The impact of shade netting on the microclimate of a citrus orchard and the tree's physiology

By

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Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the authorship owner thereof and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: December 2018

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Summary

This study aimed to quantify the change in microclimate under shade netting and its effect on the leaf physiology of 'Nadorcott' mandarin (*Citrus reticulata* Blanco) in a winter rainfall production area. The first experiment quantified the change in orchard microclimate. The reduction in solar radiation affected the ambient, soil temperature and the number of hours for specific physiological and phenological temperature ranges under the shade netting. A reduced wind speed under the netting potentially led to less removal of moisture from the air and slightly increased relative humidity leading to a decrease in vapour pressure deficit (VPD) of the air. Therefore, the atmospheric evaporative demand was reduced and increased the volumetric soil water content. The second experiment focused on how the changes in microclimate affected a citrus leaf's physiology. A reduction in VPD, especially in summer, led to increased stomatal conductance and resulted in increased CO_2 assimilation rates of leaves under the shade netting. Therefore, the shade netting did not influence the leaf physiology negatively. Thirdly the total carbohydrate assimilation and distribution of different carbohydrate components, i.e., reducing sugars, polysaccharides, and starch, in leaves and roots were investigated as glucose equivalents. The most notable change under the shade netting in carbohydrate levels was in leaves, with an increased starch content especially noted after harvest. Root carbohydrates showed some differences between treatments, however, these could not only be attributed to the shade net treatment. The fourth experiment investigated how the change in microclimate under shade netting influenced the tree water status or tree water potential as well as fruit and canopy growth. This was accomplished by conducting pre-dawn pressure chamber readings of the main treatments as well as additional irrigation treatments. An increased tree water potential for trees under the shade netting was recorded as well as increased tree canopy volume and final fruit size. It can, therefore, be concluded that 20% white shade netting altered the orchard microclimate without negatively affecting the leaf physiology associated with photosynthesis in 'Nadorcott' mandarin trees. Leaves under the shade netting favoured the production of storage carbohydrates and the reduction in solar radiation and increased soil water content that led to a less water stressed

environment, increasing the tree water potential. Further research should include how the microclimate can be altered in citrus production areas with different climatic conditions in South Africa.

Opsomming

Die gebruik van skadunette in sitrusverbouing is besig om 'n populêre tegnologie te word om die mikroklimaat van 'n boord te verander. Sonstraling is 'n belangrike faktor wat verskeie klimaat parameters asook fisiologiese prosesse in die plant beheer. Die verlaging van sonstraling kan dus die mikroklimaat verander en 'n direkte of indirekte impak maak op die boomfisiologie wat geassosieer is met die fotosintese van 'n sitrusboom. Vier eksperimente was uitgevoer om die moontlike veranderinge in mikroklimaat deur skadunette te dokumenteer en die invloed van dié veranderinge op die fisiologie van 'Nadorcott' mandaryn in 'n winter reënval area te bepaal. Die eerste eksperiment was gemik om die verandering in mikroklimaat onder die skadunet waar te neem. Die opvallende verlaging in sonstraling het veranderinge in lug-, grondtemperatuur asook die hoeveelheid ure van temperatuur reekse vir belangrike fisiologiese en fenologiese prosesse te weeg gebring. 'n Vermindering in windspoed onder die skadunet het gelei tot minder lugvermenging en vogverwydering en kon die verhoging in relatiewe humiditeit (RH) en verlaging in waterdampdruk verskil (WDV) veroorsaak het. Dus is die atmosfeer se aanvraag tot vog verlaag onder die skadunet en dit het daartoe gelei dat 'n verhoogde voginhoud in grond oor die twee seisoene waargeneem was. Die tweede eksperiment was gefokus om die veranderinge van klimaatparameters wat normaalweg as beperkend beskou word vir 'n sitrusblaar se fisiologie te dokumenteer en vas te stel hoe hierdie verandering fotosintese impakteer. Die resultate dui daarop dat die skadunet 'n verlaging in WDV veroorsaak het, veral in die somer maande, wat gelei het tot verhoogde huidmondjiegeleiding en as gevolg daarvan 'n verhoogde CO_2 assimilasie tempo. Die skadunet het dus nie die blaar fisiologie negatief beïnvloed nie. Die totale koolhidraat-assimilasie en verspreiding van reduserende suikers, polisakkariedes en stysel in blare en wortels, was ondersoek. Verhoogde styselvlakke in die blare onder skadunet was veral waarneembaar na-oes. Wortelkoolhidrate het in spesifieke periodes veranderinge getoon, maar dit kon nie alleenlik aan die skadunet behandeling toegeskryf word nie. Laastens was ondersoek hoe die skadunet die boom se waterstatus asook vrug en vegetatiewe groei beïnvloed het deur drukbomlesings voor dagbreek te neem. Die resultate toon dat die bome onder die

skadunet 'n verhoogde, minder negatiewe, stamwaterpotensiaal gehad het. Die blaredak asook die finale vruggrootte onder die skadunet was verhoog. Daar kan dus 'n voorlopige gevolgtrekking gemaak word dat 20% wit skadunet die mikroklimaat van 'n boord verander het met geen negatiewe uitwerking op blaarfisiologie wat met fotosintese van die 'Nadorcott' mandaryn boom verband hou nie. Die blare onder die skadunet het die produksie van reserwe koolhidrate begunstig en die verhoogde voginhoud in die grond dui op 'n laer vogstremming wat die boom se waterpotensiaal verhoog. In verdere navorsing kan verskillende produksie areas en klimaatstoestande waar sitrus in R.S.A verbou word ingesluit om verskille in die fisiologiese respons te bepaal.

This thesis is a compilation of chapters, starting with a literature review, followed by four research papers. Each paper is prepared as a scientific paper for submission to *Southern African Journal for Plant and Soil*. Repetition or duplication between papers might therefore be necessary.

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Chapter 1: General introduction

Citrus is produced in various climatic regions in the northern and southern hemispheres, with different climatic constraints for each production region. With increasing competition in global trade of citrus, citrus producers are using technologies to not only increase yield, but also to increase fruit quality to remain competitive. Shade netting is a technology used in agriculture to protect fruit and trees from adverse and extreme climatic events such as high solar radiation, hailstorms, high wind speeds, and is primarily focused on increasing return of investment (Wachsmann et al., 2014). In addition to these functions, shade netting is used in citrus production to physically prevent bees from cross-pollinating flowers and thereby to produce high-value seedless fruit (Talon et al., 1997). High quality mandarin fruit are produced in the Western Cape Province of South Africa, a region characterised as a Mediterranean-type climate, with warm, dry summers and winter rainfall. These climatic conditions ensure good internal quality as well as good rind colour development of fruit. However, it is unknown to what extent shade netting will impact citrus physiology, growth patterns and fruit quality of 'Nadorcott' mandarin under these conditions.

By reducing solar radiation and acting as a synthetic windbreak, shade netting can result in modifications to the microclimate through changes in air and soil temperature, relative humidity and soil water content. Changes in orchard microclimate have been shown in various horticultural crops and in different climatic regions of the world by using shade netting with different percentile shading and colours (Kalcsits et al., 2017; Lobos et al., 2013; Nicolás et al., 2008; Smit, 2007; Stamps, 2009).

Due to the impact on microclimate, shade netting can potentially be used to improve the photosynthetic rate (Jifon and Syvertsen, 2003) as 30% of full sunlight saturates the photosynthetic apparatus of citrus (Syvertsen, 1984). It could, therefore, be beneficial to reduce solar radiation to reduce photo-inhibition and increase CO_2 assimilation as the result of potentially increasing stomatal conductance during midday depression (Jifon and Syvertsen, 2003).

Citrus' carbohydrate assimilation pattern throughout a year follows climatic and phenological trends. If changes in CO_2 assimilation occur due to shade netting, the carbon allocation and distribution within a tree could potentially be altered and thereby affect tree phenology. This aspect could affect fruit development, fruit size and, potentially, yield. The importance of carbohydrates has been shown in various growth stages in a citrus tree, and it is unknown to what extent the carbohydrate status under shade netting would change throughout a fruiting cycle in different seasons.

With increasing global temperatures and disruptions to normal weather patterns, e.g. drought occurring more often in certain regions, citrus producers are using shade nets to alter not only the above ground microclimate but also the soil environment to increase the water use efficiency in an orchard (Alarcón et al., 2006; Kalcsits et al., 2017). A increase in soil water content accompanied by above ground changes under shade netting could potentially lead to less water-stressed trees and, as a result, increase stomatal conductance throughout the day to improve photosynthesis (Nicolás et al., 2008). Changes in soil water content have been shown to affect the vegetative and reproductive growth of citrus (Ginestar and Castle, 1996). Therefore, if the soil water content is altered by shade netting, it could result in changes in reproductive and vegetative growth.

Therefore, it is necessary to investigate how the commercial use of shade netting would influence a high-value crop such as 'Nadorcott' mandarin. This study was constructed to document changes and to evaluate the microclimate under 20% white shade netting and how these changes affect tree physiology associated with photosynthesis. The following aspects were specifically evaluated to increase the understanding of shade netting in a Mediterranean-type climate:

1.) Quantification of the impact of 20% shade netting on orchard microclimate with regards to solar radiation ($MJ·m^{-2}$), ambient air temperature (°C), soil temperature (°C), relative humidity (%), vapour pressure deficit of the air, wind speed ($m·s^{-1}$) and volumetric soil water content ($m^3·m^{-3}$) over two seasons.

2.) Evaluation of the changes in microclimate mostly affecting leaf physiology associated with CO_2 assimilation throughout a fruiting season.

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3.) Investigation of the carbohydrate accumulation and distribution patterns during key phenological stages of citrus fruit development.

4.) The impact of shade netting and different irrigation volumes on tree water potential and the effect on tree canopy and fruit development.

This study forms part of a larger citrus industry and Department of Trade and Industry funded project in which the impact of shade netting on the phenology of citrus trees, efficacy of plant growth regulators (Brown, 2018), and fruit quality (Botes, 2018) were quantified and documented.

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Chapter 2: Literature review: The role of netting on microclimate and physiology in horticulture focused on citrus

1. Introduction

Citrus production occurs in more than 30 countries and is generally located between the 20° and 40° North-South latitudes (Davies, 1997). These latitudes cover most of the sub-tropical regions on the six continents but include large variation in climatic conditions between citrus production regions of the southern and northern hemispheres, with some microclimates being too cold for citrus production.

Global production of citrus in the 2015/2016 season was estimated around 120 million tons (Food and Agriculture organization of United Nations, 2016), with oranges contributing 63% and mandarins 21%. The global export of citrus for the 2015/2016 season was a total of 12 million tons (FAO, 2016). South Africa is the second largest exporter of fresh citrus with a share of 28% of world exports (CGA, 2016; FAO, 2016). Production alternates between the southern and northern hemispheres, resulting in citrus (Citrus spp.) fruit being available throughout the year. The importance of citrus in the global food trade is due to the dietary benefits of supplying sugars, fibre, nutrients, etc. for a balanced diet. The fruit are also a source of ascorbic acid (vitamin C) which is of importance in human health to improve antioxidant properties for protection against chronic diseases (Liu et al., 2012).

2. Citrus climatic requirements

According to Srivastava and Singh (2002) there are three principal requirements for successfully cultivating citrus, i.e. climate, water and fertile soil. Climate is the most important factor controlling tree and fruit growth, yield and fruit quality. The concept of heat units is used in citrus production to calculate the amount of heat the tree receives within a year, and is correlated with tree and fruit growth, fruit quality and in determining possible harvest dates (Davies and Albrigo, 1994). Despite the limitations of climate to cultivate citrus fruit, i.e. climatic areas with an average minimum temperature range smaller than 9 °C, citrus is able to adapt to a wide range of climatic conditions, from tropical to arid desert (Syvertsen and Lyde, 1981 cited in Srivastava and Singh, 2002). Tropical growing regions are situated within the latitudes 23.5° North and South of the equator and have

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average annual temperatures above 18 °C and minimum temperatures at highest elevation rarely falling below 0 °C (Davies and Albrigo, 1994). Due to differences in topography and elevations, microclimates of tropical growing conditions are subdivided into low, middle and high tropics (Davies, 1997). These subdivided regions differ with regards to the climatic conditions such as temperature, rainfall, relative humidity and interception of solar radiation (Davies and Albrigo, 1994).

Production of citrus in sub-tropical regions is a great contributor to global citrus production. Southern Africa has great diversity in climatic regions, ranging from semi-tropical to Mediterranean-type regions with winter rainfall. The production areas in South Africa have six main climatic production categories and are sub-divided into 40 sub-regions which are classified by the prevailing temperatures of the region (Barry et al., 2011). The differences in climatic conditions determine the cultivars suited to an area.

Sub-tropical climates can be further divided into categories of semi-arid and humid conditions, with South Africa being characterised as a semi-arid climate (Davies and Albrigo, 1994). Semi-arid is sometimes described as a Mediterranean-type climate with a distinct dry, warm summer and winter rainfall. This type of climate with warm summers and cool winters results in good colour development and production of high quality fruit. Gradual change in climates due to global warming, higher temperatures, ambient CO_2 concentrations and changing precipitation patterns are thought to occur in some areas (Res et al., 1998). In Mediterranean-type climates of citrus production in the Western Cape Province of South Africa, such as Citrusdal, Clanwilliam and Sandveld, as well as increases in temperatures and changes in winter rainfall patterns could lead to stressed conditions and thus lower the quality of fruit (McCaskill et al., 2016). With changes in climatic conditions and an increase in global citrus production, the demand for high quality citrus fruit is increasing. Therefore, a solution is needed to produce high quality fruit. An option to reduce the risk of loss in production quality is to develop tolerant conditions, valid as a long-term solution. Shade netting is one technology used to protect the fruit against increasing temperatures by reducing direct solar radiation on fruit (Racsko and Schrader, 2012).

However, the use of shade netting on commercial field-grown citrus is relatively new compared to other horticultural crops. Thus, there is a lack of understanding of the direct effect of shade netting on the orchard microclimate and how it affects a citrus tree's physiology and fruit growth due to the possible change in microclimate. In-depth research on a large scale is, therefore, needed to determine if shade netting has a positive or negative impact in citrus in terms of sustainable production and export of fruit.

3. Citrus phenology

The genus Citrus belongs to the Rutaceae family and is characterised by evergreen trees with their evolutionary origin being a complex taxonomy due to hybridization; therefore, it is suggested that true citrus fruit originated from tropical and semi-tropical regions of south-east Asia (Goldschmidt and Koch, 1996; Spiegel-Roy and Goldschmidt, 1994). One of the main climatic considerations in deciding if a region is suitable for citrus production is the prevalence of low temperatures as the citrus tree and fruit are sensitive to frost damage. Freezing temperatures $< 2\ °C$ not only affect the fruit's internal and external quality, but also the tree's vegetative and reproductive balance. Climatic requirements for citrus reproductive and vegetative phenology are important to understand in order to know how possible change in microclimate can affect not only the physiology of a tree but also the phenology as well. Changes in temperature range requirements and the amount of time at that temperature range during critical phenological stages can change the physiology of a citrus tree grown under shade netting.

3.1 Vegetative development

The optimum temperature range for growth is 23 to 34 °C, minimum 12.5 – 13 °C, while maximum temperatures of $\geq 37\ °C$ limits vegetative growth (Mendel, 1969). Citrus trees undergo periods of growth, called flushes of vegetative, reproductive and root growth cycles under control of environmental and endogenous factors (Bevington and Castle, 1985). Vegetative growth of citrus occurs in a number of flushes throughout the growing season with the number of flushes varying from two to five depending on the climate and water availability (Mendel, 1969). In tropical conditions,

there is no definite growth flush period and growth flushes continue throughout the year, compared to trees grown in sub-tropical conditions where a rest period or type of endodormancy occurs when growth stops due to cold temperatures or water stress. The spring shoots emerge after a period of cold (winter) and are short with many buds sprouting in contrast to summer flushes that have fewer buds sprouting but the shoot growth is longer due to the warmer prevailing temperatures (Mendel, 1969). More flushes occur under warm temperatures that are favourable for growth, such as normally found in warm subtropical regions of the world, i.e. Florida and Brazil (Spiegel-Roy and Goldschmidt, 1994). The growth rate of citrus shoots is primarily influenced by temperatures above the biological zero, i.e. 13 °C (Mendel, 1969).

3.2 Root development and growth

Root growth and development is periodic and alternates with shoot growth. The timing and intensity of root growth are is determined by soil temperature and water content, as well as endogenous factors such as carbohydrates, nutrient competition between plant organs and also hormones. Root growth and development occurs at soil temperatures above 13 °C but is restricted in functionality and growth rate at temperatures below 22 °C. Optimum functionality and growth is at 26 °C, but is restricted at temperatures above 36 °C (Bevington and Castle, 1985). When temperatures are above 22 °C a positive correlation exists between root growth and soil temperature, if there is adequate soil water availability. The alternating pattern of root/shoot growth in a season is due to competition for nutrients and carbohydrates in a plant. Young growing shoots are stronger sinks for assimilates and nutrients and cause roots to cease growing (Bevington and Castle, 1985; Syvertsen, 1994). Hormones influence the alternating pattern of root growth due to the production of auxins in young growing meristems, during flushes, being transported basipetally to the roots and inhibiting further growth (Monselise, 1947; Taiz et al., 2015). Root growth could potentially take place continuously throughout the year with ideal soil temperatures and water availability; however, it is interrupted by above ground shoot growth during successive flushes throughout a season. The

vegetative balance of citrus tree is important because the following year's flowers are initiated on the new shoots.

3.3 Reproductive development

Flower induction in citrus occurs during an induction period, normally triggered by cold temperatures or water stress. Floral induction is the first visible microscopic change within the bud that leads to the development of a reproductive organ (Monselise and Halevy, 1964). Cold temperatures and a period of water stress have the same effect on flowering due to an interruption of growth during the year (Goldschmidt and Koch, 1996). Water stress is normally used to successfully induce flowers in warm winter regions in South Africa. Temperature is the most important factor inducing flower buds. If soil and air temperatures are between 15 °C and 20 °C for a prolonged period (three months), growth of the trees stops and the tree goes into a type of endo-dormancy or quiescence that causes the buds to change from vegetative buds to reproductive buds (García-Luís et al., 1992). Monthly mean air temperatures of below 15 °C will induce flowering and will lead to heavy bloom in spring when soil temperatures rise (Moss, 1976).

Flower differentiation occurs close to the end of the cold period when conditions are favourable for growth. At this time, only morphological differences between vegetative and reproductive buds can be observed (Iglesias et al., 2007). After a citrus tree has experienced a period of cold, buds break in spring in subtropical regions. During bud differentiation, carbohydrate content at the tree level is a prerequisite but is not the only limiting factor controlling this process (Goldschmidt, 1999).

The spring flush, often referred to as the most important flush in terms of fruit set for commercial production, gives rise to both vegetative and reproductive shoots (Spiegel-Roy and Goldschmidt, 1994). Different types of reproductive shoots develop and could be purely reproductive or mixed (reproductive and vegetative). Shoots that are purely reproductive form one or more flowers from axillary buds, i.e. white bloom, and normally sprout first. Green bloom flowers, i.e. leafy inflorescences are on a small shoot with one or more flowers at the tip of shoots or distributed throughout a shoot (Davenport, 1990). Leafy inflorescences have a better chance of setting fruit,

possibly due to leaves that supply photoassimilates and due to enhanced sink strength and the production of gibberellic acid (Goldschmidt and Monselise, 1977; Iglesias et al., 2007).

Between bud break and anthesis, the rate of flower development is related to heat units accumulated throughout the year, with higher prevailing temperatures giving rise to faster accumulation of heat units above the threshold temperatures and faster bloom (Lovat et al., 1984).

3.4 Fruit development

After anthesis, fruit set, fruit growth, and maturation are described in three successive phases of fruit development. After floral formation, fruit set is phase I, which comprises cell division and development of fruitlets (Goldschmidt, 1999). A number of factors influence fruit set with one of the most limiting factors being carbohydrate availability, due to the high demand for energy during flowering and fruit set.

During phase I of fruit development, cell division takes place after anthesis and commences during fruitset (Bain, 1958). The fruitlets start to produce the cellular structures, which mostly comprise of rind and reaches maximum thickness before phase II (Bain, 1958). The fruit enlargement phase II starts after cell division stops and the fruit increases in volume due to water uptake and cell enlargement. During this phase, pulp growth occurs as juice sacs enlarge, but no further cell division occurs. Fruit grow continuously from final fruit set until harvest and the juice segments enlarge with sugar content increasing close to the end of phase II. With the increase of pulp volume, the rind thickness decreases.

During maturation (phase II of fruit development), a change in temperature, especially day-night temperature differences, is very important for colour development in citrus. Warm temperatures tend to produce less carotenoids in the fruit rind and affect the chromoplast conversion of chloroplasts exposing carotenoid pigments, which give the fruit its orange colour. Cool autumn air and soil temperatures promote the onset of colour change in citrus and enhance the development of the desired colour (Young and Erickson, 1961). Cold night temperatures also lower the fruit respiration rate and

lower the amount of sugars being used in the process. This is important for acid and sugar ratios for better quality fruit, especially in mandarins and oranges.

4. Citrus physiology related to photosynthesis and carbohydrate accumulation

4.1 Carbohydrate accumulation

Citrus is an evergreen tree and does not shed leaves at the onset of autumn. This enables citrus leaves to photosynthesize throughout the year if climatic conditions are favourable. However, evergreen trees, including citrus, experience abscission of older leaves, but leaves can stay photosynthetically active for one to three years. The rate of photosynthesis of Citrus is lower than that of deciduous horticultural crops with higher rates of 20-30 ($\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$) (Kriedemann, 1971).

Photosynthesis is a fundamental physiological process occurring in a plant's chloroplast. It enables the plant to convert light energy into chemical energy by using carbon dioxide (CO_2) and water (H_2O) to produce carbohydrates. Carbohydrates formed during photosynthesis are used for energy and/or building blocks for new growth. The main products formed from photosynthesis are sucrose (non-reducing and the main transport sugar), glucose and fructose, the hexose-reducing sugars that are intermediate sugars (Taiz et al., 2015).

The CO_2 assimilation rates in Citrus leaves range from 4 to 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Syvertsen and Lloyd, 1994) and reach the light saturation point at a relatively low irradiance of approximately one-quarter to one-third (30%) of full sunlight, i.e. 600 to 700 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic active radiation (PAR). These levels are normally attained for short periods during the day especially in the outer canopy of the tree. According to Kriedemann (1968), citrus thrives in hot dry conditions, such as found in Citrusdal, South Africa. However, photosynthesis is optimum at relatively low temperatures (25 °C to 30 °C) whereas temperatures higher than 35 °C leads to a reduction in photosynthesis due to a reduction in stomatal conductance.

Under conditions of high irradiance resulting in higher leaf temperatures, a large reduction in photosynthesis can occur due to stomata's high sensitivity to increasing leaf to air vapour pressure deficit (VPD) (Goldschmidt and Koch, 1996). Kriedemann (1968) demonstrated the importance of relative humidity (RH%) and the interplay with high temperatures on photosynthesis. The rate of carbon assimilation declined from increasing temperatures from 21 to 31 °C at a constant RH of 50-60%. However, at 85% RH with temperatures $\geq$ 31 °C, an increase of stomatal conductance occurred due to a lower VPD between leaf and atmosphere, which resulted in a higher CO_2 assimilation rate. Therefore, if the humidity is increased at the same time as temperature, photosynthesis could continue for longer or at a higher rate due to higher stomatal conductance at higher RH.

Citrus is sensitive to soil water deficit and stomatal closure often occurs at physiological midday because of high evaporative demand and the difficulty to supply water to leaves. The closure of stomata in midday leads to a decrease in photosynthesis due to the impairment of gas exchange between the atmosphere and leaf (Spiegel-Roy and Goldschmidt, 1996). Without CO_2 entering the leaf the first enzyme in carboxylation fixation process, ribulose bisphosphate carboxylase (RuBisco), is impaired and no photosynthesis occurs in C3 plants (Taiz et al., 2015). Brakke and Allen (1995) demonstrated the midday depression of citrus seedlings under various climatic effects. The maximum CO_2 assimilation rate was obtained with moderate ambient temperatures and VPD (29 °C /2.4 kPA) as well as soil water content above 50% water requirement. In contrast, the midday depression in the study occurred typically when seedlings were exposed to high temperatures and VPD (37 °C/3.6 kPA) and soil water content below 50%. In addition, the different VPD and temperature regimes showed no midday depression when soil water content was >50% (Brakke and Allen, 1995). Thus, a change in VPD and soil water content have a significant effect on how the stomata function in a given environment and affect gas exchange for photosynthesis.

In addition to the diurnal pattern of photosynthesis within the canopy, differences also occur between the eastern and western side of the tree due to different peaks of PAR throughout the day. The photosynthesis of a grapefruit tree peaked on the eastern side corresponding to the peak of solar

radiation whereas the western side of the tree peaked only later in the day due to an increase in solar radiation. It was concluded that the photosynthetic rate of leaves being exposed to adequate PAR (eastern side) was greater than the leaves on the western side in the afternoon (Fishler, 1985 cited by Spiegel-Roy and Goldschmidt, 1994 p.58).

In sub-tropical growing regions when the winter temperatures drop below the optimum for photosynthesis or when overcast conditions prevail, the photosynthesis rate drops to undetectable levels although evergreen trees can photosynthesize during the winter when conditions are favourable (Syvertsen et al., 1997). This characteristic enables the plant to build carbohydrate reserves for the following spring flush, although at a reduced rate. These low CO_2 assimilation rates could be explained by the reduced activity of Rubisco enzyme thereby decreasing the carboxylation reaction of carbon metabolism (Ribeiro and Machado, 2007). Photosynthesis rates increase in spring when soil and air temperatures increase, normally above a threshold of 20 °C, which coincides with the investment in energy/sink demand needed for new growth.

Table 2 Summary of environmental conditions that influence the rate of photosynthesis in various Citrus ssp.

Citrus Cultivar	Light (PAR/PPFD μmol·m^{-2}·s^{-1})	CO$_2$ (ppm)	Temp. (°C)	VPD (kPa)	Ac (μmolCO$_2$·m^{-2}·s^{-1})	Literature cited
Carrizo citrange	Not given	330	29	2.4	14.5	Brakke and Allen (1995)
Citrus. sinensis	1677	Ambient	35.5	5.3	10.5	Jifon and Syvertsen (2001)
C. paradisi	1594	Ambient	37.5	4.8	10.2	Jifon and Syvertsen (2001)
C. sinensis	1200-1500	±360	27	>2	8-9	Jifon and Syvertsen (2003)
C. paradisi	1200-1500	±360	27	>2	7-8	Jifon and Syvertsen (2003)
C. sinensis	1207	366-383	34	3.81	6.5	Nebauer (2013)
C. sinensis	700-1000	Ambient	26	0.8	11.9	Khairi and Hall (1976)
C. sinensis	700-1000	Ambient	38	4.1	6.9	Khairi and Hall (1976)
Citropis gabunensis Engl Swing	700-1000	Ambient	26	0.8	5.2	Khairi and Hall (1976)

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Citropis gabunensis Engl Swing	700-1000	Ambient	38	4.1	2.2	Khairi and Hall (1976)
Eremocitrus glauco Lindh Sing X C limon L Burm	700-1000	Ambient	26	0.8	10.5	Khairi and Hall (1976)
Eremocitrus glauco Lindh Sing X C limon L Burm	700-1000	Ambient	38	4.1	6.5	Khairi and Hall (1976)
C. limon L Burm. f	700-1000	Ambient	26	0.8	9.6	Khairi and Hall (1976)
C. limon L Burm. f	700-1000	Ambient	38	4.1	5.9	Khairi and Hall (1976)

4.2 Carbohydrate distribution

Sucrose is not only the main product formed by photosynthesis but is also the main form in which carbohydrates are transported in woody trees, including citrus, because of its non-reducing properties (Loescher et al., 1990). Plant organs acting as sinks are interacting factors competing for resources such as carbohydrates, minerals, water, etc. Sucrose is transported from leaves, the source, to plant organs that are metabolically active with a high demand of energy, called sinks. Sink strength is determined by the size of the sink multiplied by the sink activity or metabolic state of the plant organ, with the latter being the deciding factor if the source is non-limiting (Gifford and Evans, 1981). Sink capacity is, therefore, associated with the regulation of dry matter partitioning in the whole tree. Regarding sink strength, seed and fruit are generally the strongest followed by vegetative development (shoots/leaves), root growth and storage of supplied carbohydrates.

