly, drought during stage 3 (cell expansion, Connors, 1919) of fruit development of an early maturing peach variety increased TSS and decreased fruit relative water loss (Mercier et al., 2009). Lopez et al. (2016) found that deficit irrigation 9 to 15 days before harvest resulted in greater TSS than full irrigation in nectarines. Similarly, Lopez et al. (2011) reported that peach TSS, TTA, fruit firmness, crispness, and sourness increased

without irrigation; however, sweetness, juiciness, and flavor intensity decreased with irrigation.

Current fertilizer recommendations utilized by growers in the southeastern United States are believed to be based on studies performed in the 1950-60s (Gammon Jr. and Shoemaker, 1963; Smith and Taylor, 1952). They may not reflect the nutritional needs associated with modern production systems. Fertilization practices can affect trees in multiple ways, including fruit quality, since a good amount of nutrients are allocated to form a fruit (Policarpo et al., 2002).

Vashisth et al. (2017) tested different fertilization levels on subtropical peaches and found no differences in TTA and TSS/TTA ratio, with inconsistent results on TSS. Similarly, Dolinski et al. (2018) found no differences in peach fruit TSS, TTA, and TSS/TTA ratio in response to a wide range of N levels in the first two years of their experiment. In the third year, differences were found in TSS and TSS/TTA ratio, with the results following a quadratic response based on the treatment level. Fruits of the lowest (0 and 40 $kg \cdot ha^{-1}$ N) and highest (200 and 240 $kg \cdot ha^{-1}$ N) levels of nitrogen had lower values of TSS and TSS/TTA ratio, while the medium level (120 $kg \cdot ha^{-1}$ N) had higher values. Crisosto et al. (1997) found no differences in skin gas exchange (permeability of CO_2 and C_2H_4) among three different fertilization levels during postharvest quality assessments of nectarines. However, the fruit cuticle density was reduced as the fertilization levels increased. Likewise, fertilization can affect diseases and pests, which may compromise fruit quality, rendering the fruits non-marketable. Daane et al. (1995) found that higher levels of fertilizer boosted the incidence of brown rot (*Monilinia*

fructicola) in nectarine fruits and increased the percentage of fruit infested with peach twig borer (*Anarsia lineatella*) and oriental fruit moth (*Grapholita molesta*).

Most of the work on irrigation and its effect on peach fruit quality has been conducted in regions where irrigation is a must (*e.g.*, regions with Mediterranean-like climate). The most common comparisons made in those studies were deficit irrigation versus full irrigation. The common irrigation practices for those regions aim to meet the evapotranspiration needs and optimize the water used by trees without creating negative effects on fruit yield and quality. On the contrary, in the southeastern United States, peach producers do not necessarily irrigate young peach trees. Furthermore, no research has been done to understand fruit quality characteristics of peaches from trees that were irrigated or non-irrigated since establishment. In addition, we believe that current fertilizer recommendations utilized by growers in the southeastern United States are based on studies performed several decades ago. Potential improvements to reflect the nutritional needs associated with modern production systems are needed.

We hypothesized that 1) trees irrigated (supplemental irrigation) since planting will have a similar or better fruit quality than non-irrigated (rainfall only) trees, 2) irrigation system (drip vs. micro-sprinkler) will not induce differences in peach fruit quality, and 3) different fertilizer levels will not affect the peach fruit quality. The specific objective of this research was to evaluate the effects of irrigation levels (irrigated vs. non-irrigated), irrigation systems (drip vs. micro-sprinkler), and fertilization levels (25%, 50%, 100%, and 200%) applied during the first three years after planting on fruit weight, size, and quality of peach trees that were harvested during the second and third year after planting.

Material and methods

Plant material and field characteristics

The experiment was conducted at the Dempsey Farm, University of Georgia, Griffin, GA (Cecil sandy loam 33°14' 55" N, 84°17' 57" W). Soil amendments for P, K, and lime (to reach pH 6.0) were made according to recommendations (Horton et al., 2015) from soil analyses of samples collected the previous year before planting. In July-Aug. 2015, trees of 'Julyprince' peach cultivar grafted onto 'Guardian™' were planted (358 trees/ha, with tree spacing of 4.6 m within rows and 6.1 m between rows). 'Julyprince' yields abundantly, slightly-late in the season, and its fruit have great size, being attractive and very-firm (Okie and Layne, 2008). 'Guardian™' is the most utilized rootstock in the region, being vigorous (Reighard et al., 1997) and tolerant to peach tree short life and resistant to most root-knot nematodes (Nyczepir and Beckman, 2000). Pest and disease management followed the recommended guidelines published in the Southeastern Peach, Nectarine, and Plum Pest Management and Culture Guide (Horton et al., 2015). Precipitation records of the closest weather station of the experiment for 2016, 2017, 2018, and the historical normal 1981-2010 are presented in Table 2.1.

Experimental design and treatments

Field set-up and experimental design is described in the material and methods section of Chapter 2. Briefly, two irrigation levels (irrigated vs. non-irrigated), two irrigation systems (drip vs. micro-sprinkler irrigation), and four fertilization levels (25%, 50%, 100%, and 200%) were tested. Irrigated trees were either drip- or micro-sprinkler-

irrigated (supplemental irrigation). The irrigation was controlled using a network of sensors and solenoid valves. The irrigation was automatically activated as needed to maintain the soil's volumetric water content (VWC) above the set thresholds as follows: 1) Irrigation off from January to early-May; 2) VWC threshold of 25% from early-May to early-August; 3) VWC threshold of 20% from early-August to mid-September; 4) VWC threshold of 15% from mid-September to late-September; and 5) irrigation off from late-September to December. Drip irrigation delivered an average of 2,959 L/tree across fertilizer levels for the three years of experiment; whereas micro-sprinkler irrigation delivered 4,743 L/tree, measured using flow meters. Non-irrigated trees received water only from rain events. Figures 2.2 and 2.3, show the soil VWC for the different treatment combinations and the rain events.

The fertilizer levels were based on the current recommendations from the Southeastern Peach, Nectarine, and Plum Pest Management and Culture Guide (Horton et al., 2015). The recommendations for N are 65, 95, and 98 $kg \cdot ha^{-1}$ for 1-, 2-, and 3-year-old trees, respectively. Hereafter, the levels above will be referred as 100% level. Based on this 100% level, other three levels were adjusted per year equivalent to 25%, 50%, and 200%, creating a total of four fertilizer levels. The fertilizer applications were done by hand using granular fertilizer (one application of 10.0N-4.4P-8.3K [March] and two applications of 15.5N-0P-0K [May and July] for 1- and 2-year-old trees; and one application of 10.0N-4.4P-8.3K [March] and one application of 15.5N-0P-0K [August] for 3-year-old trees).

Variables measured

Fruit measurements. Fruit for analysis was harvested on July 14, 2017 and in July 10 and July 16, 2018. For each harvest, fruit were separated into commercial and non-commercial (comprising small, damaged, and/or misshaped fruit) categories. Total weight was recorded for each category. A sub-sample of 10 fruits per data tree was collected from the commercial category and placed in a plastic bag. The fruit was stored in a cooler with ice until further analyses in the Peach Research and Extension laboratory at the University of Georgia in Griffin Campus, Griffin, GA. Fruit was processed the same day or on the day after harvesting. Fruit weight, fruit size, I_{ad} index, TSS, TTA, and TSS/TTA ratio were measured.

Average fruit weight was estimated by weighing a 10-fruit sub-sample per tree. From this sub-sample, five fruits were randomly selected to measure other fruit parameters as described below. Fruit size (longitudinal fruit diameter on the equatorial portion of the fruit) was measured using a fruit sizer (Cranston Machinery Co.; Oak Grove, OR). Fruit maturity was assessed by measuring the index of absorbance difference between two wavelengths (670 and 720 nm) - I_{ad} index - using a DA-Meter (Sintéleia; Bologna, Italy). This measurement has a direct correlation with the chlorophyll *a* content in the outer fruit mesocarp. The DA-Meter was used to measure the same fruit twice, one measurement on the blush side and another on the opposite side of the fruit. The device immediately averaged the values giving one value per fruit. The I_{ad} index ranges from 0 to 2.2, being 0 to ~0.4 ripe, ~0.6 just ripe, ~0.8 commercial ripe, and ~1.2 to 2.2 green fruit (adapted from Gasic et al., 2015). Finally, fruits were sliced and stored inside 1-quart storage bags at -80 °C freezer until juice processing.

Juice measurements. Fruit slices were thawed at room temperature and blended in individual blender cups (Ultima Blender BL810 30 Ninja; Newton, MA) using the "pulse" setting until reaching a homogenous puree. Approximately 33 g of the puree were centrifuged at 20,133 g_n for 20 min at 5 °C (Model 5810R Eppendorf; Hauppauge, NY). The supernatant juice was filtered through a two-layered cheesecloth and stored in 15 mL conical tubes at -20 °C until further analysis.

Juice samples were thawed at room temperature and vortexed. TSS was assessed on 300 µL of juice using a digital hand-held refractometer (Palette PR-32 Atago; Bellevue, WA, USA) and expressed as °Brix. TTA (expressed as % malic acid) was measured using an automated titrator (Model Easy Pro Mettler Toledo; Columbus, OH, USA) as follows: a volume of 6 mL of juice sample diluted in 50 mL of deionized H_2O was titrated with 0.1 N NaOH to an endpoint pH of 8.2. The ratio between TSS:TTA was calculated.

Statistical analysis

SAS 9.4 (SAS Institute Inc., Cary, NC) was used to compare treatment means and interactions, using PROC GLIMMIX. Means were separated using Tukey's Honest Significant Difference method with significance level at $P \leq 0.05$.

Results

Figures 2.2 and 2.3, represent the VWC of the irrigated and non-irrigated treatments in 2017 and 2018. Based on the stem water potential measurements, no evidence of drought stress was found in 2017 and 2018 (Table 2.2).

In 2017, no significant differences were found among any of the treatments tested for the average fruit weight ($\bar{x}$ = 180.8 g), fruit diameter ($\bar{x}$ = 70.9 mm), TTA ($\bar{x}$ = 0.72%), and TSS/TTA ratio ($\bar{x}$ = 13.9) ($P > 0.05$, Tables 3.1 and 3.2). The I_{ad} index was not affected by the irrigation systems ($P = 0.45$) or fertilizer levels ($P = 0.06$); however, it was affected by the irrigation levels ($P = 0.01$). Non-irrigated (rainfall only) trees had a slightly-advanced fruit ripeness stage (I_{ad} index = 0.54) than irrigated trees (I_{ad} index = 0.67) (Table 3.1). Total soluble solids (TSS) was affected by irrigation systems (P = 0.04), with micro-sprinkler-irrigated trees having greater TSS than drip-irrigated trees (10.2 vs. 9.5 °Brix). However, fertilizer levels or irrigation levels did not affect TSS ($\bar{x}$ = 10.0 °Brix, $P = 0.11$ and 0.44, respectively) (Table 3.2).

In 2018, micro-sprinkler-irrigated trees had greater fruit weight than drip-irrigated trees (293.8 vs. 270.2 g, $P = 0.03$, Table 3.3). However, no differences were found in average fruit weight among fertilization levels ($\bar{x}$ = 279.0 g, $P = 0.77$) or between irrigation levels ($\bar{x}$ = 279.0 g, $P = 0.38$, Table 3.3). Fruit diameter ($\bar{x}$ = 80.8 mm), I_{ad} index ($\bar{x}$ = 0.49) and TSS/TTA ratio ($\bar{x}$ = 13.6) were not affected by the treatments tested ($P > 0.05$, Tables 3.3 and 3.4). No significant differences were found in TSS between drip- vs. micro-sprinkler-irrigated trees ($P = 0.89$) or among the fertilization levels (P = 0.11). However, irrigation levels affected TSS ($P = 0.01$), with non-irrigated trees having greater TSS values (11.26 vs. 10.69 °Brix) than irrigated trees (Table 3.4). Similarly, irrigation systems or fertilization levels did not affect TTA ($P = 0.17$ and 0.24, respectively). TTA was affected by irrigation levels with non-irrigated trees having greater TTA than irrigated trees (0.83 vs. 0.79 %) (Table 3.4).

Discussion

During the two years of this research, rainfall was considered normal compared to historical data. Trees had access to water from rainfall. Based on the stem water potential results of our experiment (Chapter 2), trees did not experience drought stress throughout fruit growth and ripening periods (Table 2.2). Further, no major differences were found in photosynthetic net assimilation between irrigation levels or systems (Tables 2.5). Despite the lack of effects on physiological measurements, different irrigation levels and systems had some effects on fruit quality.

Significant differences in I_{ad} index in 2017, and TSS and TTA in 2018 were found when comparing irrigated vs. non-irrigated trees. The more advanced maturation stage of non-irrigated trees in 2017, based on the I_{ad} index, is related to a more intense peach color (more yellow and red instead of green), and might be a result of the advanced bud-break progression reported in non-irrigated trees in 2017 (Chapter 2). This result is consistent with Mercier et al. (2009), who reported advanced peach fruit maturity of peaches with water restriction. Although an advanced stage of maturation based on the I_{ad} index was reported in 2017, changes in TSS, TTA, and TSS/TTA ratio were not seen that year, agreeing with Costa et al. (2009). Minas et al. (2018) in a review of peach fruit quality, reported that changes on the I_{ad} index were not necessarily associated with changes in the traditional harvest indexes, such as flesh firmness and TSS.

In 2018, non-irrigated and irrigated trees displayed similar values of I_{ad} index. However, TSS and TTA were slightly greater for non-irrigated trees compared to irrigated trees, agreeing with Alcobendas et al. (2013) and Lopez et al. (2011). They reported greater TSS and TTA for non-irrigated trees or trees under RDI in comparison

to fully irrigated trees. Fruit from non-irrigated trees tend to have higher sugars and acids concentrations in comparison to irrigated trees, likely because of the decreased water content, which concentrates the solutes in the fruit (Lopez et al., 2010) and increases its osmotic adjustment (Lo Bianco et al., 2000). Seasonal differences may have had a strong influence on sugars and acids, as noticed by the inconsistent differences. Bryla et al. (2005) and Lopez et al. (2016) also reported variable fruit quality parameters accordingly to different years of evaluation. Fruit weight was not affected by irrigation levels in either year of evaluation. On the contrary, Alcobendas et al. (2012) reported greater fruit weight for full irrigation treatment in comparison with RDI treatment. As previously stated, our non-irrigated trees received typical amounts of rain, and there was no clear evidence of drought in either year.

Irrigation systems (drip and micro-sprinkler) had a small effect on fruit quality. In 2017, micro-sprinkler-irrigated trees displayed higher TSS than drip-irrigated trees, disagreeing with Bryla et al. (2005), who tested different irrigation methods and did not find differences in TSS among the treatments in a 3-year project. Drip irrigation has a smaller wetting zone where the root will proliferate, in comparison with a bigger wetting zone provided by micro-sprinkler irrigation. Thus, drip-irrigated trees are more efficient absorbing water than micro-sprinkler-irrigated trees, because roots grow closer to where the water is in the soil (Tagliavini et al., 1996).

In 2018, micro-sprinkler-irrigated trees produced fruit with greater fruit weight than drip-irrigated trees. However, we do not believe this difference was really caused by differences in irrigation systems. In March of 2018, an advective freeze hit the orchard during bud break, negatively affecting the drip-irrigated trees because of the

landscape of the experiment. We hypothesize that flower buds that were in an advanced stage of development before the advective freeze hit, were severely affected and killed. Thus, mostly late buds were able to develop. Late buds experience a shorter fruit development period in comparison to early buds. A shorter fruit development period results in smaller fruit weight, as reported by Wert et al. (2009).

Different fertilizer levels did not affect any of the variables evaluated in either year. Further, no differences in physiological parameters were found among the fertilizer levels (Chapter 2). These results agree with Vashisth et al. (2017) who reported no differences in TTA and inconclusive results for TSS across several fertilizer levels and two peach cultivars evaluated. Similarly, Dolinski et al. (2018) reported no differences in peach fruit quality parameters in two out of three years of data collection. Daane et al. (1995) also did not find any changes in peach fruit TTA and TSS when testing different N levels.

Conclusions

Our results suggest that irrigating young peach trees could potentially decrease TTA, TSS, and increase I_{ad} index in comparison with non-irrigated trees. However, the differences between irrigation levels are small and variable, depending on the year of evaluation. Likewise, irrigation systems did not cause consistent differences. While irrigation can lead to decreased fruit quality, some benefits were observed when trees are irrigated during a period of drought stress. Increased canopy and trunk size, photosynthetic activity, and water status were reported in irrigated trees vs. non-irrigated trees during the 2016 season (Chapter 2). Different fertilizer levels did not

induce any changes in fruit quality, corroborating past literature. There is a common belief that excessive fertilization tends to reduce fruit quality; however, we were not able to confirm this. Thus, proper irrigation management that allows superior tree growth and increases fruit yield of young peach trees without affecting fruit quality, should be the aim of peach growers. Further, a reduction in fertilizer use has little impact on growth, yield, or fruit quality of young peach trees, decreasing production costs in an environmentally-sound way.

Literature cited

Alcobendas, R., J.M. Mirás-Avalos, J.J. Alarcón, and E. Nicolás. 2013. Effects of irrigation and fruit position on size, colour, firmness and sugar contents of fruits in a mid-late maturing peach cultivar. Sci. Hort. 164.

Alcobendas, R., J.M. Mirás-Avalos, J.J. Alarcón, F. Pedrero, and E. Nicolás. 2012. Combined effects of irrigation, crop load and fruit position on size, color and firmness of fruits in an extra-early cultivar of peach. Sci. Hort. 142:128–135.

Belisle, C., U.T.X. Phan, K. Adhikari, and D.J. Chavez. 2018. A fruit quality survey of peach cultivars grown in the southeastern United States. HortTechnology 28:189–201.

Bryla, D.R., E. Dickson, R. Shenk, R.S. Johnson, C.H. Crisosto, and T.J. Trout. 2005. Influence of irrigation method and scheduling on patterns of soil and tree water status and its relation to yield and fruit quality in peach. HortScience 40:2118–2124.

Chatzitheodorou, I.T., T.E. Sotiropoulos, and G.I. Mouhtaridou. 2004. Effect of nitrogen, phosphorus, potassium fertilisation and manure on fruit yield and fruit quality of the peach cultivars ‘Spring Time’ and ‘Red Haven’. Agron. Res. 2:135–143.