Transport of photosynthates from source to sinks is through mass flow in phloem tissue, driven by an osmotically generated pressure gradient between the source and sink (Taiz et al., 2015). Sucrose transport in phloem tissue is not defined by gravity but to sink strength and can be either basipetally, towards the base of the tree, or acropetally, upwards. Kriedemann (1970) used ^{14}C labeled isotopes to determine the pattern of carbohydrate assimilation within a fruiting citrus branch. He concluded that leaves from the previous growth cycle support terminal fruit growth to a lesser extent, whereas the photoassimilates derived from the same season's growth are mainly transported acropetally to terminal fruits. Vegetative laterals do not transport carbohydrates acropetally but rather support fruit growth basipetally. During the growth cycle of a citrus tree, there are phenological stages resulting in changes in allocation pattern in order to maintain a reproductive and structural balance within the tree.

During the spring bloom, photosynthesis is in high demand with the previous season's growth supporting the vegetative and reproductive growth of new flushes (Powell and Krezdorn, 1977). The new vegetative flush at this stage is considered a sink (high respiration rate) for up to three months because its photosynthetic rate is still too low to accommodate the high demand of energy (Syvertsen,

1994). After the leaf has hardened off, it becomes a source of sugars, generally from November onwards under South African conditions.

At the stage when leaves start to export sugars to sinks, the concentration of exportable carbohydrates is high during the day and reserve carbohydrates, i.e. starch, are low. But leaves produce starch during the day in leaf chloroplasts to sustain cell activity at night or during midday depression of photosynthesis when respiration is the main source of energy. Goldschmidt et al. (1991) showed the daily fluctuation of soluble carbohydrates and starch of 'Wilking' mandarin leaf. The soluble sugars content had less fluctuation in diurnal cycles than the starch content of the leaf providing a continuous supply of photosynthates to different sink types. Starch is produced in the leaves during the day and maximum levels are to be found after sunset. At night when photosynthesis is absent, leaves respire and break starch down via mitochondrial respiration. This lowers the starch content in leaves throughout the night until morning when leaves start to photosynthesize and produce photoassimilates.

Annual changes in a Citrus tree's carbohydrate levels are closely correlated with phenological trends and environmental effects (Goldschmidt, 1998). After full bloom and the first vegetative flush, the citrus tree will undergo a root growth flush. The roots become a sink for carbohydrates for a short period and the levels of reducing and non-reducing sugars increase in these organs due to new growth (Goldschmidt and Koch, 1996). In summer when the sink demand is high from developing fruit and roots, starch levels in old leaves decline as starch is mobilized and in the form of sucrose allocated to sinks. At the onset of winter the reduced sink activity, above and below ground, leads to a build-up of sucrose in leaves. Sucrose is then converted to starch, the main storage carbohydrate in citrus and is not readily mobilized (Erickson, 1968). Starch is stored in older leaves and roots throughout autumn and winter, which is used as sources of reserve carbohydrates. These stored carbohydrates are very important to sustain new growth and fruit development at the beginning of a new growth cycle in spring, which then reduces starch in old leaves and roots (Jones and Steinacker, 1951). However, during times of high sink demand for carbohydrates for new growth, some carbohydrates are still

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exported to storage organs indicating the importance of reserve resources for the following growing season (Goldschmidt and Koch, 1996).

Carbohydrate utilization in citrus trees

In spring the tree mobilizes stored carbohydrates, i.e. starch and other non-structural carbohydrates, and utilizes it for new growth as the demand for photosynthates becomes high in developing organs and cannot be met at this stage by the leaves (Syvertsen and Loyd, 1994). Carbohydrates in citrus fruit are very important for two stages of fruit development, viz. fruit set and fruit enlargement, and is referred to as the most limiting factor during these two stages (Goldschmidt, 1998). The first stage of fruit development after anthesis is a period of rapid cell division and an active sink for photosynthates. In carbohydrate deficient trees abscission of developing ovaries and fruitlets occurs after bloom (Goldschmidt, 1999). Fruitlet abscission is said to be a self-thinning mechanism for trees to regulate fruit number to match the bearing potential of the tree (Spiegel-Roy and Goldschmidt, 1996). Iglesias et al. (2003) showed the dependence of carbohydrates during fruit set by defoliating trees, thereby reducing the source and sucrose supplementation to alter the carbohydrate pool. Defoliation of 66% of leaves 35 days after anthesis resulted in increased fruitlet abscission from 26% to 43% and in trees supplemented with sucrose the abscission decreased by 15%.

After fruitset the second phase of fruit development involves cell enlargement resulting in increased fruit size due to an increase in fruit volume. At this stage of fruit development, the amount of fruit that abscise is lower but due to the fruit-to-fruit competition, the demand for assimilates increases. If the crop load on either a tree or single shoot is high, the competition between fruit for assimilates are high and as a result of lower available assimilates smaller fruit will be produced. The effect of reduced sink competition by girdling or defruiting has been shown to increase fruit size (Spiegel-Roy and Goldschmidt, 1996). It is important to note that the control of fruit growth is not only by carbohydrates but also affected by other endogenous and exogenous factors which fall outside the scope of this review. It can, therefore, be said that by altering the environment in which the tree

grows, i.e. to change the photosynthetic response or manipulate the source-sink balance to create increased carbohydrates availability during fruit development, higher production per hectare could be expected.

5. Effect of shade netting on physiology of citrus

5.1 Photosynthesis/CO_2 assimilation

The photosynthesis system of a citrus leaf saturates at relatively low irradiance of about one-quarter to one-third of full sunlight (Kriedemann, 1968; Syvertsen 1984). It can, therefore, be beneficial to place shade netting over citrus trees to lower irradiance and heat load in production areas with high irradiance, such as Citrusdal, South Africa, and potentially increase photosynthesis. Leaves adapt anatomically and physiologically to their light environment. Sun and shaded leaves have different characteristics to optimize photosynthesis (Taiz et al., 2015). Sun acclimated leaves normally have higher CO_2 assimilation at high irradiance, but the quantum efficiency of shaded leaves is greater at lower irradiance. This enables the shaded leaf to photosynthesize more efficiently at lower light levels and accumulate enough carbohydrates to survive. Syvertsen (1984) demonstrated this aspect in potted grapefruit and orange trees. Leaves of trees grown at high light levels had higher CO_2 assimilation rates and the opposite for shaded leaves. But the quantum efficiency of shaded trees were higher for both cultivars. Excess solar radiation creates heat stress and impairs photosynthesis due to stomatal closure, enzymatic impairment and photoinhibition of photosystem II (PSII) in the light reaction (Sinclair, 1982; Taiz et al., 2015). Shade netting reduces light levels and could prevent photosynthesis to be impaired due to excess solar radiation (Jifon and Syvertsen, 2003). Leaf temperatures can be 9 °C higher than ambient temperatures when exposed to high solar radiation (Syvertsen and Albrigo, 1980). By reducing radiation with 50% shade net, midday leaf temperatures were reduced by as much as 8 °C (Jifon and Syvertsen, 2003).

A reduction in the orchard temperature and increased RH would reduce vapour pressure deficit (VPD), decrease the transpiration demand and in return increase CO_2 assimilation rates as the stomata stay open longer during the day. Jifon and Syvertsen (2001) showed a significant increase of

grapefruit and orange leaf photosynthesis during midday by temporary draping trees with 50% shade net. Nicolás et al. (2008) found contrasting results with photosynthesis not being affected by 40% shading. Most research done on citrus photosynthesis was done using high percentile shading (40% to 50%). The reduction of solar radiation is an important factor as it influences various climatic factors which affect physiological processes in the leaves and fruit. In the afternoon or at midday when temperatures are high and RH low, the resulting high VPD will led to stomata closure (Brakke and Allen, 1995). Shade netting could be effective in reducing midday depression seen as stomata close and reducing photosynthesis, due to lower solar radiation and reduced leaf temperatures (Jifon and Syvertsen, 2001; Medina et al., 2002). Photosynthesis impairment due to photoinhibition caused by excess light can be counteracted by the use of shade netting as shown by Jifon and Syvertsen (2003), especially during midday photoinhibition.

5.2 Transpiration

The transpiration stream delivers solutes and water via the xylem to sustain growth of leaves and fruit. In addition, it is involved in phloem loading and unloading for photoassimilates from sources to sinks. Water loss from within a leaf to the atmosphere happens by means of diffusion and is driven by differences in water vapour pressure between the leaf and the atmosphere, i.e. VPD (Taiz et al., 2015). Changes in ambient air and/or leaf temperature and surrounding RH can have a great effect on the VPD between the leaf and atmosphere, the driving force behind transpiration.

Shade netting modifies the orchard microclimate by reducing leaf temperature and thereby reducing the transpiration demand due to lower VPD (Nicolás et al., 2008; Stamps, 1994). In many cases it was found that transpiration rates of citrus leaves under shade nets were not as affected as other physiological parameters (Alarcón et al., 2006; Jifon and Syvertsen, 2003). Nicolás et al. (2008) had contradicting results to the latter authors and found a decrease in transpiration, which was related to a reduction in VPD. Medina et al. (2002) indicated an increase in transpiration rates due to higher stomatal conductance. As discussed earlier, wind speed is reduced by shade netting and causes an increase of RH due to less air mixing resulting in lower VPD. Reduced wind also maintains the

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boundary layer, which is the more humid air adjacent to the leaf, thereby creating a lower VPD gradient around the leaf and thereby reducing transpiration (Taiz et al., 2015). Citrus is very sensitive to very high VPD, which leads to stomatal closure and impairs gas exchange needed for photosynthesis (Spiegel-Roy and Goldschmidt, 1996). Leaf temperature, which is higher than ambient temperatures due to direct solar radiation, creates a large VDP between the leaf and air (Syvertsen and Albrigo, 1980). By placing shade net over the tree, the radiation and leaf temperatures are reduced, thereby decreasing VPD between the air and the leaf. This can result in higher stomatal conductance and yet lower transpiration rates (Alarcón et al., 2006; Jifon and Syvertsen, 2001; Jifon and Syvertsen 2003; Medina et al., 2002; Nicolás et al., 2008).

5.3 Stomatal conductance

The control of citrus stomata, mainly found on the abaxial side (bottom) of a leaf, responds to various environmental stimuli such as heat, water, excess light and changes in humidity (Levy, 1980). Citrus stomatal conductance (g_s) decreases when VPD increases and tree or leaf water potential decreases as a result of stomatal closure (Syvertsen, 1982). The decrease in stomatal conductance automatically lowers the photosynthetic rate, because of a lowered gas exchange - this normally occurs in citrus during the midday depression of photosynthesis (Brakke and Allen, 1995). Therefore, if the change in microclimate under shade netting affects stomatal conductance, a change in photosynthesis is expected.

The modified light regime under netting is expected to increase stomatal conductance and lower the effect of high VPD during midday depression. But it was also noted by Medina et al. (2002) that lower light levels under nets negatively affected stomatal conductance in the morning and also reduced assimilation rates due to limited CO_2 exchange. If transpiration demand is high due to high VPD, the tree cannot supply enough water from the roots via the xylem. As a result of water stress, abscisic acid will be synthesized in roots and transported to the stomata where it triggers stomatal closer (Taiz et al., 2015). Low water supply also triggers stomatal closure due to insufficient transpiration and the plant's adaptive nature to conserve water (Gomes et al., 2004). Lowered radiation

under netting can lower the evaporation from soil and enable the soil water to be more freely available for use and reduce transpiration demand due to increased humidity and lowered VPD. Stomata can then be open for longer and increase net photosynthesis throughout the day. The trend in the use of high percentage aluminized netting for citrus showed a reduced heat stress environment, affected VPD and increased stomatal conductance. This was found in some cases or by some authors who noted an increase only in midday when citrus stomata as a norm shut down (Alarcón et al., 2006; Jifon and Syvertsen, 2001; Jifon and Syvertsen 2003; Medina et al., 2002; Nicolás et al., 2008).

5.4 Water use efficiency

Water use efficiency (WUE) is an important concept in agriculture and indicative of the right crop water requirement to produce a crop. WUE is related to the amount of biomass produced relative to the amount of water consumed (Turner, 2004). Therefore, it is important for the plant to consume water without applying water stress but still produce maximum dry matter of fruit and leaves (Tejero et al., 2011). If the transpiration rate of a citrus tree is lowered and the carbon assimilation stays unaffected, WUE can increase, because less water is used for the same amount of carbohydrates produced. The use of shade netting can reduce transpiration demand, due to a lower VPD, and if photosynthesis is not negatively affected by shading of leaves the crop could potentially have an increased WUE. This was found in studies on citrus where assimilation rates under netting remained unaffected and transpiration was reduced (Jifon and Syvertsen, 2001; Jifon and Syvertsen, 2003). In some cases, it was found that an improved WUE was due to an increased carbon assimilation rate along with increased transpiration rates (Medina et al., 2002). By reducing transpiration demand under netting an increased yield per hectare could be expected for the same amount of water applied or realise the same yield with less water. Furthermore, if soil and air temperatures were to be reduced less water would be required due to lower evaporation, and further increasing WUE. If shade netting were to increase stomatal conductance during the midday depression of photosynthesis, more water could be lost via transpiration, but the tree could photosynthesize for longer during the day thereby producing more carbohydrates for partitioning within the plant. However, the change in RH and

possibly temperature can reduce VPD and in return reduce transpiration demand of the atmosphere. Therefore it can be summarised that, shade netting is a technology that can be used to manipulate the orchard microclimate to favour physiological processes in order to potentially increase yield or decrease water use per hectare.

6. Cultural factors influencing photosynthesis and carbohydrate accumulation in citrus

6.1 Cultural aspects

In agriculture there is a general saying that a producer harvests sunlight because it drives physiological processes such as photosynthesis, which is essential for growth of the plant. In this section of the review the focus will fall on how to manipulate photosynthesis at orchard level. In well-managed orchards receiving optimum mineral nutrition and water supply, solar radiation interception becomes the critical factor limiting growth (Wheaton et al., 1978).

High-density plantings with a spacing of approximately 4.5-5 m $\times$ 2 m are used in mandarin production to increase trees density in order to potentially achieve earlier break-even with production by increased early tonnage per hectare (Barry et al., 2011). The increased plant densities initially increase yield per hectare but also allow less solar radiation to go to waste by more interception by trees compared to low-density orchards. The concept of leaf area index (LAI) is used to describe the amount of foliage per hectare and the potential for carbon fixation. A larger LAI increases sunlight received by leaves thereby potentially increasing photosynthesis and CO_2 uptake to produce more photosynthates per ground area (Cohen et al., 1987). High-density plantings normally have higher yields in the first few years of production, but trees can become overshadowed and production declines if the orchard is not managed well (Wheaton et al., 1997). As the empty spaces between trees are filled in high-density plantings, trees tend to overshadow one another compared to lower density plantings. One option is to remove every second tree and replant it elsewhere or to maintain adequate light distribution in the tree canopy by pruning.

Selective pruning of old and non-productive shoots enhances light penetration within the canopy and is essential in modern citriculture. Hedging of trees by mechanical pruning can be used to let sunlight penetrate the tops of trees, or branches can be selectively pruned by hand to create "windows" for light penetration. Tree shape affects interception and distribution within the tree. Cohen et al. (1987) evaluated the penetration of light in three different tree shapes and the amount of CO_2 being assimilated in light created boundaries within the tree. Row orientation did not affect light interception, but the amount of light penetrating triangle shaped trees per area was higher than rectangular shapes. This higher PAR led to a higher amount of CO_2 (g/m^2/day) fixed within the triangular-shaped tree, in contrast to rectangular trees that only had maximum light at the top of the canopy, thus a smaller leaf area that is productive to supply the carbohydrate demand. The net photosynthesis rate of the treatments did not differ significantly, but light distribution was better in North-South orientated rows with triangular-shaped trees leading to a more productive tree.

North-South planting direction can maximize the amount of solar radiation intercepted by the tree whereas, with East-West rows, the south-facing side of the tree is overshadowed and relies on morning sun and scattered light through the canopy. In South Africa, northern facing slopes are warmer due to more solar radiation hours accumulating throughout the day. These factors, i.e. high PAR and temperatures, could potentially have a great effect on the photoassimilates being produced and partitioned within a tree making it more productive.

Water supply to any given crop is important to maintain transpiration during the day and physiological processes to prevent physiological disorders. Water is lost through transpiration of the leaves and fruit, evaporation of soil and is collectively termed evapotranspiration. Water loss through transpiration happens predominantly through the stomata of a leaf. If the transpiration demand is not met by adequate water uptake by roots, turgor pressure, which is essential to physiological processes such as stomatal control, is lost (Taiz et al., 2015). In the summer months during high evapotranspiration demand, stomata close early during the day to conserve water. The stomata being closed for the larger part of the day leads to reduced gas exchange of CO_2 and water vapour between

the tree and atmosphere and a lower photosynthetic rate. During fruit set and growth, water is critical in order to ensure open stomata for development (Spiegel-Roy and Goldschmidt, 1996). If transpiration is too low during fruit set and photosynthesis stops due to stomatal closure, there is partial loss of available photoassimilates to be partitioned between fruitlets. This causes fruit to abscise due to carbohydrate and water shortages that triggers endogenous factors influencing abscission (Iglesias et al., 2007). Thus, water supply is important to maintain turgor pressure in order for photosynthesis to operate through gas exchange for carbon assimilation.

Soil type is an important factor in water supply and demand in the orchard. Soil structure determines the amount of water that needs to be irrigated due to different water holding capacities, drainage and soil water hydraulic conductivity. With sandy soil, normally found in Citrusdal, South Africa, pores are large (macro-pores) and water drains easily. If a soil contains more clay, the water holding capacity increases due to capillary forces in the meso- and micro-pores and can have water available for longer periods to supply the roots, but drainage is lower compared to sandy soils and if over-irrigated, can cause waterlogged conditions. Citrus roots are very sensitive to waterlogged conditions, generally caused by poor drainage and over-irrigation. Waterlogged or anaerobic conditions causes photosynthesis to be impaired due to stomatal closure, as abscisic acid is produced by the roots and is transported to stomata (Rodríguez-Gamir et al., 2011). Therefore, accurate scheduling of irrigation incorporating soil structure and type is important to maximize water uptake during critical phenological stages in order to sustain physiological processes, i.e. photosynthesis and transpiration.

6.2 Biological aspects

Photosynthesis is highly regulated by sink strength (sink activity × sink size) and citrus fruit are strong sinks that assimilate photosynthates upon fruit set from photosynthesis and not from storage carbohydrates. In case of a disruption of the balance between sink and source, citrus trees or branches can experience alternate bearing, with a high yield of small fruit ("on" year) in one year, followed by a lower yield of large fruit ("off" year). Some cultivars are regular bearers, yields are more or less

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constant year to year, but can be triggered to alternate bearing due to stressful growing conditions or conditions that promote excessive fruit set (Monselise and Goldschmidt, 1981). In cultivars with a regular bearing habit the feedback mechanism is more prominent to control yield, year to year, compared to alternate bearing cultivars that don't easily shed reproductive parts (Goldschmidt and Monselise, 1977).

In an "on" year the fruit sink strength is high due to the excessive crop load. This results in fruit-to-fruit competition and small fruit, and if stored reserves are depleted during spring it could lead to whole tree collapse. The increased sink size affects the photosynthesis demand and according to Taiz et al. (2015) will lead to an increased rate of photosynthesis. In comparison, "off" trees produce large fruit and accumulate storage carbohydrates in a tree structure (Goldschmidt and Koch, 1997). Increased fruit load increases the amount of available reducing sugars and lowers the amount of starch produced in citrus leaves (Lenz and Küntzel, 1974). Lower fruit load reduces the need for assimilates and in return can lower photosynthesis due to an accumulation of photosynthates in leaves and other storage organs. Monselise et al. (1981) noted how storage carbohydrates (starch) increased in different organs of "off" trees and indicated that this accumulation could lower photosynthesis. Some authors concluded that a reduced rate of photosynthesis is due to an accumulation of starch granules that restrict CO_2 diffusion within the leaf.

The partitioning of assimilates to plant organs could influence the photosynthetic potential of leaves by feedforward or feedback inhibition. The latter is when carbon partitioning to different organs is low due to low sink activity, i.e. low fruit number in an "off" year or imbalance of leaves to fruit. In citrus the feedback inhibition mechanism is still unclear in terms of what product of photosynthesis causes the inhibition (Nebauer et al., 2011), although accumulation of carbohydrates is thought to be the cause (Goldschmidt and Monselise, 1977) in order to prevent an oversupply. Nebauer et al. (2011) studied how non-structural carbohydrates affect the feedback inhibition of photosynthesis by girdling branches with and without fruit and manipulating leaf-to-fruit ratio. Girdled vegetative shoots showed accumulation of soluble sugars and starch and a decrease in carbon

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assimilation rates compared to girdled reproductive shoots and in ungirdled vegetative shoots photosynthetic rate remained unaffected. Therefore, fruit remained a strong enough sink for carbon so that there was no net effect on photosynthesis of the girdled fruiting branches. The girdled vegetative shoot had no sink and could not transport photosynthates to other sinks, and therefore carbohydrates had to be stored in leaves. These shoots became chlorotic due to sugar turnover to starch which started to accumulate within chloroplasts (Stander et al., 2017). The low sink demand and excess stored carbohydrates lowered the rate of photosynthesis. Reducing sugars accumulated in response to a high leaf:fruit ratio but had no significant effect on photosynthesis while accumulation of starch lowered photosynthesis and caused feedback inhibition in citrus leaves (Iglesias et al., 2002; Nebauer et al., 2011). Roots seem to be a strong unsaturable sink if other sinks are not available to compete for photosynthates (Goldschmidt and Koch, 1996). Thus, if roots are not limited by girdling, assimilates will accumulate in roots and photosynthesis will not be limited by low sink strength aboveground (Nebauer et al., 2013).

Carbohydrate differences, pattern and economy

Citrus trees store carbohydrates during the winter to be used during bloom in spring when the low temperatures adversely affect photosynthesis. During an "off"-year with a low crop load, more storage carbohydrates accumulate, and the inverse for "on" years with lowered accumulation in plant structures (Goldschmidt and Golomb, 1982). The "off"-year pattern of accumulation starts in spring when there are not enough carbohydrates to support growth flushes due to the previous "on"-year and becomes a trigger for alternate bearing (Monselise and Goldschmidt, 1982). With low sink availability, photoassimilates are partitioned to fruit and produce large, unmarketable fruit. When the rate of photosynthesis increases in spring, carbohydrates become abundant and photosynthates are partitioned to new developing leaves and reproductive structures (Goldschmidt and Golomb, 1982; Goldschmidt, 1999). This is when an increase of starch is observed. In sub-tropical regions where root growth does not occur continuously throughout the year due to low soil temperatures in winter (Bevington and Castle, 1985), starch starts to build up in the leaves as the roots are inactive and no

longer functions as a sink (Schaffer et al., 1986). In a low cropping year, leaves can become chlorotic due to an excess build-up of starch in chloroplast which rupture (Stander et al., 2017). In the following spring after an "off"-year, stored carbohydrates are mobilized by enzymatic conversion to sucrose and other transportable sugars and results in a high fruit set (high flower count) which creates the "on"-year cycle. However, the large sink reduces the amount of carbohydrates available for the following season's growth (Goldschmidt, 1999). In citriculture, alternate bearing is managed by thinning fruit either chemically or by hand, and girdling during fruit set in an expected "off"-year to make more carbohydrates available in the tree (Monselise and Goldschmidt 1981; Wheaton 1997). Gibberellic acid is known to have a negative effect on flower induction, but at fruit set seeds normally produce large amounts of GA and tend to set better. Therefore, the application of synthetic GA's is used in citriculture to set more fruit, especially fruit with a low seed count. GA can, therefore, be used in controlling alternate bearing by increasing fruit set during an "off"-year and minimize the effects of alternate bearing.

The use of shade netting could potentially be a technology used to manipulate photosynthesis and carbohydrate accumulation and partitioning in the tree, which could reduce the negative impact of alternate bearing cycles.

7. Horticultural use of shade nets

The use of shade netting in horticulture is becoming an important technology to potentially increase yield and fruit quality for a better financial return to producers. Crops are generally covered with shade nets to protect fruit from environmental hazards such as hail, high radiation, wind and pests. However, netting is being used more often to modify a crop's morphology and physiology by altering the light quality and quantity that reaches the tree (Stamps, 2009). Covering a crop with shade netting will effect microclimatic variables such soil and air temperatures, relative humidity and wind speed. Temperatures change due to a lowered irradiation and this will directly affect the humidity and variables controlled by temperatures, i.e. vapour pressure deficit (VPD).

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Changing the spectrum of light alters ratios of light, i.e. red to far-red ratio, and causes changes in growth and development (Stamps, 2009) due to the detection of a type of light by specific photoreceptors (Oren-Shamir et al., 2001). Lobos et al. (2013) investigated the effect of red, black and white nets at different shade intensities (25%, 50% and 75%) on blueberries by placing the respective nets over the crop after fruit set. No significant increase in yield or productivity was seen but only a delayed harvest date which could have a significant advantage to growers by producing fruit in a different time slot. The highest shade percentage in the study delayed harvest in the first year by 7-13 days compared to the control but had a negative impact on the return bloom. The intermediate shade percentage of red and white netting also delayed harvest but did not have a negative impact on the return bloom the following season. In contrast Retamales et al. (2008) reported an increase in yield by using white, grey and red nets with two different shade percentages (35% and 50%), which was attributed to a greater number of fruit that set. It was thought that a possible higher

Fig. 3. Spectra of transmittance of different colour shade netting (Oren-Shamir et al., 2001).

carbohydrate and water availability, as a result from less photoinhibition from high irradiation and heat stress, impacted fruit set. By altering the light spectrum with the use of netting, a crop could potentially allocate carbohydrates to different organs causing an imbalance in the reproduction and vegetative growth. Retamales et al. (2008) indicated that netting which reduced photosynthetic active radiation (PAR) above 47% significantly increased shoot and internode length, whereas the use of red, grey and white netting that only reduced the PAR by <41% had no significant effect on these parameters compared to the open in blueberry cultivar.