Connors, C.H. 1919. Growth of fruits of peach. N.J. Agr. Expt. Sta. Annu. Rpt. 40:82–88.

Conrad II, C.E. and P. Knox. 2016. The southeastern drought and wildfires of 2016, National Oceanic and Atmospheric Association Southeast Regional Climate Center. Chapel Hill, NC.

Costa, G., M. Noferini, G. Fiori, and P. Torrigiani. 2009. Use of Vis/NIR spectroscopy to assess fruit ripening stage and improve management in post-harvest chain. Fresh Prod. 3:35–41.

Crisosto, C.H., R.S. Johnson, T. DeJong, and K.R. Day. 1997. Orchard factors affecting postharvest stone fruit quality. HortScience 32:820–823.

Crouch, J. 2017. Bad news for Southeast peaches: Something freezing this way came. 7 July 2019. <https://www.climate.gov/news-features/blogs/beyond-data/bad-news-southeast-peaches-something-freezing-way-came>.

Daane, K.M., R.S. Johnson, T.J. Michailides, C.H. Crisosto, J.W. Dlott, H.T. Ramirez, G.Y. Yokota, and D.P. Morgan. 1995. Excess nitrogen raises nectarine susceptibility to disease and insects. California Agr. 49:13–18.

Dolinski, M.A., J.W. de O. Dangelo, F.L. Cuquel, A.C.V. Motta, and L.L.M. de Mio. 2018. Quality peach produced in fertilizer doses of nitrogen and green pruning. Bragantia 77:134–140.

Fonsah, E.G., K.C. Taylor, and F. Funderburk. 2007. Enterprise cost analysis for middle Georgia peach production. Univ. Georgia Coop. Ext. Serv. Athens, GA.

Gammon Jr., N. and J.S. Shoemaker. 1963. Effect of fertilizers on mineral levels in the leaves of Flordawon peach. Florida State Hort. Soc. 1746:380–384.

Gasic, K., G.L. Reighard, J. Windham, and M. Ognjanov. 2015. Relationship between fruit maturity at harvest and fruit quality in peach. Acta Hort. 1084:643–648.

Giorgi, M., F. Capocasa, J. Scalzo, G. Murri, M. Battino, and B. Mezzetti. 2005. The rootstock effects on plant adaptability, production, fruit quality, and nutrition in the peach (cv. ‘Suncrest’). Sci. Hort. 107:36–42.

Horton, D., P. Brannen, B. Bellinger, D. Lockwood, and D. Ritchie. 2015. 2015 Southeastern peach, nectarine and plum pest management and culture guide. Uni. Georgia Coop. Ext. Bul. 1171. Athens, GA.

Lo Bianco, R., M. Rieger, and S.-J.S. Sung. 2000. Effect of drought on sorbitol and sucrose metabolism in sinks and sources of peach. Phys. Plant. 108:71–78.

Lopez, G., A. Arbones, J. del Campo, M. Mata, X. Vallverdu, J. Girona, and J. Marsal. 2008. Response of peach trees to regulated deficit irrigation during stage II of fruit development and summer pruning. Spanish J. Agr. Res. 6:479–491.

Lopez, G., M.H. Behboudian, X. Vallverdu, M. Mata, J. Girona, and J. Marsal. 2010. Mitigation of severe water stress by fruit thinning in 'O'Henry' peach: Implications for fruit quality. Sci. Hort. 125:294–300.

Lopez, G., G. Echeverria, J. Bellvert, M. Mata, M.. Behboudian, J. Girona, and J. Marsal. 2016. Water stress for a short period before harvest in nectarine: Yield, fruit composition, sensory quality, and consumer acceptance of fruit. Sci. Hort. 211:1–7.

Lopez, G., M. Hossein Behboudian, G. Echeverria, J. Girona, and J. Marsal. 2011. Instrumental and sensory evaluation of fruit quality for 'Ryan's sun' peach grown under deficit irrigation. HortTechnology 21:712–719.

Mercier, V., C. Bussi, F. Lescourret, and M. Génard. 2009. Effects of different irrigation regimes applied during the final stage of rapid growth on an early maturing peach cultivar. Irr. Sci. 27:297–306.

Minas, I.S., G. Tanou, and A. Molassiotis. 2018. Environmental and orchard bases of peach fruit quality. Sci. Hort. 235:307–322.

Nyczepir, A.P., T.G. Beckman. 2000. Host status of guardian peach rootstock to *Meloidogyne* sp. and *M. javanica*. HortScience 35:772.

Okie, W.R. and D.R. Layne. 2008. 'Scarletprince' and 'Julyprince' Peaches. HortScience 43:1603–1605.

Okie, W.T. 2016. The Georgia peach: Culture, agriculture, and environment in the American south. Cambridge University Press, New York, NY.

Orazem, P., F. Stampar, and M. Hudina. 2011. Quality analysis of 'Redhaven' peach fruit grafted on 11 rootstocks of different genetic origin in a replant soil. Food Chem. 124:1691–1698.

Perea, R.G., A. Daccache, J.A. Rodríguez Díaz, E. Camacho Poyato, and J.W. Knox. 2018. Modelling impacts of precision irrigation on crop yield and in-field water management. Precision Agric. 19:497–512.

Policarpo, M., L. Di Marco, T. Caruso, P. Gioacchini, and M. Tagliavini. 2002. Dynamics of nitrogen uptake and partitioning in early and late fruit ripening peach (*Prunus persica*) tree genotypes under a mediterranean climate. Plant Soil 239:207–214.

Reighard, G.L., W.C. Newall Jr., T.G. Beckman, W.R. Okie, E.I. Zehr, and A.P. Nyczepir. 1997. Field performance of *Prunus* rootstock cultivars and selection on replants soil in South Carolina. Acta Hort. 451 243–249.

Smith., C.B., and G.A. Taylor. 1952. Tentative optimum leaf concentration of several elements for Elberta peach and Stayman apple in Pennsylvania orchards. Proc. Amer. Soc. Hort. Sci. 60:33–41.

Sotiropoulos, T., I. Therios, and N. Voulgarakis. 2010. Effect of various foliar sprays on some fruit quality attributes and leaf nutritional status of the peach cultivar 'Andross'. J. Plant Nutr. 33:471–484.

Tagliavini, M., D. Scudellazi, B. Marangoni, and M. Toselli. 1996. Nitrogen fertilization management in orchards to reconcile productivity and environmental aspects, p. 161-170. In: C. Rodriguez-Barrueco (ed.). Fertilizer and Environment. Developments in Plant and Soil Sciences. Springer, Dordrecht, Netherlands.

Vashisth, T., M.A. Olmstead, J. Olmstead, and T.A. Colquhoun. 2017. Effects of nitrogen fertilization on subtropical peach fruit quality: Organic acids, phytochemical content, and total antioxidant capacity. J. Amer. Soc. Hort. Sci. 142:393–404.

Wert, T.W., J.G. Williamson, J.X. Chaparro, E.P. Miller, and R.E. Rouse. 2009. The influence of climate on fruit development and quality of four low-chill peach cultivars. HortScience 44:666–670.

Zhang, X., E.A. Davidson, D.L. Mauzerall, T.D. Searchinger, P. Dumas, and Y. Shen. 2015. Managing nitrogen for sustainable development. Nature 528:51–59.

Zhou, H., F. Zhang, K. Roger, L. Wu, D. Gong, N. Zhao, D. Yin, Y. Xiang, and Z. Li. 2017. Peach yield and fruit quality is maintained under mild deficit irrigation in semi-arid China. J. Integrative Agric. 16:1173–1183.

Table 3.1. Effects of fertilizer levels, irrigation levels, and irrigation systems on physical fruit parameters of 'Julyprince' peaches at harvest in July 2017.

Treatment	Fruit weight (g)	Fruit diameter (mm)	I_{ad} index
Fertilizer level			
25%	174.8	70.7	0.58
50%	184.8	71.2	0.50
100%	189.7	71.7	0.65
200%	173.7	69.7	0.69
Irrigation level			
Irrigated	179.3	71.2	0.67 a[z]
Non-irrigated	182.3	70.5	0.54 b
Irrigation system			
Micro-sprinkler	183.1	72.0	0.65
Drip	175.4	70.4	0.70
P values			
Fertilizer level	0.16	0.31	0.06
Irrigation level	0.60	0.37	**0.01**
Irrigation system	0.39	0.28	0.45
Fertilizer level*	0.34	0.56	0.61

Irrigation level			
Fertilizer level*	0.21	0.13	0.10
Irrigation system			

[z] Means followed by the same letter within a column and treatment effect are not significantly different by Tukey's honestly significant different test, $P \leq 0.05$. Means without letters indicate non-statistical differences, $P > 0.05$.

Table 3.2. Effects of fertilizer levels, irrigation levels, and irrigation systems on chemical fruit parameters of 'Julyprince' peaches at harvest in July 2017.

Treatment	TSS (°Brix)	TTA (% Malic acid)	TSS/TTA Ratio
Fertilizer level			
25%	9.5	0.72	13.2
50%	10.2	0.71	14.5
100%	9.8	0.71	13.8
200%	10.3	0.74	14.1
Irrigation level			
Irrigated	9.8	0.73	13.5
Non-irrigated	10.0	0.71	14.3
Irrigation system			
Micro-sprinkler	10.2 a[z]	0.73	14.2
Drip	9.5 b	0.74	12.8
P values			
Fertilizer level	0.11	0.75	0.26
Irrigation level	0.44	0.16	0.10
Irrigation system	**0.04**	0.73	0.12
Fertilizer level*	0.12	0.46	0.50

Irrigation level			
Fertilizer level*	0.53	0.41	0.35
Irrigation system			

[z] Means followed by the same letter within a column and treatment effect are not significantly different by Tukey's honestly significant different test, $P \leq 0.05$. Means without letters indicate non-statistical differences, $P > 0.05$.

Table 3.3. Effects of fertilizer levels, irrigation levels, and irrigation systems on physical fruit parameters of 'Julyprince' peaches at harvest in July 2018.

Treatment	Fruit weight (g)	Fruit diameter (mm)	I_{ad} index
Fertilizer level			
25%	277.1	80.2	0.51
50%	281.2	80.8	0.45
100%	273.9	80.4	0.49
200%	283.6	81.7	0.50
Irrigation level			
Irrigated	282.0	81.2	0.48
Non-irrigated	275.9	80.4	0.49
Irrigation system			
Micro-sprinkler	293.8 a[z]	82.0	0.43
Drip	270.2 b	80.3	0.53
P values			
Fertilizer level	0.77	0.50	0.70
Irrigation level	0.38	0.26	0.82
Irrigation system	**0.03**	0.16	0.12

Fertilizer level* Irrigation level	0.46	0.45	0.32
Fertilizer level* Irrigation system	0.24	0.62	0.54

[z] Means followed by the same letter within a column and treatment effect are not significantly different by Tukey's honestly significant different test, $P \leq 0.05$. Means without letters indicate non-statistical differences, $P > 0.05$.

Table 3.4. Effects of fertilizer levels, irrigation levels, and irrigation systems on chemical fruit parameters of 'Julyprince' peaches at harvest in July 2018.

Treatment	TSS (°Brix)	TTA (% Malic acid)	TSS/TTA Ratio
Fertilizer level			
25%	10.7	0.80	13.4
50%	10.9	0.83	13.2
100%	11.0	0.79	13.9
200%	11.4	0.83	13.8
Irrigation level			
Irrigated	10.7 b[z]	0.79 b	13.6
Non-irrigated	11.3 a	0.83 a	13.6
Irrigation system			
Micro-sprinkler	10.7	0.77	14.0
Drip	10.7	0.81	13.2
P values			
Fertilizer level	0.11	0.24	0.26
Irrigation level	**0.01**	**0.03**	0.91
Irrigation system	0.89	0.17	0.07
Fertilizer level*	0.39	0.35	0.62

Irrigation level			
Fertilizer level*	0.78	0.19	0.30
Irrigation system			

[z] Means followed by the same letter within a column and treatment effect are not significantly different by Tukey's honestly significant different test, $P \leq 0.05$. Means without letters indicate non-statistical differences, $P > 0.05$.

CHAPTER 4

NITROGEN PARTITIONING IN YOUNG 'JULYPRINCE' PEACH TREES GROWN WITH DIFFERENT IRRIGATION AND FERTILIZATION PRACTICES IN THE SOUTHEASTERN UNITED STATES [1]

[1] Casamali, B, M.W. van Iersel, and D.J. Chavez. To be submitted to *HortScience.*

Abstract

Fertilizer recommendations for peach cultivation in the southeastern United States were developed decades ago and may not reflect the peach trees' needs under current cultivation practices. Adequate fertilization for young peach trees induces a balanced vegetative/reproductive growth, saves economical resources, and is environmentally sound. Additionally, droughts in the region are becoming more common. Supplemental irrigation for peaches from field establishment serves as an insurance in case drought conditions occur and can increase/advance yield of young peach trees. Our objective was to determine the influence of different fertilizer levels (25, 50, 100, and 200%), irrigation levels (irrigated vs. non-irrigated), and irrigation systems (drip vs. micro-sprinkler) on nitrogen partitioning and concentration in different organs of young peach trees. The cumulative N removal was not affected by the different fertilizer levels, with an average removal of 583.7 g/tree after three years. Most of the N allocation was accounted for by summer pruning and defoliation (68% of the total N removed). Irrigated trees had higher cumulative N removal after three years (656.7 g/tree) than non-irrigated trees (510.6 g/tree), with differences between irrigated vs. non-irrigated trees in most vegetative removal events (winter and summer pruning, and defoliation). Drip-irrigated trees had higher cumulative N removal after three years (720.9 g/tree) than micro-sprinkler-irrigated tress (592.6 g/tree), with differences in N removal found in vegetative and reproductive removal events. Differences in N removal were driven mainly by differences in dry weight of material rather than N concentration in the organs. These results suggest that different fertilizer levels did not alter the N partitioning in young peach trees, indicating that reduction in fertilizer applications can

be done without negative effects. Further, irrigation induces greater vegetative growth, especially under drought conditions, which may result in greater canopy volume and fruit yield compared to non-irrigated trees. Differences between irrigation systems are not consistent; however, drip is more efficient than micro-sprinkler irrigation, with ~38% water savings.

Additional index words. *Prunus persica*, allocation, nitrogen concentration, dry weight, pruning, defoliation, fruit yield

Introduction

The state of Georgia has experienced below average rainfall (drought) in the past few years (NDMC, USDA, and NOAA, 2019). These droughts can hinder peach tree growth and development. Some growers in the southeastern United States do not irrigate young peach trees until the third year after field establishment. This creates a situation where the non-irrigated trees can experience periods of drought and subsequent stress. As reported in Chapter 2, lack of water during the first years of field establishment can be detrimental (Abrisqueta et al., 2008; Cockroft and Olsson, 1972; Williamson and Coston, 1990). During the first years, young trees' root system and canopy develop to support future growth and fruit yield. Supplemental irrigation from the time of orchard establishment can be an option to overcome periods of drought. Additionally, it induces greater fruit yield, bringing additional revenue for growers.

Similarly, interest in optimizing fertilization management has increased. Reasons include: a) concerns regarding over-fertilization and its effects on the balance between

vegetative and reproductive growth; b) environmental pollution by fertilizer runoff; and c) inefficient use of financial resources (Albornoz, 2016). The Southeastern Peach, Nectarine, and Plum Pest Management and Culture Guide (Horton et al., 2015) provides fertilizer recommendations for peach trees at different ages. However, there are no reports of how current fertilizer recommendations were developed. They may be based on studies half-century-old (Gammon Jr. and Shoemaker, 1963; Smith and Taylor, 1952). The current research project was started to understand the current needs of peach trees and to update the fertilizer recommendations. Reductions in fertilizer use will result in cost savings and reduction of the orchard's environmental impact.

This research tested the effect of supplemental irrigation on young peach trees since establishment and whether different fertilizer rates would impact the trees. Results of physiological, growth, fruit yield, and fruit quality parameters to assess the performance of trees with different irrigation and fertilization treatments were previously reported (Chapters 2 and 3). In this chapter, we investigate how nitrogen (N) was partitioned among different tree organs in response to irrigation and fertilization treatments. Dry weight (DW) and N concentration of all tree material removed during pruning, thinning, harvest, and leaf drop were assessed.

Prior research has focused on the allocation and partitioning of nutrients in fruit crops in response to N availability, irrigation (regulated deficit), ripening season, and fertilizer source. Rufat and DeJong (2001) studied the seasonal N accumulation patterns in organs of mature 'O'Henry' peaches grown in either low (0 $kg \cdot ha^{-1}$ N) or high (200 $kg \cdot ha^{-1}$ N) annual N fertilization. They reported that the organs' dry weight and N concentration increased with N fertilization. Fertilization greatly affected vegetative

growth of the current year in comparison with other organs being evaluated. Furthermore, annual organs, like fruits, current-year stems, and leaves, had greater responses to N fertilization than perennial organs, like branches and trunk. In another study, Baldi et al. (2010) tested the effects of mineral and organic fertilization on nutrient uptake and partitioning in 1-year-old, potted 'Stark RedGold' peach trees. The different mineral and organic fertilizers did not induce differences in dry weight of leaves, shoots, stems, or coarse roots. Further, N concentration in fine roots, leaves, and total tree were not different across treatments. Dichio et al. (2007) studied the effects of post-harvest regulated deficit irrigation (RDI) on N partitioning and tree growth of 'Springcrest' peach trees. Moderate and severe post-harvest RDI reduced the growth of watersprouts and lateral shoots. However, fruiting shoots were not affected by the post-harvest RDI treatments in comparison with the control 100% crop evapotranspiration (ET_c) irrigation. Severe post-harvest RDI induced higher N concentrations in roots, branches, and shoots; however, no differences were found in leaves. Further, severe post-harvest RDI reduced fruit yield. Zhou and Melgar (2019) investigated how different peach ripening seasons (early, mid, and late) affected nutrient partitioning. They found that mature fruits from early-season cultivars have higher N concentration in comparison with mid- and late-season cultivars. Further, pruning material is where early-season cultivars partition a greater amount of N, followed by defoliated leaves, then harvested fruit. For mid- and late-season cultivars, pruning is still where the trees partition a greater amount of N; however, harvested fruits received more N than defoliated leaves. Policarpo et al. (2002) studied N partitioning in early- and late-season peach cultivars throughout the growing season. They found that leaves are the main sinks of N during the entire

growing season (Feb. 15 to Oct.19) of early-season cultivars. Differently, for the late-season cultivar, fine roots are the main N sinks early in the season, followed by leaves, which dominate the sinks until the end of the season (Mar. 23 to Sept. 14). In summary, fertilization, irrigation, and different ripening seasons can affect the trees, inducing differential N allocation patterns. In Georgia, there are no reports of such studies to understand the N partitioning in different organs and removal events.

We performed this study to better comprehend how different irrigation and fertilization treatments induce changes in N allocation in young peach trees since field establishment. The specific objective was to determine how much N is removed from trees during pruning, fruit thinning, harvesting, and fall defoliation. We hypothesized that 1) trees receiving more fertilizer will have greater vegetative growth (as indicated by plant material removed through pruning and fall defoliation); 2) trees irrigated since establishment will grow faster and thus will have more N removed than trees without irrigation; and 3) different irrigation systems will not impact N partitioning.

Material and methods

Plant material and field characteristics

The experiment was conducted using trees of ‘Julyprince’ peach grafted onto ‘Guardian™’. Both varieties are widely used in the southeastern United States. Trees were planted in 13 July 2015 at a spacing of 4.6 m within rows and 6.1 m between rows (358 trees/ha rate). The experiment was located at the Dempsey Farm, University of Georgia, Griffin, GA (33°14’ 55” N, 84°17’ 57” W), with the soil being a Cecil sandy loam with slope of 2-6% and organic matter 1.5%. Prior to planting, amendments of

potassium, phosphorus, and lime (to reach pH 6.0) were made following the recommended guidelines published in the Southeastern Peach, Nectarine, and Plum Pest Management and Culture Guide (Horton et al., 2015). Additionally, pest management also followed the above-cited guidelines. In Apr. 2016, soil samples (0-40 cm depth) were taken from the experimental plot to measure the soil fertility level baseline (Table 4.1). In Apr. 2017, April 2018, and May 2019 soil samples were taken to assess the fertility of the soil in the different fertilizer treatments at two soil depths (0-20 and 20-40 cm), with exception of May 2019 when only 0-20 cm samples were taken (Table 4.2). In April 2018, samples were taken two days after fertilizer application, resulting in higher values relative to values of 2017 and 2019. This sampling was used to quantify the immediate effect of the fertilizer treatments on the level of nutrients in the soil right after fertilization. Annual precipitation records from the Dempsey Farm weather station for 2016, 2017, 2018, and the historical normal 1981-2010 are shown in Table 2.1.

Experimental design and treatments

Experimental design and field set-up are described on the Material and Methods section of Chapter 2. Briefly, the experiment was comprised of three factors: 1) irrigation levels (non-irrigated vs. irrigated); 2) irrigation systems (drip vs. micro-sprinkler irrigation); and 3) fertilization rates (25%, 50%, 100%, and 200%). Non-irrigated trees only received water from rain events. Irrigation was controlled automatically by an irrigation network that would activate the irrigation as needed to keep the soil moisture levels above the thresholds established as follows: 1) Irrigation off from January to

early-May; 2) VWC threshold of 25% from early-May to early-August; 3) VWC threshold of 20% from early-August to mid-September; 4) VWC threshold of 15% from mid-September to late-September; and 5) irrigation off from late-September to December. Each combination of fertilizer levels and irrigation systems was irrigated separately. Figures 2.1-2.3 show the VWC of the soil for different treatments and the rain events for 2016, 2017, and 2018, respectively. To test different irrigation systems, trees were either drip or micro-sprinkler-irrigated.

Four fertilizer levels were defined based on the current recommendations. Currently, the Southeastern Peach, Nectarine, and Plum Pest Management and Culture Guide (Horton et al., 2015) recommends 65, 95, and 98 $kg \cdot ha^{-1}$ N for 1-, 2-, and 3-year-old trees, respectively. These rates are denoted as 100% in this experiment. Three other rates (25%, 50%, and 200%) were chosen based on the recommended rate, generating four N fertilizer rates for the experiment: 16, 33, 65, and 129 $kg \cdot ha^{-1}$ N for 1-year-old trees; 23, 48, 95, and 191 $kg \cdot ha^{-1}$ N for 2-year-old trees; and 24, 49, 98, and 195 $kg \cdot ha^{-1}$ N for 3-year-old trees. Granular fertilizer was applied by hand: one application of 10.0N-4.4P-8.3K (in March) and two applications of 15.5N-0P-0K (in May and July) for 1- and 2-year-old trees; and one application of 10.0N-4.4P-8.3K (in March) and one application of 15.5N-0P-0K (in August) for 3-year-old trees. Fertilizer 10.0N-4.4P-8.3K had N form as 10.0% ammoniacal nitrogen (Farmers Favorite Fertilizer; Agri-AFC, Evergreen, AL), and fertilizer 15.5N-0P-0K had N form as 1.0% ammoniacal nitrogen + 14.5% nitrate nitrogen (Yara Liva Tropicote; Yara, Tampa, FL).

Sample collection and tissue analyses

A total of 64 trees representing all treatment combinations were randomly selected (2 irrigation systems × 2 irrigation levels × 4 fertilization levels × 4 blocks). Each treatment combination had one tree evaluated per block. For each tree's removal event (pruning, fruit thinning, harvesting, and defoliation), the fresh weight (FW) and the DW of the material were either measured or calculated (see detailed description below) for the 64 trees. DW was measured after drying the material at 65 ºC until constant weight was achieved. The total amount of N removed by removal events was calculated by multiplying DW by N concentration in the tissues of the material. Nitrogen concentration from all tissues was assessed by a commercial laboratory (Waters Agricultural Laboratories; Camilla, GA). Each removal event is described below.

Winter pruning. Trees were winter pruned following commercial procedures for an open-vase training system in Feb. 2017 and Feb. 2018. In Feb. 2017, pruned material FW, DW and N concentration were assessed. In Feb. 2018, trees were winter pruned following the same procedure described for the winter pruning in Feb. 2017, with one modification due the trees' size. Total pruned material FW was recorded, and a sub-sample of known weight was taken and dried instead of all pruned material as in 2017. The FW, DW, N concentration were determined for this subsample and estimated for the total pruned material based on its FW.

Fruit thinning. Fruit were thinned following commercial procedures. In Apr. 2017, trees were thinned following commercial standards (three or four fruits per fruiting twig at a spacing of 15-20 cm). Fresh weight, DW and N concentration were assessed. In

2018, trees were not thinned because of an advective freeze. The number of fruitlets left on the trees after the freeze did not merit additional thinning.

Summer pruning. Trees were summer-pruned following commercial procedures for an open-vase training system in July 2016, June 2017, and June 2018. In July 2016. Six samples out of the 64 trees were randomly selected to determine the proportion of leaves vs. stems (temporary branches of 1- or 2-year-old) in the pruning material. The stems and leaves from these samples were separated. The percentage of leaves was 57.1% and stems was 42.9%. FW and DW of leaves and stems were recorded. These proportions were also used for summer pruning of 2017 and 2018 seasons calculations. The FW of pruned material was recorded for the 64 trees for all seasons. In 2016, all 64 trees had the fresh-pruned material dried to estimate the dry weight. In 2017 and 2018, subsamples of the total pruned material were dried. Based on the proportion of leaves vs. stems estimated (as described above), the DW of leaves and stems was calculated. Dried samples of leaves and stems were submitted for N analysis.

Harvested fruit. Fruit were harvested following commercial procedures. In July 2017 and 2018, mature fruit were harvested, and FW recorded. A sub-sample of fruit of known weight was taken, and DW and N concentration were assessed.

Defoliation. Different techniques were utilized every year because of the increasing size of the trees. Further, samples for nutritional analysis were taken before leaf natural senescence. Therefore, the values of N concentration reported in this study likely overestimated the real N concentration of defoliated leaves. Zhou and Melgar (2019) reported values of N concentration in peaches around 1.5% after leaves have senesced. In Oct. 2016, to estimate the N removed via fall defoliation, one significant

scaffold (permanent large branches coming from the trunk) of each 64 trees was selected and the total number of leaves in the scaffold was counted. FW, DW, and N concentration were assessed on a sub-sample of 100 leaves of each 64 trees. In Oct. 2017, one scaffold per tree from a subsample of eight trees (one tree from each irrigation level*fertilizer level treatment combination) was selected and the total number of leaves in the scaffold was counted. An average number of leaves per scaffold was calculated based on the eight trees. Additionally, the number of scaffolds per tree for all 64 trees were counted for both years. An estimate of the total number of leaves per tree was obtained by multiplying the number of leaves in one scaffold by the number of scaffolds in a tree. FW, DW, and N concentration were assessed on a sub-sample of 100 leaves and calculated for the total number of leaves of each tree. In Oct. 2018, all 64 trees had the number of scaffolds counted. Following that, seven trees were randomly selected. Their canopy was wrapped with bird nets to collect all the fallen leaves. FW of the fallen leaves for each tree were recorded. The total number of leaves on those seven trees was estimated based on a correlation between the total FW and the FW of a 100-leaf sub-sample. This 100-leaf sub-sample was dried, and the average DW of one leaf was estimated. An average number of leaves per scaffold was determined based on the seven trees. The total number of leaves per tree on the 64 trees were estimated by multiplying the number of scaffolds per tree by the average number of leaves per scaffold. By knowing the total number of leaves per trees and the average DW of a single leaf, the total DW of the pruning material for the 64 trees was estimated. A sub-sample of 100 leaves taken in November 2018 from all 64 trees were sent for N concentration analysis.

Physiological measurements

Leaf and stem water potential were measured using a pressure chamber (1505D-EXP PMS Instrument Company; Albany, OR), following the protocol described in Material and Methods, Chapter 2.

Statistical analysis

SAS 9.4 (SAS Institute Inc., Cary, NC) was used to compare treatment means and interactions, using PROC GLIMMIX. Means were separated using Tukey's Honest Significant Difference method with significance level at $P \leq 0.05$. Interactions between factors are not shown because most were not significant. When present, the interactions did not have biological meaning. Therefore, only main factors are reported.

Results

Fertilizer levels

The soil analyses performed in 2017, 2018, and 2019 show the nutritional status of the soil during the research period (Table 4.2). Variations in P and K were also reported because the first fertilizer application of each year (in March), was an application of 10.0N-4.4P-8.3K. The second and third fertilizer application were 15.5N-0P-0K (as described in the material and methods section). In 2017 and 2019, the analyses were performed before fertilization, indicating less variation in the soil nutritional levels among different fertilizer levels. In 2017, soil receiving 200% fertilizer had the highest amount of NO_3-N (48.3 kg·ha^{-1}) and P (105.4 kg·ha^{-1}). Soils receiving 25 and 50% of fertilizer had the lowest amount of NO_3-N, whereas soil receiving 25% of

fertilizer had the lowest amount of P (Table 4.2). Samples taken from 0-20 cm had higher amount of P (107.9 kg·ha^{-1}) and K (161.1 kg·ha^{-1}) than 20-40 cm samples (46.2 and 137.5 kg·ha^{-1} for P and K respectively). In 2018, soil samples were taken after fertilization, resulting in remarkable differences in soil nutritional levels among the highest and lowest fertilizer levels. Soil from 200% fertilizer level had 123.0, 63.6, 165.8, and 322.6 kg·ha^{-1} of NH_4-N, NO_3-N, P, and K respectively, whereas soil form 25% fertilizer level had 15.5, 37.3, 69.7, and 183.3 kg·ha^{-1} of NH_4-N, NO_3-N, P, and K respectively (Table 4.2). When present, differences between sample depths showed that nutrients were more present in the top layer than in the deep layer of the soil (Table 4.2). In 2019, soil receiving 200% of fertilizer had the highest P amount (194.0 kg·ha^{-1}) and soil receiving 25% of fertilizer the lowest P amount (89.9 kg·ha^{-1}) (Table 4.2).

No differences were found among fertilizer levels for the total amount of N removed from trees during the three years of the experiment (P = 0.167). The average removal across the fertilizer levels was 583.7 g/tree (Figure 4.1). Analysis of the percentage of N partitioning in the three years revealed that defoliation and summer pruning were where most of the N was allocated (68% of the total N removed). For the individual removal events, differences in N removal among fertilizer treatments were found only for summer pruning in June 2017 and winter pruning in February 2018 (Figure 4.1). For the summer pruning in June 2017, the greatest amount of N was removed from trees receiving 100% of fertilizer (55.3 g/tree), followed by trees receiving 200% and 25% of fertilizer (51.5 and 47.3 g/tree, respectively), and lastly by trees receiving 50% of fertilizer (42.1 g/tree). For the winter pruning in February 2018, trees receiving 100% of fertilizer had the greatest amount of N removed (83.8 g/tree),

followed by trees receiving 200% of fertilizer (70.1 g/tree), and then followed by trees receiving 25 and 50% of fertilizer (66.2 and 57.9 g/tree, respectively) (Figure 4.1). For the specific cases of summer pruning in June 2017 and winter pruning in February 2018, significant differences were found for DW of the material (Table 4.3). The means separation followed a very similar pattern than those from the total amount of N removed from those two removal events - greater values for 100%, followed by 200 and 25%, and lastly by 50% (Table 4.3). The N concentration analysis among fertilizer rates within every removal event indicated significant differences only for the leaves removed from the summer pruning in July 2016 ($P = 0.013$) and fruits harvested in July 2017 ($P < 0.001$) (Table 4.3).

Irrigation levels and systems

Figures 2.1-2.3 represent the VWC of the soil and how the Sensorweb system maintained the VWC above the thresholds established through irrigation (see materials and methods). Additionally, rain events are represented. In 2016, non-irrigated trees had lower water potential than irrigated trees, because of drought stress caused by a lower than normal rainfall (Table 2.2). In 2017 and 2018, no major differences were found between irrigated vs. non-irrigated trees, because the precipitation was similar to the historical average, not inducing drought stress in the trees.

A greater amount of N was removed from the irrigated trees (656.8 g/tree) when compared with the non-irrigated trees (510.6 g/tree) ($P < 0.001$, Figure 4.2). Defoliation and summer pruning were where the trees partitioned most of the N in a given year. Irrigation induced greater N removal than non-irrigation for most of the removal events

(Figure 4.2). Differences were found within all the pruning practices (winter or summer) and most of the defoliations. The amounts of N removed from events related to fruits (thinned and harvested fruits) were not different between irrigated and non-irrigated trees (Figure 4.2). Irrigating trees induced greater DW removal from most of the events, with the exception of fruit thinning, fruit harvesting, and defoliation in 2017, and fruit harvesting in 2018 (Table 4.4). Non-irrigated trees had higher N concentration than irrigated trees for summer pruning in June 2018 (P = 0.034 and 0.014 for leaves and stems, respectively) and fruit harvested in 2018 (P = 0.043, Table 4.4).

Drip irrigation delivered 2,959 L/tree of water, averaged over fertilizer levels and totaled over the three years of the experiment, whereas micro-sprinkler irrigation delivered 4,743 L/tree. This accounts for a reduction of 38% by using drip-irrigation. Drip-irrigated trees had more N removed (720.9 g/tree) in comparison with micro-sprinkler-irrigated trees (592.6 g/tree) (P = 0.004) (Figure 4.3). These differences were mainly driven by differences found in the amount of N removed from defoliation in 2016 and 2018, summer pruning in 2018, fruit harvested in 2017 and 2018, and fruit thinning 2017 (Figure 4.3). Similar to the other treatments, most of the material removed each year was from summer pruning and defoliation. Drip irrigation induced greater DW removal than micro-sprinkler irrigation for fruit thinning in Apr. 2017 (P = 0.028) and summer pruning in June 2018 (P = 0.019 and 0.004 for leaves and stems, respectively) (Table 4.5). In contrast, micro-sprinkler irrigation induced greater DW removal for harvested fruit in 2018 (P = 0.036). Drip-irrigated trees had higher N concentration than micro-sprinkler-irrigated trees for the defoliation in 2016 (P = 0.016) and 2018 (P = 0.002), and stems from summer pruning in 2018 (P = 0.009), (Table 4.5).

Discussion

Fertilizer levels

The total amount of N removed per tree is a combination of the DW of the tree material removed and the N concentration of those tissues. Different fertilizer level rates did not affect the total amount of N removed by the trees in the three years of the experiment. This disagrees with findings of Rufat and DeJong (2001), who reported an increased N content (greater potential of N removed) in fertilized peach trees vs. trees not fertilized for three years before the experiment. In that study, the lack of fertilization significantly reduced the N in the trees, negatively affecting the DW and the N concentration. In contrast, our lowest fertilizer level, the 25% rate, did not affect the DW or the N concentration in comparison to the recommended rate. This basal fertilizer application was able to maintain tree growth and development. One of the reasons for this maintenance capacity is the recycling of nutrients in the orchard (Zhou and Melgar, 2019). Most of the removal events (pruning, fruit thinning, and defoliation) do not remove the nutrients from the orchard. The fresh material is removed from the trees but immediately returned to the soil surface. There the material can be mowed, decomposed, and re-incorporated into the soil profile, allowing for nutrient recycling. Therefore, the only removal event that actually removes material and nutrients from the orchard is harvesting. Zhou and Melgar (2019) estimated that N removal from harvesting was 19.0, 20.8, and 27.4 $kg \cdot ha^{-1}$ for early-, mid-, and late-cultivars of peaches grown in South Carolina. Tagliavini et al. (1996) reported that N removal in fruit from mature peach trees in Italy ranges from 12 to 36 $kg \cdot ha^{-1}$. In our research, N removal in harvested fruits averaged 8.7 $kg \cdot ha^{-1}$ in 2017 and 8.4 $kg \cdot ha^{-1}$ in 2018. It is

important to highlight that 2017 was the first year of harvesting, and in 2018 trees were severely affected by a late freeze, which reduced the fruit load. Our research is the first report of N partitioning for young peach trees in the southeastern United States.

Evaluations of individual removal events showed differences in N removal among fertilizer levels only for winter or summer pruning events. The differences were caused solely by variations in DW of the material and not related to the N concentration in the tissues. These findings agree with Rufat and DeJong (2001), who reported greater DW of peach leaves and branches (which can be associated with pruning) in treatments receiving 200 $kg \cdot ha^{-1}$ N vs. 0 $kg \cdot ha^{-1}$ N. Additionally, 200 $kg \cdot ha^{-1}$ N treatment induced slightly greater N concentration in leaves, but no differences between treatments were found for the N concentration in branches. Statistical differences in N concentration among fertilization treatments were found only for leaves from the summer pruning in July 2016 and fruit harvested in 2017. However, they were not consistent throughout the experiment and may not hold a biological meaning. Nevertheless, the result of greater N concentration in leaves in July 2016 in higher fertilizer levels agrees with Rufat and DeJong (2001).