Anti-hail netting reduced PAR by 18.4% and significantly increased average fruit weight at harvest of both 'Royal Gala' and 'Fuji' apples in Brazil (Amarante et al., 2011). Under netting the fruit developed less physiological disorders and reduced symptoms caused by pests such as fruitfly. Time of day also plays a role to what extent shading will affect the tree. Temporal shading in the morning hours seems to have a greater negative effect on fruit yield and growth than when shaded in

the afternoon (Miller et al., 2015). This could be due to the fact that optimum temperatures and solar radiation for photosynthesis prevail during early hours and as a result have high photosynthesis rates, but by reducing the amount of light intercepted the leaf cannot function at its optimum (Miller et al., 2015). The reduction in photosynthesis in return lowers the amount of carbohydrates available for growth and physiological processes. White hail netting reduced the PAR from 1300 µmol·m^{-2}·s^{-1} to 1000-1100 µmol·m^{-2}·s^{-1} and reduced photosynthesis, but photosynthesis under black hail net was increased (Stampar et al., 2002). The higher photosynthetic rate was attributed to higher crop load of the different trees and not due to differences between treatments, i.e. white and black hail nets. In addition, there was no significant difference in yield, however, crop production efficiency could be influenced by the use of shade nets.

With recent developments to reduce the reliance on the use of chemicals in horticulture, 90% shade netting was applied over 'Imperial Gala' apple trees to determine if shade net could be used as a thinning mechanism instead of chemicals (Zibordi et al., 2009). Temporary shading of trees with 90% netting for 30 days after full bloom, until there was a peak in fruit drop, caused the initial fruit drop under shade net trees to be 7 days slower than chemical thinning trees but the rate equalled at 7 weeks after full bloom, and final fruit weight did not differ significantly. Therefore, temporary shade nets of high shade percentages could be used as a mechanical thinning mechanism due to reduced availability of carbohydrates as a result of lowered photosynthesis, causing fruit to abscise. Abscission or thinning of fruit could potentially be done with the use of extreme shading percentages, but if the timing and period of shading is wrong, the increased shading could impact the tree and important fruit postharvest parameters (McArtney et al., 2004).

Nets are not only to be used for crop protection purposes against physical damage, but also to reduce the incidence of pests, such as the effects of different coloured nets on aphids and whiteflies, and the amount of virus transmitted by these vectors on tomatoes (Ben-Yakir et al., 2012). Under pearl/white and yellow nets the incidence of both the vectors was two- to three- times lower than with

the use of black and red nets and the appearance of virus symptoms was also lower. Physical protection was not the only characteristic that affected the incidence of the pests but also the change of the light spectrum. Whiteflies and aphids have photoreceptors that peak at wavelengths of 520-530 nm, the region of green to yellow light (Coombe, 1982) Thus the red net only absorbed wavelengths shorter than red light, scattered red and far-red light and had no inhibitory effect on the ability of vectors to see the host plants (Ben-Yakir et al., 2012*).* By understanding shade netting, under specific cultural conditions, one could manipulate the crop as well as the biological aspect in order to be beneficial to a producer for a more profitable practice.

8. Current production of citrus under netting

Citrus is produced in a range of climatic conditions of the northern and southern hemispheres. South Africa, Peru, Chile, Australia, New Zealand, Brazil, Argentina and Uruguay compete with regards to their export window in the market chain overlapping within the southern hemisphere's citrus supply period. This overlapping of production creates a demand for high-quality fruit that exceeds that of competitors. To succeed in the export market both the external (colour and appearance) and internal (sugar acid ratio) quality parameters are critically important.

8.1 Advantages of shade netting in Citriculture

Seedless citrus fruit, particularly of mandarins, is more valuable than seeded fruit due to consumer preference (Iglesias et al., 2007). Thus, the tendency to produce seedless fruit, especially in the production segment of mandarins, is becoming the norm in citriculture. Parthenocarpic cultivars produce seedless fruit due to ovules abscising after pollination (Spiegel-Roy and Goldschmidt, 1996). However, some cultivars are self-incompatible but can produce seeds if the ovules are fertilized by another cultivar's viable pollen carried by insects. Self-incompatible cultivars will produce seedless fruit if not cross-pollinated, but the ability to set fruit is lower due to the lack of gibberellins (GA) produced by seeds. This can be overcome by applying synthetic GA to fruitlets to overcome fruit abscission at petal fall (Iglesias et al., 2007). It is possible to plant compatible cultivars that can cross-pollinate one another far apart, but when it is not possible, a physical barrier such as shade

netting can be used to prevent bees from cross-pollinating. An added advantage in preventing cross-pollination is physically preventing birds, animals, potential phytosanitary pests and vectors of diseases from entering the orchard.

The physical properties of netting are important in order to protect the tree from dramatic climatic events such as hailstorms, strong wind and high solar radiation. Hailstorms are commonly found in the northern production areas of South Africa and could be devastating to the crops and trees. The thickness of the thread gives strength to netting and will determine its longevity (Castellano et al., 2008). High wind speeds can be damaging to the tree as well as the fruit and is considered a problem in citriculture around the world and especially in South Africa, Australia, Florida and California. The fruit is most susceptible to wind damage/scarring during the initial eight weeks after bloom (spring) when the small fruitlet rubs against other fruit and/or leaves and branches (Albrigo, 1976). Permeability of the net to air movement depends on the porosity and resistance to let air through. The smaller the mesh value, the less permeable the netting (Mistriotis and Castellano, 2012). Several authors have noted that the use of shade netting can lower prevailing wind speeds. Wind speed was found to be reduced from 9% above an apple canopy (Tanny et al., 2008) to 70% in a citrus orchard (Wachsmann et al., 2014). In addition to lower above canopy wind speeds, it was noted that within the canopy wind speed can be reduced up to 95% (Tanny et al., 2008). Shade netting could, therefore, provide a good synthetic windbreak protecting fruit and new vegetative flushes against high winds.

Solar radiation and PAR are factors that affect all physiological and morphological processes within a plant, directly and indirectly (Tiaz et al., 2015). Reducing the amount of solar radiation incident on the orchard can have significant effects on soil, air and canopy temperatures, which in return will have an effect on relative humidity (Stamps, 1994). An obvious note is that the higher the percentage shade net used, the less solar radiation passes through to the orchard floor. The cause of sunburn on citrus fruit is still being investigated, however, it is known that solar radiation and high temperatures are important factors, as characterised by heat waves. Shade netting could be useful

during periods of heat stress to reduce the occurrence of sunburnt fruit by a lowered fruit surface temperature due to lower irradiation.

In a few citrus production regions in South Africa low average temperatures of the coldest month of the year (<2 °C) may cause frost damage to fruit and trees. Shade netting could possibly be used to change the microclimate, to reduce stress of extreme heat as well as cold extremes. The use of shade netting was shown to elevate night temperatures by 0.5 °C in a study on apples done by Tanny et al. (2008). Shade netting can, therefore, potentially be used to manipulate the microclimate of a citrus orchard.

Citrus nurseries use shade netting to shorten production cycles and to increase land use per hectare. Budded nursery trees are placed in shade houses covered with black or white nets, to protect the young plants as well as vegetative flushes from unfavourable, high light growing conditions to enhance maximum growth by using either 30% or 50% shade depending on climatic conditions. In nursery trees, rapid vegetative development can lead to a higher production rate, and Raveh et al. (2003) showed the effect of different shading on vegetative development of young citrus trees. They concluded that the shoot:root ratio is altered by the use of netting in favour of the shoots, producing longer shoots compared to the control. Nets can, therefore, be used to lower the environmental limitations and possibly increase productivity in nurseries.

9. Conclusions

Citrus is produced in diverse climatic regions around the world, with differences between hemispheres, continents, and even within countries. Production regions of citrus are mostly limited by insufficient water supply, inadequate soil or climatic constraints, such as low prevailing winter temperatures. However, the prevailing climate is the most important factor determining whether citrus could be commercially produced within a given region (Spiegel-Roy and Goldschmidt, 1996).

Plants normally adapt their physiological processes to their environment in order to survive. In different studies of different crops and shade net types it has been used to illustrate how shade could affect return on investment for producers. The changed microclimate by the use of photoselective

netting could lead to an increased packout per hectare due to less blemishes on fruit such as sunburn, wind scaring and potentially insect damage (Stamps, 2009).

By placing shade nets over a crop the microclimate is altered. This change in microclimate could in turn potentially have a positive or negative effect on the phenology and physiology of a tree. An important change in microclimate is the reduction in solar radiation. Irradiation has a great impact on other environmental factors, such as temperature, and causes direct and indirect changes to different environmental parameters. These changes in environmental parameters can have great effects on physiological processes, such as photosynthesis, transpiration, stomatal conductance and water use efficiency. In citrus it has been shown that these physiological parameters are improved, in some cases not affected, and to a lesser extent negatively affected. These studies on citrus were mostly done at tree canopy level by temporarily placing netting over the trees and measuring the changes in physiology.

The effect of permanent netting over citrus has not been studied extensively in terms of physiology, phenology and fruit quality citrus. If the long-term physiology is affected by netting, differences could occur in the carbohydrate assimilation, distribution and utilization thereof. This would affect yield and quality, therefore a warranting an in-depth study on a commercial scale to evaluate the effect of the changed microclimate on the tree's physiological pattern throughout a season under shade netting.

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Chapter 3: Quantification of the influence of permanent shade netting on the microclimate of a 'Nadorcott' mandarin orchard in a Mediterranean-type climate

Abstract

Shade netting is a technology used in agriculture to alter light quality and quantity to increase return on investment. Currently, shade netting in citrus production is mainly used to prevent cross-pollination of flowers and to produce seedless fruit. However, by reducing solar radiation and adding resistance to wind a change in microclimate is also expected. The evaluation of the modification of the microclimate in a citrus orchard in a Mediterranean-type climate was done in Citrusdal, South Africa, after enclosing the treatment blocks with a 20% white permanent shade netting structure. The study showed differences between the shade netting and open treatments in terms of seasonal variations in different parameters. Solar radiation was reduced by 17% over the two fruiting seasons. The shade netting barely affected average ambient air temperature across the entire season, but a change was seen in the amount of hours experienced within a specific physiological temperature range. Changes in monthly air temperatures under shade netting within the canopy led to an increase in effective heat units (EHU) accumulated throughout a season and could have an effect on vegetative and fruit growth. A high reduction of wind speeds was observed for both seasons, as well as increased soil water content under the netting. Lowered windspeed reduced air mixing under shade netting and led to a slight increase in relative humidity. These small changes in temperature and RH resulted in a decrease in vapour pressure deficit (VPD) under the netting. Soil temperatures changed under shade netting with a higher daily average temperature; however, this was due to less diurnal fluctuation between the maximum and minimum temperatures. Citrus trees in an orchard covered by 20% shade netting experienced a modification of the microclimate which could lead to a reduction in superficial damage and possibly improved carbon accumulation and water use efficiency.

Keywords: Shade netting, microclimate, physiology, phenology, *Citrus reticulata* Blanco

Introduction

Solar radiation is the main source of energy for plants and chlorophyll-containing organisms that have the ability to convert solar radiation into carbohydrates which can then be used by the plant for energy. Photosynthesis in Citrus trees is saturated at low irradiance, normally 30 percent of full sunlight is required (Syvertsen, 1984). By altering the quantity of light, light interception by a plant would change resulting in different carbon assimilation potential. In addition to carbohydrate balance, solar radiation is also an important factor that influences climatic parameters, such as temperature and humidity.

Fruit can be damaged by severe climate events, e.g. hailstorms, wind and high solar radiation resulting in sunburn (Shahak et al., 2004). In agriculture, the use of shade netting as a technology to change the light quality and quantity is primarily focused on increasing return on investment by reducing the occurrence of damage to fruit. In citriculture in South Africa, however, shade netting is predominantly used to produce seedless fruit by physically preventing bees from entering an orchard and cross-pollinating two cultivars (Talon et al., 1997). Recently there is also an increased use of shade netting in citrus production to reduce the impact of adverse climatic events.

By constructing a permanent shade net structure over an orchard the microclimate is expected to be modified to a certain extent resulting in a reduction in light, and a reduction and/or increase in temperature, relative humidity (RH), wind speed, soil temperature and soil water content. Alteration of these parameters could also affect vapour pressure deficit (VPD) and evapotranspiration in the plant-soil-water continuum and, therefore, water use efficiency of an orchard. Changes in these climatic parameters are known to directly affect the primary and secondary physiological processes in a fruit tree. A reduction of the air temperature would be the first aspect possibly affected by shade netting, due the reduction in solar radiation and it is also noted that leaves exposed to direct solar radiation can have leaf temperature up to 9 °C higher than the ambient temperature (Syvertsen and Albrigo, 1980) which could result in heat damage. By the reduction of solar radiation of an apple tree under netting, the daily temperature at canopy level was decreased by 6 °C without changes in the

mean daily ambient temperature (Shahak et al., 2004). Comparative results obtained with tomatoes and spinach showed a reduction of 3.2 °C (Kittas et al., 2012) and 1 to 5 °C (Meena et al., 2014), respectively, in daily mean temperature. In the afternoon, maximum air temperature is normally experienced in an orchard due to excess solar radiation, and if light were to be reduced, changes in maximum temperatures would be expected.

In research done in Israel no significant differences were obtained in mean air temperature when comparing shade net with the unnetted control environment; however, there was a significant reduction in maximum air temperature (Nicolás et al., 2008; Wachsmann et al., 2014). Shahak et al. (2004b) reported a lowering of the maximum temperature by up to 5 °C under shade nets of different colours and light penetration. It is therefore expected that by shading trees a reduction of leaf temperature and heat load would be experienced in an orchard (Jifon and Syvertsen, 2003; Kalcsits et al., 2017).

The reduction in wind speed and altering of the temperature under netting also affected the RH (Tanny et al., 2006). Elad et al. (2007) showed an increase of 20 to 30% RH under 25% black shade nets and resulted in a lowered VPD. Many authors rather report VPD than RH, due to the large effect VPD has on different physiological processes in a plant; these authors noted the change in RH (Alarcón et al., 2006; Jifon and Syvertsen 2003; Kalcsits et al., 2017; Nicolás et al., 2008). Therefore, in general, a higher RH is expected under shade netting due to lower air movement and lower air and leaf temperature (Shahak et al., 2004; Stamp et al., 2009).

The impact of temperature and RH on VPD forms part of various studies due to the direct effect on carbon assimilation and water balance in the tree as VPD affects stomatal conductance. Citrus stomata are very sensitive to high temperatures which result in a large VPD causing their closure, and a reduction of photosynthesis due to the reduction in gas exchange (Brakke and Allen, 1995). Even if the VPD is decreased by a small amount under shade nets, this can lead to large physiological changes as a higher potential for carbon accumulation exists. It has been reported that no notable reduction in ambient temperature occurred under shade net covered citrus orchards. However, it was

found that the vapour pressure between the air and leaf was reduced due to lowered leaf temperatures (Jifon and Syvertsen, 2003; Nicolás et al., 2008).

In addition to these microclimatic changes within the tree canopy, it can be expected that soil temperatures would be altered and thereby possibly change the root environment. These changes in the temperature ranges in an orchard covered by shade netting can affect the physiological processes impacting the vegetative-reproductive balance of a citrus tree in addition to the damages to fruit.

Shade netting also acts as an effective windbreak, reducing wind speed but this would depend on the net properties, i.e. weave thickness and mesh value (Mistriotis and Castellano, 2012; Stamps et al., 2009). Tanny et al. (2008) measured a 9% reduction in wind speed in an apple orchard, and Wachsmann et al. (2014) measured up to 70% (0.5 to 0.8 m·s^{-1}) reduction for the maximum mean wind speed. This reduction of maximum wind speed directly impacts on fruit quality, especially during the first 8 weeks after full bloom when fruitlets are most susceptible to wind scarring (Albrigo, 1976).

This research was initiated due to a lack of comprehensive data on the changes to the microclimate under shade netting in citrus. In this study done in a Mediterranean-type climate, viz. Citrusdal, Western Cape Province, South Africa, the aim of the research was to measure and describe those changes occurring in a 'Nadorcott' mandarin orchard after enclosing it with 20% white shade net. It was hypothesised that 20% permanent white shade net would alter the orchard microclimate and growing conditions in terms of ambient and soil temperature, soil water content, solar radiation, relative humidity, and wind speed.

Materials and Methods

Site, plant material and shade net properties

The experiment was conducted in Citrusdal (32° 35′ 22″ S, 19° 0′ 53″ E), Western Cape Province, South Africa, in a commercial orchard of 'Nadorcott' mandarin (*Citrus reticulata* Blanco.) budded onto 'Carrizo' citrange rootstock. 'Nadorcott' mandarin is late maturing and harvested from July until August under Citrusdal conditions. Trees were planted in 2012 at a spacing of 5.5 x 2.5 m

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in uniform soil. All trees received the same commercial cultural practices, i.e. nutrition and irrigation, unless otherwise specified. A permanent netting structure according to commercial standards was constructed over the orchard as follows. The area was divided into eight equal size blocks of 25×75 m. Thereafter the two treatments, i.e. open (control) and netting, were randomly allocated to four blocks each (Fig. 1). A 20% white shade net (Plusnet, Randfontein, Gauteng, South Africa) with a shade factor of 12 to 17% was used and constructed horizontally at 5.5 m height over four uniform treatment blocks (25×75 m) a month before full bloom in September 2015. To accommodate the randomised block layout within an existing commercial and allow for statistical comparisons orchard, the nets separating the blocks only extended from the roof to the 1.5 m above soil level into the tree canopy of the affected row. This practical solution, which allow for a randomised block layout, was decided on even if it was known the effect on the climate would have been less compared to a fully covered commercial block.

Above canopy microclimatic evaluation

Microclimatic parameters were quantified using two Campbell Scientific weather stations (GRWS100, Stellenbosch, South Africa); one weather station was placed under the shade netted area and the other in an open block above the tree canopy. Logging of the following parameters occurred on an hourly basis on a CR1000 data logger: solar radiation ($MJ \cdot m^{-2}$) [LI-COR LI-200R Pyranometer, LI-COR, Lincoln, NE, USA] , wind speed ($m \cdot s^{-1}$) [Anemometer 03001, R.M. Young, Michigan, U.S.A] air and soil temperatures (°C) [CS215-L probe, Campbell Scientific, Utah, U.S.A] relative humidity (%) [CS215-L probe, Campbell Scientific, Utah, U.S.A] and soil water content ($m^3 \cdot m^{-3}$) [CS650 Soil water content reflectometer, Campbell Scientific, Utah, U.S.A]. All above ground sensors and instruments were positioned to measure at 4 m height above the soil and 1 m below the net. Soil temperature probe were placed in the first 20 cm of soil as well as the volumetric soil water content probe which not installed at dripper wetting zone not close to a rooting pot. The data were interpreted as differences between the open (control) and netted area during phenological stages and fruit growth stages II and III as classified by Bain (1958). Due to method development and

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optimisation of the equipment and installation during the first season, only certain parts of the data will be presented, i.e. 9 Feb. until 24 Oct. 2016 (Season 1) and for the second season from 16 Jan. to 27 Aug. 2017.

The data were used to calculate and compare temperature ranges relevant to citrus physiology and phenology: hours below 0 °C which can cause low temperature or freeze damage to fruit and can cause dieback to a tree; hours affecting flower induction (11-15 °C optimum and 16-20 °C sub-optimum). In addition, the total hours accumulated between 26-30 °C which is considered to be optimum for photosynthesis as well as above 35 °C, known to lead to a decrease in photosynthesis due to stomatal closure, were calculated. Due to the importance in optimum fruit developmental stages, the Effective Heat Units (E.H.U) were calculated from 19 Feb. until Mar. 2016 and from 16 Jan. until 31 Mar. 2017 by using the following equation:

E.H.U = (Monthly average – 13 °C) × days in the month   [Eq. 1]

The optimum soil temperatures needed for root growth and function range from 21-25 °C and 26-30 °C, and the total hours within those ranges were calculated accordingly. The irradiation data was calculated from 0600_{HR}-2000_{HR} throughout the two seasons, and wind speed was measured and converted from m.s^{-1} to km.h^{-1} and maximum speeds were analysed over both seasons.

VPD was calculated from the following equations to compare the impact of changes in all these climatic parameters on the tree's physiology:

Saturation vapour pressure (e_{sat}) = 0.6108 × Exp (17.5028 × Temp. (°C) / (Temp. (°C) + 240.97)   [Eq. 2]

Actual vapour pressure (e_a) = Relative humidity / 100 × e_{sat} [Eq. 3]

Vapour Pressure Deficit (kPa) = $e_{sat - e_a}$ [Eq. 4]

Within tree canopy climatic measurements

In addition to the data from the weather stations, collected above the canopy, additional air temperature data were gathered in each replicate (n=4) by the use of TinyTag Plus 2 TGP-4510 data loggers (Gemini Data Loggers, Chichester, UK). In order to quantify possible differences within the tree canopy the loggers were placed within the tree at 1 to 1.5 m above ground level and fixed to the

main branch out of direct sunlight. Calculations, as made for the weather stations for air and soil temperatures, were also done with the data from the TinyTag loggers only with different time frames due to data being more complete during the critical growth stages of citrus. Total E.H.U were calculated (Eq. 1) from July 2016 until Aug. 2017 and expressed as a monthly value as well as the total during the three critical growth phases; I, II and III (Bain, 1958). The air and soil temperatures were further used to calculate the total hours for specific temperature ranges during seasons, critical phenological stages, and physiological processes as described for the microclimate evaluation above the canopy.

Statistical analysis for within-canopy temperatures

STATISTICA data analysis software version 13 (Dell Inc. 2015, Round Rock, TX, USA) was used to analyse the data. Analysis of variance (ANOVA) was used or repeated-measures ANOVA was performed when responses were repeated on the same respondent. Mean separations were carried out using Fisher's least significant difference test, where applicable, at $P \leq 0.05$ and 0.1. The experimental design was a randomised complete block design (RCBD) with four blocks per treatment (n = 4), i.e. control and shade netting. Where indicated, some data captured were analysed as a one-way ANOVA with values of $P \leq 0.05$ considered significant.

Results

Solar radiation (MJ·m^{-2})

Over both seasons, the average daily radiation was reduced under the shade netting by 18% (1.00 to 0.82 MJ·m^{-2}) in the first season and 16% (0.68 to 0.57 MJ·m^{-2}) in the second season (Fig. 2 A and C). Maximum radiation levels followed the same trend as the average levels under the shade netting with a 17% reduction in the first season and a 12.5% reduction in the second season (Fig. 2 B and D) from 2.4 to 2.0 MJ·m^{-2} and 2.4 to 2.1 MJ·m^{-2} respectively. Seasonal differences were also recorded; throughout the summer (Jan. to Mar.) the shade netting reduced radiation levels by 12% (1.6 to 1.4 MJ·m^{-2}) and 9% (1.1 to 1.0 MJ·m^{-2}) in the first and second seasons, respectively. During

the cooler part of the year, i.e. autumn to spring, the shade netting had a greater effect with a 17 to 19% reduction in light levels.

Ambient air temperature (°C)

In both seasons, average air temperature above the canopy showed a low degree of variation between the shade netting and the open areas (Fig. 3 A and D) with a reduction of 0.7 °C and 0.1 °C under shade netting in the two seasons. Small differences in minimum (Fig. 3 C and F) and maximum (Fig. 3 B and E) daily temperature were recorded. Despite minimal differences in the lower temperature ranges under the shade netting the total hours below 0 °C in July 2017 was decreased by 22 hours (Table 1). Shade netting reduced the maximum temperature above the canopy by 0.7 °C (3.3% reduction) in the first season and 1.03 °C (3.6% reduction) in the second season. However, even though temperature averages did not differ to a large extent, the shade netting reduced the temperatures classified for this study as extreme, i.e. > 35 °C, by 18% (12 hours) in the first season and by 20% (54 hours) in the second season (Table 1).

The temperature ranges 11-15 °C and 16-20 °C measured above the canopy differed between seasons as well as between treatments. However, contrasting results were seen with the shade net accumulating less hours for these two ranges in 2017 compared to 2016. In addition, for the temperature range of 26-30 °C, there was a 7.3% decrease in 2016 and only 1% in 2017 from 0600 to 1800$_{HR}$. Expressing the data on a seasonal basis, this temperature range indicates that the shade netting accumulated more hours during the summer months, but the reverse occurred at the onset of winter with the open accumulating more hours until spring 2017 (Table 1).

Within the tree canopy, the average air temperatures throughout the year showed significant differences between the treatments within months and between months (Fig. 4A). From Nov. 2016 until Mar. 2017 there was a significant increase of 0.56 °C in average air temperatures within the tree canopy under shade netting. The same pattern occurred for the average monthly maximum temperatures with a significant difference between the month and treatments within a month ($P= 0.003$) (Fig. 4B). From Nov. 2016 until Feb. 2017 monthly maximum temperatures within the canopy

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under shade netting (Fig. 4B.) was significantly higher than the open. However, the total hours above 35 °C and between 26 and 30 °C (Table 1) throughout the year showed no significant increase. In the spring, summer, autumn and winter no significant differences were observed between treatments (Table 1). Minimum air temperatures (Fig. 4C) did not differ between the treatments throughout the season, however, an increasing trend of a minimum values is evident, especially during the coldest period (June to July).

Effective Heat Units (E.H.U)

Total E.H.U calculated for the first season above the canopy resulted in similar values for the two treatments (Table 2). Only a 22 and 1.9 unit higher accumulation occurred in the open treatments during the first season and second seasons, respectively. When comparing E.H.U at the different fruit growth stages, the same response was observed with low variation between treatments. However, within the tree canopy measurements showed a significantly higher total monthly E.H.U accumulation under netting compared to the open block (Table2; Fig. 5). This increase in E.H.U from spring until the end of summer led to a significant increase in the total E.H.U accumulated under the shade netting before harvest (May 2017).

Relative Humidity (RH %)

When comparing the RH in 2016, the average daily RH was reduced by 0.5% RH under the netting (Fig. 6A), however, in the second season the shade netting had a 6% higher RH (Fig. 6C). The minimum RH in 2016 (Fig. 6B) was only 1% RH higher in the open, and for the second season there was an increase of 3.4% RH under shade netting (Fig. 6D). These changes were more evident during the summer months when temperatures were higher.

Vapour Pressure Deficit (VPD)

The average maximum daily VPD during the summer months was reduced by 0.024 kPa in 2016 (Feb. – Mar.), however in 2017 (Jan. – Mar.) a larger reduction in VPD was recorded (0.212 kPa) resulting in a 38% difference between treatments (Fig. 7B and D). In the first season, the shade

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netting reduced the average daily VPD by 2.3% (0.0941 kPa), and in the second season by 15% (0.189 kPa) (Fig. 7A and C).

Soil temperatures (°C)

Mean daily soil temperature under shade netting during the two seasons was increased by 0.5 °C and 2 °C, respectively (Fig. 8A and D). Even though the averages were higher, the maximum daily soil temperatures were reduced by 4% (1 °C) in the first and by 23% (8 °C) in the second season (Fig. 8B and E). A higher average minimum soil temperature was recorded under shade net resulting in a 1.3 °C (15%) and 4.1 °C (44%) increase compared to the control in the two seasons (Fig. 8C and F). Soil temperatures under the shade netting therefore had less daily fluctuation between the maximum and minimum temperatures compared to the open orchard's soil temperature. This reduction in fluctuation of temperatures resulted in notably higher amount of accumulation of hours in the optimum root growth ranges of 21-25 °C and 26-30 °C (Table 3). The second season (2017) being a warmer year overall, more hours in these temperature ranges for both the open and shade netting treatments were accumulated compared to the first season leading to a 33% increase in the second season compared to 14% in the first season.

Volumetric soil water content (m³·m⁻³)

The volumetric soil water content ($m^3 \cdot m^{-3}$) and the average daily water availability under the shade netting were increased by 10% and 12% in the two seasons (Fig. 9A and 10A). The minimum soil water before the start of a new irrigation cycle supports this increased average soil water content, as these values where increased by 7% (2016) and 17% (2017) (Fig. 9C and 10C). Maximum daily water content (Fig. 9B and 10B) in general was also higher under shade netting for both years by 17% and 7% for 2016 and 2017 respectively.

Wind speed (km·h⁻¹)

The shade netting treatment reduced the total wind hours during the first season, with the average maximum daily wind speed being reduced by 21% from 7.4 to 4.8 km·h⁻¹ (Fig. 11 B). Furthermore, from Sept. until Oct., which coincides with the period when major wind damage to

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fruitlets occurs, a reduction in wind speed of 40% (3.4 km·h^{-1}) occurred. During 2017, the same trend occurred with the maximum wind speeds being reduced up to 80% from 7.3 to 1.8 km·h^{-1} (Fig. 11D).

Discussion

By constructing a permanent shaded environment over a citrus orchard, light quantity was altered and changes in orchard microclimate were expected. These changes in canopy microclimate could affect different primary and secondary physiological processes of a citrus tree. The 20% shade netting treatment in Citrusdal reduced the mean daily solar radiation levels above the canopy by~17% over two seasons. During summer when higher solar radiation levels occur, the reduction in solar radiation level was less at 12%, but a greater reduction in irradiation was observed throughout the autumn, winter and spring. This could be due to prevailing overcast conditions as Citrusdal is in a winter rainfall area. Similar reduction in irradiation was recorded by several authors using netting of different shade percentages and colours (Jifon and Syvertsen, 2003, Shahak et al., 2004a; Shahak et al., 2004b; Stamps, 2009). Importantly, the netting reduced the maximum daily solar radiation level that could induce stress in leaf physiological processes and/or fruit physiological responses, such as sunburn to fruit. The shade factor should be taken into consideration when constructing shade netting over an orchard, as the orchard topography and aspect, i.e. flat or sloped orchard, north or south facing slopes, and prevailing macroclimate (sub-tropical vs. Mediterranean-type climate), could create a more shaded environment if overcast conditions persist or the topography further reduces solar radiation. The reduced irradiation levels under shade netting, therefore, lowered the amount of energy entering the orchard that would normally be lost through conversion into heat energy that could potentially be stressful to a citrus tree. Solar radiation is an important climatic parameter that has a direct and indirect effect on other climatic parameters affecting leaf physiological responses.

Although irradiation levels were reduced, only small differences occurred in mean daily temperatures above the canopy throughout the two years, with the largest reduction of 1 °C found under shade netting in the second season; although there were differences in maximum temperature on hot, summer days. These results concur with previous studies (Nicolás et al., 2008; Kittas et al.,

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2012). However, regarding air temperatures under shade netting contradictory results were reported of either an increase (Arthurs et al., 2013; Solomakhin and Blanke, 2010; Wachsmann et al., 2014) or no significant differences (Retamales et al., 2006). It is important to note that most of these studies were not done on citrus and mostly high percentage shade netting (>20%) and different coloured nets were used.

The complexity of altering microclimate by shade netting was illustrated in the contrasting air temperature results above and within the canopy. Despite a reduction in solar radiation under the shade netting, air temperatures within the canopy were higher under the shade netting. It is thought that this could be the result of the reduction in wind speed and less energy loss from the orchard floor, as seen in the higher soil temperature. This finding, however, warrants further investigation to elucidate the effect of the shade netting within the tree. The complexity added by plant structure thereby reducing wind speed within the increased canopy development under netting which could have reduce heat loss within a plant by convection (Larcher, 1995). Within the tree canopy, the ambient air temperature from Nov. 2016 until Mar. 2017 was 0.56 °C higher under the shade netting compared to the control. Comparing the above-canopy measurements with the within-tree canopy measurements, the latter temperatures were higher for specific months. Maximum temperatures were higher within the canopy but were not significantly different between treatments due to a large block variation. Minimum air temperatures were higher by 1 °C on a monthly basis, especially during the winter months which is in agreement with Wachsmann et al. (2014) who reported an increase of 0.5 °C for within canopy minimum air temperatures.

To quantify the impact of a certain temperature range on tree physiology, the total hours in a given temperature range known to affect physiological and phenological processes for citrus were calculated. Temperatures above 35 °C are considered to be stressful to citrus and are correlated with the shutdown of photosynthesis due to stomatal closure (Syvertsen, 1984). The shade net reduced the total hours accumulated above 35 °C in both seasons by 18% which could indicate that the trees potentially experienced less stressful conditions under shade netting throughout the summer months,

which is critical for fruit growth (Bain, 1958). The reduction in stressful conditions for physiological processes could increase stomatal conductance or extend the time that stomata are open during the day. In return, more carbohydrates could be assimilated due to an increase in photosynthesis or its extended duration.

The optimum temperature for a citrus tree's photosynthesis is between 25 and 30 °C at 30% full sunlight (Syvertsen, 1984). The shade net only reduced the average daily irradiation by 17% and it can, therefore, be reasoned that the leaves were still light saturated with adequate sunlight and that temperature played the major role in determining the rate of photosynthesis. Although there were more hours for optimum photosynthesis accumulated from 0600_{HR} to 1800_{HR} in the control treatment (open) over both seasons, this difference was attributed to more hours accumulated in autumn, winter and spring. Therefore in summer, when the temperature rises above those optimal for photosynthesis, the reduction in temperature under the shade netting could increase the total hours for optimal photosynthesis during a day. Within the canopy, the same trend occurred with more hours for optimal photosynthesis in total; however, no differences between the shade netting and the control were recorded. During this period, i.e. summer, carbohydrate assimilation and distribution are important for fruit growth and sugar accumulation, and if it is possible that a tree can photosynthesise longer during this phenological stage, it could support more reproductive and vegetative growth.

In terms of flower induction, temperatures between 10 and 15 °C in autumn are considered optimum with temperatures between 16 and 20 °C inductive for meristems to differentiate (Davenport, 1990; Moss, 1976). The temperature ranges within the canopy showed contrasting results to the above canopy which accumulated more hours under shade netting than within the canopy for the latter phenological temperature ranges. The within-canopy data during flower induction may be more important than above canopy data because it is within the tree itself where these inductive temperatures are required. It is possible that these changes could have an impact on the amount of reproductive vs. vegetative buds developing in spring, which is the focus of an additional part of the current study.

Temperatures below 0 °C can result in freeze damage to fruit and the tree causing dieback of shoots if too low temperatures persist for extended periods. In the first season, a relatively warm winter was experienced and the data showed a small increase in temperature of netting on low temperatures compared to the second year. In the second season, the shade netting buffered the effect of low temperatures and reduced the number of hours below 0 °C in June to July by 22 compared to the open treatment. This reduction in duration at low temperatures is of importance for late-maturing cultivars such as 'Nadorcott' mandarin which is harvested after the coldest period of the winter in the Western Cape.

The RH values were to a large extent contradictory over the two seasons; however a trend of a higher RH was recorded, as expected, under shade netting due to less air movement and mixing (Tanny et al., 2006). Minimum RH occurred after midday when a decrease in photosynthesis is expected due to stomatal closure as a result of a too large water potential gradient between the leaf and the atmosphere (Brakke and Allen, 1995). The 2.7% increase in minimum RH in summer under the shade netting in the second season possibly affected the physiology of the tree through increased stomatal conductance of leaves. More important than the RH and temperature values are the mean and maximum VPD, which were reduced for both seasons under the shade netting treatment. The VPD difference between treatments for the first season was low; however, in the warm, dry second season, the shade netting treatment showed a reduction of 15% in the mean and 12% for the maximum VPD. This indicates that the atmosphere's demand for water was reduced under the 20% shade netting and could influence the plant's transpiration rate as well as possibly increase or prolong stomatal conductance during a day.

Changes in soil temperature have not been included in most studies on the impact of shade netting, but it is suspected to have an impact on root growth and function of a citrus tree (Bevington and Castle, 1985; Taiz et al., 2015). The shade netting treatment in this study increased mean soil temperature in both seasons, as well as decreased daily fluctuations between the maximum and minimum temperatures. This is in agreement with Solomakhin and Blanke (2010) who reported a 0.9

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°C increase in soil temperature at 5 cm depth under white shade netting in an apple orchard. In this study minimum daily soil temperatures were higher by 1.3 °C and 4.2 °C in the two seasons, and maximum soil temperature on average higher for the first season by 0.9 °C, with larger differences throughout the summer. Throughout the second year the maximum temperature was reduced by up to 7.9 °C. However, a reduction in mean, maximum, and minimum soil temperatures at 20 cm and 40 cm depth under different colour and percentage shade netting in an apple orchard has been reported (Kalcsits et al., 2017).

This reduction in soil temperature is suspected to have an effect not only on the growth and function of roots but also on respiration affecting root carbohydrates (Goldschmidt and Koch, 1996), and should be included in future studies on shade netting. Bryla et al. (2001) showed that roots experienced fewer daily fluctuations in temperature and acclimated earlier to environmental conditions with a lower respiration rate compared to roots that experienced large daily fluctuations. The reduced temperature fluctuation under shade netting increased the total hours for optimal root growth and function for citrus (Bevington and Castle, 1985) by 614 and 106 hours, respectively. In addition to the improved temperature range in the root zone, the increase in volumetric soil water content of 11.7% and 9.6% could have a large effect on root proliferation and elongation under shade netting. The possibility for more intense root growth under shade netting, between vegetative flushes, could lead to a higher production of cytokinins by the roots (Taiz et al., 2015) and could, therefore, result in more vegetative growth in summer and autumn. If more roots are produced under shade netting due to more optimal hours for growth and function, it is possible that the tree could absorb water and nutrients more effectively under netting compared to the open. However, it should be noted that with the increase of soil water, there is also the possibility for nutrient leaching. The change in water relations is an important aspect of shade netting indicating the possibility of reducing the irrigation volume under netting.

By constructing a shade net structure above trees, resistance to wind reduces wind speed (Tanny et al., 2006). This reduction in wind speed is of particular importance from bloom until 8 weeks after

full bloom when citrus fruitlets are most sensitive to wind blemishes (Albrigo, 1976). The reduction in wind speed under shade netting coincided with this period and can therefore be of large financial importance for the production of fruit with fewer wind blemishes and higher packout percentage.

It is concluded that by constructing permanent 20% white shade netting over a commercial 'Nadorcott' mandarin orchard in Citrusdal, the structure changed the orchard microclimate with regard to some important climatic parameters affecting citrus physiology. In return the horticultural response could potentially be of significant financial importance for citrus growers. In addition, the lack of new resources, i.e. arable land, water, and optimum growing climate, could lead to the use of shade netting in order to establish successful orchards in areas previously deemed marginal. An additional aspect to take into consideration for future research on shade netting would be the impact of these microclimatic changes documented in this study on the pest and disease pressures in a citrus orchard.

Table and figures

Table 1. Temperature ranges in a 'Nadorcott' mandarin orchard in Citrusdal as affected by 20% white shade netting. The values are cumulative hours at the specified temperature ranges (°C) for above canopy (4 m) and within canopy measurements as well as the seasonal effect on optimal photosynthetic temperature range.

| | Treatment | Air temperature ranges (°C) | | | | | Optimal photosynthesis temperature range (26 - 30°C) | | | |
		< 0 (Low temperature damage)	11 -15 (Optimal range for flower induction)	16 - 20 (Sub-optimal range for flower induction)	26 -30 (Optimal range for *Ac)*	> 35 (Stomatal closure)	Summer	Autumn	Winter	Spring
	Above canopy									
2016	Control	5	520	290	382	68	36	127	73	145
	Shade netting	6	510	277	354	56	37	134	66	118
2017	Control	26	584	480	553	268	187	310	11	--[z]
	Shade netting	4	559	556	548	214	205	316	72	--
	Within tree canopy									
	Control	14ns[y]	558ns	563ns	1119ns	580ns	364ns	315ns	88ns	299ns
	Shade netting	9	575	580	1063	701	359	292	67	277
	P-Value	*0.2351*	*0.4556*	*0.5474*	*0.1696*	*0.0979*	*0.7941*	*0.4598*	*0.3636*	*0.1193*

[z] Data not recorded

[y] Mean values are not significant different between treatments (*P ≤ 0.05*) (n = 4)

Table 2. The effect of 20% white shade netting on E.H.U based on a 4 m height as well as within tree canopy E.H.U during fruit growth stages I, II and III of a 'Nadorcott' mandarin orchard in Citrusdal.

Above canopy	I	II	III	Total
2016				
Control	--[z]	298	366	665
Shade netting	--[z]	289	352	642
2017				
Control	--[z]	714	310	1024
Shade netting	--[z]	711	311	1022
Within tree canopy				
Control	876a[y]	1354a	-29ns[x]	2233a
Shade netting	921b	1402b	-44ns	2314b
P-value	*0.0011*	*0.0147*	*0.4142*	*0.0266*

[z] Data not recorded

[y] Mean values within a column with different letters differ significantly ($P \leq 0.05$) (n = 4)

[x] Indicates no significant difference between treatments

Table 3. Soil temperature ranges at 5 cm depth as affected by 20% white shade netting in a 'Nadorcott' mandarin orchard in Citrusdal. The values are cumulative hours for temperature ranges optimum for root growth and function.

Soil temperature ranges (°C)

	Treatment	21-25	26-30	Total
2016	Control	461	204	665
	Shade netting	553	238	771
2017	Control	811	425	1236
	Shade netting	971	879	1850

Fig. 1. Randomised complete block design of the shade netting experiment situated in Citrusdal, Western Cape Province, South Africa. Treatments were allocated randomly in four blocks, and consisted of the control (open) and 20% white shade net.
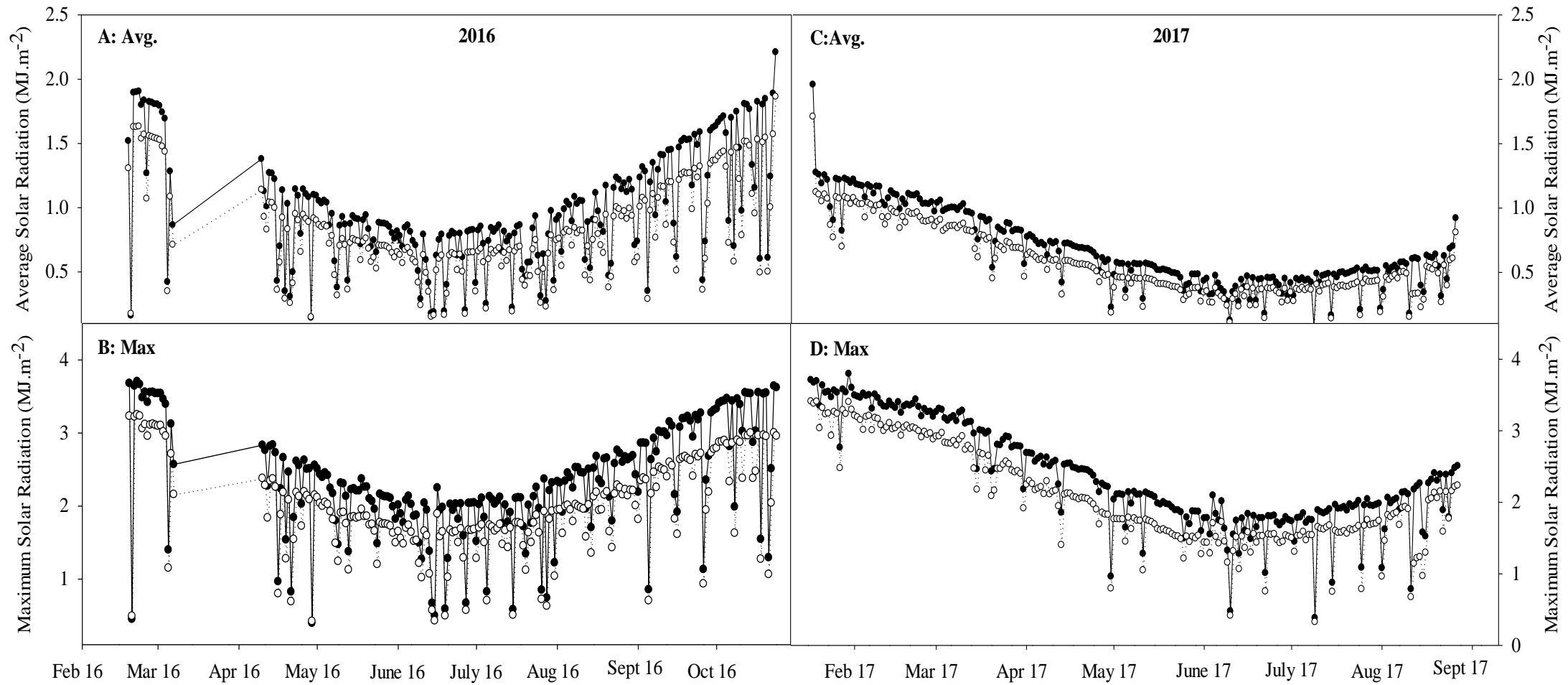
Fig. 2. The effect of 20% white shade netting on average (A and C) and maximum (B and D) daily solar radiation at a height of 4 m in a 'Nadorcott' mandarin orchard situated in Citrusdal; (○ Shade net; ● Control).
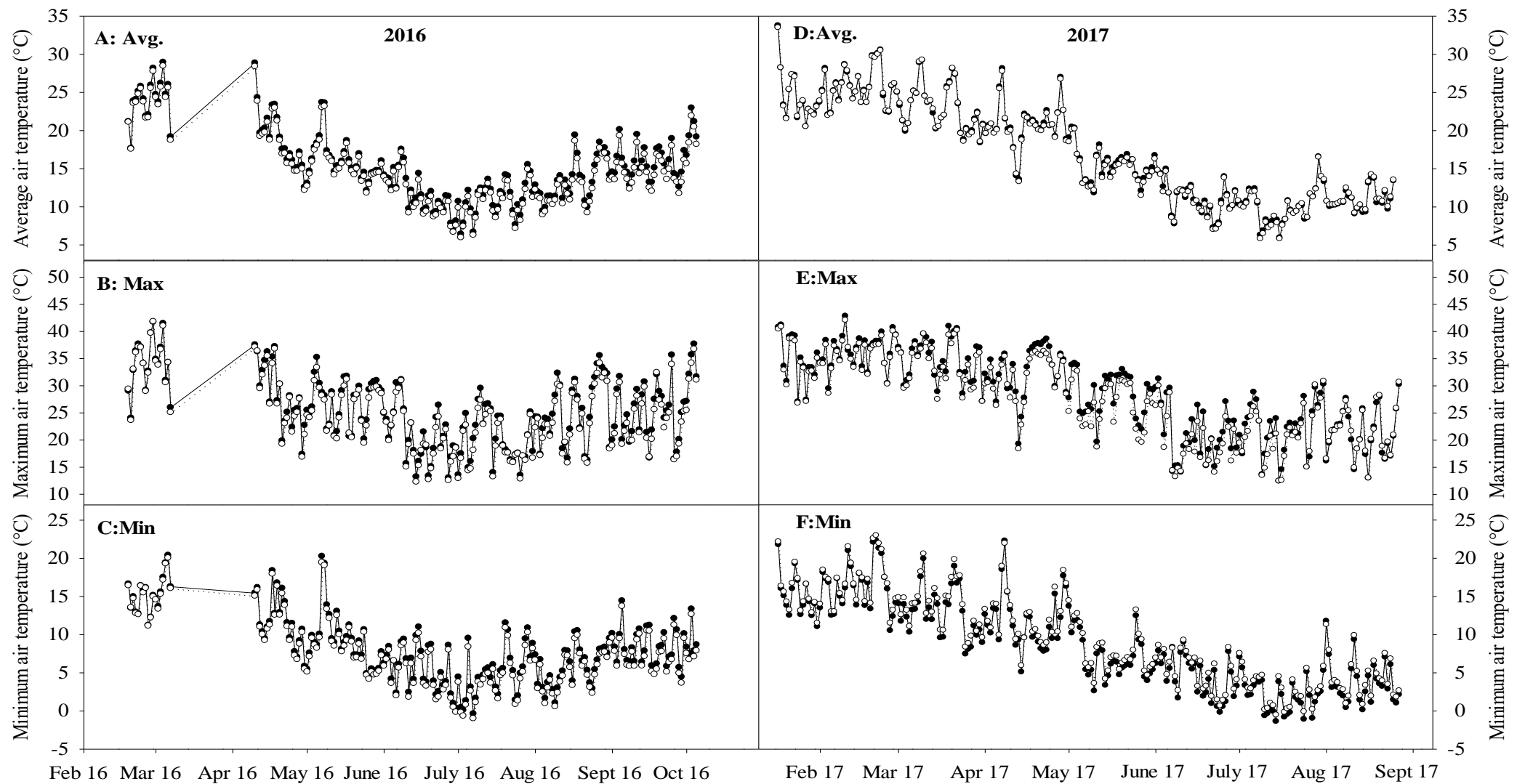
Fig. 3. The effect of 20% white shade netting on average (A and D), maximum (B and E) and minimum (C and F) temperatures at 4 m height above the canopy from ground level in a 'Nadorcott' mandarin' orchard situated in Citrusdal in 2016 and 2017; (○ Shade net; ● Control).
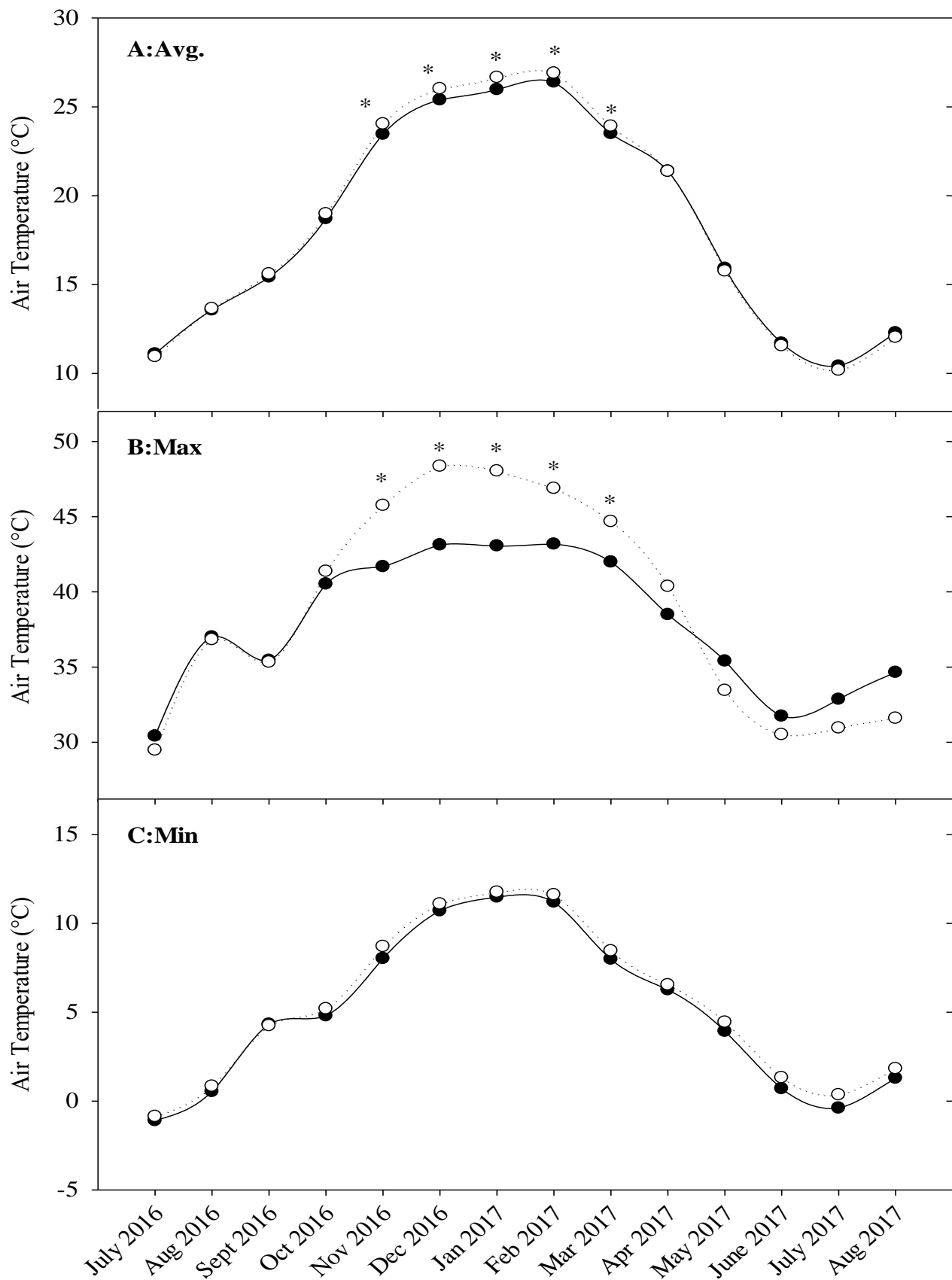
Fig. 4. The effect of 20% white shade netting on within-canopy average (A), maximum (B) and minimum air temperatures (°C) on a monthly basis from July 2016 until Aug 2017 in a 'Nadorcott' mandarin orchard in Citrusdal; (○ Shade net; ● Control). * Indicates mean values within a month differ significantly ($P \leq 0.05$) (n = 4).
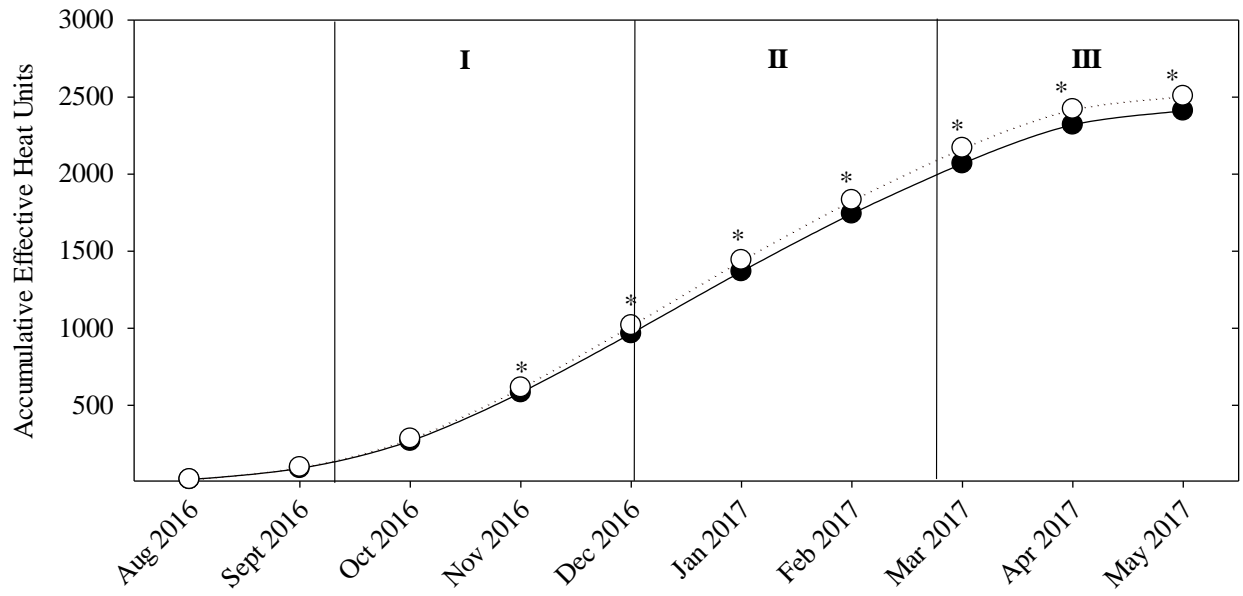
Fig. 5. The effect of 20% white shade netting on within tree canopy accumulative effective heat units during critical fruit growth stages I, II and III in a 'Nadorcott' mandarin orchard in Citrusdal; (○ Shade net; ● Control). * Mean values within a month differs significantly between treatments (*P < 0.05*) (n = 4); *Month*Treatment P < 0.0001.*
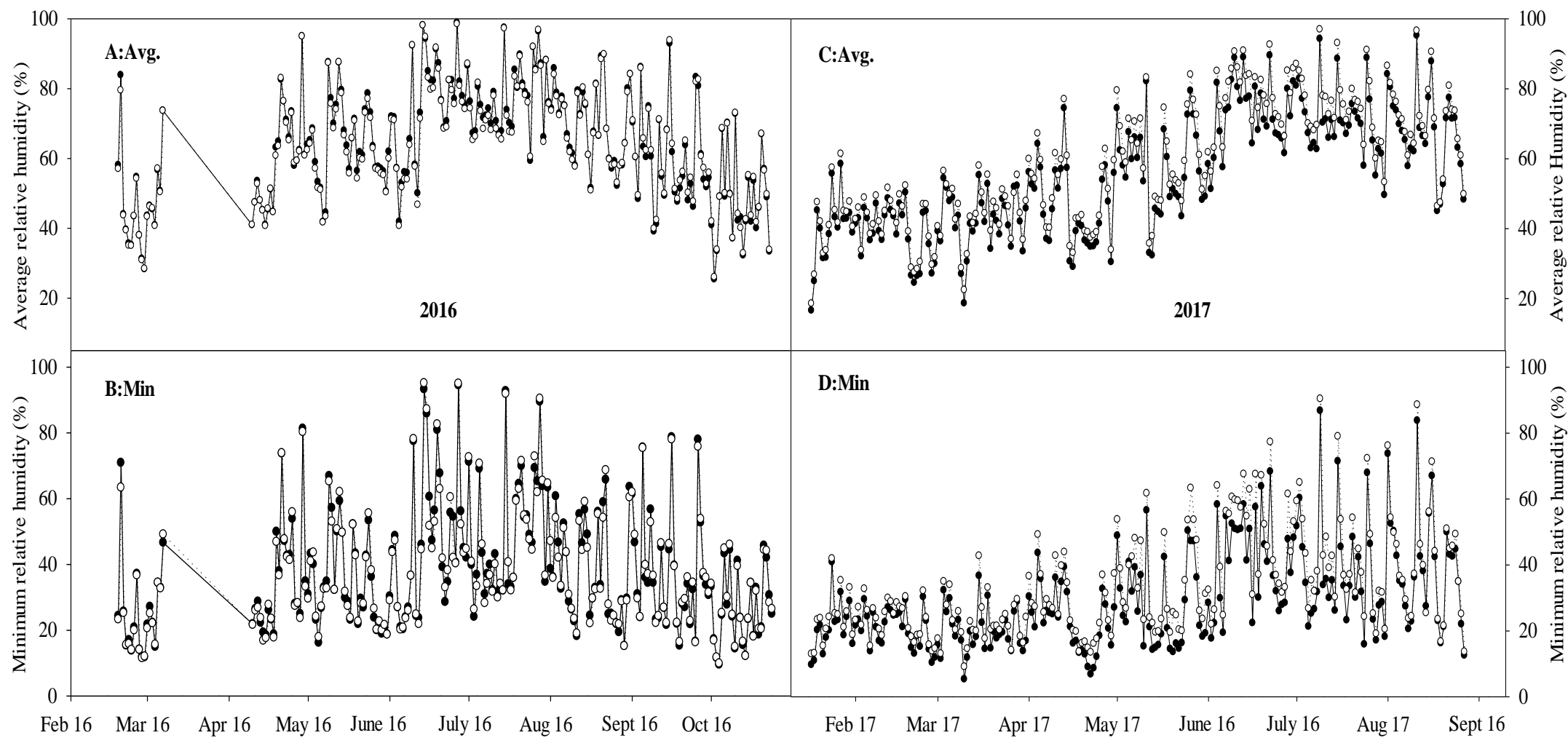
Fig. 6. The effect of 20% white shade netting on daily average (A and C) and minimum (B and D) relative humidity (%) in a 'Nadorcott' mandarin orchard in Citrusdal measure 4 m above canopy from ground level (○ Shade net ● Control).