Irrigation levels and systems

Water potential measurements indicated that non-irrigated tees were exposed to drought in 2016, but not in 2017 or 2018 (see chapter 2).

The total amount of N removed from irrigated trees was greater than that of non-irrigated trees for the three years. This difference started in the 2016 season and continued in the 2017 and 2018 seasons. In 2016, a severe drought took place during

most of the spring and summer seasons, as shown by the lack of rain in comparison with the historical average. Evaluation of the individual removal events reveals that most of the events induced greater N removal for irrigated vs. non-irrigated trees. Analysis of canopy volume and trunk cross-sectional area (Chapter 2) indicated that tree growth was reduced by the drought and trees did not recover during the three years of research. All other pruning events and defoliation in 2018 also showed more N removed from irrigated vs. non-irrigated trees. This illustrates how one year of drought (2016) can negatively affect the peach tree growth and development in following years. Differences in total N removed between irrigated and non-irrigated trees are the result of differences in DW of removed material. Irrigated trees had more DW removed for most events in comparison with non-irrigated trees. This agrees with findings of Steinberg et al. (1990) for peach stems and leaves dry weight. Boland et al. (2000) reported reductions in summer, but not winter, pruning FW when peach trees experienced deficit irrigation. This distinct behavior might be attributed to the different composition of summer and winter pruning material. We estimated that for summer pruning, leaves accounted for 57.1% and stems for 42.9%, while winter pruning material consists 100% dormant stems. The N concentration of the removal events was slightly affected by the irrigation levels. Summer pruning material and harvested fruit from 2018 had greater N concentration if trees were non-irrigated. This response was not observed in 2016 and 2017, therefore it may not hold a biological meaning.

Drip irrigation resulted in greater N removal from trees than micro-sprinkler irrigation. Differences between drip and micro-sprinkler irrigation were found in N removal from fruit thinning, summer pruning, harvested fruit, and defoliation events. For

most of the cases, drip irrigation induced higher N removal than micro-sprinkler irrigation. These differences were driven majorly by differences on DW, with exception of defoliation, where drip-irrigated trees had higher N concentration than micro-sprinkler-irrigated trees. The difference in N removal between irrigation systems differs from the other main treatments (fertilization or irrigated vs. non-irrigated) where differences in total N removal were driven mainly by differences in N removal from pruning and defoliation events (vegetative material) instead of fruit thinning and harvest fruits (reproductive organs). Differences in DW between drip vs. micro-sprinkler for individual removal events were reported only for fruit thinning in 2017, and summer pruning and harvested fruits in 2018. The differences in fruit thinning DW can be explained by the greater canopy volume found in drip-irrigated trees in Sept. 2016 (Chapter 2). Greater canopy volume allows for a greater fruit production area, which was the case in our study (Chapter 2). A greater number of fruits was produced in drip-irrigated trees and some were removed during fruit thinning in 2017. For the case of summer pruning in 2018, a possible explanation comes from the advective freeze that affected the orchard in Mar. 2018. The freeze killed a significant number of flowers and fruitlets from the drip-irrigated trees only, because of the topography of the experimental plot. After having a great number of flowers and fruitlets removed, the competition for photoassimilates was reduced. Thus, the dry matter and nitrogen partitioning was shifted towards vegetative growth instead of reproductive growth. Similarly, Zhou and Melgar (2019) and Policarpo et al. (2002) reported greater vegetative growth after harvest in early-season cultivars in comparison to late-season cultivars, because of the removal of fruits (sinks). The reduced number of fruits in drip-irrigated trees in

comparison to micro-sprinkler-irrigated trees possibly resulted in increased N concentration in the stems in June 2018 and in the leaves from defoliation in 2018, since the nutrients did not have to be allocated to fruit production. Differences in N concentration between irrigation systems were found for defoliation in 2016, possibly because of the severe drought in 2016. In Sept. 2016, drip-irrigated trees had greater canopy volume than micro-sprinkler-irrigated trees (Chapter 2). Therefore, drip-irrigated trees were growing at a different rate than micro-sprinkler-irrigated trees. It is possible that because of the differences in growth, micro-sprinkler-irrigated trees were already reallocating nutrients from leaves to permanent organs in preparation for fall defoliation.

Conclusions

Different fertilization rates did not show any negative effects in terms of shifting of DW and N concentration among different organs of peach trees. This suggests that young peach trees need less fertilizer than what is currently recommended. Peach trees receiving reduced levels of fertilizer can still allocate enough assimilates and N for satisfactory tree growth and development. At the same time, reductions in fertilization are environmentally sound and decrease the production costs. The long-term sustainability of reduced fertilization is being monitored. Irrigation induces greater vegetative growth in general, especially under drought conditions. The greater canopy did not translate to greater DW of harvested fruit or nitrogen removed in harvested fruit. However, it is important to highlight that fresh fruit yield was greater for irrigated vs. non-irrigated trees in 2017 (Chapter 2). In our experiment, differences between drip and

micro-sprinkler irrigation appears to be more related to the environment than solely because of a treatment effect.

Literature cited

Abrisqueta, J.M., O. Mounzer, S. Álvarez, W. Conejero, Y. García-Orellana, L.M. Tapia, J. Vera, I. Abrisqueta, and M.C. Ruiz-Sánchez. 2008. Root dynamics of peach trees submitted to partial rootzone drying and continuous deficit irrigation. Agr. Water Mgt. 95:959–967.

Albornoz, F. 2016. Crop responses to nitrogen overfertilization: A review. Sci. Hort. 205:79–83.

Baldi, E., M. Toselli, and B. Marangoni. 2010. Nutrient partitioning in potted peach (*Prunus persica* L.) trees supplied with mineral and organic fertilizers. J. Plant Nutr. 33:2050–2061.

Beckman, T.G., W.R. Okie, A.P. Nyczepir, G.L. Reighard, E.I. Zehr, and W.C. Newall. 1997. History, current status and future potential of Guardian™ peach rootstock. Acta Hort. 451:251–258.

Boland, A.M., P.H. Jerie, P.D. Mitchell, I. Goodwin, and D.J. Connor. 2000. Long-term Effects of restricted root volume and regulated deficit irrigation on peach: I. Growth and mineral nutrition. J. Amer. Soc. Hort. Sci. 125:135–142.

Cockroft, B. and K.A. Olsson. 1972. Pattern of new root production in peach trees under irrigation. Austral. J. Agr. Res. 23:1021–1025.

Dichio, B., C. Xiloyannis, A. Sofo, and G. Montanaro. 2007. Effects of post-harvest regulated deficit irrigation on carbohydrate and nitrogen partitioning, yield quality and vegetative growth of peach trees. Plant Soil 290:127–137.

Gammon Jr., N. and J.S. Shoemaker, 1963. Effect of fertilizers on mineral levels in the leaves of Flordawon peach. Florida State Hort. Soc. 1746:380–384.

Horton, D., P. Brannen, B. Bellinger, D. Lockwood, and D. Ritchie. 2015. 2015 Southeastern peach, nectarine and plum pest management and culture guide. Uni. Georgia Coop. Ext. Bul. 1171. Athens, GA.

National Drought Mitigation Center, U.S. Department of Agriculture, and National Oceanic and Atmospheric Association. 2019. United States drought monitor. 11 June 2019. <https://droughtmonitor.unl.edu/Data/Timeseries.aspx>.

Okie, W.R. and D.R. Layne. 2008. 'Scarletprince' and 'Julyprince' peaches. HortScience 43:1603–1605.

Policarpo, M., L. Di Marco, P. Gioacchini, and M. Tagliavini. 2002. Nitrogen use efficiency and N partitioning in early and late fruit ripening peach varieties. Acta Hort. 592 543–547.

Reighard, G.L., W.C. Newall Jr., T.G. Beckman, W.R. Okie, E.I. Zehr, and A.P. Nyczepir. 1997. Field performance of *Prunus* rootstock cultivars and selection on replants soil in South Carolina. Acta Hort. 451 243–249.

Rufat, J. and T.M. DeJong. 2001. Estimating seasonal nitrogen dynamics in peach trees in response to nitrogen availability. Tree Physiol. 21:1133–1140.

Smith., C.B., and G.A. Taylor. 1952. Tentative optimum leaf concentration of several elements for Elberta peach and Stayman apple in Pennsylvania orchards. Proc. Amer. Soc. Hort. Sci. 60:33–41.

Steinberg, S.L., J.C. Miller, and M.J. McFarland. 1990. Dry matter partitioning and vegetative growth of young peach trees under water stress. Austral. J. Plant Physiol. 17:23–36.

Tagliavini, M., D. Scudellazi, B. Marangoni, and M. Toselli. 1996. Nitrogen fertilization management in orchards to reconcile productivity and environmental aspects, p. 161–170. In: C. Rodriguez-Barrueco (ed.). Fertilizer and Environment. Developments in Plant and Soil Sciences. Springer, Dordrecht, Netherlands.

Williamson, J.G. and D.C. Coston. 1990. Planting method and irrigation rate influence vegetative and reproductive growth of peach planted at high density. J. Amer. Soc. Hort. Sci. 115:207–212.

Zhou, Q. and J.C. Melgar. 2019. Ripening season affects tissue mineral concentration and nutrient partitioning in peach trees. J. Plant Nutr. Soil Sci. 182:203–209.

Table 4.1. Soil analysis from the experimental field (0-40 cm depth) at the Dempsey Farm, University of Georgia, Griffin, GA. Samples taken in April of 2016 before any fertilizer treatments were applied (n=48).

	P	K	Mg	Ca	B	Zn	Mn	Fe	Cu	NO_3-N	NH_4-N	CEC	OM
pH	$kg \cdot ha^{-1}$											$cmol \cdot kg^{-1}$	%
5.95	58.9	188.4	184.8	866.4	0.5	3.3	22.4	66.2	1.4	17.0	5.4	4.84	1.51

Table 4.2. Soil analyses for NH_4-N, NO_3-N, P, and K for the different fertilizer levels (25, 50, 100, and 200%) and sample depth (0-20 and 20-40 cm) in 2017, 2018, and 2019 from the Dempsey Farm, University of Georgia, Griffin, GA. In 2017 and 2019 soil samples were taken before fertilizer application (recommended practice). In 2018, soil samples were taken two days after fertilizer application, displaying elevated values relative to samples taken in 2017 and 2019, because of the sampling time.

	Fertilizer level				Sample depth (cm)		*P* value	
	25%	50%	100%	200%	0-20	20-40	Fertilizer level	Sample depth
	kg·ha^{-1}							
Nutrient	April 2017							
NH_4-N	2.3	2.4	4.7	7.6	4.8	3.7	0.091	0.523
NO_3-N	17.2 b[z]	22.4 b	30.9 ab	48.3 a	31.4	28.0	**<0.001**	0.533
P	57.7 c	60.0 bc	85.0 ab	105.4 a	107.9 a	46.2 b	**<0.001**	**<0.001**
K	155.1	144.1	141.6	156.4	161.1 a	137.5 b	0.223	**<0.001**
	April 2018							
NH_4-N	15.5 c	43.5 bc	47.7 b	123.0 a	86.1 a	28.7 b	**<0.001**	**<0.001**

NO_3-N	37.3 b	45.6 ab	47.7 ab	63.6 a	45.0	52.1	**0.019**	0.230
P	69.7 c	87.5 c	123.2 b	165.8 a	155.3 a	67.8 b	**<0.001**	**<0.001**
K	183.3 c	224.6 bc	245.2 b	322.6 a	289.4 a	198.5 b	**<0.001**	**<0.001**
				May 2019[y]				
NH_4-N	7.3	6.7	7.9	8.4	7.6		0.592	
NO_3-N	4.4	2.9	6.7	2.1	4.0	N/A	0.058	N/A
P	89.9 c	104.8 bc	138.3 b	194.0 a	131.8		**<0.001**	
K	146.7	151.1	163.0	160.9	155.4		0.548	

For the fertilizer levels, n=32 in 2017 and 2018, and n=16 in 2019. For the sample depths, n=64.

[z] Means followed by the same letter within a row and treatment effect (fertilizer levels or sample depth) are not significantly different by Tukey's honestly significant different test, $P \leq 0.05$. Means without letters indicate non-statistical differences, $P > 0.05$.

[y] Samples were collected only from the 0-20 cm soil profile.

Table 4.3. Dry weight (DW) and N concentration in the different removal events for the different fertilizer levels (25, 50, 100, and 200%) for trees of 'Julyprince' peaches in 2016, 2017, and 2018.

Removal event	Dry weight (g/tree)				*P* value
	25%	50%	100%	200%	
Pruning Leaf July 2016	360.9	382.6	355.3	340.9	0.888
Pruning Stem July 2016	270.8	287.5	270.1	256.0	0.895
Defoliation Oct. 2016	1080.9	1121.4	980.6	1413.8	0.181
Pruning Feb. 2017	875.2	992.4	811.4	1055.0	0.342
Thinning Apr. 2017	39.6	41.3	38.0	41.2	0.978
Pruning Leaf June 2017	968.6 ab[z]	854.9 b	1149.7 a	1054.6 ab	**0.028**
Pruning Stem June 2017	798.8 ab	725.8 b	965.8 a	894.9 ab	**0.026**
Harvesting July 2017	1778.3	1616.1	1642.7	2017.3	0.179
Defoliation Oct. 2017	2070.4	2003.2	2355.0	2204.7	0.124
Pruning Feb. 2018	3169.4 ab	2761.9 b	3901.7 a	3400.5 ab	**0.007**
Pruning Leaf June 2018	3766.1	3159.9	3944.2	3425.1	0.326
Pruning Stem June 2018	3138.3 ab	2523.7 b	3650.7 a	2805.6 ab	**0.038**

Harvesting July 2018	1829.7	2276.7	1929.3	2014.4	0.752
Defoliation Nov. 2018	3622.9	3557.6	3606.6	3639.2	0.986
Total	23769.9	22304.9	25601.0	24563.0	0.121

	N concentration (% DW)				
	25%	50%	100%	200%	*P* value
Pruning Leaf July 2016	3.29 b	3.46 ab	3.43 ab	3.59 a	**0.013**
Pruning Stem July 2016	0.96	0.99	0.93	0.97	0.891
Defoliation Oct. 2016	2.92	2.96	2.95	2.99	0.808
Pruning Feb. 2017	1.33	1.29	1.37	1.36	0.369
Thinning Apr. 2017	2.78	2.92	2.82	3.05	0.201
Pruning Leaf June 2017	3.97	3.98	3.93	4.00	0.662
Pruning Stem June 2017	1.11	1.11	1.09	1.07	0.709
Harvesting July 2017	1.41 a	1.30 b	1.44 a	1.35 ab	**0.001**
Defoliation Oct. 2017	3.43	3.51	3.43	3.52	0.335
Pruning Feb. 2018	2.11	2.08	2.12	2.07	0.807

Pruning Leaf June 2018	3.95	3.89	3.97	3.94	0.808
Pruning Stem June 2018	1.08	1.06	1.08	1.09	0.697
Harvesting July 2018	1.16	1.14	1.24	1.16	0.262
Defoliation Nov. 2018	2.89	2.96	2.88	2.95	0.646

[z] Means followed by the same letter within a row (removal event) are not significantly different by Tukey's honestly significant different test, $P \leq 0.05$. Means without letters indicate non-statistical differences, $P > 0.05$.

Table 4.4. Dry weight (DW) and N concentration in the different removal events for irrigated vs. non-irrigated for trees of 'Julyprince' peaches in 2016, 2017, and 2018.

Removal event	Dry weight (g/tree)		*P* value
	Irrigated	Non-irrigated	
Pruning Leaf July 2016	428.3 a[z]	291.6 b	**<0.001**
Pruning Stem July 2016	323.2 a	219.0 b	**<0.001**
Defoliation Oct. 2016	1502.3 a	796.0 b	**<0.001**
Pruning Feb. 2017	1350.0 a	517.0 b	**<0.001**
Thinning Apr. 2017	42.4	37.6	0.441
Pruning Leaf June 2017	1274.5 a	739.5 b	**<0.001**
Pruning Stem June 2017	1070.1 a	622.6 b	**<0.001**
Harvesting July 2017	1870.3	1656.9	0.136
Defoliation Oct. 2017	2258.2	2058.4	0.074
Pruning Feb. 2018	3709.4 a	2907.4 b	**<0.001**
Pruning Leaf June 2018	4067.6 a	3080.1 b	**0.004**
Pruning Stem June 2018	3404.3 a	2654.9 b	**0.010**
Harvesting July 2018	2119.9	1905.2	0.481
Defoliation Nov. 2018	3818.7 a	3394.4 b	**0.011**
Total	27239.1 a	20880.4 b	**<0.001**
	N concentration (% DW)		
	Irrigated	Non-irrigated	*P* value
Pruning Leaf July 2016	3.45	3.43	0.695

Pruning Stem July 2016	0.98	0.94	0.452
Defoliation Oct. 2016	2.98	2.93	0.365
Pruning Feb. 2017	1.32	1.35	0.384
Thinning Apr. 2017	2.94	2.85	0.351
Pruning Leaf June 2017	3.93	4.00	0.098
Pruning Stem June 2017	1.09	1.11	0.420
Harvesting July 2017	1.38	1.37	0.507
Defoliation Oct. 2017	3.46	3.48	0.600
Pruning Feb. 2018	2.08	2.11	0.600
Pruning Leaf June 2018	3.88 b	3.99 a	**0.034**
Pruning Stem June 2018	1.05 b	1.11 a	**0.014**
Harvesting July 2018	1.14 b	1.21 a	**0.043**
Defoliation Nov. 2018	2.96	2.87	0.081

[z] Means followed by the same letter within a row (removal event) are not significantly different by Tukey's honestly significant different test, $P \leq 0.05$. Means without letters indicate non-statistical differences, $P > 0.05$.

Table 4.5. Dry weight (DW) and N concentration in the different removal events for drip- vs. micro-sprinkler-irrigated for trees of 'Julyprince' peaches in 2016, 2017, and 2018.