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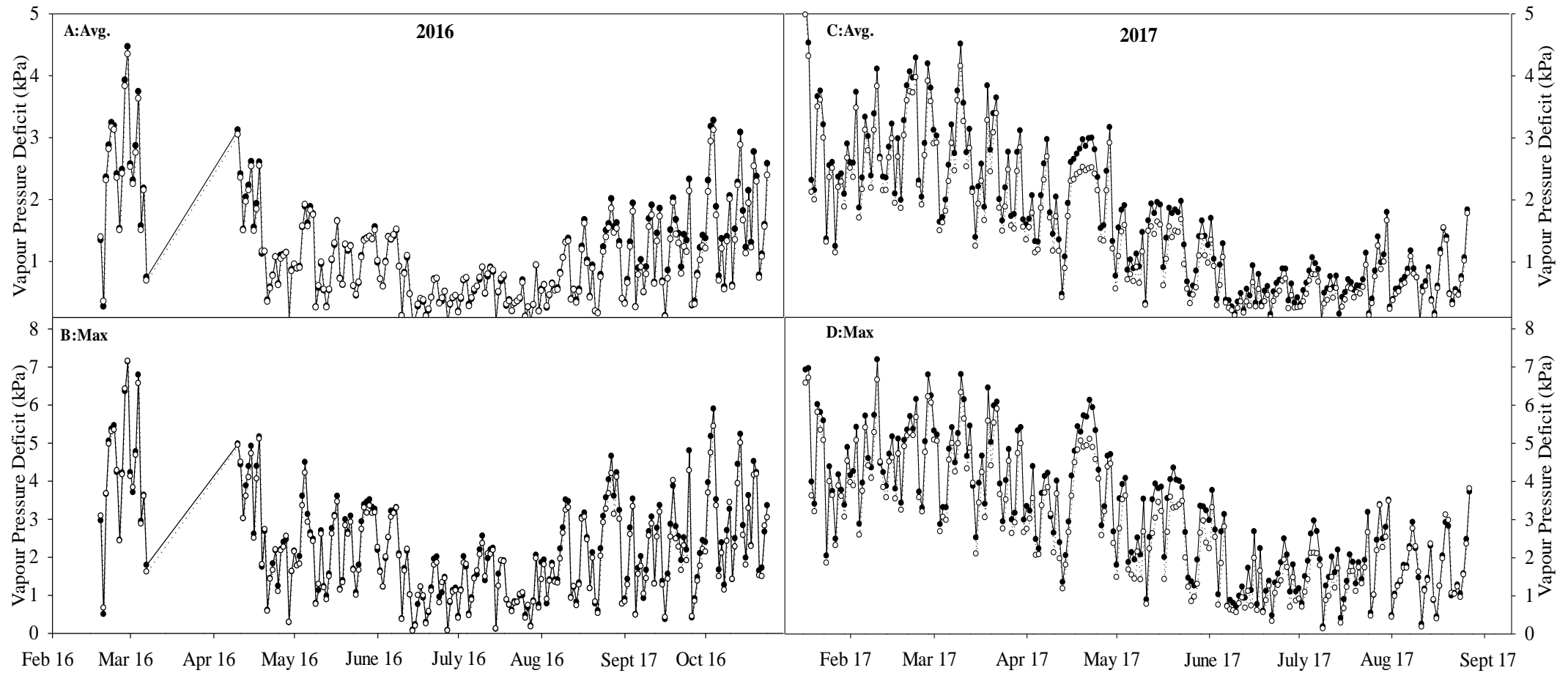
Fig. 7. The effect of 20% white shade netting on the daily average (A and C), and maximum (B and D) vapour pressure deficit (kPa) of a 'Nadorcott' mandarin orchard in Citrusdal 4 meters above the canopy from ground level(○ Shade net ● Control).
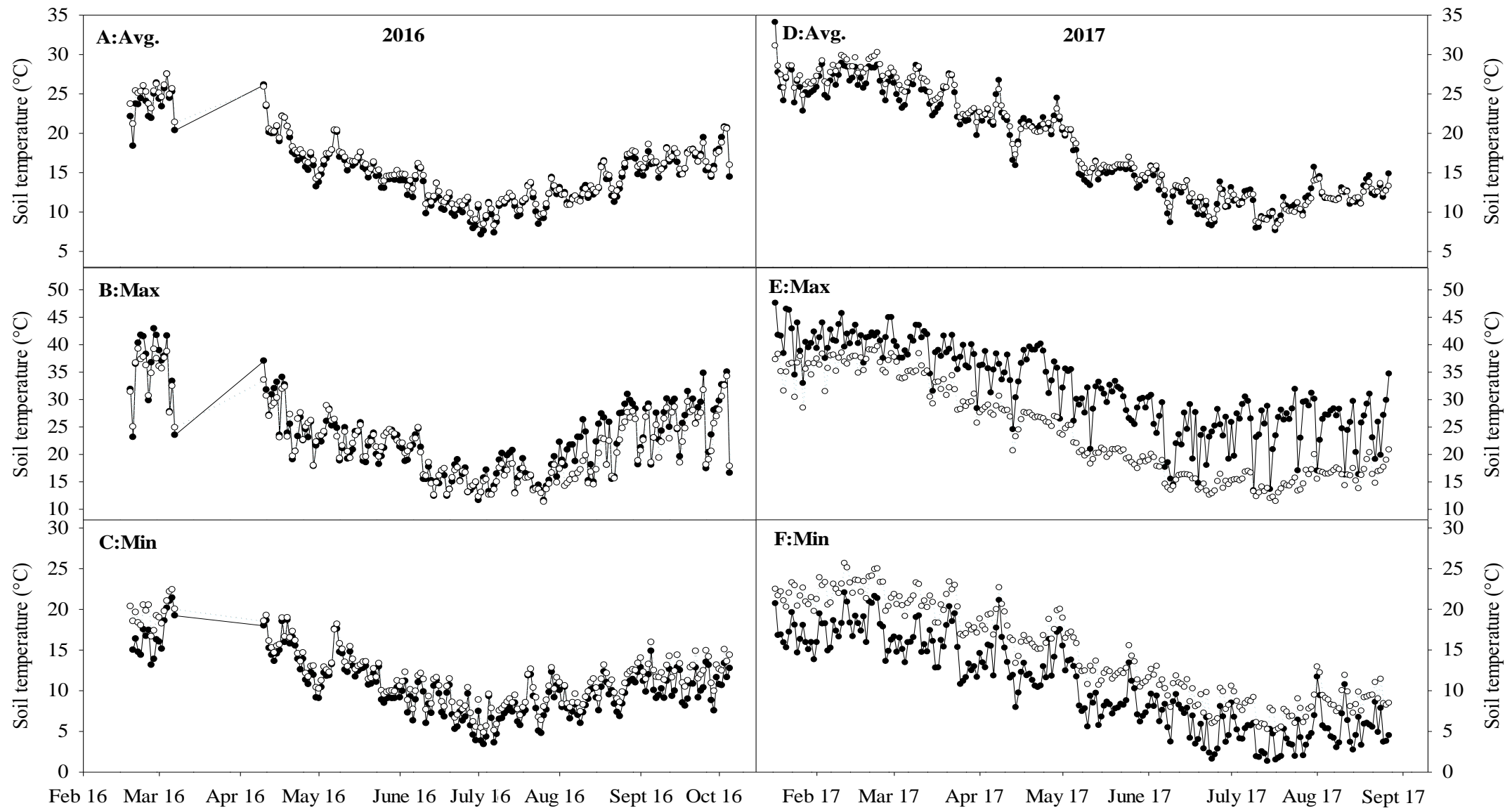
Fig. 8. The effect of 20% white shade netting on the average daily (A and D), maximum (B and E) and minimum (C and F) soil temperatures of a 'Nadorcott' mandarin orchard in Citrusdal (○ Shade net, ● Control).
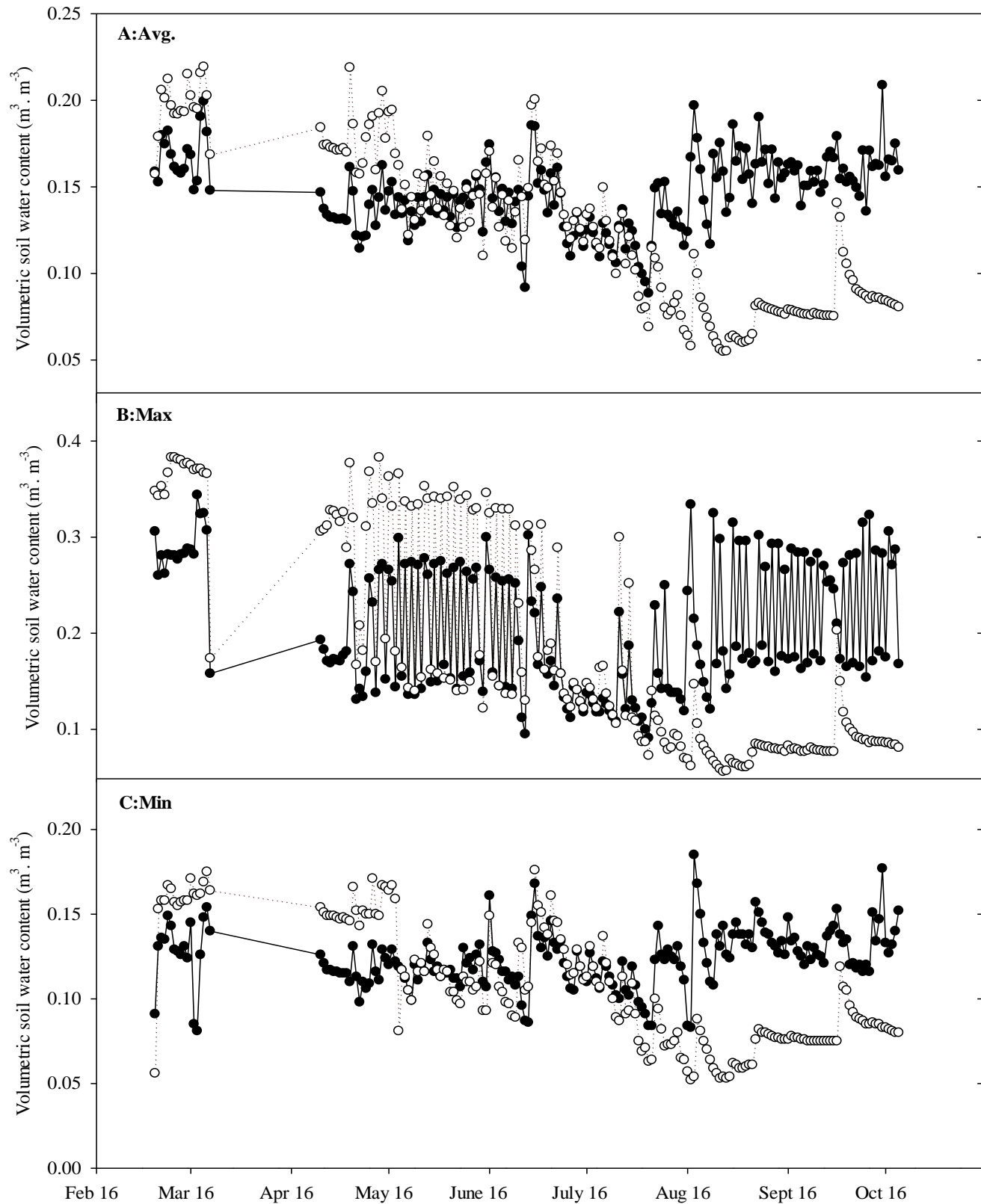
Fig. 9. Daily mean (A), maximum (B) and minimum (C) volumetric soil water content (m³·m⁻³) in 2016 and the effect of 20% white shade netting on the water availability in a uniform clay-loam soil in a 'Nadorcott' mandarin orchard in Citrusdal Need to provide labels (○ Shade net ● Control).

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Fig. 10. Daily mean (A), maximum (B) and minimum (C) volumetric soil water content (m^3·m^{-3}) in 2017 and the effect 20% white shade netting affect the water availability on a uniform clay-loam soil in a 'Nadorcott' mandarin orchard in Citrusdal (○ Shade net ● Control).

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Fig. 11. The effect of 20% white shade netting on total hourly wind speed daily average wind speed and maximum wind speed during the 2016 and 2017 seasons in a 'Nadorcott' mandarin orchard in Citrusdal (○ Shade net ● Control).

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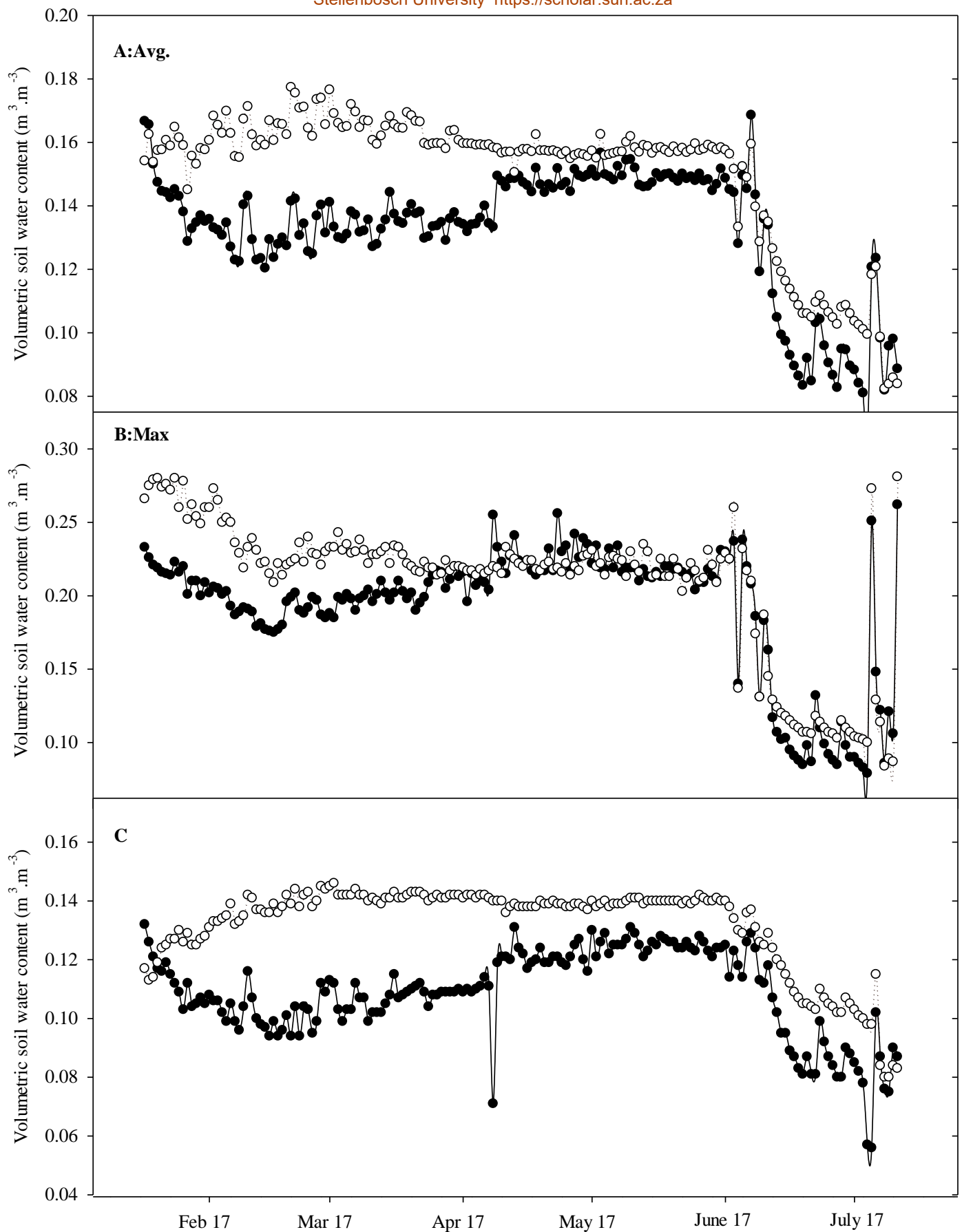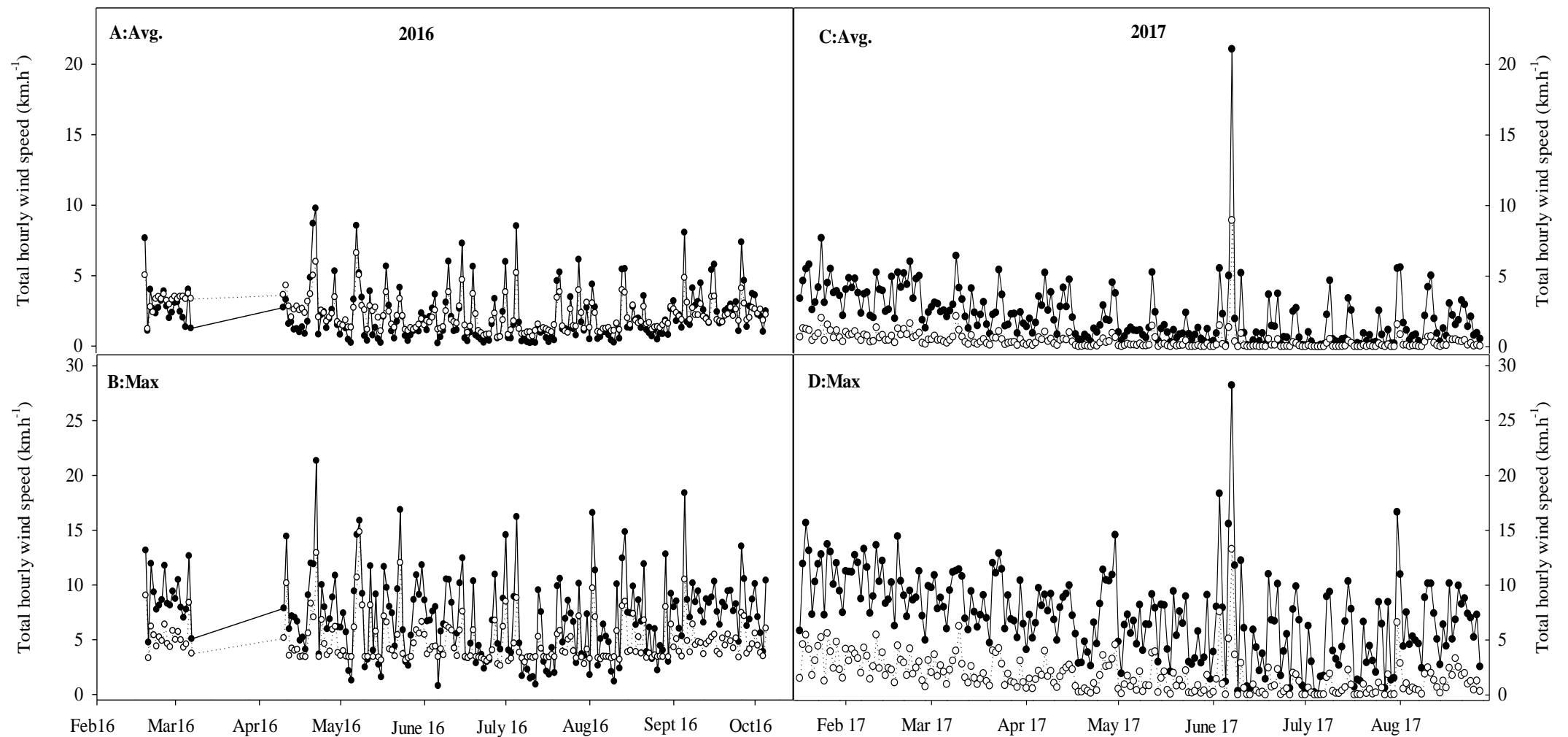
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Chapter 4: The influence of shade netting on 'Nadorcott' mandarin tree physiology during a fruiting season

Abstract

Shade netting is used in agriculture to protect fruit and trees from dramatic climate events, and in citrus production to prevent cross-pollination and seed formation which reduces the value of fruit produced. An advantage of shade netting in citriculture is the reduction in solar radiation, which can thereby decrease the development of sunburn lesions. However, the reduction in solar radiation can potentially alter the microclimate and affect the physiology of a citrus tree. This paper aimed to quantify to what extent 20% white shade netting would affect the physiology of a tree throughout the season in relation to its effect on the orchard microclimate (reported in Chapter 3). The relative humidity and air temperature were continuously logged in addition to monthly leaf physiology measurements were taken with a closed chamber infra-red gas analyser. The shade netting did not affect the air temperature but a higher trend in RH resulted in VPD being influenced during the summer. VPD was lowered by the shade net in Jan. 2017 from 2.4 to 2.1 kPa, which in turn resulted in a 15% increase in CO_2 assimilation rate due to the higher stomatal conductance. This increase in stomatal conductance and CO_2 assimilation rates under the shade netting was observed throughout the middle of the summer when the trees were subjected to the highest level of heat stress. Shade netting positively affected the climatic parameters involved in carbon assimilation during the summer months which could potentially lead to a higher sugar accumulation.

Keywords: Microclimate, CO_2 assimilation, stomatal conductance, transpiration, water use efficiency

Introduction

Photosynthesis is the essential physiological process in a plant to ensure carbohydrates are available for energy, in the form of ATP, and for building blocks to sustain growth (Taiz et al., 2015). The photosynthetic rate of citrus, a C_3 plant, can vary between 4 to 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and is lower than

for deciduous fruit crops with rates of 15 to 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Kriedemann, 1971). The rate of CO_2 assimilation is dependent on various climatic factors such as solar radiation, air and leaf temperature, relative humidity, vapour pressure deficit, soil water availability and water status of a tree. Photosynthetic rates of 4-10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ are considered to be normal in citrus trees subjected to photosynthetically active radiation (PAR) levels of between 600 and 700, i.e. ±30% full sunlight in summer (Kriedemann, 1971; Syvertsen and Lloyd, 1994).

Environmental cues directly or indirectly affect assimilation rates via the impact on the stomata aperture. Photosynthesis in citrus trees occurs at its optimum rate between temperatures of 25 and 30 °C. In general, in citrus stomatal conductance is impaired above 35 °C air temperature due to the lack of gas exchange between the atmosphere and leaf (Kriedemann, 1968). Temperatures below 15 °C reduce photosynthesis due to an increased resistance to CO_2 diffusion into the leaf as a result of lower stomatal conductivity (Vu and Yelenosky, 1987). The citrus stomata are therefore sensitive to environmental changes and close due to too high vapour pressure deficit (VPD) between the leaf and atmosphere which is affected by leaf and/or air temperatures and relative humidity (Brakke and Allen, 1995). Changes in the microclimate of an orchard could have an effect on stomatal conductance and transpiration rate which could cascade into affecting CO_2 assimilation.

During winter in Mediterranean-type growing regions of South Africa, the rate of photosynthesis could drop to undetectable levels due to the low ambient temperatures and or as a result of insufficient PAR during overcast periods. However, it is known that evergreen trees, including citrus, can photosynthesize during the winter when the conditions are favourable (Syvertsen et al., 1997). These lowered CO_2 assimilation rates during winter are also affected by the reduced activity of the Rubisco enzyme which decreases the carboxylation reaction of the carbon metabolism (Vu, 1999). The continuance of photosynthesis during cooler months, albeit at a lower rate, enables citrus trees to assimilate carbohydrate reserves required for vegetative and reproductive development the following growing season. The rate of photosynthesis increases again to optimum in the spring

when soil and air temperatures increase, normally above a threshold of 20 $^{\circ}$C which coincides with the investment of energy or as sink demand increases.

In citriculture, shade netting is a developing technology used to alter, and possibly improve, the microclimate in an orchard to improve productivity and reduce physical blemishes. By reducing the amount of solar radiation, a leaf adapts anatomically and physiologically to the more shaded environment (Syvertsen, 1984), which enables photosynthesis at lower light levels due to the alteration of the light compensation point (Kriedemann, 1968). The quantum efficiency of shaded leaves tends to be steeper at lower light levels, enabling the leaf to assimilate CO_2 more efficiently at lower light levels (Taiz et al., 2015). It is, therefore, possible that the reduced radiation under shade netting can be beneficial for citrus photosynthesis due to the negation of the high heat load and irradiance (Kriedemann, 1968, Sinclair, 1982, Syvertsen, 1984; Taiz et al., 2015). Conditions of heat stress and high irradiance are typically found from Nov. to Mar. in the Mediterranean-type citrus production region of South Africa, for example Citrusdal, Western Cape Province.

High radiation levels can increase leaf temperatures up to 8 °C (Syvertsen and Albrigo, 1980); however, 50% shade netting was effective in reducing leaf temperature by 9 °C (Jifon and Syvertsen, 2000). A lower leaf temperature will reduce the VPD_{Leaf} and thereby the evaporative demand which will increase stomatal conductance. Jifon and Syvertsen (2001) reported that the 50% shade netting used in their study improved photosynthesis, especially during the midday depression typically found in citrus, and led to a significant increase in photosynthesis of 5 µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ in both orange and grapefruit leaves. In contrast Nicolás et al. (2008) found that CO_2 assimilation remained unaffected after the application of 40% shade netting over lemon trees.

It could, therefore be construed that the rate and duration of photosynthesis under shade netting would be positively influenced by manipulating the light interception of a citrus orchard. For this study the aim was to quantify the effect of 20% white shade netting on the seasonal photosynthetic and transpiration rates of 'Nadorcott' mandarin trees. If carbohydrate accumulation and levels could be improved, there could be potential economic value to citrus producers due to increased yield under

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shade nets. It was hypothesized in this study that 20% white shade net would affect physiological parameters associated with photosynthesis and thereby the carbohydrate accumulation throughout a growing season.

Materials and Methods

Site, plant material and shade net properties

The experiment was conducted in Citrusdal (32° 35′ 22″ S, 19° 0′ 53″ E), Western Cape Province, South Africa, in a commercial orchard of 'Nadorcott' mandarin (*Citrus reticulata* Blanco.) budded onto 'Carrizo' citrange rootstock. 'Nadorcott' mandarin is late maturing and harvested from July until August under Citrusdal conditions. Trees were planted in 2012 at a spacing of 5.5 x 2.5 m in uniform soil. All trees received the same commercial cultural practices, i.e. nutrition and irrigation, unless otherwise specified. A permanent netting structure according to commercial standards was constructed over the orchard as follows. The area was divided into eight equal size blocks of 25×75 m. Thereafter the two treatments, i.e. open (control) and netting, were randomly allocated to four blocks each (Fig. 1). A 20% white shade net (Plusnet, Randfontein, Gauteng, South Africa) with a shade factor of 12 to 17% was used and constructed horizontally at 5.5 m height over four uniform treatment blocks (25×75 m) a month before full bloom in September 2015. To accommodate the randomised block layout within an existing commercial and allow for statistical comparisons orchard, the nets separating the blocks only extended from the roof to the 1.5 m above soil level into the tree canopy of the affected row. This practical solution, which allow for a randomised block layout, was decided on even if it was known the effect on the climate would have been less compared to a fully covered commercial block.

Method development

Three preliminary studies were done in order to optimise photosynthesis measurements and thereby reduce variation in the values. Firstly, the time of optimal photosynthesis during the day was determined by measuring from 0830_{HR} – 1530_{HR} on a cloudless day (Fig. 2). Secondly, the leaves at various positions within the canopy, viz. north–south and east–west, were measured, and lastly the

optimum position of the leaf on a shoot for measurements was determined (Fig. 3). These data were analysed using a one-way ANOVA by using STATISTICA [Dell Inc. 2015, Dell Statistica version 13] where applicable.

Since photosynthesis increased from 0900_{HR} – 1130_{HR} and onwards until the midday depression occurred (Fig. 2), subsequent physiological measurements were done from 0900_{HR} until 1200_{HR}. As there were no statistical differences in photosynthesis rate in the various canopy positions within the tree, i.e. north, east and south as well as between the top, middle and bottom of the tree (data not shown), all subsequent measurements were conducted on leaves situated on the outside and in the middle of the canopy and on the middle of a shoot.

Variation in the rate of photosynthesis was recorded within a shoot (Fig. 3); the terminal leaf had higher values but with a large degree of variation. The bottom leaf had lower rates of photosynthesis but also high variation. Both positions one and three had variations larger than 1 $\mu mol \cdot m^{-2} \cdot s^{-1}$, whereas the middle leaf (position 2) only varied by 0.55 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Therefore all subsequent measurements were standardised in terms of time ($0900 – 1200_{HR}$) and position of leaf, i.e. middle leaves of a shoot on a single tree canopy, in order to reduce variation in measurements of photosynthesis rate.

Microclimate evaluation

Ambient air temperature (°C) and relative humidity (% RH) within the canopy were measured hourly using TinyTag Plus 2 TGP-4510 data loggers (Gemini Data Loggers, Chichester, UK) one hour (0800_{HR}) before physiological measurements started until the end of measurements (1200_{HR}). One TinyTag data logger was used per replicate and the values were used to calculate the vapour pressure deficit (VPD) [Eq. 2,3,4, p. 53].

Physiological measurements

Physiological measurements of CO_2 assimilation, stomatal conductance and leaf transpiration were done using two uniform sun-exposed leaves in the middle of an eastern facing canopy position for each replicate (n = 4). Measurements were done between 0900_{HR} and 1200_{HR} on cloudless days

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on a monthly basis (30-day interval) from the start of the season (July 2016) until the end of the season before harvest (June 2017). The CO_2 assimilation rate (A_c, expressed as $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$), leaf stomatal conductance (g_s, expressed as $mmol \cdot m^{-2} \cdot s^{-1}$) and leaf transpiration (E, expressed as $mmol\ H_2O \cdot m^{-2} \cdot s^{-1}$) data were measured with a closed chamber infra-red gas analyser (IRGA) (Li-6400, LI-COR, Lincoln, NE, USA). The flow rate of air was set at 200 $\mu mol \cdot s^{-1}$ and air CO_2 concentration was set at 400 ppm, photosynthetic photon flux density (PPFD) at 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and leaf temperature maintained at 25 °C, with the RH% controlled manually to ensure the VPD was maintained below 2 kPa. From the assimilation and transpiration values the photosynthetic water use efficiency (WUE) was calculated ($\mu mol\ CO_2 \cdot m^{-2}\ mmol\ H_2O \cdot m^{-2}$).

Physiological measurements in different climate zones: During the start of the season in spring 2016 (Sept.), once-off physiological measurements were done during full bloom in semi-arid (Kakamas) and sub-tropical (Nelspruit) production regions of South Africa to determine comparative values for these parameters under different climatic conditions on 'Nadorcott' mandarin planted on *Carrizo citrange* rootstocks. Both orchards used were mature 7-8 year commercial orchards. The shade netting properties and cultivar used in these two sites were similar to those of the principal trial site, i.e. 20% white shade netting, as described above for both of the sites. The IRGA measurements were done as described for seasonal physiological measurements based in Citrusdal. The aim was to make a comparison with full bloom measurements of the physiological response of the same cultivar at the same stage under shade netting in Citrusdal in Oct 2016.

Kakamas: Five uniform trees were used, and physiological measurements were taken at three-time periods 0900-1030HR, 1030-1300HR and 1400-1600HR from three mature uniform leaves per tree.

Nelspruit: Six uniform trees were identified for measurements in orchard under shade net and in the adjacent open orchard on which leaves on positions as described above on three shoots were used for physiological measurements. Measurements from three-time periods 0900-1030HR, 1100-1230HR and 1300-1430HR were pooled together.

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Statistical analysis

Physiological and climatic data were analysed using STATISTICA data analysis software version 13 (Dell Inc. 2015, Round Rock, TX, USA). Analysis of variance (ANOVA) or repeated-measures ANOVA was performed when responses were repeated on the same respondent. Mean separations were carried out using Fisher's least significant difference test, where applicable, at $P \leq$ *0.05* and *0.1*. The experimental design was a Randomised Complete Block Design (RCBD) with four blocks per treatment (n = 4), i.e. control and shade net.

Statistical analysis for climate zones: Since these measurements were done in a commercial orchard in which treatments could not be randomly allocated, data were analysed as a completely randomised design with pseudo-replicates. Repeated measures analysis (RMANOVA) was used to analyse different physiological parameters that were repeated within a day. Mean separation was done using Fisher's least significant difference test (Fisher's LSD), where values $P \leq 0.05$ were considered to be significant.

Results

The air temperature (°C) between 0800-1200$_{HR}$ did not differ significantly over the season on the day of physiological measurements (Fig. 4A). In general, low temperatures were observed in winter, increasing in spring and reaching a maximum in the summer months of Jan. to Feb. 2017, where after both the shade net and control temperature declined throughout autumn to winter. Relative humidity (RH) was 1.6% higher under the shade netting during the season, but RH was not significantly different for the whole season (data not shown). However, in Jan. 2017 the shade netting environment had a significantly higher RH of 48.3% compared to the control of 44.4% (Fig. 4B). In Feb. 2017, RH was at the lowest for both the shade netting and control, before increasing towards June. Even though there was no difference in air temperatures between treatments, the slightly higher RH values under shade netting, especially during the summer months, affected the VPD during this period.

Differences between treatments during the summer months, especially in Jan. and Feb. 2017, occurred with the shade netting significantly reducing VPD in Jan. 2017 from 2.4 to 2.1 kPa (13% reduction) and in Feb. 2017 by 10% (from 3.9 to 3.5 kPa). In Mar. 2017 a smaller reduction of 8% (from 2.5 to 2.3 kPa) occurred (*P = 0.0669*). From Apr. 2017 until June 2017 no differences between treatments were observed and VPD was lower due to low temperatures and high RH during winter (Fig. 4C).

Throughout the season the physiological response of the 'Nadorcott' mandarin trees were only moderately affected by the shade netting (Table 1) with the A_c (*P = 0.1069*) and g_s (*P = 0.0819*) being higher under the shade netting over the season. Even though not significant at 95% confidence levels, A_c was 9% higher under the shade netting, raising assimilation rates from 5.23 to 5.75 $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$. Stomatal conductance was also higher throughout the season, but E and WUE remained unaffected over the whole season.

The aim of the monthly measurements over the season was to document not only the seasonal effect on the 'Nadorcott' mandarin trees' physiology, but also to evaluate the effect of the shade netting within months over the season and, therefore, the critical development stages (Fig. 6).

In order to interpret how the changes in microclimate, in general, can influence the physiology of 'Nadorcott' mandarin, the shade netting and control measurements were pooled (Fig. 5). Monthly differences occurred in A_c rates and g_s indicating seasonal effects (Fig. 5A and B). July 2016 had the lowest rate with a gradual increase until Oct. 2016 to Jan. 2017 which differed from Feb. and Mar. 2017 due to prevailing high temperatures resulting in an increased VPD. During autumn (Apr. 2017 and May 2017) A_c rates increased again until declining during the winter (June 2017).

In general, there are three different levels over the season which do not correspond with the same changes in climate (Fig. 6). The first level for A_c and g_s was in the period from winter (July 2016) to spring (Sept. 2016) when temperatures were sub-optimum and restricted photosynthesis physiology ($\pm$ 3 $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$). The second level, ranging from 5 - 6 $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$, were Oct.

2016, Feb. 2017, Mar. 2017 and June 2017, with the highest rates greater than 6 µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ in Nov. 2016, Jan. 2017, Apr. 2017 and May 2017. In summer (Feb. to Mar. 2017) the g_s and A_c were reduced from Jan. 2017 due to the large increase in VPD and temperature experienced in this period, whereas in June 2017 with the same A_c and g_s the prevailing low temperature was considered to be the limiting factor. The highest values for these two parameters (Nov. 2016, Jan. 2017, Apr. 2017 and May 2017) were as a result of non-restricting VPD in these months.

Transpiration rates followed the same pattern as stomatal conductance (g_s) throughout the season (Fig. 5C). Photosynthetic WUE expressed as ratio A_c:E was not influenced by the shade netting treatment (Table 1), however comparing months, photosynthetic WUE differences occurred as a result of changes in prevailing climate (Fig. 5D).

From Nov. 2016 under the shade netting a higher trend in A_c, g_s, and E started to occur and persisted until Apr. 2017 (Fig. 6A to C). From May 2017 onwards this trend became less apparent as the climate changed after summer. There was no distinct trend of a higher or lower WUE between the shade netting and control during the season (Fig. 6D). Due to the higher trend for the physiological measurements, the impact of the shade netting was analysed in more detail for the summer months (Jan. to Mar. 2017) and at the onset of autumn (Apr. 2017) (Fig. 7).

In Jan. 2017 the trees under shade netting had a higher A_c rate ($P = 0.0465$) compared to the control (7.08 vs. 8.35 µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$). Similar differences occurred in Feb. 2017 and Mar. 2017 with higher A_c rates by 1.16 µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ ($P = 0.07$) and 1.12 µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ ($P = 0.081$), respectively. At the beginning of autumn (Apr. 2017), no differences were obtained between the shade netting and control (Fig. 7A). Stomatal conductance followed the same trend as A_c with higher conductance during these months, with Jan. 2017 being 21% ($P = 0.0038$) higher under the shade netting (0.11 vs. 0.14 mmol$\cdot m^{-2} \cdot s^{-1}$(Fig. 7B). In mid-summer (Feb. 2017) g_s was the lowest for all measurements, however, the shade netting treatment had higher g_s by 0.02 mmol$\cdot m^{-2} \cdot s^{-1}$ compared to the control ($P = 0.0922$). Thereafter, no further differences were observed between treatments for g_s. During Jan. 2017, E rates were 0.3 mmol $H_2O \cdot m^{-2} \cdot s^{-1}$ higher under the shade netting ($P = 0.0791$)

compared to the control trees (Fig. 7C); however after that only numerical increases occurred. With differences in A_c rates and minimal differences in E experienced in these months, WUE remained high but unaffected by the shade netting for Jan. 2017 to Mar. 17. In Apr. 2017 WUE was 0.6 µmol·CO_2 mmol H_2O^{-1} higher (from 3.9 to 4.5 µmol·CO_2 mmol H_2O^{-1}) under the shade net ($P = 0.0488$) compared to the control (Fig. 7D). This difference was due to higher A_c and not a reduction in E for the specific month.

During full bloom in Citrusdal, which experiences a Mediterranean-type climate, A_c rate of 5.58 µmol CO_2·m^{-2}·s^{-1} (Fig. 6) was recorded which was higher than Kakamas, a semi-arid area (3.41 µmol CO_2·m^{-2}·s^{-1}) (Table 2) and Nelspruit, a subtropical area (2.51 µmol CO_2·m^{-2}·s^{-1}) (Table 3). However, in none of these production regions were significant differences between treatments observed. In both Kakamas and Nelspruit, shade netting did not affect A_c, g_s, and E throughout the day at full bloom. However, it is noted that in Nelspruit the time of the measurements had a significant reduction in above-mentioned parameters later in the day whereas in Kakamas leaves did not show a midday depression. In the morning in Nelspruit, physiological parameters associated with CO_2 assimilation were at their highest, then decreasing until midday (Table 3).

Discussion

The 20% shade netting reduced solar radiation by 17% during the study and affected leaf photosynthesis and stomatal conductance during the summer months. Citrus leaves are sensitive to high temperatures and high VPD and stomata quickly closes to reduce less water loss (Brakke and Allen, 1995; Khairi and Hall, 1976). However, a citrus tree responds to high VPD by reducing stomatal conduce and, as a result, a reduction of A_c rates is observed (Goldschmidt, 1999). The increase in VPD in Feb. and Mar 2017 had a negative effect on stomatal conductance and photosynthesis causing stomatal closure and a decrease in CO_2 assimilation rates. During the summer, i.e. Jan. to Mar. 2017, the shade net positively affected the photosynthetic physiology of the

'Nadorcott' mandarin tree in addition to a trend of higher A_c and g_s whereas E and photosynthetic WUE remained relatively constant.

The differences in VPD during the middle of summer between the control and the shade netted treatments could be due to an increased RH within the shade net structure, possibly due to the lower wind speeds removing less moisture from the area (Stamps, 1994). The lower VPD under the shade netting was during summer, reducing the atmosphere's demand for water and creating a less water-stressed environment. In Jan. 2017, VPD was reduced by 34% under the shade netting and resulted in a 15% higher A_c rate due to an increased g_s facilitating better gas exchange between the leaf and the atmosphere. These higher rates of assimilation under the shade netting were less marked in Feb. and Mar. 2017.

In Feb. and Mar. 2017, A_c rates were lowest for both treatments due to the high VPD experienced, especially in Feb. 2017, however, the shade netting had higher possible A_c rates due to a reduction of VPD that led to an increase in g_s. Other research on citrus using higher percentage shade nets (40 – 50%) as well as different coloured nets showed an increase in A_c and g_s of citrus leaves due to the positive impact of either the temperature, VPD or both (Alarcón et al., 2006; Cohen et al., 2005; Jifon and Syvertsen 2001; Jifon and Syvertsen, 2003; Nicolás, 2008,). In other fruit crops an increase in A_C rates under shade netting has also been reported (Nicolás, 2005; Smit, 2007) which indicates the possibility of higher carbon assimilation during the season.

These higher rates of photosynthesis coincide with phase II of fruit development as well as summer vegetative flush development. At that time there is an increase in sink strength from the fruit and shoots, and by modifying the microclimate enable higher carbon assimilation rate could be beneficial to production efficiency. In production regions such as Citrusdal that experience high heat load during summer when fruit enlargement takes place, a decrease in photosynthesis can occur which would negatively affect fruit growth and eventual fruit size. A study by Wachsmann et al. (2014) on 'Orri' mandarin reported a higher yield per tree under the shade nets, but this was not due to an increase in fruit size for different nets but rather due to the bearing potential. Stampar et al. (2002)

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reported a reduction of photosynthesis under white shade netting on apples (cv. 'Jonagold') with an increased yield per tree which was attributed to the increased fruiting potential of experimental trees. In citrus, girdling of a shoot results in more carbohydrates being available to fruit and as a result increases fruit size (Cohen, 1981). If higher A_c rates occur at each stage critical for fruit enlargement and vegetative growth, the increased availability of carbohydrates could enhance fruit growth and possibly explaining the enhanced vegetative growth seen under shade netting (Stamps, 2009).

Furthermore, it has been established that by closing an orchard with a high percentage shade netting, leaf temperature can be lowered in the day affecting physiological parameters, i.e. photosynthesis, stomatal conductance, and transpiration (Alarcón et al., 2006; Cohen et al., 2005; Jifon and Syvertsen 2001; Jifon and Syvertsen, 2003; Medina et al., 2002; Nicolás 2008; Smit, 2007). It could, therefore, be possible that the 20% shade netting used in this experiment did not affect leaf temperatures during milder conditions in autumn and winter resulting in less of an impact on the tree's physiology during these seasons. In subsequent studies shade nets of higher factors should be included in order to assess at which level of shading the leaves will acclimate anatomically and if the impact on the tree physiology would be negative during colder months.

A lack of change in the physiological parameters under shade netting during the morning hours has been mentioned (Medina et al., 2002; Stampar et al., 2002); they only noted an increased A_c during midday as compared to the control which was due to a reduction in leaf temperature, directly reducing the VPD and increasing stomatal conductance. These changes could potentially result in an increased carbohydrate pool due to extended A_c under shade netting at midday. Therefore, further studies can include the effect of shade netting on photosynthesis throughout a day, especially during high heat stress periods in the summer months. From these results it is evident that during heat stress periods during the summer (from, Jan. until Mar. 2017), shade netting significantly reduced VPD, and possibly reduced midday depression and enhanced photosynthesis during this part of the day (Brakke and Allen, 1995). If this is true, the trees under shade netting would be less dependent on reserve

carbohydrates, i.e. starch, during midday due to respiration and would have more photoassimilates available for new growth.

The lack of differences during full bloom under the shade netting compared to the control in Kakamas and Nelspruit could firstly indicate that no negative effect occurs due to the shade netting. This period is however not typically a high stress period in these production regions. In future research it could be valuable to measure the effect of netting during physiological fruit drop (Nov. to Dec.) as there is a high demand for carbohydrates to set fruit whereas full bloom is more hormonally-controlled and less dependent on assimilates (Iglesias et al., 2007). A reduction in VPD at that stage of fruit development could potentially affect fruit set and fruit size. The second period which measurements could be taken is during Jan. to Mar. (Stage II of fruit development) to determine whether during this stage the same lowered rate of assimilation is experienced as seen in this study, in order to evaluate if the shade netting has the same effect on VPD in a humid area such as Nelspruit during the same period and increasing carbon assimilation.

Transpiration rates were only higher in Jan. 2017 under the shade netting when stomatal conductance was at the highest due to the reduction in VPD. However, a trend of higher values under the shade netting was observed. This could be due to a large variation in E within monthly readings as the RH within the IRGA is manually controlled to keep VPD within the chamber below 2 kPa. WUE under the shade netting remained fairly constant throughout the season with the only difference between treatments in Apr. 2017. This increased WUE is attributed to a higher A_c rate and not a reduction in E under the shade netting. Smit (2007) stated that VPD lowered the transpiration demand in an apple orchard covered by black nets even when stomatal conductance was increased. Jifon and Syvertsen (2003) observed a significant increase in WUE in their study and it was attributed to higher A_c with the E remaining unaffected in the shaded leaves. A study done to illustrate how to improve WUE during summer months on lemon trees reported a reduction of transpiration but WUE was mitigated by an increase of A_c (Alarcón et al., 2006). Furthermore Nicolás et al., (2008) had contradicting results to the former author by increasing WUE under shade netting without affecting

A_c rates rather by decreasing the E of the leaves. Medina et al. (2002) found an increased E which was linked to an increase in g_s; however, the WUE was higher under the nets due to greater carbon assimilation. These results indicate a potential to reduce irrigation in a 'Nadorcott' mandarin orchard due to the possibility of using water more efficiently under shade netting.

To conclude, a permanent 20% white shade net in a 'Nadorcott' mandarin orchard in Citrusdal, reduced the solar radiation and changed other microclimate parameter but did not negatively affect physiological parameters associated with photosynthesis. Furthermore during heat stress periods in the summer the shade netting increase stomatal conductance due to a lowered VPD, and in return increased photosynthesis. It can be condluded that the shade netting did not negatively affect physiological parameters related to photosynthesis throughout a season and could, in fact, increase carbohydrate assimilation during high heat stress periods in the season.

Table and Figures

Table 1. The effect of 20% white shade netting on the rate of CO_2 assimilation (A_c, µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$), leaf stomatal conductance (g_s, mmol$\cdot m^{-2} \cdot s^{-1}$), leaf transpiration (E, mmol $H_2O \cdot m^{-2} \cdot s^{-1}$) and photosynthetic water use efficiency (WUE) (µmol$\cdot CO_2$ mmol H_2O^{-1}) over a season between 0900 – 1200_{HR} in a 'Nadorcott' mandarin orchard in Citrusdal.

Treatment	A_c	g_s	E	WUE
Control	5.23ns[z]	0.0669ns	1.18ns	4.20ns
Shade netting	5.75	0.0745	1.25	4.35
P-values				
Treatment	*0.1069*	*0.0819*	*0.2089*	*0.3369*
Month	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*
*Treatment*Month*	*0.3170*	*0.2371*	*0.5582*	*0.7117*

[z] Indicating no significant difference between treatments for the specific physiological parameter at 95 % significant level as determined by Fisher's LSD test (n = 4).

Table 2. The effect of 20% white shade net on the rate of CO_2 assimilation (A_c, µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$), leaf stomatal conductance (g_s, mmol $\cdot m^{-2} \cdot s^{-1}$) and leaf transpiration (E, mmol $H_2O \cdot m^{-2} \cdot s^{-1}$) during full bloom between 0900 – 1030$_{HR}$, 1030-1300$_{HR}$, and 1400-1600$_{HR}$ in a 'Nadorcott' mandarin orchard in an arid climate in Kakamas.

Effect	A_c	g_s	E
Treatment			
Control	3.24ns[z]	0.0325ns	1.018ns
Shade netting	3.58	0.0363	1.093
Time			
09:00-10:30	3.69ns	0.0355ns	0.918ns
10:30-13:00	3.45	0.0361	1.100
14:00-16:00	3.09	0.0317	1.148
P-Value			
Treatment	*0.2374*	*0.3284*	*0.5077*
Time	*0.0921*	*0.1583*	*0.1378*
*Treatment*Time*	*0.6814*	*0.3573*	*0.4682*

[z] Mean values within a column do not differ significantly at 95 % confidence levels as tested by Fisher LSD test (n = 5).

Table 3. The effect of 20% white shade net on the rate of CO_2 assimilation (A_c, µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$), leaf stomatal conductance (g_s, mmol $\cdot m^{-2} \cdot s^{-1}$) and leaf transpiration (E, mmol $H_2O \cdot m^{-2} \cdot s^{-1}$) during full bloom between $0900 - 1030_{HR}$, $1100 - 1230_{HR}$, and $1300 - 1430_{HR}$ in a 'Nadorcott' mandarin orchard in a sub-tropical climate in Nelspruit.