Removal event	Dry weight (g/tree)		
	Drip	Micro-sprinkler	*P* value
Pruning Leaf July 2016	486.7	369.9	0.135
Pruning Stem July 2016	365.7	280.6	0.153
Defoliation Oct. 2016	1716.1	1288.6	0.086
Pruning Feb. 2017	1505.8	1194.2	0.392
Thinning Apr. 2017	53.3 a[z]	31.5 b	**0.028**
Pruning Leaf June 2017	1272.2	1276.7	0.973
Pruning Stem June 2017	1058.4	1081.7	0.825
Harvesting July 2017	2076.6	1663.9	0.087
Defoliation Oct. 2017	2315.0	2201.5	0.432
Pruning Feb. 2018	3962.9	3455.8	0.116
Pruning Leaf June 2018	4674.7 a	3460.6 b	**0.019**
Pruning Stem June 2018	4074.4 a	2734.2 b	**0.004**
Harvesting July 2018	1586.4 b	2653.3 a	**0.036**
Defoliation Nov. 2018	3933.0	3704.5	0.360
Total	29081.2 a	25397.0 b	0.015

	N concentration (% DW)		
	Drip	Micro-sprinkler	*P* value

Pruning Leaf July 2016	3.53	3.38	0.190
Pruning Stem July 2016	0.99	0.98	0.929
Defoliation Oct. 2016	3.16 a	2.80 b	**0.016**
Pruning Feb. 2017	1.28	1.37	0.072
Thinning Apr. 2017	2.93	2.95	0.912
Pruning Leaf June 2017	3.93	3.94	0.922
Pruning Stem June 2017	1.09	1.08	0.752
Harvesting July 2017	1.45	1.32	0.109
Defoliation Oct. 2017	3.48	3.43	0.484
Pruning Feb. 2018	2.11	2.05	0.587
Pruning Leaf June 2018	3.92	3.83	0.410
Pruning Stem June 2018	1.16 a	0.94 b	**0.009**
Harvesting July 2018	1.15	1.13	0.611
Defoliation Nov. 2018	3.09 a	2.84 b	**0.002**

[z] Means followed by the same letter within a row (removal event) are not significantly different by Tukey's honestly significant different test, $P \leq 0.05$. Means without letters indicate non-statistical differences, $P > 0.05$.

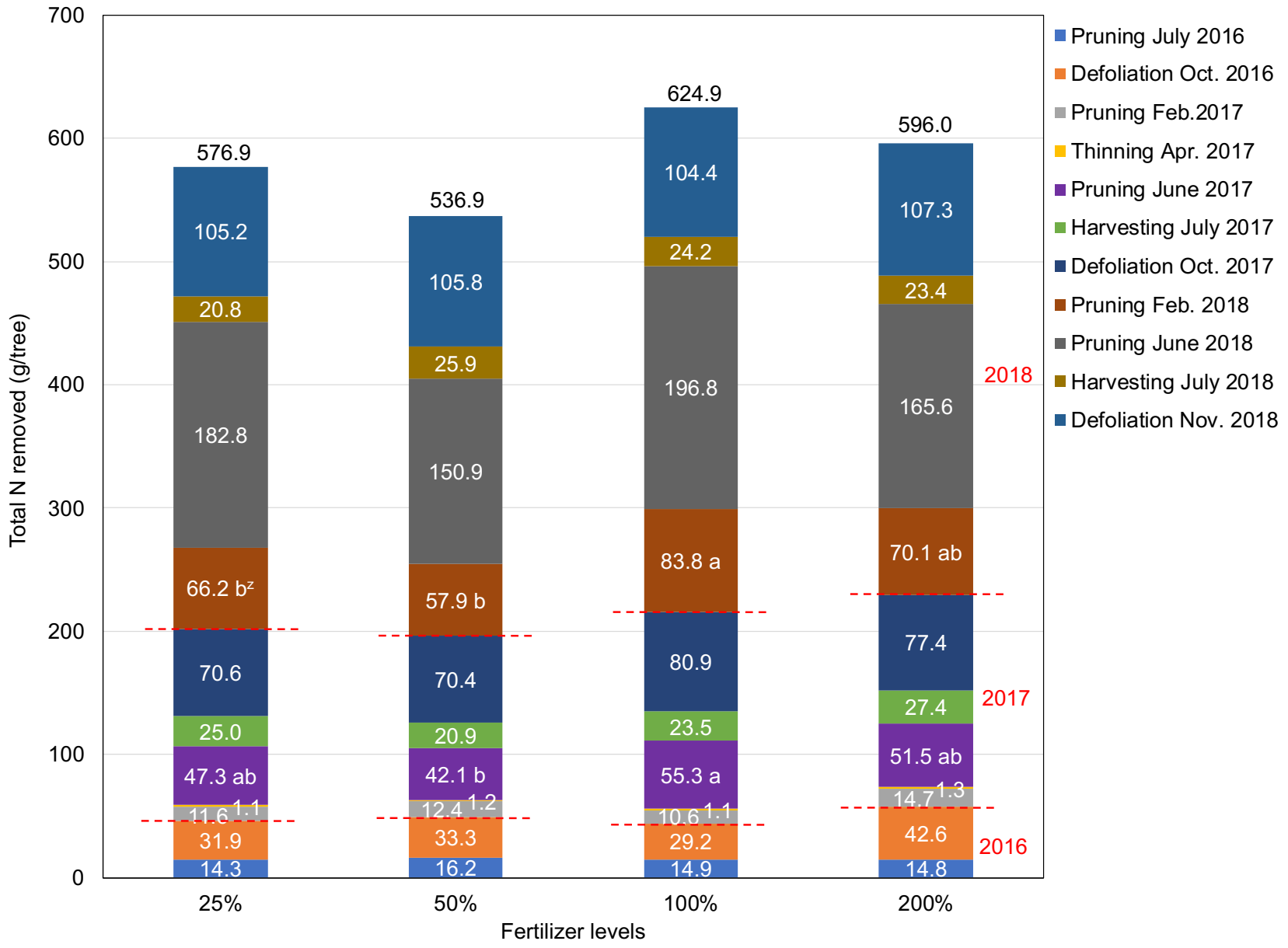
700
600
500
400
300
200
100
0
Total N removed (g/tree)
576.9
536.9
624.9
596.0
105.2
20.8
182.8
66.2 bz
70.6
25.0
47.3 ab
11.6
1.1
31.9
14.3
105.8
25.9
150.9
57.9 b
70.4
20.9
42.1 b
12.4
1.2
33.3
16.2
104.4
24.2
196.8
83.8 a
80.9
23.5
55.3 a
10.6
1.1
29.2
14.9
107.3
23.4
165.6
70.1 ab
77.4
27.4
51.5 ab
14.7
1.3
42.6
14.8
2018
2017
2016
25%
50%
100%
200%
Fertilizer levels
Pruning July 2016
Defoliation Oct. 2016
Pruning Feb.2017
Thinning Apr. 2017
Pruning June 2017
Harvesting July 2017
Defoliation Oct. 2017
Pruning Feb. 2018
Pruning June 2018
Harvesting July 2018
Defoliation Nov. 2018

Figure 4.1. Total N removed per tree for the different fertilizer levels (25, 50, 100, and 200%) of ‘Julyprince’ peaches in 2016, 2017, and 2018. For each removal event (different colors), the means are shown inside the bars. [z] Means followed by different letter within a removal event are significantly different by Tukey’s honestly significant different test, $P \leq 0.05$. Means without letters indicate non-statistical differences, $P > 0.05$.

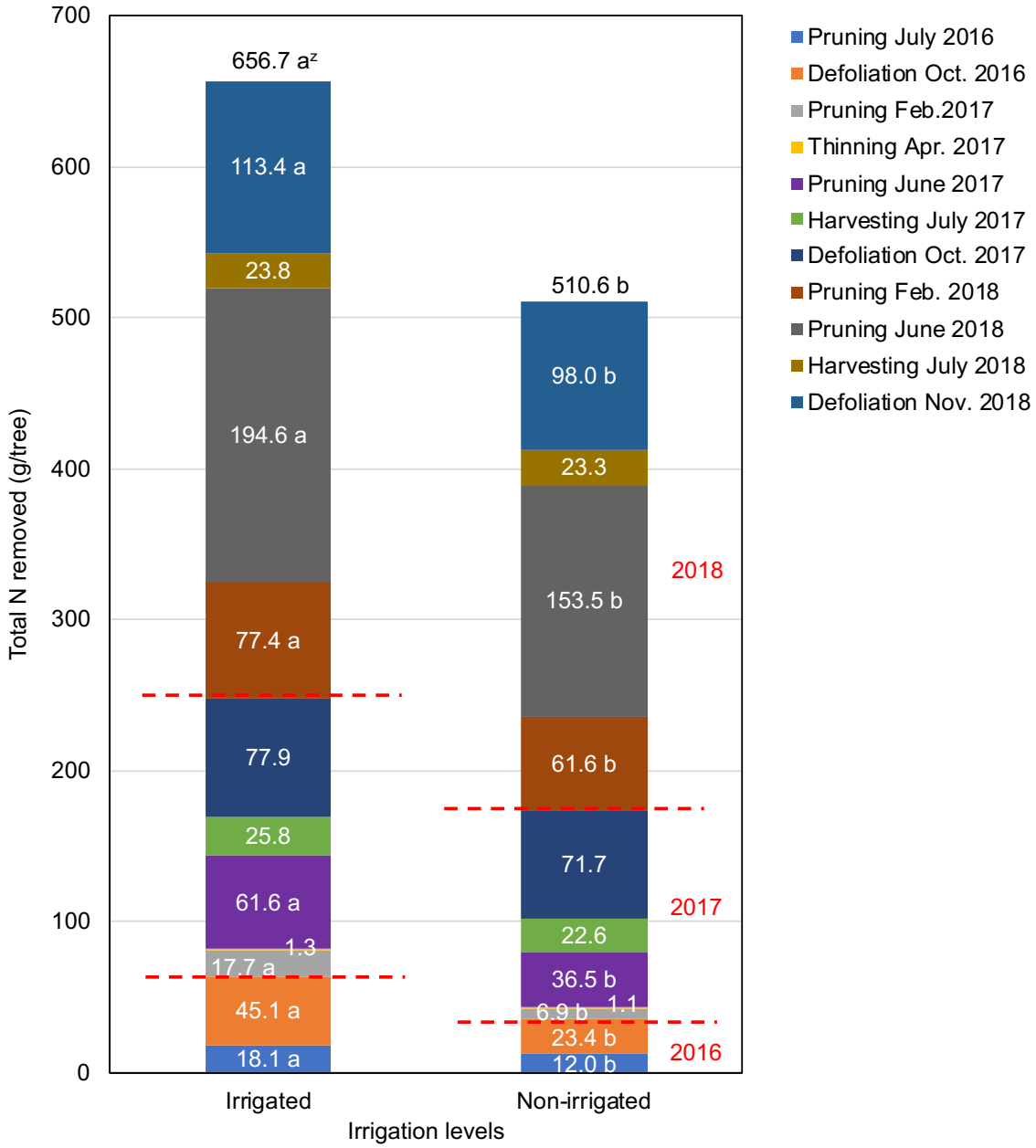

Figure 4.2. Total N removed per tree for the different irrigation levels (irrigated vs. non-irrigated) of 'Julyprince' peaches in 2016, 2017, and 2018. For each removal event (different colors), the means are shown inside the bars. [z] Means followed by different letter within a removal event are significantly different by Tukey's honestly significant different test, $P \leq 0.05$. Means without letters indicate non-statistical differences, $P > 0.05$.

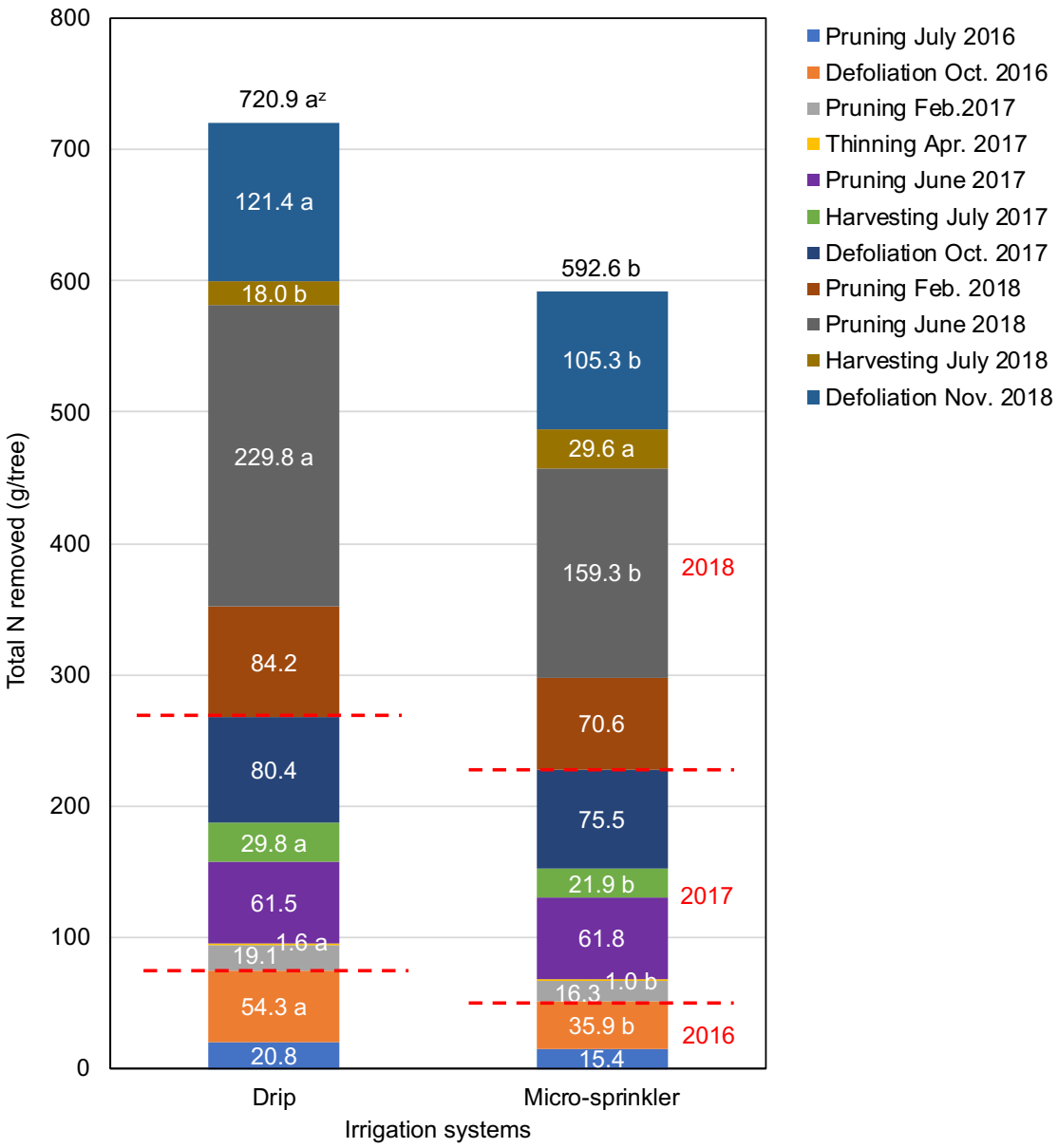

Figure 4.3. Total N removed per tree for the different irrigation systems (drip vs. micro-sprinkler) of 'Julyprince' peaches in 2016, 2017, and 2018. For each removal event (different colors), the means are shown inside the bars. [z] Means followed by different letter within a removal event are significantly different by Tukey's honestly significant different test, $P \leq 0.05$. Means without letters indicate non-statistical differences, $P > 0.05$.

CHAPTER 5

DIFFERENTIAL GENE EXPRESSION OF FIELD-GROWN YOUNG PEACH TREES IN RESPONSE TO IMPROVED IRRIGATION AND FERTILIZATION MANAGEMENT IN THE SOUTHEASTERN UNITED STATES [1]

[1] Casamali, B, A. Malladi, M.W. van Iersel, and D.J. Chavez. To be submitted to *Journal of the American Society for Horticultural Science.*

Abstract

Drought stress for peach trees is detrimental for tree growth and fruit production. In the southeastern United States, traditionally, peach trees are grown without supplemental irrigation until the third or fourth year after field planting. This practice, combined with drought conditions, can negatively affect the trees. Furthermore, recommended fertilization practices for peach production were created decades ago. A comprehensive study was conducted to assess the effects of different irrigation and fertilization practices on young peach trees after establishment. The specific objective of this research was to test the expression of genes associated with drought stress and nitrogen metabolism in field-grown peach trees exposed to different irrigation levels (irrigated vs. non-irrigated trees), different irrigation systems (drip- vs. micro-sprinkler irrigated trees), and four fertilizer levels (25%, 50%, 100%, and 200%; being 100% the current recommendation). Non-irrigated trees, which suffered drought stress as supported by diverse physiological parameters measured, had higher relative expression of genes related to ABA biosynthesis (*ChlH* and *PpCYP707A3*). Genes related to osmoregulation (*SIP1* and *P5SC)* and reactive oxygen species scavenging (*POD*) had remarkable increased relative expression in non-irrigated vs. irrigated trees. Genes related to dehydration and aquaporins (*PpDhn3* and *Pp-δTIP1*) had decreased relative expression in non-irrigated vs. irrigated trees. No major differences were found between relative expression of drip- and micro-sprinkler-irrigated trees or among different fertilizer levels.

Introduction

Peach production in the southeastern United States has been faced by multiple weather related challenges, such as early-season freezes, low chill accumulation (Crouch, 2017), and drought stress (Conrad II and Knox, 2016). Droughts are becoming more common (NDMC, USDA, and NOAA, 2019) and are predicted to occur even more frequently (Bhargava and Sawant, 2013).

In the southeastern United States, young peach trees are not commonly irrigated from planting until three years later, when they start producing fruit. During this period of initial plant growth and development, peach roots explore the soil and the canopy is being formed. However, the only source of water comes from rainfall events. The lack of supplemental irrigation for young trees results in suboptimal conditions for tree growth and fruit production (B. Casamali et al., unpublished data, Chapters 2, 3, and 4).

Drought is a major abiotic factor caused by water deficiency or insufficient access to water (Eldem et al., 2012). Terminal drought conditions, when there is a progressive depletion of the soil's water, will cause premature tree death. Intermittent drought conditions will affect trees growth and development, but generally do not lead to tree death. Under these conditions, trees will have subsistence yields, which are much lower than yields of irrigated trees (Bhargava and Sawant, 2013). To overcome environmental stresses, trees acclimate by inducing expression or repression of a series of target genes (Xiao et al., 2006). This differential gene expression triggers changes in a variety of mechanisms at molecular and morphological levels, aiming to allow the tree to withstand stresses and remain alive (Eldem et al., 2012). Chaves et al. (2003) described several plant responses to drought stress, e.g.: improved mobilization of

reserves for fruit production, minimized water loss by closing stomata, improved water uptake by increased root production, osmotic adjustment to maintain cell turgor, and morphological or molecular changes to avoid/dissipate excess light.