Effect	A_c	g_s	E
Treatment			
Control	2.55ns[y]	0.0181ns	0.478ns
Shade netting	2.51	0.0195	0.445
Time			
09:00-10:30	3.31a[z]	0.02303a	0.494ns
11:00-12:30	2.46b	0.01918b	0.4670
13:00-14:30	1.82c	0.01421c	0.4233
P-Value			
TMT	*0.9049*	*0.5752*	*0.5757*
Time	***<0.0001***	***0.0002***	*0.3114*
*TMT*Time*	*0.9477*	*0.8825*	*0.8541*

[z] Mean values within the same column with different letters differ significantly ($P \leq 0.05$).

[y] Mean values within a column do not differ significantly at 95 % confidence levels as test by Fisher LSD test (n = 6).

Fig. 1. Randomised complete block design of the shade netting experiment based in Citrusdal, Western Cape Province, South Africa. Treatments were allocated randomly in four blocks and consisted of the control (open) and 20% white shade netting.
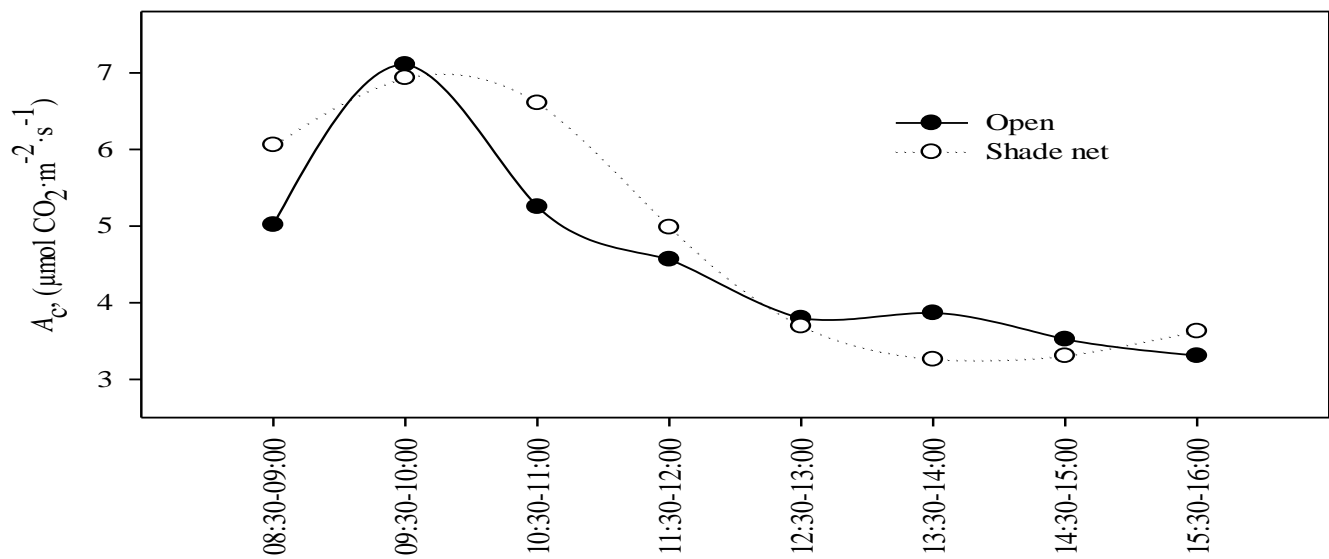
Fig. 2. Daily photosynthesis during the day for shade netting and open treatments of 'Nadorcott' mandarin in Citrusdal, Western Cape Province, South Africa (n = 8).
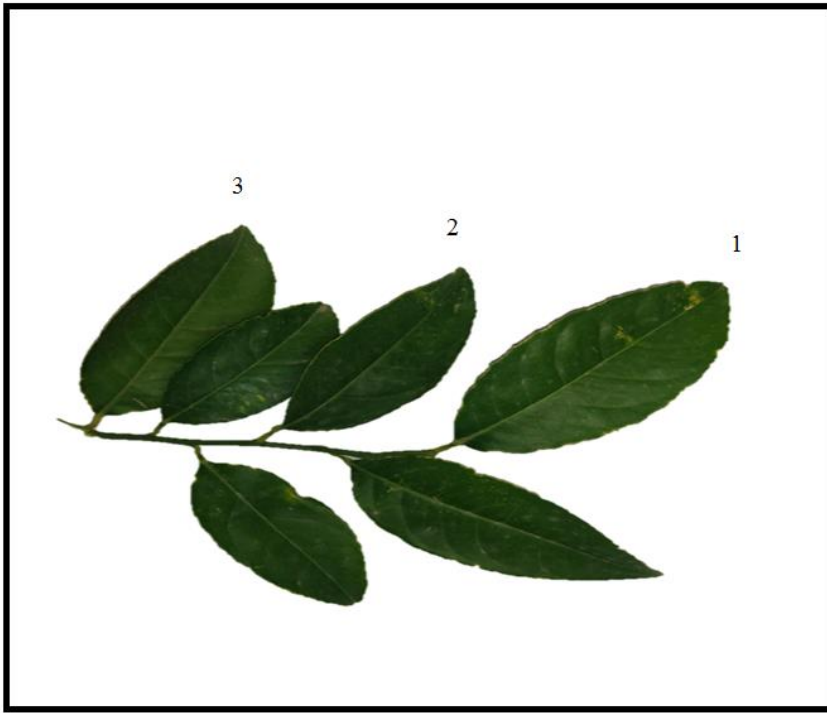
Fig. 3. Preliminary results for the position of an "off"-shoot to be used in monthly physiological measurements throughout the year on a 'Nadorcott' mandarin tree on the farm Houtkaprug in Citrusdal. 1 Terminal leaf 2. Middle leaf 3. Bottom leaf.
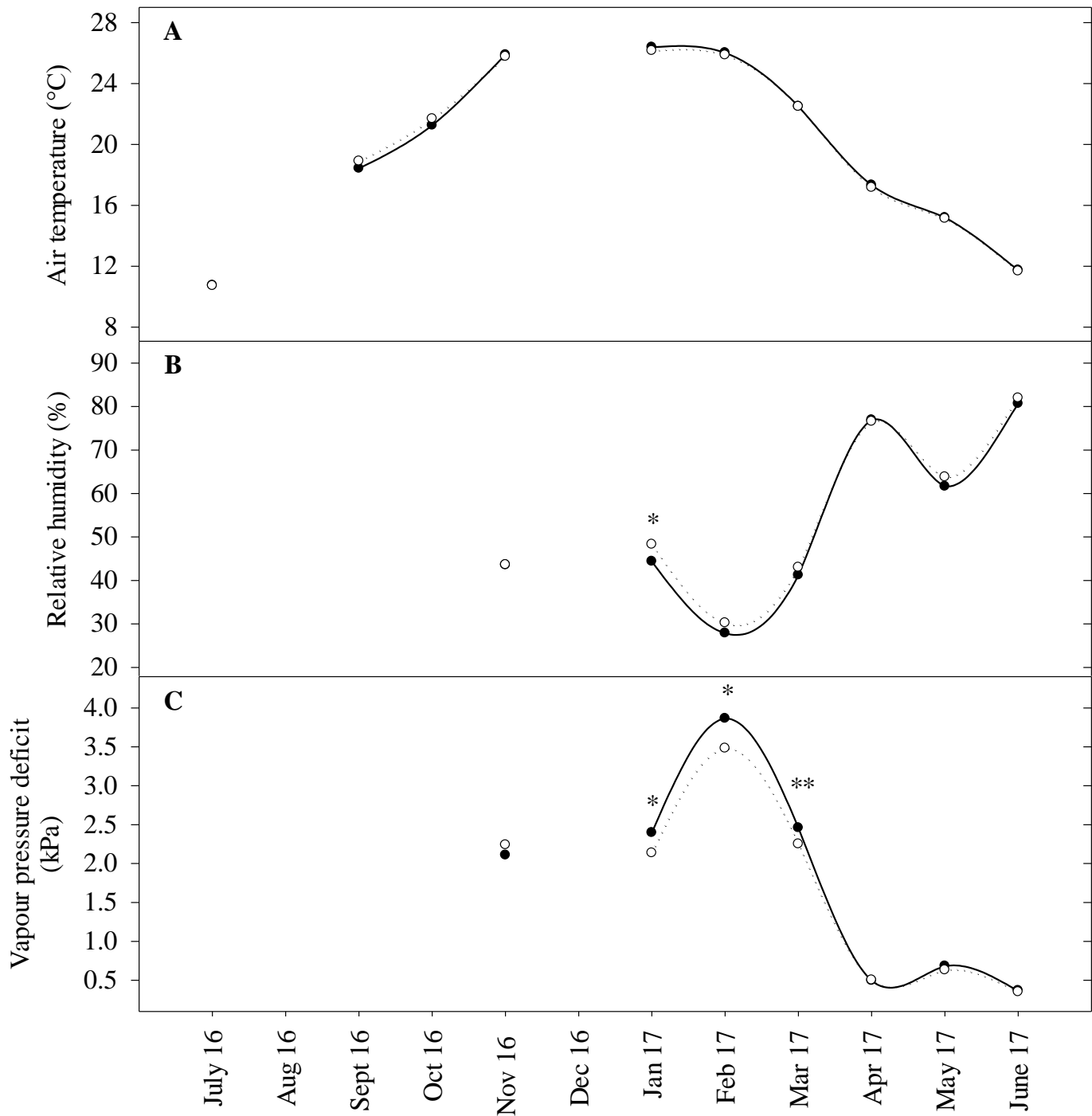
Fig. 4. Changes in important physiological climatic regulatory parameters, i.e. A: Air temperature (°C), B: Relative humidity (%), and C: Vapour pressure deficit, on the same day as physiological measurements were made between 0800_{HR} and 1200_{HR} under 20% shade net in a 'Nadorcott' mandarin orchard (n = 4).

*Indicates significant differences between treatments within a month as test by Fisher LSD test ($P \leq 0.05$).

**Indicates significant differences between treatments within a month as test by Fisher LSD test ($P \leq 0.10$).
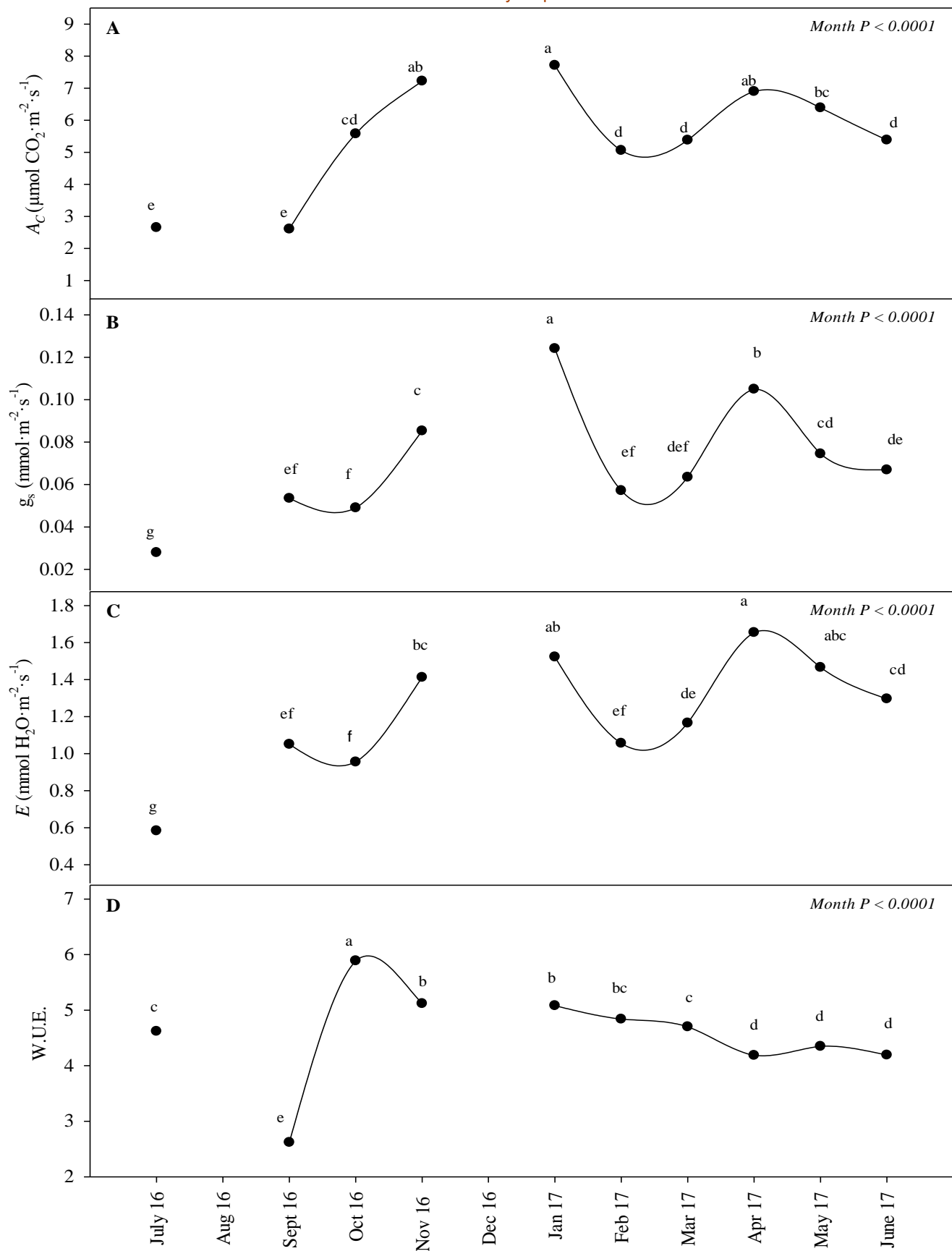
Fig. 5. Seasonal effect on A_C (A), g_s (B), E (C) and WUE (D) in a 'Nadorcott' mandarin orchard in Citrusdal from July 2016 until June 2017. Monthly mean values with different letters differ significantly from one another ($P \leq 0.05$) as tested by LSD test (n = 8).
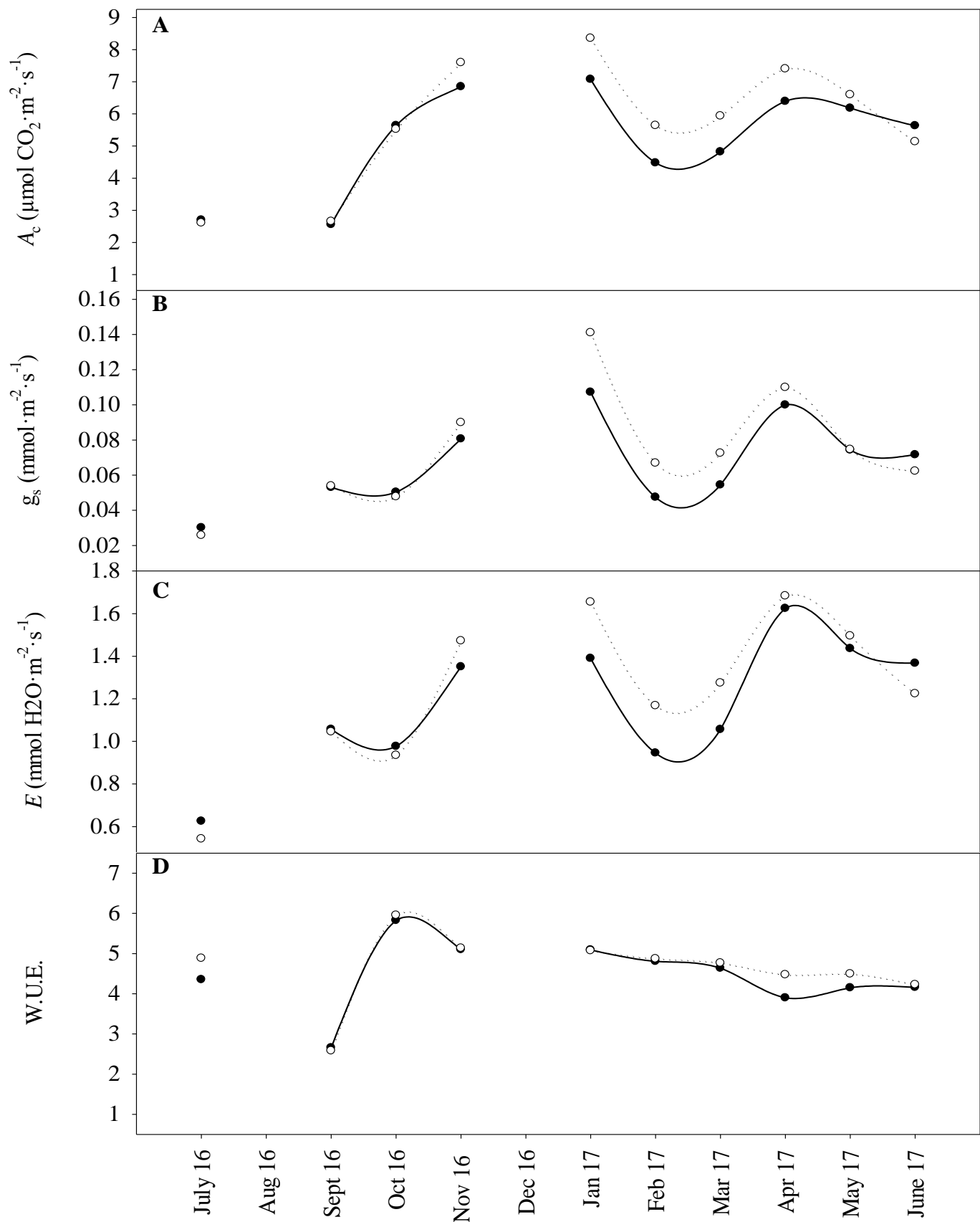
Fig. 6. Leaf physiological parameters A_C (A), g_s (B), E (C) and WUE (D) trend of a 'Nadorcott' mandarin under 20% white shade net (○) compared to the control (●) on a monthly basis in Citrusdal (n = 4).
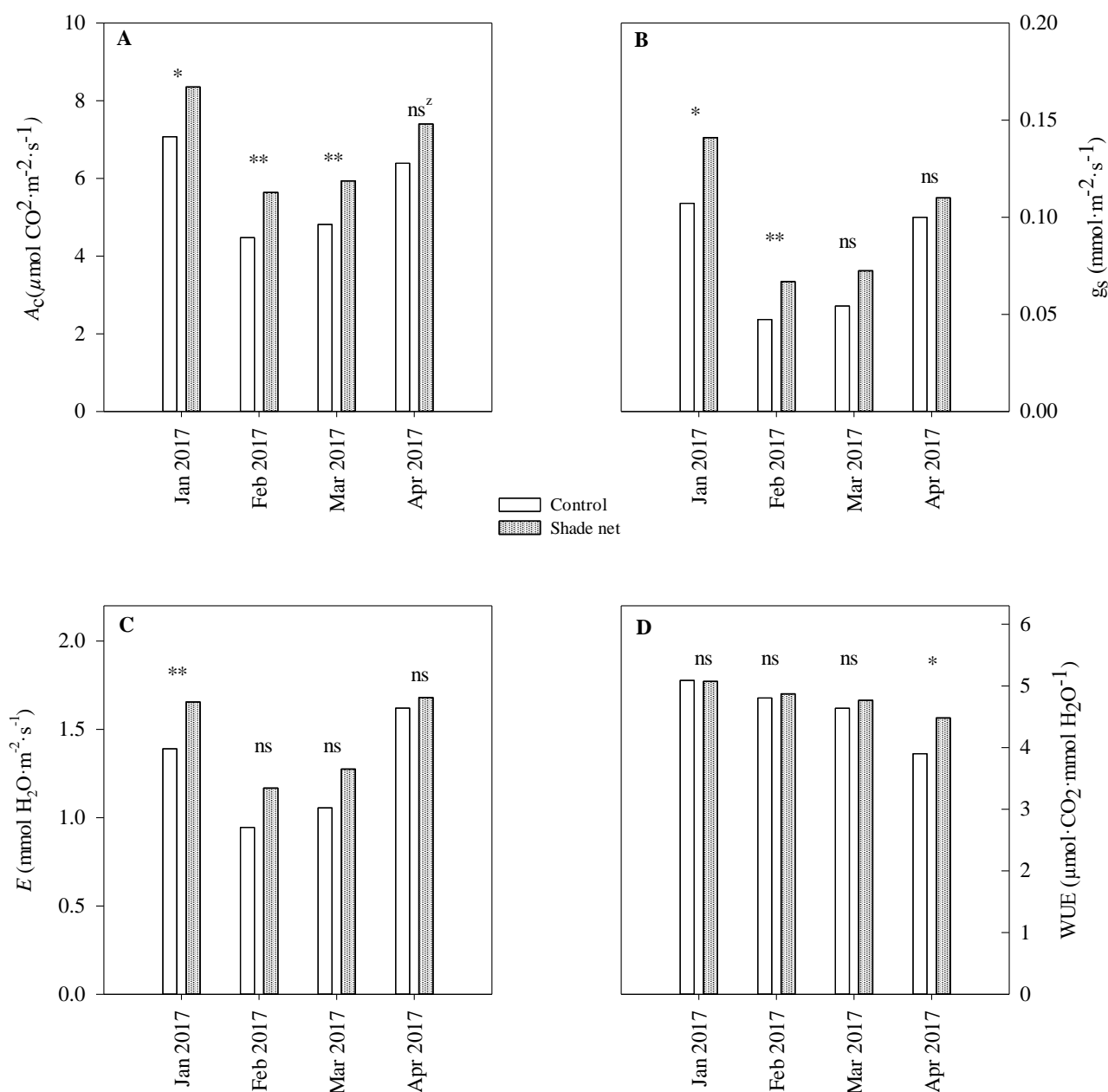
Fig. 7. The effect of 20% white shade netting during the summer months on physiological parameters of a 'Nadorcott' mandarin tree physiology, i.e. A_C (A), g_s (B), E (C) and WUE (D); (n = 4).

* Indicates significant differences between treatments within a month as tested by Fisher's LSD test ($P \leq 0.05$).

** Indicates significant differences between treatments within a month as tested by Fisher's LSD test ($P \leq 0.10$).

z Indicates no significant differences between treatments within a month.

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Chapter 5: The effect of shade netting on carbohydrate accumulation of 'Nadorcott' mandarin

Abstract

Shade netting is used to protect agricultural crops from dramatic climatic events that could potentially lower their value. Shade netting alter the light quality and quantity, thereby affecting the carbon assimilation and carbohydrate partitioning of the crop. 'Nadorcott' mandarin trees planted in 2012 in Citrusdal, Western Cape Province, South Africa, were covered with 20% white shade netting before full bloom of the 2015 season. Possible changes in carbohydrate levels of mature, fully expanded leaves on non-bearing shoots as well as fibrous roots (diameter <0.5 mm) were evaluated. Some notable differences were observed between open and shade netted treatments for root and leaf carbohydrates. Seasonal variation was observed in the reducing sugar and polysaccharide patterns. The higher starch concentration of shade netted trees throughout the two seasons indicates a preference for the production of non-structural carbohydrates possibly due to an extended carbon assimilation. Netting of 'Nadorcott' mandarin with white shade netting increased the starch levels, which may either have an influence on the final fruit set after physiological drop in November or lead to increased reproductive and vegetative growth.

Keywords: Non-structural carbohydrates, shade netting, microclimate, *Citrus reticulata* Blanco, physiology, phenology

Introduction

Shade netting is a controlled-environment agricultural technology used to protect high-value crops from dramatic climatic events such as hail, sunburn and wind damage. This technology has recently been applied in citrus (*Citrus* spp.) orchards as it may be beneficial in counteracting harsh environmental conditions, dramatic within season climatic changes as well as for the expansion of citrus orchards to climatically-limiting regions. Constructing shade netting over the crop alters the amount of light intercepted at canopy level (Wachsmann, 2014), thereby creating a constant semi-

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shaded environment. The altered microclimate that results is thought to affect the plant's physiological processes such as transpiration and possibly the source-sink balance as well as photosynthesis.

Photosynthesis converts light energy into usable energy in the form of carbohydrates. The soluble carbohydrate pool is composed of sucrose, a non-reducing sugar, as well as fructose and glucose, two hexose-intermediate sugars (Taiz et al., 2015). These fixed carbohydrates in the form of sucrose are transported in the phloem by a pressure gradient between source (leaves) and sinks, i.e. vegetative and reproductive growth. The pattern of carbohydrate assimilation within a fruiting branch of citrus was determined by Kriedemann (1970) using ^{14}C-labelled isotopes. Leaves from the previous growth cycle were shown to support terminal fruit growth to a lesser extent, while the photoassimilates derived from growth in the current season were transported acropetally to terminal fruits. Vegetative lateral shoots do not transport carbohydrates acropetally but rather support fruit, shoot and root growth basipetally. It is noted that soluble solute levels in the leaf remain low throughout the season due to the high assimilate demands of developing fruit during summer and autumn (Goldschmidt, 1998).

Photoassimilates are used by the plant for energy to sustain growth and as intermediates used as building blocks. If assimilates cannot be used, they are converted to starch which is the main storage carbohydrate in woody plants (Loescher et al., 1990). Starch is a polysaccharide consisting of glucose monomers and in citrus leaves the levels show diurnal fluctuations (Goldschmidt and Koch, 1996). The starch is compartmentalised to prevent photo-inhibition and used during respiration when photosynthesis is absent. Starch is stored in the leaves and roots of a citrus tree during autumn and at the onset of winter due to a lowered sink activity as a result of lowered temperatures (Goldschmidt and Koch, 1996; Kriedemann, 1968). The accumulation of starch in leaves can also be seen with the absence of fruit in an "off" year, during an alternate bearing cycle (Stander et al., 2017), or when branches were girdled (Iglesias et al., 2002).

Seasonal changes in citrus tree-carbohydrate levels are closely correlated with the phenological trends and the ambient environment (Goldschmidt, 1998). In spring, non-structural carbohydrates, i.e. starch and other polysaccharides, are mobilised and used for new reproductive and vegetative growth. During this stage, starch levels decrease, and the soluble sugar content increases in leaves and roots, supplementing the demand for carbohydrates that cannot be met by photosynthesis alone. If the demand is not met during bloom, flowers and fruitlets abscise, indicating the importance of carbohydrates to set fruit (Goldschmidt, 1999). This dependence on carbohydrates during fruit set has been shown by altering the source-sink relations either by defoliation or girdling (Cohen et al., 1981; Iglesias et al., 2003). After fruit set, the second phase of fruit growth involves cell enlargement, a reduction in fruit abscission, and increased demand for assimilates due to fruit to fruit competition. If the crop load on either a tree or single shoot is high, this results in a reduction in average fruit size. Fruit size can be increased by reducing sink competition by girdling or defruiting (thinning) (Spiegel-Roy and Goldschmidt, 1996).