In peaches, some research has been done in the past to understand molecular mechanisms and the plant's responses to drought stress. Further, research was done to identify peach genotypes with improved drought tolerance (Bielsa et al., 2018; Jiménez et al., 2013; Ksouri et al., 2016). These studies generated basic information about how peaches are physiologically affected by drought stress and how specific genes have differential gene regulation. This information can be utilized in breeding programs to develop drought tolerant cultivars.

In peaches, Haider et al. (2018) reported upregulation of genes related to superoxide dismutase (*SOD*), catalase (*CAT*), peroxidase (*POD*), ascorbate peroxidase (*APX)*, pheophorbide a oxygenase (*PAO*), *WRKY* transcription factors (TF), and dehydration-responsive element binding proteins (*DREBs*) when potted-trees suffered severe drought stress. Some of those genes (*SOD*, *CAT*, *POD*, and *APX*) are related to reactive oxygen species (ROS) that are overproduced by trees under stress. Other (*PAO*, *WRKY* TF, and *DREBs*), related to defense responses to drought.

Ksouri et al. (2016) performed high-throughput sequencing (RNA-seq) to measure transcriptome changes in peach roots and leaves affected by drought stress. They reported subsets of 500 differentially expressed genes (DEGs) in roots and 236 in leaves associated with 99 metabolic pathways. Most of the receptor kinases and transcription factors found in the roots were strongly downregulated under drought stress. Calcium uniporters, protein kinases and phospholipases, DEGs associated with

electron transporters, and the ABA synthesis pathway, were upregulated under drought stress. Other hormonal pathways were generally downregulated under drought stress.

The expression of genes related to osmolyte accumulation was studied by Jiménez et al. (2013). The authors reported that drought stress can upregulate the expression of raffinose synthase *(SIP1)* in leaves and roots of peach trees. However, ∆-1-pyrroline-carboxyllate synthase *(P5SC)* expression was not altered in leaves but was overexpressed in roots under drought stress. On the other hand, sorbitol 6-phosphate dehydrogenase *(S6PDH)* expression was generally not changed under drought stress. Artlip and Wisniewski (1997) conducted research testing a dehydrin gene, which is associated with cellular dehydration and can be affected by drought or low temperatures. They reported greater expression of *ppdhn*1 dehydrin gene in leaves, bark, and xylem of peach trees subjected to water deficit compared to well-watered control.

Another important topic that has received increased attention lately is the effects of over-fertilization on plants and the environment (Albornoz, 2016). In the southeastern United States, peach growers have been following the same fertilizer recommendations (Horton et al., 2015) for decades. Overfertilization can lead to excessive vegetative growth creating shade to fruiting wood, indirectly affecting flowering, fruit set, fruit growth, and ripening. Further, pathological and physiological disorders, insect pests, and diseases can be affected by overfertilization (Weinbaum et al., 1992). Little work has been done to assess the differential gene expression of fruit trees in response to different fertilizer treatments. Zhang et al. (2016) investigated nitrogen metabolism associated genes responses to foliar application of 0.5% (w/v) urea in peach trees.

They reported that glutamine synthase (*GS*) and nitrite reductase (*NiR*) genes had reduced expression after application. However, glutamate dehydrogenase (*GDH*), asparagine synthetase (*AS*), and nitrate reductase (*NR*) genes had increased expression after foliar urea application. Working with citrus trees, Liao et al. (2019) tested the effect of five different nitrogen (N) levels on the relative expression of the same five genes (*GS*, *NiR*, *GDH*, *AS*, and *NR*) than Zhang et al. (2016). Further, they tested the expression of these genes from 60 to 360 days after anthesis. For all of the genes, the most relevant changes in expression happened 120 days after anthesis. Generally, the lowest and highest N levels had lower gene expression in comparison with the three middle N levels, which had the highest gene expression.

Although there are studies evaluating how drought stress affects the gene expression of peach trees, most studies were performed in potted trees grown in greenhouse conditions. None of the studies were carried out in field conditions in the southeastern United States. Further, very limited information is available about how different fertilizer levels affect expression of nitrogen metabolism related genes of peach trees. By conducting research with irrigation levels and systems, and fertilizer levels for young peach trees established in an orchard in Georgia, we can generate information to help filling those voids.

The specific objective of this research was to test the expression of genes associated with drought stress and nitrogen metabolism in field-grown peach trees with different irrigation levels (irrigated vs. non-irrigated trees), different irrigation systems (drip- vs. micro-sprinkler-irrigated trees), and four fertilizer levels (25%, 50%, 100%, and 200%). The hypotheses were that: 1) non-irrigated trees (affected by drought stress)

would have increased activity of genes related to osmoregulation, ROS production, and ABA synthesis in comparison with irrigated trees (supplemental irrigation), and 2) genes related to nitrogen metabolism would have low activity under low fertilization levels in comparisons with higher fertilizer levels.

Material and methods

The experiment was conducted during the 2016 season. The precipitation was below historical normal from Mar. until Aug. 2016 (Table 2.1). Drought stress was present in our orchard, in particular in non-irrigated trees in comparison with irrigated trees (supplemental irrigation). Canopy volume and trunk cross-sectional area of non-irrigated trees were negatively affected in comparison with irrigated trees, as reported and discussed on Chapter 2.

Plant material, field characteristics, experimental design, and treatments

The full description of the plant material and field characteristics, and experimental design and treatments are described in Chapter 2. Briefly, scions of 'Julyprince' peach grafted onto 'Guardian™' were planted on 13 Jul. 2015 (358 trees/ha at a spacing of 4.6 m within rows and 6.1 m between rows) at the Dempsey Farm, University of Georgia, Griffin, GA. The soil is a Cecil sandy loam, slope of 2-6%, pH ~5.9, and organic matter ~1.5%. Soil amendments for K, P, and lime (to reach pH 6.0) were made prior planting as recommended (Horton et al., 2015) based on soil samples taken before planting.

The experiment was designed with three main effects: 1) Irrigation levels (irrigated vs. non-irrigated trees); 2) irrigation systems (drip- vs. micro-sprinkler-irrigated trees); and 3) fertilizer levels (25, 50, 100, and 200%). Irrigated trees were either drip- or micro-sprinkler irrigated. Drip-irrigated trees had four emitters (SB-20 Bowsmith; Exeter, CA) placed in a circle at ~45 cm around the trunk, with each emitter delivering 7.6 $L \cdot h^{-1}$, for a total of 30.4 $L \cdot h^{-1}$ per tree. Micro-sprinkler-irrigated trees had one micro-sprinkler (QN-08 Rain Bird; Azusa, CA) located at ~10 cm away from the trunk, delivering 30.4 $L \cdot h^{-1}$ per tree. The irrigation control was done individually for each fertilizer level within each irrigation system (drip or micro-sprinkler). Irrigation volume was measured using flow meters. In 2016, drip irrigation delivered an average of 1,852 L/tree across fertilizer treatments for the three years of experiment; whereas micro-sprinkler irrigation delivered 2,560 L/tree. Non-irrigated trees received water only from rain events.

Four fertilization levels were tested (referred as 25%, 50%, 100%, and 200%). The current recommendation is referred as 100% and is based on Horton et al. (2015). The amount of nitrogen in each level was as follows: 16, 33, 65, and 129 $kg \cdot ha^{-1}$ N for 1-year-old trees; 23, 48, 95, and 191 $kg \cdot ha^{-1}$ N for 2-year-old trees; and 24, 49, 98, and 195 $kg \cdot ha^{-1}$ N for 3-year-old trees.

Data was collected from four replicate trees (n=4) for all 16 treatment combinations: irrigated vs. non-irrigated; drip- vs. micro-sprinkler-irrigated; and fertilizer levels (25, 50, 100, and 200%), totaling 64 data trees.

Measurements of water potential, photosynthetic net assimilation (A_n) and water use efficiency (WUE)

Following the protocols described in Chapter 2, mid-day leaf water potential was assessed in July of 2016, and mid-day stem water potential was measured in Aug. of 2016. In June, July, and Aug. 2016, (A_n) and leaf transpiration (E) were measured, and leaf WUE was estimated.

Gene expression

Genes selected for this study are based on previous research in peaches (Bassett et al., 2009; Haider et al., 2018; Jiménez et al., 2013; Wang et al., 2016; Yooyongwech et al., 2008; and Zhang et al., 2016). A list of these genes and their primer sequences and annealing temperatures are presented in Table 5.1. Several genes with possible response to drought stress were selected: *Raffinose synthase (SIP1), Δ-1-pyrroline-carboxylate synthase (P5SC), Magnesium-chelatase subunit H (ChlH), Peroxidase dismutase (POD), Superoxide dismutase (SOD; Cu-Zn), Transcription factor WRKY70 (WRKY70), Dehydrin PpDhn3 (PpDhn3), Aquaporin Pp-δTIP1 (Pp-δTIP1),* and *Putative (+)-abscisic acid 8'-hydroxylase 3 (PpCYP707A3).* Two genes related to nitrogen metabolism were selected: *Glutamate dehydrogenase (GDH)* and *Nitrite reductase (NiR).*

Sample collection and storage

Leaf samples from the actively growing terminal portion of young branches were collected in May 20, Aug. 5, and Sept. 26, 2016. From each data tree, five leaves were collected from different sides of the tree's canopy, put inside 15 mL conical centrifuge

tubes, and immediately flash frozen and stored in liquid nitrogen (LN). After freezing, sample tubes were stored in -80 ºC freezer until RNA extraction.

RNA extraction

RNA was extracted from peach leaves using the Spectrum™ Plant Total RNA Kit (Sigma-Aldrich, St. Louis, MO), following the kit's instructions with the addition of polyvinylpyrrolidone (PVP) as a modification. The detailed protocol is described below. Frozen sample tubes were removed from the -80 ºC freezer and placed in LN for handling during extraction process. A cryo-station (2600 Cryo-station; Spex SamplePrep, Metuchen, NJ) and cryo-blocks (2663 Cryo-block; Spex SamplePrep, Metuchen, NJ) were used during sample preparation and grinding, until tissue lysis. Approximately 100 mg of leaf tissue was taken from the 15 mL sample tubes using a disposable polypropylene spatula and placed inside a 2 mL centrifuge vial with two 4.5 mm metal beads. Samples were ground using a Geno/Grinder (2010 Geno/grinder; Spex SamplePrep, Metuchen, NJ) for 3 min at 1100 rpm. After grinding, a mixture of 700 µL of lysis solution + 7 µL of 2-ME + 14 mg PVP was added to each 2 mL sample vial and thoroughly vortexed. Samples were incubated at 56 ºC for 5 min, and after centrifuged at 21,130 g_n for 5 min (Model 5424; Eppendorf, Hauppauge, NY). The lysate supernatant was transferred to a filtration column seated on a new 2 mL centrifuge tube and centrifuged at 21,130 g_n for 1.5 min. Filtration column was disposed and 750 µL of binding solution was added to the filtered lysate following by a brief vortex at medium speed. The lysate was transferred to a binding column seating on a new 2 mL centrifuge tube and centrifuged at 21,130 g_n for 1 min. The RNA was bound to the binding column and the flow-through liquid was discarded. After, 500 µL the washing

solution #1 was added to the binding column and centrifuged at 21,130 g_n for 1 min, having the flow-through liquid discarded. Then, 500 µL of washing solution #2 was added to the column and centrifuged at 21,130 g_n for 30 s, having the flow-through liquid discarded – this whole step was repeated twice. After discarding all the flow-through liquid, the binding column was centrifuged at 21,130 g_n for 1 min to dry. The binding column was transferred to a new 2 mL centrifuge tube and 50 µL of elution solution was added carefully on the center of the binding column filter. Samples were allowed to sit at room temperature for 1 min before being centrifuged at 21,130 g_n for 1 min to elute. The binding column was disposed, and the purified RNA was in the flow-through eluate inside the 2 mL centrifuge tube.

RNA quantification and quality analysis

Immediately after RNA extraction, the RNA concentration and purity were measured by adding 2 µL of purified RNA in a Nanodrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE). Values of RNA yield and 260/280 nm absorbance ratios were recorded. Purified RNA samples were stored in a -80 ºC freezer until further analysis. To determine the quality of the RNA, RNA was separated on denaturing 1-1.5% agarose gel stained with ethidium bromide, using gel electrophoresis.

DNase treatment and reverse transcription

Genomic DNA contamination was removed by treating 1 µg of total RNA with DNase (RQ1 RNase-free DNase; Promega, Madison, WI), incubating in the thermocycler at 37 ºC for 34 min. Reverse transcription of the DNase-free RNA was done using ImProm-II Reverse Transcriptase (Promega, Madison, WI), Oligo(dT) 18 Primer (Thermo Scientific; Wilmington, DE), and dNTPs (Promega, Madison, WI) in a

20 µL reaction volume. The reverse transcriptase was performed through an incubation at 42 ºC for 75 min followed by another incubation at 75 ºC for 15 min. After, 100 µL of nuclease-free water were added to the samples to make a 6-fold diluted cDNA template solution. cDNA templates were stored in a -20 ºC freezer until further analysis.

Quantitative PCR (qPCR)

All qPCR reactions were performed in a reaction volume of 12 µL total, comprised of 6 µL of PowerUP SYBR Master Mix (Applied Biosystems, Vilnius, Lithuania), 1.5 µL of cDNA template, 3 µL of primer mix [0.2 µM] (IDT Integrated DNA Technologies, Coralville, IA), and 1.5 µL nuclease-free water. Four biological replications (same as the ones used for the water potential measurements) were used. The qPCR reactions were performed using a QuantStudio 3 Real-Time PCR System (Applied Biosystems, Foster City, CA). The qPCR thermal cycling conditions varied among the different genes of interest (GOI) used, because of the different annealing temperatures of each GOI. The list of genes utilized (including the three reference genes), the primers' sequences, and the annealing temperatures are listed on Table 5.1. Thermal conditions were: 50 ºC for 2 min; 95 ºC for 10 min; and 40 cycles of 95 ºC for 40 s + GOI annealing temperature for 40 s + 72 ºC for 1 min. Primer specificity was confirmed by using a melt curve analysis after qPCR, following the thermal conditions of: 95 ºC for 15 s; 60 ºC for 1 min; and 95 ºC for 15 s. For each GOI qPCR run, non-template controls were added to the plate. Fluorescence values from all samples were baseline-corrected and mean PCR efficiency was determined for each gene using LinRegPCR (Ruijter et al., 2009). The next steps followed procedures described by Rieu and Powers (2009). The relative quantity of template in the samples (RQ) was

calculated using the number of cycles at a threshold and the PCR efficiency. Then, the RQ values were normalized to three internal reference genes (*β-actin, KyActin1, and* RNA polymerase II (*RP II*)), providing the normalized RQ (NRQ). Following, the NRQ values where log_2 transformed, and the resulting values were submitted to an analysis of variance followed by means separation. For data presentation, treatments were compared to a calibrator sample (control): irrigated trees (when comparing irrigated vs. non-irrigated); drip irrigated trees (when comparing drip- vs. micro-sprinkler-irrigated trees); and 100% fertilizer (when comparing 25, 50, 100, and 200% fertilizer levels).

Statistical analysis

SAS 9.4 (SAS Institute Inc.; Cary, NC) was used to compare treatment means and interactions, using PROC GLIMMIX. Means were separated using Tukey's Honest Significant Difference test with significance level at $P \leq 0.05$. Result were the average of four biological replicates (n=64). For the gene expression data, interactions between factors are not shown because most were not significant. When present, the interactions did not have biological meaning. Therefore, only main factors are reported.

Results

Volumetric water content (VWC), water potential, A_n, and WUE

Chapter 2 describes the results of VWC, water potential, A_n, and WUE. Briefly, the rain events and the VWC of the different treatments combinations [drip vs. micro-sprinkler irrigation with different fertilizer rates (25, 50, 100, and 200%)] from May to Oct. 2016 were shown on Figure 2.1. Although we did not measure the VWC of the soil

for the non-irrigated trees to compare with the irrigated trees, we measured the leaf and stem water potential, describing the water status of the trees.

Mid-day leaf water potential was measured in July 2016 and mid-day stem water potential was measured in Aug. 2016. For both, non-irrigated trees had lower water potential than irrigated trees ($P < 0.001$) (Table 2.2). No differences were found among fertilizer or irrigation system (drip vs. micro-sprinkler) levels. Further, A_n and WUE were measured in June, July, and Aug. 2016. Over the three months, irrigated trees had almost twice as more A_n than non-irrigated trees ($P < 0.001$) (Table 2.5). Non-irrigated trees had greater WUE than irrigated trees ($P < 0.001$) for the same period. Irrigation systems and fertilizer levels had minor effects on A_n and WUE; however, they were not consistent throughout the period of evaluation (Table 2.5).

Gene expression

Irrigation levels

Genes related to osmoregulation (*SIP1* and *P5SC* are genes that codify enzymes responsible for raffinose and proline biosynthesis) had increased relative expression in non-irrigated trees vs. irrigated trees for most of the evaluation dates (Fig. 5.1A and B). Magnesium-chelatase subunit H *(ChlH)* is a gene that encodes an enzyme (magnesium-protoporphyrin IX), which is responsible for initiating chlorophyll biosynthesis and also mediates ABA signaling in guard cells, by binding ABA. Significant change in relative expression was found only in August, with upregulation in non-irrigated trees (Fig. 5.1C). Genes that play a role scavenging free radicals (*POD* and *SOD; Cu-Zn*) had mixed results. While *POD* was upregulated in non-irrigated trees

(remarkably in August and September), *SOD; Cu-Zn* was upregulated in May and then downregulated in August and September (Fig. 5.1D and E). Transcription factor *WRKY70* belongs to a group of positive regulators of plant tolerance to osmotic stress. *WRKY70* was downregulated in May and August in non-irrigated in comparison to irrigated trees, but similar expression in September (Fig. 5.1F). Dehydrin genes, such as *PpDhn3*, are linked to cold acclimation and other factors related to dehydration, such as salt or drought stress. Similarly, aquaporin genes (such as *Pp-δTIP1*) encode proteins related to water movement across membranes. Dehydrin *PpDhn3* and aquaporin *Pp-δTIP1* had similar relative expression responses. In May, relative expression of *PpDhn3* increased in non-irrigated trees in comparison with irrigated trees, while no differences were found for *Pp-δTIP1*. In August and September, both genes had increased relative expression in irrigated vs. non-irrigated trees (Fig. 5.1G and H). Abscisic acid catabolism increases with overexpression of genes from the CYP707A group (such as *PpCYP707A3*). *PpCYP707A3* was downregulated in May and upregulated in September in non-irrigated vs. irrigated trees (Fig. 5.1I). Genes related to nitrogen metabolism (*GDH* and *NiR*) had distinct responses. In August, *GDH* was downregulated and *NiR* was upregulated in non-irrigated trees in comparison with irrigated trees (Fig. 5.1J and H).

Irrigation systems and fertilizer levels

No major differences were found for most of the genes and evaluation dates when comparing irrigation systems (drip vs. micro-sprinkler) or fertilizer levels (25, 50, 100, and 200%) (Table 5.2). Gene expression was calculated relative to control drip-irrigated trees when comparing drip- vs. micro-sprinkler-irrigated trees, and relative to

control 100% fertilizer level when comparing 25, 50, 100, and 200% fertilizer levels. When present, the differences were not consistent throughout the period of evaluation (results not shown).

In May, *GDH* was downregulated on micro-sprinkler irrigated trees in comparison with drip-irrigated trees (relative expression of 0.73 vs. 1.00, $P = 0.019$) (Table 5.2). In August, micro-sprinkler-irrigated trees had increased *SIP1* gene expression in comparison with drip-irrigated trees (relative expression of 2.13 vs. 1.00, $P = 0.025$) (Table 5.2). In September, micro-sprinkler-irrigated trees had greater gene expression of *P5SC* (relative expression of 1.33 vs. 1.00, $P = 0.009$) and *WRKY70* (relative expression of 1.57 vs. 1.00, $P = 0.001$) in comparison with drip-irrigated trees (Table 5.2). For the comparisons between fertilizer treatments, differences were found among treatments for *PpDhn3* in September ($P < 0.001$) and *NiR* in August ($P = 0.044$). For *PpDhn3*, treatments receiving 25 and 50% fertilizer were upregulated (relative expression of 1.52 and 1.47, respectively) when compared to treatments receiving 100 and 200% fertilizer (relative expression of 1 and 0.99, respectively). For *NiR*, trees receiving 100% fertilizer level had the greatest relative expression (1.00) and trees receiving 25% fertilizer level had the lowest relative expression (0.52). Trees receiving 50 and 200% had intermediate values (0.67 and 0.80, respectively), statistically similar to values of both 100 and 25% fertilizer levels (Table 5.2).

Discussion

Severe drought was reported in 2016 in the southeastern United States (Conrad II and Knox, 2016). When comparing the 2016 total precipitation amount with the

historical normal 1981-2010, a 20% reduction in total rain was recorded. The period from March through August had remarkable below normal precipitation. Further, measurements of leaf and stem water potential were conducted to assess the water status of the trees grown in different irrigation and fertilization treatments, since leaf and stem water potential are related to the soil water content and weather conditions (Mahhou et al., 2005). Our results indicated that non-irrigated trees suffered severe drought stress in the two months we measured the water potential. Irrigated trees had the drought stress intensity alleviated because of the supplemental irrigation applied. These results were corroborated by past research in which was reported an increased water potential on irrigated trees when compared with trees subjected to deficit irrigation regimes (Goldhamer et al., 1999; Mercier et al., 2009). Measurements of A_n and WUE were also conducted to characterize how the different treatments, especially irrigated vs. non-irrigated, affected the tree's carbon assimilation. Irrigated trees had significantly higher A_n than non-irrigated trees. Over the three-month period, A_n rate of irrigated trees was ~70% higher than non-irrigated rates, which demonstrates the severity of the drought stress. Water use efficiency was also affected by the lack of supplemental irrigation, resulting in ~35% higher WUE for non-irrigated trees vs. irrigated trees over the three-month period, agreeing with results reported by Haider et al. (2018). It is important to highlight that the increase in WUE was driven by reduction in E and not by increase in A_n. Reduction in E is one of the first signs of trees suffering drought stress. It is a result of stomata closure, which is directly induced by drought stress through diverse hormonal and metabolic signaling pathways (Agurla et al., 2018). Fertilizer

levels and irrigation systems did not induce constant changes in water potential, A_n, and WUE during the three-months period of the study.

Genes related to osmoregulation (*SIP1* and *P5SC*) were upregulated in non-irrigated trees, especially in May and August, stimulating osmotic adjustment. Months of May through August had very minimal precipitation, likely inducing the differences observed. In September, differences were less pronounced. September samples were taken six days after a major precipitation event, which may have alleviated the drought stress in non-irrigated trees. Osmotic adjustment is an important mechanism to protect plants from drought stress. *SIP1* plays a role in sugars production and *P5SC* is involved in proline biosynthesis. Trees can accumulate osmolytes, such as proline, raffinose, mannitol, sorbitol, carnitine, and fructans to protect the integrity of cell membranes, avoiding disintegration (Jiménez et al., 2013; Mahajan and Tuteja, 2005). The results of *SIP1* agree with (Jiménez et al., 2013), who reported higher relative expression of *SIP1* in leaves and roots of drought-stressed (non-irrigated) peach trees. In the same research, the authors reported no differences in *P5SC* gene expression in leaves between irrigated and non-irrigated trees. However, significant differences were found in roots. One possible explanation for the diverging results of *P5SC* in leaves between our results and those from Jiménez et al. (2013) is likely the duration of the study/drought stress. While our trees were suffering some sort of drought stress from March until the sampling dates, Jiménez et al. (2013) exposed young peach trees to decreasing daily soil VWC for 16 days only, starting with fully irrigated trees. Therefore, the short exposure to drought likely was not enough to trigger overexpression of *P5SC* in leaves, differently than roots, which are the first organs to sense and respond to drought stress.

Magnesium (Mg)-chelatase is an enzyme complex composed of three subunits: CHL I, CHL D, and CHL H (Rissler et al., 2002). One of the enzyme complex's functions is to chelate Mg into protoporphyrin IX, resulting in magnesium-protoporphyrin IX, which is responsible for the biosynthesis of chlorophylls *a* and *b* (Jia et al., 2011). Haider et al. (2018) investigated the effects of mild and severe drought stress in Mg-chelatase subunit H (*ChlH*) gene of peach leaves. They reported that mild stress did not affect the expression of *ChlH*; however, severe stress downregulated the activity of *ChlH.* Our results are not consistent with those from Haider et al. (2018). In May and September, the gene expression was not different between irrigated vs. non-irrigated trees. In contrast, *ChlH* gene expression was upregulated in non-irrigated vs. irrigated trees in August. One possible explanation for these differences is that magnesium-protoporphyrin IX can bind ABA, acting on a signal transduction pathway of ABA in guard cells, increasing ABA activity. Because of this dual-function of magnesium-protoporphyrin IX, the response in relative expression of the *ChlH* gene in our research might not have been associated with chlorophyll *a* and *b* biosynthesis. Rather, it was likely associated with ABA signaling, since the chlorophyll biosynthesis and ABA signaling processes may be distinct (Du et al., 2012). As previously discussed, reductions in A_n and increases in WUE in non-irrigated vs. irrigated trees were reported in our research. This indicates that reductions in gas exchanges occurred because of stomatal closure, which is a result of the effect of ABA in guard cells.

Still related to ABA metabolism, we reported a small decrease in relative expression of *PpCYP707A3* in May and a small increase in September in non-irrigated vs. irrigated tress. *PpCYP707A3* is responsible for ABA catabolism (Wang et al., 2016).

The onset of drought was in May and non-irrigated trees may have responded by decreasing gene expression of *PpCYP707A3*. This may result in higher quantities of ABA to trigger stomata closure. In contrast, in September, *PpCYP707A3* was overexpressed because trees had received a significant amount of water through rain; therefore, trees were likely trying to increase ABA catabolism to avoid stomata closure.

Transcription factor (TF) families are involved in the trees' perception of environmental cues, leading to transcriptional reprograming and tree adaptation. *WRKY70* is a key component working in the salicylic and jasmonic acids pathways, enhancing the tolerance to osmotic stress (Li et al., 2013). A small decrease in *WRKY70* relative expression was observed in May and a decrease in August when comparing non-irrigated vs. irrigated trees. Our results diverge from Haider et al. (2018), who reported upregulation of this gene under mild and severe stress. The divergent results could be because of the different lengths of drought stress between the two studies. Haider et al. (2018) exposed the plants to up to six days of drought stress and measured the expression of *WRKY70*, while our research exposed the plants to four to five months of drought stress and measured the activity months after the drought stress onset. Li et al. (2013) discusses how *WRKY70* is only involved in early responses to osmotic stress, trying to suppress the ABA effects in stomata closure. However, as the drought stress continues over time, *WRKY70* relative expression seems to be suppressed, allowing for ABA-responsive genes to act, inducing stomata closure. Shang et al. (2010) reported that the protein ChlH interacts with a group of WRKY TFs, repressing their activity to allow for ABA responses. This research makes an important

bridge connecting our results of increased *ChlH* relative expression in August and decreased *WRKY70* relative expression in the same period.

Reactive oxygen species (e.g. H_2O_2 and O_2^-) are produced by plants in response to diverse stresses, causing cell damage/loss. Trees can deploy mechanisms to alleviate the damage through scavenger enzymes, which will detoxify the ROS (Bhargava and Sawant, 2013). We investigated the responses of two scavenger genes, *POD* and *SOD; Cu-Zn,* and how they responded in irrigated vs. non-irrigated peach trees. While *POD* had increased relative expression in non-irrigated vs. irrigated trees from May through September, *SOD; Cu-Zn* had a slightly increased relative expression in May and then decreased in August and September. The results for *POD* agree with Haider et al. (2018); however, the results for *SOD; Cu-Zn* are divergent. Although our results were different from Haider et al. (2018), they match reports of Eldem et al. (2012). The authors reported that under drought stress, a miRNA (miR398) that regulates the expression level of two *SODs; Cu-Zn* was downregulated in peaches. In the same research, the authors discuss how *SOD; Cu-Zn* genes can be upregulated or downregulated, depending on the species. *SOD; Cu-Zn* is involved in the first line of defense against ROS (Alscher et al., 2002), and are responsible for the first steps in ROS detoxification (Blokhina et al., 2003). This possibly explains why there was an increase in relative expression in May (early stress) in our research. At the same time (May), *POD* relative expression in non-irrigated trees in comparison with irrigated trees was slightly higher. Over time, *POD* relative expression in non-irrigated trees increased significantly, likely suppressing the *SOD; Cu-Zn* activity. *PODs* are responsible for ROS detoxification subsequently *SODs* on their metabolic pathway (Blokhina et al., 2003).

This suggests that peach trees can fine tune the scavenger enzymes activities accordingly to the detoxification needs, keeping an equilibrium. Alscher et al. (2002) states that ROS are produced even when trees are not under stress, and for this specific situation, the ROS production and detoxification are in balance.

Genes related to dehydration and aquaporins (*PpDhn3* and *Pp-δTIP1*), which are related to water movement across membranes, had similar patterns of relative expression when comparing non-irrigated with irrigated trees. *PpDhn3* encodes proteins that are responsible for membranes, enzymes, and nucleotides stabilization when plants are suffering abiotic stress (Yu et al., 2018). Aquaporins are water channel proteins responsible for water movement across the cell membrane (Yooyongwech et al., 2008). In May, *PpDhn3* was slightly overexpressed in non-irrigated trees, likely because trees were still transitioning from a period of available water (April) to a dry period (May). Artlip and Wisniewski (1997) found that desiccation of tissues increased the accumulation of *PpDhn1* in peach leaves, stating that the synthesis of dehydrin is tightly associated with the onset of stress. After months under drought stress (August and September), the non-irrigated peach trees displayed downregulation of *PpDhn3,* likely because the stress had severely affected the trees. Further, different dehydrins may have different responses at different time intervals, acting in distinct stages of the drought stress (Yu et al., 2018). Downregulation of *Pp-δTIP1* in non-irrigated trees was also found in August and September, agreeing with results of *TIP* aquaporins reported by Afzal et al. (2016) in *Vitis sp.*, Ksouri et al. (2016) in peaches, and Pawłowicz et al. (2017) in *Festuca sp.* This reduces the water movement across membranes. Aquaporins have reduced expression under drought stress because they are highly

correlated with stomatal conductance. When stomata are closed, water movement in the soil-plant-atmosphere continuum is reduced, to conserve water in the cells instead of lose water to the atmosphere. This is a mechanism for co-regulation of photosynthesis and water loss at cellular level (Pou et al., 2013). Although a significant precipitation event occurred six days before the measurements in September, the *Pp-δTIP1* relative expression remained lower in non-irrigated vs. irrigated trees, agreeing with Pou et al. (2013) on *Vitis sp*. They reported that *TIP1* relative expression in leaves of *Vitis sp.* trees submitted to drought and then re-watering, remained lower even after 7 days of re-watering.

Nitrogen assimilation by trees is mediated by a series of enzymes that enable the different N forms to be taken up, e.g.: nitrate reductase (NR), nitrite reductase (NiR), glutamine synthetase (GS), and glutamate dehydrogenase (GDH). Each enzyme is encoded by a different gene of similar name. Our results showed that gene expression related to N metabolism is rather stable during drought stress and had similar trends in May and September (no differences between treatments), but opposite responses in August (while *GDH* was downregulated in non-irrigated vs. irrigated trees, *NiR* was upregulated). GDH catalyzes the reversible reaction occurring between glutamate and 2-oxoglutarate + ammonium. *GDH* gene was reported to be downregulated when *Brassica juncea* were exposed to osmotic stress (Goel and Singh, 2015). Working with pakchoi, Xiong et al. (2018) reported that drought caused an increase in NH_4^+ accumulation, because of an increase in NR activity and decrease in GS activity. They did not measure the activity of NiR; however, it is expected that the activity would have increased as well since they are part of the same pathway. These findings corroborate

our results of upregulation of *NiR* in non-irrigated vs. irrigated trees. By having a source of N intermediates to generate amino acids, plants under drought stress can utilize these amino acids for osmotic regulation, reducing dehydration (Xiong et al., 2018).

Fertilizer levels induced changes in *NiR* relative expression. The highest relative expression was for trees grown with 100% fertilizer level, decreasing towards the lowest (25%) and highest (200%) fertilizer levels. This result agrees with Liao et al. (2019) who reported quadratic-style responses among different nitrogen rates in citrus leaves when testing the relative expression of *AS*, *GS*, *NR*, *NiR*, and *GDH*. They concluded that increased N fertilization enhances the activity of N metabolism genes; however, excessive fertilization acts an inhibitory factor on the same genes, similar to our results on *NiR.* Irrigation systems did not have impact on gene expression of trees likely because both systems kept the VWC of the soil around the established values (as described on the materials and methods section), resulting in similar water potentials of the plants.

Conclusions

The drought stress reported in 2016 greatly affected the relative expression of the genes tested in young peach trees. On the other hand, irrigation systems and fertilizer levels had none to minor effects on the same genes. While genes related to ABA biosynthesis were upregulated in non-irrigated trees, trees activated mechanisms of self-protection against drought stress, such as osmoregulation, scavenging of ROS, and dehydration avoidance. This research is one of the first reports of differential gene expression in field-grown young peach trees affected by drought stress for an extended

period. Most previous research was conducted in greenhouse and for very brief periods. Negative physiological responses associated with the lack of supplemental irrigation since establishment for peach trees were reported in previous chapters. This differential gene expression study gives us an insight in the biological reasons behind the physiological responses.

Literature cited

Afzal, Z., T.C. Howton, Y. Sun, and M.S. Mukhtar. 2016. The roles of aquaporins in plant stress responses. J. Dev. Biol. 4:1–22.

Agurla, S., S. Gahir, S. Munemasa, Y. Murata, and A.S. Raghavendra. 2018. Mechanism of stomatal closure in plants exposed to drought and cold stress, p. 289–320. In: M. Iwaya-Inoue, M. Sakurai, and M. Uemura (eds.). Survival Strategies in extreme cold and desiccation. Advances in experimental medicine and biology. Springer, Singapore.

Albornoz, F. 2016. Crop responses to nitrogen overfertilization: A review. Sci. Hort. 205:79–83.

Alscher, R.G., N. Erturk, and L.S. Heath. 2002. Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. J. Exp. Bot. 53:1331–1341.

Artlip, T., M. Wisniewski, 1997. Tissue-specific expression of a dehydrin gene in one-year-old 'Rio Oso Gem' peach trees. J. Amer. Soc. Hort. Sci. 122:784–787.

Bassett, C.L., M.E. Wisniewski, T.S. Artlip, G. Richart, J.L. Norelli, and R.E. Farrell. 2009. Comparative expression and transcript initiation of three peach dehydrin genes. Planta 230:107–118.

Bhargava, S., K. Sawant, 2013. Drought stress adaptation: Metabolic adjustment and regulation of gene expression. Plant Breeding 132:21–32.

Bielsa, B., S. Hewitt, S. Reyes-Chin-Wo, A. Dhingra, and M.J. Rubio-Cabetas. 2018. Identification of water use efficiency related genes in 'Garnem' almond-peach rootstock using time-course transcriptome analysis. PLoS One 13:1–24.

Blokhina, O., E. Virolainen, and K. V. Fagerstedt. 2003. Antioxidants, oxidative damage and oxygen deprivation stress: A review. Annu. Bot. 91:179–194.

Chaves, M.M., J.P. Maroco, and J.S. Pereira. 2003. Understanding plant responses to drought - from genes to the whole plant. Functional Plant Biol. 30:239–264.

Conrad II, C.E., and P. Knox. 2016. The southeastern drought and wildfires of 2016, National Oceanic and Atmospheric Association Southeast Regional Climate Center. Chapel Hill, NC

Crouch, J. 2017. Bad news for Southeast peaches: Something freezing this way came. 7 July 2019. <https://www.climate.gov/news-features/blogs/beyond-data/bad-news-southeast-peaches-something-freezing-way-came>.

Du, S.-Y., X.-F. Zhang, Z. Lu, Q. Xin, Z. Wu, T. Jiang, Y. Lu, X.-F. Wang, and D.P. Zhang. 2012. Roles of the different components of magnesium chelatase in abscisic acid signal transduction. Plant Mol. Biol. 80:519–537.

Eldem, V., U. Çelikkol Akçay, E. Ozhuner, Y. Bakır, S. Uranbey, and T. Unver. 2012. Genome-wide identification of miRNAs responsive to drought in peach (*Prunus persica*) by high-throughput deep sequencing. PLoS One 7:e50298.

Goel, P. and A.K. Singh. 2015. Abiotic stresses downregulate key genes involved in nitrogen uptake and assimilation in *Brassica juncea* L. PLoS One 10:e0143645.

Goldhamer, D.A., E. Fereres, M. Mata, J. Girona, and M. Cohen. 1999. Sensitivity of continuous and discrete plant and soil water status monitoring in peach trees subjected to deficit irrigation. J. Amer. Soc. Hort. Sci. 124:437–444.

Haider, M.S., M.M. Kurjogi, M. Khalil-ur-Rehman, T. Pervez, J. Songtao, M. Fiaz, S. Jogaiah, C. Wang, and J. Fang. 2018. Drought stress revealed physiological, biochemical and gene-expressional variations in 'Yoshihime' peach (*Prunus Persica* L) cultivar. J. Plant Interactions 13:83–90.

Horton, D., P. Brannen, B. Bellinger, D. Lockwood, and D. Ritchie. 2015. 2015 Southeastern peach, nectarine and plum pest management and culture guide. Uni. Georgia Coop. Ext. Bul. 1171. Athens, GA.

Jia, H.-F., Y.-M. Chai, C.-L. Li, L. Qin, and Y.-Y. Shen. 2011. Cloning and characterization of the H subunit of a magnesium chelatase gene (*PpCHLH*) in Peach. J. Plant Growth Regulat. 30:445–455.

Jiménez, S., J. Dridi, D. Gutiérrez, D. Moret, J.J. Irigoyen, M.A. Moreno, and Y. Gogorcena. 2013. Physiological, biochemical and molecular responses in four Prunus rootstocks submitted to drought stress. Tree Physiol. 33:1061–1075.

Ksouri, N., S. Jiménez, C.E. Wells, B. Contreras-Moreira, and Y. Gogorcena. 2016. Transcriptional responses in root and leaf of *Prunus persica* under drought stress using RNA sequencing. Frontiers Plant Sci. 7:1–19.

Li, J., S. Besseau, P. Törönen, N. Sipari, H. Kollist, L. Holm, and E.T. Palva. 2013. Defense-related transcription factors WRKY70 and WRKY54 modulate osmotic stress tolerance by regulating stomatal aperture in Arabidopsis. New Phytol. 200:457–472.

Liao, L., T. Dong, X. Liu, Z. Dong, X. Qiu, Y. Rong, G. Sun, and Z. Wang. 2019. Effect of nitrogen supply on nitrogen metabolism in the citrus cultivar 'Huangguogan'. PLoS One 14:e0213874.

Mahajan, S. and N. Tuteja. 2005. Cold, salinity and drought stresses: An overview. Archives Biochem. Biophys. 444:139–158.

Mahhou, A., T.M. DeJong, T. Cao, and K.S. Shackel. 2005. Water stress and crop load effects on vegetative and fruit growth of 'Elegant Lady' peach [*Prunus persica* (L.) Batch] trees. Fruits 60:55–68.

Mercier, V., C. Bussi, F. Lescourret, and M. Génard. 2009. Effects of different irrigation regimes applied during the final stage of rapid growth on an early maturing peach cultivar. Irr. Sci. 27:297–306.

Mirás-Avalos, J.M., R. Alcobendas, J.J. Alarcón, P. Valsesia, M. Génard, and E. Nicolás. 2013. Assessment of the water stress effects on peach fruit quality and size using a fruit tree model, QualiTree. Agr. Water Mgt. 128:1–12.

National Drought Mitigation Center, U.S. Department of Agriculture, and National Oceanic and Atmospheric Association. 2019. United States drought monitor. 11 June 2019. <https://droughtmonitor.unl.edu/Data/Timeseries.aspx>.

Pawłowicz, I., M. Rapacz, D. Perlikowski, K. Gondek, and A. Kosmala. 2017. Abiotic stresses influence the transcript abundance of PIP and TIP aquaporins in *Festuca* species. J. Appl. Genet. 58:421–435.

Pou, A., H. Medrano, J. Flexas, and S.D. Tyerman. 2013. A putative role for TIP and PIP aquaporins in dynamics of leaf hydraulic and stomatal conductances in grapevine under water stress and re-watering. Plant, Cell Environ. 36:828–843.

Rahmati, M., G.H. Davarynejad, M. Génard, M. Bannayan, M. Azizi, and G. Vercambre. 2015. Peach water relations, gas exchange, growth and shoot mortality under water deficit in Semi-Arid weather conditions. PLoS One 10:e0120246.

Rieu, I. and S.J. Powers. 2009. Real-time quantitative RT-PCR: Design, calculations, and statistics. Plant Cell 21:1031–1033.

Rissler, H.M., E. Collakova, D. Dellapenna, J. Whelan, and B.J. Pogson. 2002. Chlorophyll biosynthesis. Expression of a second *Chl I* gene of magnesium chelatase in Arabidopsis supports only limited chlorophyll synthesis. Plant Physiol. 128:770–779.

Ruijter, J.M., C. Ramakers, W.M.H. Hoogaars, Y. Karlen, O. Bakker, M.J.B. van den hoff, and A.F.M. Moorman. 2009. Amplification efficiency: Linking baseline and bias in the analysis of quantitative PCR data. Nucleic Acids Res. 37:1–12.

Shang, Y., L. Yan, Z.-Q. Liu, Z. Cao, C. Mei, Q. Xin, F.-Q. Wu, X.-F. Wang, S.-Y. Du, T. Jiang, X.-F. Zhang, R. Zhao, H.-L. Sun, R. Liu, Y.-T. Yu, and D.-P. Zhang. 2010. The Mg-chelatase H subunit of Arabidopsis antagonizes a group of WRKY transcription repressors to relieve ABA-responsive genes of inhibition. Plant Cell 22:1909–1935.

Tormann, H. 1986. Canopy temperature as a plant water stress indicator for nectarines. South African J. Plant Soil 3:110–114.

Wang, D., Z. Gao, P. Du, W. Xiao, Q. Tan, X. Chen, L. Li, and D. Gao. 2016. Expression of ABA metabolism-related genes suggests similarities and differences between seed dormancy and bud dormancy of peach (*Prunus persica*). Frontiers Plant Sci. 6:1–17.

Weinbaum, S.A., R.S. Johnson, and T.M. DeJong.1992. Causes and consequences of overfertilization in orchards. HortTechnology 2:112–121.

Xiao, H., M. Siddiqua, S. Braybrook, and A. Nassuth. 2006. Three grape CBF/DREB1 genes respond to low temperature, drought and abscisic acid. Plant, Cell Environ. 29:1410–1421.

Xiong, X., L. Chang, M. Khalid, J. Zhang, and D. Huang. 2018. Alleviation of drought stress by nitrogen application in *Brassica campestris* ssp. *Chinensis* L. Agronomy 8:1–15.

Yooyongwech, S., A.K. Horigane, M. Yoshida, M. Yamaguchi, Y. Sekozawa, S. Sugaya, and H. Gemma. 2008. Changes in aquaporin gene expression and magnetic resonance imaging of water status in peach tree flower buds during dormancy. Physiol. Plant. 134:522–533.

Yu, Z., X. Wang, and L. Zhang. 2018. Structural and functional dynamics of dehydrins: A plant protector protein under abiotic stress. Int. J. Mol. Sci. 19:1–15

Zhang, C.H., B.B. Zhang, M.L. Yu, R.J. Ma, Z.Z. Song, and N.K. Korir. 2016. Isolation, cloning, and expression of five genes related to nitrogen metabolism in peach (*Prunus persica* L. Batsch). J. Hort. Sci. Biotechnol. 91:448–455.

Table 5.1. List of reference genes and genes of interest, primers sequences, and annealing temperatures of the products used in the qPCR analyses, and references where the primer sequences were extracted from.

Gene name	Primer sequence 5'-3' (forward/reverse)	Annealing temperature (ºC)	Reference
β-actin[z]	GTTATTCTTCATCGGCGTCTTCG/ CTTCACCATTCCAGTTCCATTGTC	53-55.9[y]	Wang et al. (2016)
KyActin1[z]	GATTCTGGTGATGGTGTGAGT/ GACAATTTCCCGTTCAGCAGT	55	Haider et al. (2018)
RNA polymerase II *(RP II)*[z]	TGAAGCATACACCTATGATGATGAAG/ CTTTGACAGCACCAGTAGATTCC	55	Zhang et al. (2016)
Raffinose synthase (*SIP1*)	GGTGCCATCCAGTCCTTTGT/ TGCCCTCAATCCTGCAACTT	57.2	Jiménez et al. (2013)
Δ-1-pyrroline-carboxylate synthase *(P5SC)*	CGAATTGCTGTGGATGCAAAAGT/ GCGAAGGTCAACCACAAGATCA	56.7	Jiménez et al. (2013)
Magnesium-chelatase subunit H *(ChlH)*	GGGCAATCAGATGGGGTGAA/ TCTTGCAGAACCGCGAAGAT	56.9	Haider et al. (2018)

Peroxidase dismutase *(POD)*	TCCAGGGTTGTGATGGTTCG/ AGACAACACCAGGGCAAACA	57.3	Haider et al. (2018)
Superoxide dismutase *(SOD; Cu-Zn)*	GGGTCGTCACCTTAAGCCAA/ ACCCGCATGACGGATTTCAT	57.2	Haider et al. (2018)
Transcription factor WRKY70 *(WRKY70)*	TCCCATTCGCATTGTGACGA/ CATACTTGCGTGTGCATCGG	56.8	Haider et al. (2018)
Dehydrin PpDhn3 *(PpDhn3)*	TCGGGGCTTGTTTGATTTCCTG/ TGGGCTCGCAACCTACACCTG	58-60[y]	Bassett et al. (2009)
Aquaporin Pp-δTIP1 *(Pp-δTIP1)*	CTCTTGGTGGCCAAATCACT/ TCCTTGAATGGCTCCAACTC	55	Yooyongwech et al. (2008)
Putative (+)-abscisic acid 8′-hydroxylase 3 *(PpCYP707A3)*	TCACCAAGGAGACTACCACAATAGC/ CAAGGAAGCCAACATCAAAGGAGAAC	58.1	Wang et al. (2016)
Glutamate dehydrogenase *(GDH)*	TAGACATTCCAAGCCTACTTAA/ TTTCCCTGTTGATGACACCTC	54.8	Zhang et al. (2016)
Nitrite reductase *(NiR)*	GTGGGAGGCTTCTTTAGT/ TATGCCTAGTTCATCAATCA	51.4	Zhang et al. (2016)

[z] *Internal reference.*

[y] Temperature changed to improve the primer specificity.

Table 5.2. *P* values of the ANOVA comparisons of relative expression between irrigation levels (irrigated vs. non-irrigated), irrigation systems (drip vs. micro-sprinkler), and among fertilizer levels (25, 50, 100, and 200%) for each gene of interest and month of evaluation.

	May	Aug.	Sept.	May	Aug.	Sept.	May	Aug.	Sept.	May	Aug.	Sept.
Treatment	*SIP*			*P5SC*			*ChlH*			*POD*		
Irrigation levels	**<0.001**[z]	**<0.001**	0.412	**<0.001**	**<0.001**	**0.008**	0.635	**<0.001**	0.081	**0.021**	**<0.001**	**<0.001**
Irrigation systems	0.538	**0.025**	0.550	0.177	0.398	**0.009**	0.120	0.696	0.822	0.674	0.418	0.352
Fertilizer levels	0.393	0.083	0.929	0.061	0.373	0.201	0.453	0.867	0.651	0.087	0.337	0.762
	SOD; Cu-Zn			*WRKY70*			*PpDhn3*			*Pp-δTIP1*		
Irrigation levels	**0.005**	**<0.001**	**0.002**	**0.020**	**<0.001**	0.869	**0.001**	**<0.001**	**<0.001**	0.331	**<0.001**	**0.001**
Irrigation systems	0.318	0.286	0.179	0.120	0.655	**0.001**	0.888	0.322	0.646	0.813	0.573	0.624
Fertilizer levels	0.570	0.130	0.107	0.061	0.357	0.108	0.397	0.527	**<0.001**	0.887	0.420	0.169
	PpCYP707A3			*GDH*			*NiR*					
Irrigation levels	**<0.001**	0.302	**0.009**	0.268	**<0.001**	0.957	0.058	**<0.001**	0.107			
Irrigation systems	0.361	0.233	0.131	**0.019**	0.426	0.619	0.618	0.385	0.929			

Fertilizer levels	0.070	0.851	0.822	0.406	0.214	0.086	0.559	**0.044**	0.588

[z] Means were compared by Tukey's honestly significant difference test, $P \leq 0.05$.

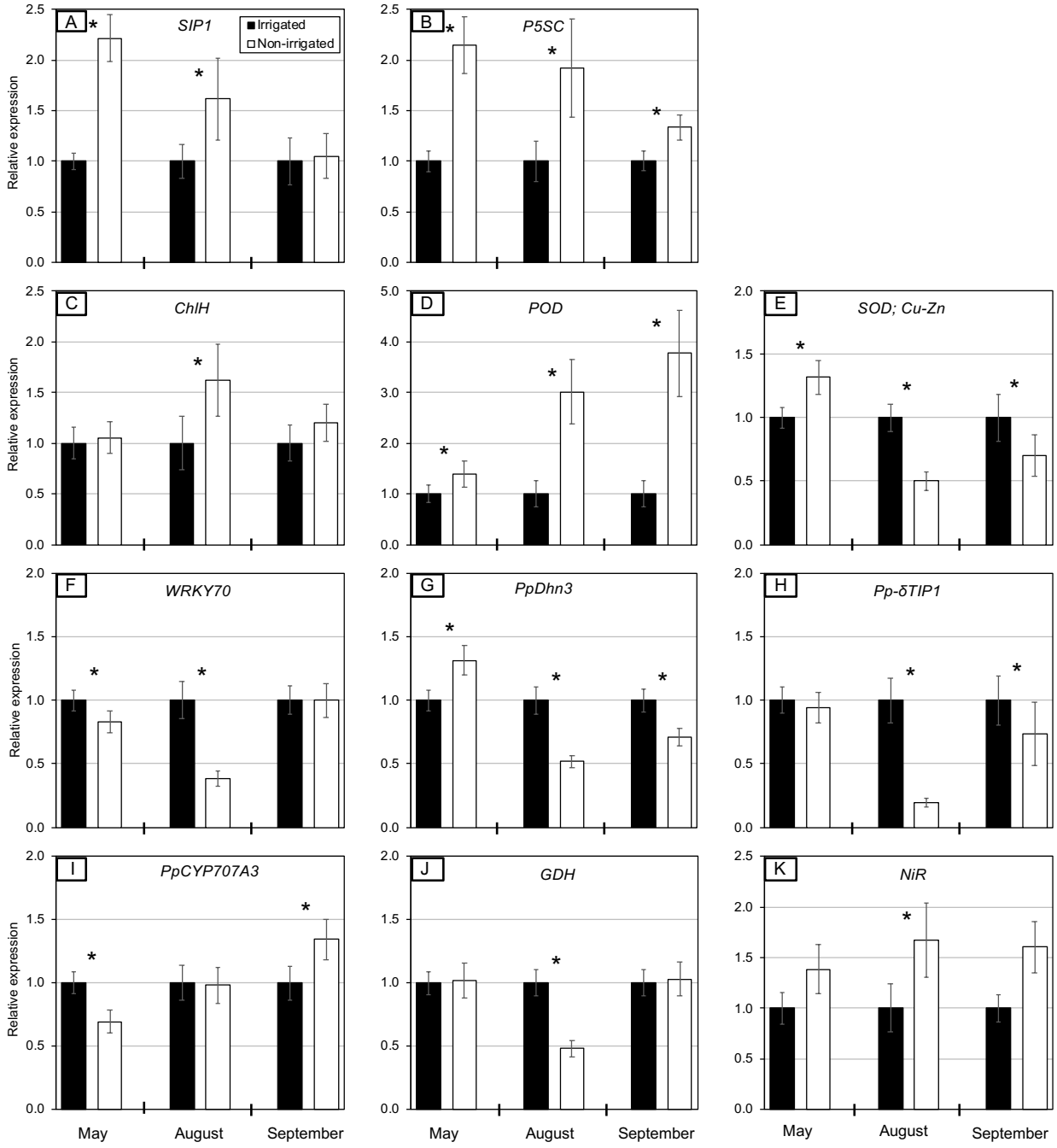

Figure 5.1. Relative expression of *SIP*, *P5SC*, *ChlH*, *POD*, *SOD; Cu-Zn*, *WRKY70*, *PpDhn3*, *Pp-δTIP1*, *PpCYP707A3*, *GDH*, and *NiR* genes from irrigated (black bars) vs. non-irrigated (white bars) trees in three different months. Gene expression is shown relative to control irrigated trees. * Indicates significant differences between irrigated vs.

non-irrigated trees within each month by Tukey's honestly significant difference test, $P \leq 0.05$. Error bars indicate standard error of the means (n=32).

CHAPTER 6

CONCLUSIONS

Droughts are becoming common and are affecting the southeastern United States during the past decades. Young peach trees are exposed to drought during the first three or four years of field establishment. Therefore, irrigation for young trees is becoming important. Greater tree growth and fruit yield can be achieved by irrigating young trees during the first years after field establishment, bringing additional revenue for growers. Drought stress induced the overexpression of genes related to ABA biosynthesis in non-irrigated trees. Concomitantly, non-irrigated trees activated mechanisms of self-protection against drought, such as osmoregulation, scavenging of reactive oxygen species, and dehydration avoidance. There is potential for a decrease in fruit quality by irrigating young trees. However, our results were not consistent and further research with several cultivars can provide a better understanding of how irrigation affects fruit quality.

Irrigation systems (drip or micro-sprinkler) did not induce consistent differences across variables and years of evaluation. However, drip irrigation resulted in ~38% of water savings in comparison with micro-sprinkler irrigation.

Fertilizer levels had little to no effect on tree growth, physiology, fruit quality, nitrogen partitioning, and gene expression. Peach trees receiving reduced levels of fertilizer can still allocate enough assimilates and nitrogen for satisfactory tree growth and development. Reductions in the recommended fertilizer levels may decrease

production costs in an environmentally sound way. The long-term sustainability of such reductions remains to be determined.