Carbohydrate demand is not restricted to fruit, as new vegetative growth occurs in three to four distinct growth flushes, acting as a sink for up to two months before the new foliage becomes a net exporter of photoassimilates (Kriedemann, 1968). Root growth can occur throughout the year when soil temperatures are favourable and when there is no other competing sinks present (Spiegel-Roy and Goldschmidt, 1996). Root growth and development is a sink for short periods during a season, normally before and during the spring flush and after the summer vegetative flush (Bevington and Castle, 1985). This alternating shoot and root growth pattern highlights the competition for nutrients and carbohydrates (Spiegel-Roy and Goldschmidt, 1996) mediated by plant hormones (Monselise, 1947).

Carbohydrate accumulation and distribution patterns are dependent on environmental and phenological cues which may be altered using shade nets. Shade netting changes the orchard microclimate by altering exposure to solar radiation, reducing wind speed, increasing relative humidity, in addition to varying the vapour pressure deficit (VPD), and the ambient air and soil

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temperatures (Alarcón et al., 2006; Jifon and Syvertsen, 2003; Lobos et al., 2013; Nicolás et al., 2008; Oren-Shamir et al., 2001; Tanny et al., 2008; Wachsmann et al., 2014; Chapter 3). These changes in microclimate may alter the leaf physiology and carbon assimilation and possibly the assimilation distribution within a plant. Furthermore, it is known that sun-acclimated leaves have different morphology and physiological characteristics than leaves that acclimated deeper within the shaded canopy (Syvertsen, 1984). Therefore, a more shaded environment can potentially change the physiology of the tree with regards to photosynthesis and other physiological parameters involved in CO_2 assimilation.

Environmental changes influence the physiological processes of a tree associated with photosynthesis, such as changes in stomatal conductance, which has a direct impact on gas exchange between the leaf and the atmosphere. Reducing solar radiation with a permanent shade net structure lowers the leaf temperature and leaf-to-air VPD, leading to an increase in stomatal conductance and the rate of photosynthesis (Jifon and Syvertsen, 2003). This may lead to an increase in carbohydrate availability, reproductive (fruit development) and vegetative growth.

It is yet to be established if shade netting would influence carbohydrate utilisation or if a change in the carbon accumulation pattern would occur. The aim of the study was to determine the effect of 20% white shade netting on the carbon accumulation pattern of 'Nadorcott' mandarin trees in Citrusdal, Western Cape Province, South Africa, over all the phenological stages of a season.

Materials and Methods

Site, plant material and shade net properties

The experiment was conducted in Citrusdal (32° 35′ 22″ S, 19° 0′ 53″ E), Western Cape Province, South Africa, in a commercial orchard of 'Nadorcott' mandarin (*Citrus reticulata* Blanco.) budded onto 'Carrizo' citrange rootstock. 'Nadorcott' mandarin is late maturing and harvested from July until August under Citrusdal conditions. Trees were planted in 2012 at a spacing of 5.5 x 2.5 m in uniform soil. All trees received the same commercial cultural practices, i.e. nutrition and irrigation, unless otherwise specified. A permanent netting structure according to commercial standards was

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constructed over the orchard as follows. The area was divided into eight equal size blocks of 25×75 m. Thereafter the two treatments, i.e. open (control) and netting, were randomly allocated to four blocks each (Fig. 1). A 20% white shade net (Plusnet, Randfontein, Gauteng, South Africa) with a shade factor of 12 to 17% was used and constructed horizontally at 5.5 m height over four uniform treatment blocks (25×75 m) a month before full bloom in September 2015. To accommodate the randomised block layout within an existing commercial and allow for statistical comparisons orchard, the nets separating the blocks only extended from the roof to the 1.5 m above soil level into the tree canopy of the affected row. This practical solution, which allow for a randomised block layout, was decided on even if it was known the effect on the climate would have been less compared to a fully covered commercial block.

Leaf and root carbohydrate sampling methods and sample preparation

Eight uniform leaves per replicate were collected monthly from non-bearing shoots from spring flush (leaf age <12 months) in the morning between 0900_{HR} and 1200_{HR} concurrently with physiological measurements. Monthly root sampling of fibrous roots (diameter <0.5 mm) were done on the same time as the leaves to minimise diurnal fluctuations in carbohydrate concentrations (Goldschmidt, 1998). Each sampling date occurred at the end of a month throughout a two-year period. Different areas around the tree were used for root sampling to avoid sampling regrowth in the previously sampled positions. The root and leaf samples were washed and left to air-dry for an hour before being stored at -80°C. The frozen samples were freeze-dried (Christ Beta 1-8 LD Freeze Dryer, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) for 48 hours. The main drying phase for the first 24_{HR} took place at -12 °C at 2.1 mBar pressure and the final drying phase for the following phase was at -51 °C at 0.04 mBar. Thereafter the samples were milled to a fine powder with an analytical grinder (Yellow line, A10, IKA-Werke, Staufen, Germany), sieved through a 200 µml sieve, vacuum sealed and stored for analysis at room temperature.

Extraction and quantification of carbohydrates

The total soluble sugars, water soluble polysaccharides and starch were extracted from 100 mg of the dried and milled plant tissue, with the same sample pellet being used for each consecutive extraction. Extractions of the different carbohydrates were done by different solvents and at different temperature ranges due to the solubility of the different molecules within the plant material.

Total soluble sugars: To extract the soluble free sugars, 4 ml 80% ethanol (80% EtOH) was dispensed into Kimix tubes with the sample, vortexed and placed on a heating block (Grant QBB4) set at 80 °C for 30 min. Thereafter, samples were vortexed and centrifuged (Eppendorf Centrifuge 5810 R) for 4 min at 3220 rcf. The supernatant was decanted into a glass tube. The residue was re-extracted two more times with 4 ml 80% ethanol at 80 °C, for 30 min and 15 min, respectively. The three 80% EtOH extracts were pooled together. The total free soluble sugars in this 80% EtOH extract was quantified using the phenol-sulphuric acid assay and expressed as glucose equivalents.

Total water-soluble polysaccharides: Four ml of 100% deionised water was dispensed into the tube containing the sample residue and, after vortexing, extracted at 80 °C for 20 hours. After centrifugation (4 min at 3220 rcf), the residue was re-extracted in a similar way, two more times. The extraction times were 20 and 24 hours respectively. The three water extracts were pooled. The total water-soluble polysaccharides in this pooled water extract was quantified using the phenol-sulphuric acid assay and expressed as glucose equivalents.

Total starch: Three ml sodium acetate buffer (5 mM; pH 4.8) was added to the sample residue, and heated for one hour at 100 °C. The sample tubes were allowed to cool to 60 °C. Three ml amyloglucosidase solution (AMG) [Sigma Aldrich (Pty) Ltd, Aston Manor, South Africa], (1 mg AMG/10 ml 5 mM acetate buffer) were added, and incubated for 16 hours at 60 °C. After the starch was hydrolysed to glucose, the AMG enzymes were denatured by heating the solution at 100 °C (tubes on heating blocks) for 15 min. The tubes were, once again, vortexed, then centrifuged (4 min at 3220 rcf), and the glucose (from starch hydrolysis) in the supernatant was quantified by the phenol-sulphuric acid assay, using glucose standards.

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Spectrophotometric, phenol-sulphuric assay: The analysis of total soluble sugars (80% ethanol + water + AMG enzyme extracts) was done using phenol-sulphuric acid assay (Brummer and Cui, 2005) at the maximum absorption wavelength of 490 nm using a spectrophotometer (Cary 50 Series, Varian, Mulgrave, Australia). A standard curve for glucose was prepared using 0.1 mg·ml^{-1} stock solution and dilution concentrations of 0, 50, 100, 150, 200 µL glucose.

The sugar content of the above-mentioned three extracts (80% ethanol, water and AMG enzyme extracts) were determined using the phenol-sulphuric acid assay (Brummer and Cui, 2005) maximum absorption wavelength of 490 nm using a spectrophotometer (Cary 50 Series, Varian, Mulgrave, Australia). A calibration curve was prepared using a 0.1 mg·ml^{-1} glucose stock solution and suitable dilutions thereof. The glucose equivalent contents of these three extracts were calculated using the calibration data.

The glucose equivalent content of the 80% ethanol extract represents the so-called "total soluble sugars". The glucose equivalent content of the water extract represents "water-soluble polysaccharides", and of the hydrolysed starch extract, represents the starch fraction.

Statistical analysis

STATISTICA data analysis software version 13 (Dell Inc. 2015, Round Rock, TX, USA) was used to analyse the data. Analysis of variance (ANOVA) or repeated-measures ANOVA was performed when responses were repeated on the same respondent. Mean separations were carried out using Fisher's least significant difference test, where applicable, at *P ≤ 0.05* and *0.1*. The experimental design was a randomised complete block design (RCBD) with four blocks per treatment (n = 4), i.e. control and shade net.

Results

The total leaf carbohydrate concentration representing the sum of soluble sugars, polysaccharides and starch was influenced by the season as well as by the shade netting treatment (Fig. 2A). Due to generally higher starch levels in both seasons under the shade netting, the

differences in total carbohydrates levels between treatments were attributed to fluctuations in soluble sugars and polysaccharide levels until July 2016. During the first season and until harvest, the total carbohydrates remained between 119.1 to 181.6 mg·g^{-1} leaf DW with regular fluctuations between months. However, at the start of fruit maturation (May 2016), the trees under shade netting had significantly higher total carbohydrate followed by a two-fold increase occurring after harvest. After harvest in the first season (July 2016), the shade netting treatment had significantly higher carbohydrate concentration compared to the control. At the start of the second season (Sept. 2016), total carbohydrate levels under the shade netting were still higher and a decreasing trend occurred with similar rates for both the treatments. The second season total carbohydrate levels remained in the same range (119.1 to 191.2 mg·g^{-1} leaf DW) as the previous season, with the same increase observed after harvest. The leaves under shade netting had higher total carbohydrates during the third flush (Apr. 2017). A similar increase of total carbohydrates was observed after harvest, but there was no significant differerence between the treatments.

Total soluble sugar (glucose equivalent) concentration in the leaves was influenced by the treatment and month, but with no significant differences between treatments in the first season (Fig. 2B). In the second season, total soluble sugar concentration decreased during fruit set (Sept. to Nov.), whereafter it increased to similar levels as in the first season towards harvest. In the second season from Apr. an increasing trend under the shade netting was seen, which decreased after harvest.

The total water-soluble polysaccharides levels (Fig. 2C), a type of storage carbohydrate due to the complexity of the structure, had significant interaction between the treatments and months, indicating changes in levels thereof during the season (*P < 0.0001*). In order to determine a possible functionality of this specific extracted substance, a pectin test was done on the polysaccharide extracts, which would indicate if these polysaccharides are structural or non-structural. The pectin concentration in these samples was undetectable, showing that non-structural polysaccharides were extracted.

The polysaccharides followed a fluctuating pattern over both seasons, with significant differences occurring between treatments within months. The polysaccharides were, in general, higher under the shade netting at the start of the season (Sept. to Nov.) and at the end of the season, especially after harvest. A decline pattern in polysaccharide concentration occurred from Sept. to Nov. 2016 but less acute. At the start of the second season (Sept. and Oct. 2016) the polysaccharides levels under shade netting remained 18 to 20% higher than that of control leaves, until the end of Nov. 2016 when no differences occurred between the two treatments.

The total leaf starch levels over the two seasons (*P = 0.0237*) were 18% higher under the shade net treatment and were also affected by seasonal changes (Fig. 2D). The most significant increase of starch levels in leaves occurred after harvest, before a subsequent decrease during the season. During the period coinciding with fruit growth, Jan. 2017 to June 2017, the same cyclic pattern as in the first season was evident until June 2017 whereafter an acute increase occurred after harvest in July (14 July 2017).

Regarding root carbohydrate components, the soluble sugars, polysaccharides and starch were affected to some extent by the shade net treatment for certain months over the two seasons (Fig. 3). Differences also occurred due to seasonal effects as a result of the citrus tree's phenological phases. In general, root carbohydrates decreased from flowering (Sept.) until fruit set (Nov.), then remained in a constant range during the season before increasing after harvest.

Total root carbohydrates, comprising of the sum of soluble sugars, water-soluble polysaccharides as well as starch, followed the same cyclic pattern as total leaf carbohydrates. However, the concentrations (mg·g^{-1} DW) were half those of leaves (Fig. 3A). The shade netting treatment affected carbohydrate content in some months; in Mar. 2016 the total carbohydrates were 24% lower (89.5 vs. 67.9 mg·g^{-1} root DW) (*P = 0.0741*). However, in the second season during the same period no differences occurred. A month before harvest, June 2017, the total carbohydrate

content of roots under shade netting was significantly higher than the open (73.6 vs. 95.2 mg·g^{-1} root DW [*P = 0.0736*]).

In Mar. 2016 the shade netting had 11.3 mg·g^{-1} root DW less soluble sugars (*P = 0.0334*) present in fibrous roots before an abrupt increase from 49.8 to 59.8 mg·g^{-1} root DW was observed in June 2016 (Fig. 3B). After that, the soluble sugar content had a cyclic pattern that ranged between 51.2 and 32.8 mg·g^{-1} root DW until a month before harvest with an abrupt increase in total soluble sugars for both shade netting and control treatments occurred.

Root total water soluble polysaccharides and starch followed the same decreasing trend as soluble sugars from after harvest and continuing during flowering in the 2016 season. Polysaccharides and starch (Fig. 3C and D), both prominent components of the total root carbohydrates, increased significantly after harvest. Before and after harvest (May 2017 – July 2017), the storage carbohydrates had an inverse pattern compared with the soluble sugars until after the 2017 harvest. This inverse pattern between reducing sugars and storage carbohydrates was more prominent in the second season after harvest. Water soluble polysaccharides only differed between treatments in Oct. 2016 (*P = 0.007*), with the roots in the shade net treatment having 33% more polysaccharides compared to the control (from 27.5 to 41.3 mg·g^{-1} root DW).

Regarding root starch content, values remained low for both treatments and only increased a month before harvest for the first season with a delayed increase in the second season. During the first season, before harvest, the control roots had higher starch content from June to Sept. 2016 with most notable differences in June 2016 (*P = 0.026*) and Sept. 2016 (*P = 0.093*) of 12.5 and 9.7 mg·g^{-1} root DW, respectively. However, in the second season during the same period, the pattern was the inverse with shade netting having two-fold more starch compared to the control.

Discussion

Shade netting affected the carbohydrate balance in 'Nadorcott' mandarin trees leading to higher leaf starch and total carbohydrate contents especially during flower formation and fruit set (phase I

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of fruit development). The importance of carbohydrate availability to sustain fruit growth is well documented, dependant on adequate energy supply during flowering, fruit set and new vegetative development - including shoot and root growth (Goldschmidt, 1999). The carbohydrate content also plays a role to maintain the reproductive and vegetative growth balance in a tree (Davenport, 1990). In terms of the seasonal pattern of carbohydrate accumulation and catabolism, a similar pattern in the shade netting and control treatments for both the leaves and roots was evident. However, the shade netting resulted in slightly higher carbohydrate levels in certain months that coincided with phenological growth stages, i.e. after harvest and during fruit set.

These cyclic patterns in carbohydrates are due to the relationship between the phenology of the tree, i.e. root and shoot flushes during a season, which influence carbohydrate storage and usage. During the second flush (summer flush) in Jan. 2016, the starch levels were significantly lower compared to Jan. 2017. However, between the last two vegetative flushes (Feb. 2017 to Mar. 2017), when root growth occurred, the carbohydrate levels remained constant. From Mar. 2017 to Apr. 2017 there was a general increase in starch levels coinciding with the third vegetative flush.

The increase in leaf starch after harvest was due to the removal of the fruit, enabling the allocation and accumulation of starch in leaves and roots (Goldschmidt and Golomb, 1982; Iglesias et al., 2002). The higher starch content of the leaves under the shade netting could be due to a cumulative increase in tree volume (Brown, 2018) and resulting higher photosynthetic capacity due to the larger leaf surface. This concurs with observations by Davenport (1990) who showed the leaf area index of a citrus tree positively influenced growth and starch accumulation. It is thought that the shade netting especially affected the rate of photosynthesis during the mid-day, as seen in the preliminary study of photosynthesis (Chapter 4, Fig. 2) thereby increasing the potential for carbon fixation. The higher carbohydrate status at this physiological stage could be due to the accumulated effect of starch from the extended carbon assimilation under the shade netting within a day (Jifon and Syvertsen, 2003).

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A higher starch content in leaves from harvest until flowering and phase I of fruit growth could be important since storage carbohydrates, such as starch, are in high demand during this period when photosynthesis rate is limited (Bustan and Goldschmidt, 1998). This aspect of higher demand for energy was evident in this study with soluble sugar levels being low during the first phase of fruit growth indicating active sink activity as flowers utilised the reduced sugars.

Fruitlet abscission or physiological fruit drop normally occurs at the end of phase I of fruit development (Nov. in Citrusdal) due to assimilate shortage that triggers a hormonal response of abscisic acid and ethylene (Goldschmidt and Koch, 1996; Iglesias et al., 2007). Inadequate carbohydrate status at this stage could therefore affect the final fruit set percentage (Schaffer et al., 1985). By girdling prior to physiological fruit drop, carbohydrates would accumulate above the girdle, leading to a reduction in fruit drop (Cohen, 1981). The higher starch concentration or carbohydrate availability in the leaves during this critical period under the shade netting could lead to less fruitlet abscission, potentially increasing fruit production (Ruiz et al., 2001; Iglesias et al., 2003). Mataa et al. (1998) noted that shading a citrus tree under a high percentage shade (50 – 55%) during physiological fruit drop did not alter the starch content in leaves but did increase the fruit drop percentage. However, reducing the photosynthetic active radiation (PAR) by 50 – 55% would likely reduce photosynthesis and lead to a lower carbohydrate supply with fruitlets abscising. It is therefore, important during the planning phase to consider how the amount of PAR will be affected under the shade net (17% in this study). In addition, the location and topography of the planned shade netting structure should be accounted for as it will influence the amount of shading and therefore the PAR.

No differences in the soluble sugar levels (glucose equivalent) in the leaves were found under shade netting compared with the open control treatment during the first season; however for three months some differences occurred in the total carbohydrate pool during the second season. As the rate of photosynthesis under the shade netting changed from Jan. to Mar. 2017 (Chapter 4, Fig. 7), changes within specific months can also be attributed to responses to phenological phase changes, leading to the metabolism of photoassimilates. The high soluble sugar levels in Apr. 2017 under the

shade netting could be ascribed to increased sink strength of the third vegetative flush (autumn flush) and hydrolysis of non-structural carbohydrates to facilitate new growth.

A short transient increase in soluble sugars was observed in the leaves under shade netting before harvest in June 2017. This could be explained by a lowered rate of photosynthesis caused by low temperatures reducing the activity of the Rubisco enzyme (Vu, 1999). These reduced CO_2 assimilation rates could increase the tree's dependence on reserve carbohydrates and causes the starch to be metabolised to sustain cell activity and new growth (Taiz et al., 2015). This reduction in storage carbohydrates being metabolised to produce soluble sugars, needed for new growth, could be a possible explanation for the higher soluble sugar concentration.

Brown (2018) reported that under shade netting, the fruit growth rate was significantly higher during phases II and III (Jan. to June) which could be the reason for the increase in soluble sugar levels and reduction in starch and polysaccharides for these months under the shade netting. The higher total carbohydrate for these months under the shade netting could, therefore, be important to facilitate fruit growth. These findings indicate the need for further research on the impact of shade netting on the respiration rate of leaves and specifically the fruit to determine the effect on carbohydrate metabolism during phenological stages.

After fruit removal at harvest the carbohydrate metabolism of citrus trees changes (Goldschmidt and Koch, 1996). However, it is relevant to note that in the shade netting treatment the total carbohydrate levels were higher in both seasons compared to the control. It could, therefore, be possible that even if a similar pattern of carbohydrates metabolism is observed under shade netting, a higher potential to sustain new growth for the following season could be realised due to a higher carbohydrate content.

Regarding root carbohydrates, starch could be most influential, as it is the principal storage form influencing root development. In the first season, from a month before harvest until the start of phase I, the shade netted trees had higher root starch levels compared to the control. However, an inverse pattern occurred during the second season. These changes could potentially indicate the start

of an alternate bearing habit, typically experienced by 'Nadorcott' mandarin trees. By altering the fruiting potential, as a result of alternate bearing, changes in storage carbohydrates in roots are expected. Although care was taken to sample uniform fibrous roots, the soil structure is a complex system, and with soil environment differences occurring, the root structure and function could potentially be different due to the position within the soil. Under the shade netting, there was the potential for increased root growth and proliferation (which was not measured in this study) due to more optimal soil temperatures and water availability (Chapter 3, Table 3 and Fig. 8 and 9) according to the temperature ranges indicated by Bevington and Castle, (1985). Therefore, it is suggested that the quantification of root growth and dry matter accumulation should be the focus of a subsequent study on the effect of shade netting in citrus production. This aspect could be a fundamental change under netting, resulting in potentially higher water and nutrient uptake under shade netting.

To conclude, shade netting influenced carbohydrate levels in the citrus trees, with emphasis on the leaves, at various phenological stages. However, the changes in microclimate under the shade netting did not change the seasonal pattern of carbohydrate accumulation in leaves or roots. The higher amount of non-structural carbohydrates in the leaves under the shade netting could result in a more sustainable cropping cycle. Furthermore, there was indication that under the shade netting the trees could assimilate more soluble sugars in the leaves which can be converted into starch, thereby favouring the production of reserve carbohydrates. This increase in stored carbohydrates in leaves under the shade netting could alter the reproductive-vegetative phenology of a tree. During fruit growth, the vegetative flushes are prioritised regarding carbohydrate allocation for a short period, causing an increase in vegetative growth. Increased fruit load reduces vegetative growth as the fruit becomes the prioritised sink. Therefore, by accumulating more carbohydrates under shade netting, fruit growth during these periods could be enhanced. In this study, more carbohydrates were available at times under shade netting and this may lead to increased fruit size. In order to further evaluate the time when carbohydrates accumulated under the shade netting, the use of molecular techniques are suggested to evaluate the gene expression profile of α-amylase which may determine the turnover

rate of starch within leaves and roots, thereby determining when starch accumulation takes place under shade nets.

Figures

Fig. 1. Randomised complete block design of the shade netting experiment situated in Citrusdal, Western Cape Province, South Africa. Treatments were allocated randomly in four blocks, and consisted of the control (open) and 20% white shade netting.
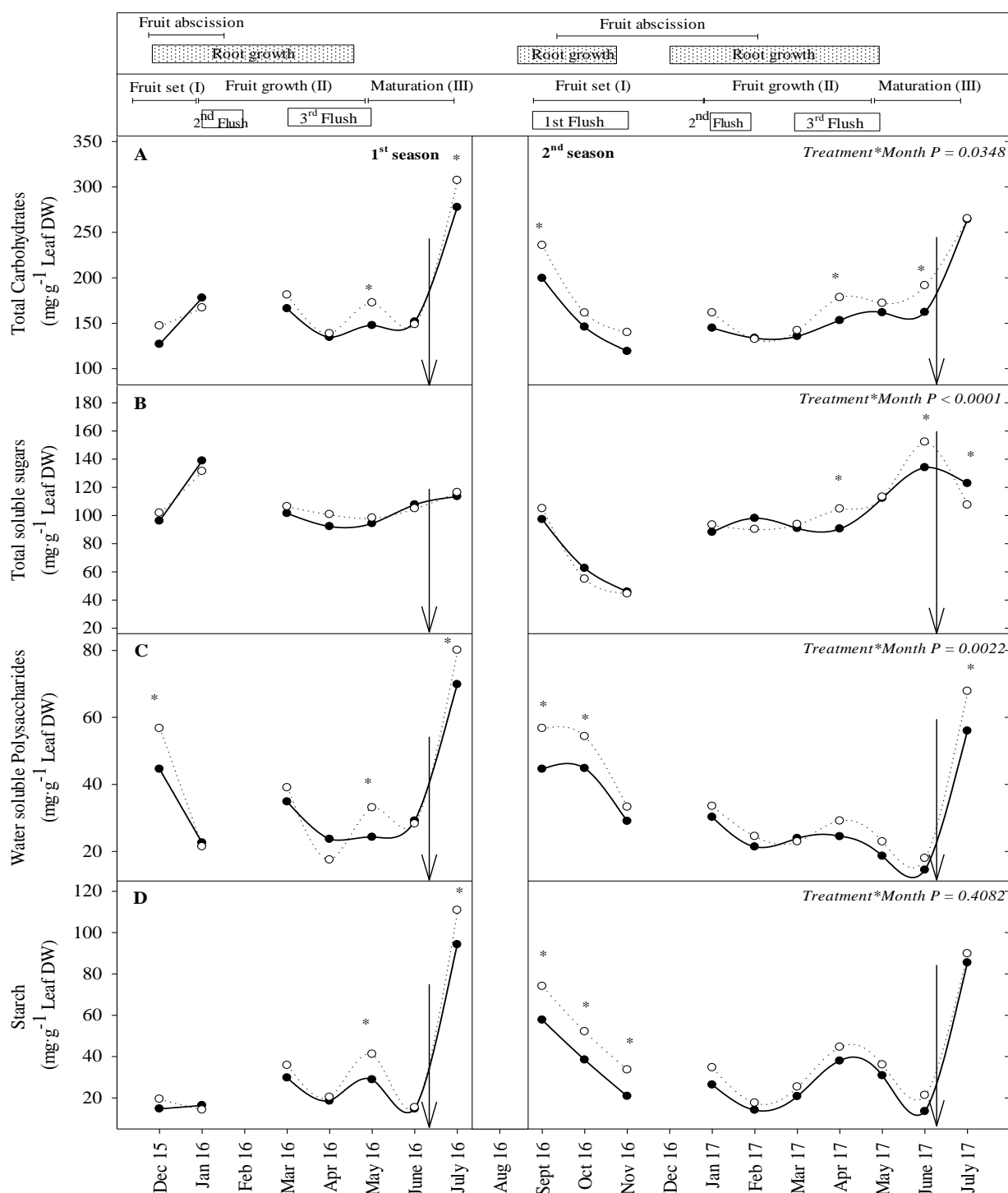
Fig. 2. The effect of 20% white shade netting on total carbohydrates (A), soluble sugars (B), polysaccharides (C), and starch (D) in leaves on an "off" shoot of 'Nadorcott' mandarin. The physiological measurements were based on samples gathered between 0900_{HR} and 1200_{HR} on a monthly (n = 4) basis during different phenological periods. The orchard was located in Citrusdal, Western Cape Province, South Africa; (○ Shade net; ● Control). *, ** Indicates significant differences between treatments within a month ($P \leq 0.05$ and $P \leq 0.10$, respectively) as tested by Fisher's LSD test; n = 4. Arrows indicate harvest date for the two seasons.

Fig. 3. The effect of 20% white shade netting on total carbohydrates (A), soluble sugars (B), polysaccharides (C), and starch (D) in fibrous roots (diameter ≤ 0.05 mm) of 'Nadorcott' mandarin. The physiological measurements were based on samples gathered between 0900_{HR} and 1200_{HR} on a monthly (n = 4) basis during different phenological periods. The orchard was located in Citrusdal, Western Cape Province, South Africa; (○ Shade net; ● Control). *, ** Indicates significant differences between treatments within a month ($P ≤ 0.05$ and $P ≤ 0.10$, respectively) as tested by Fisher's LSD test; n = 4. Arrows indicate harvest date for the two seasons.

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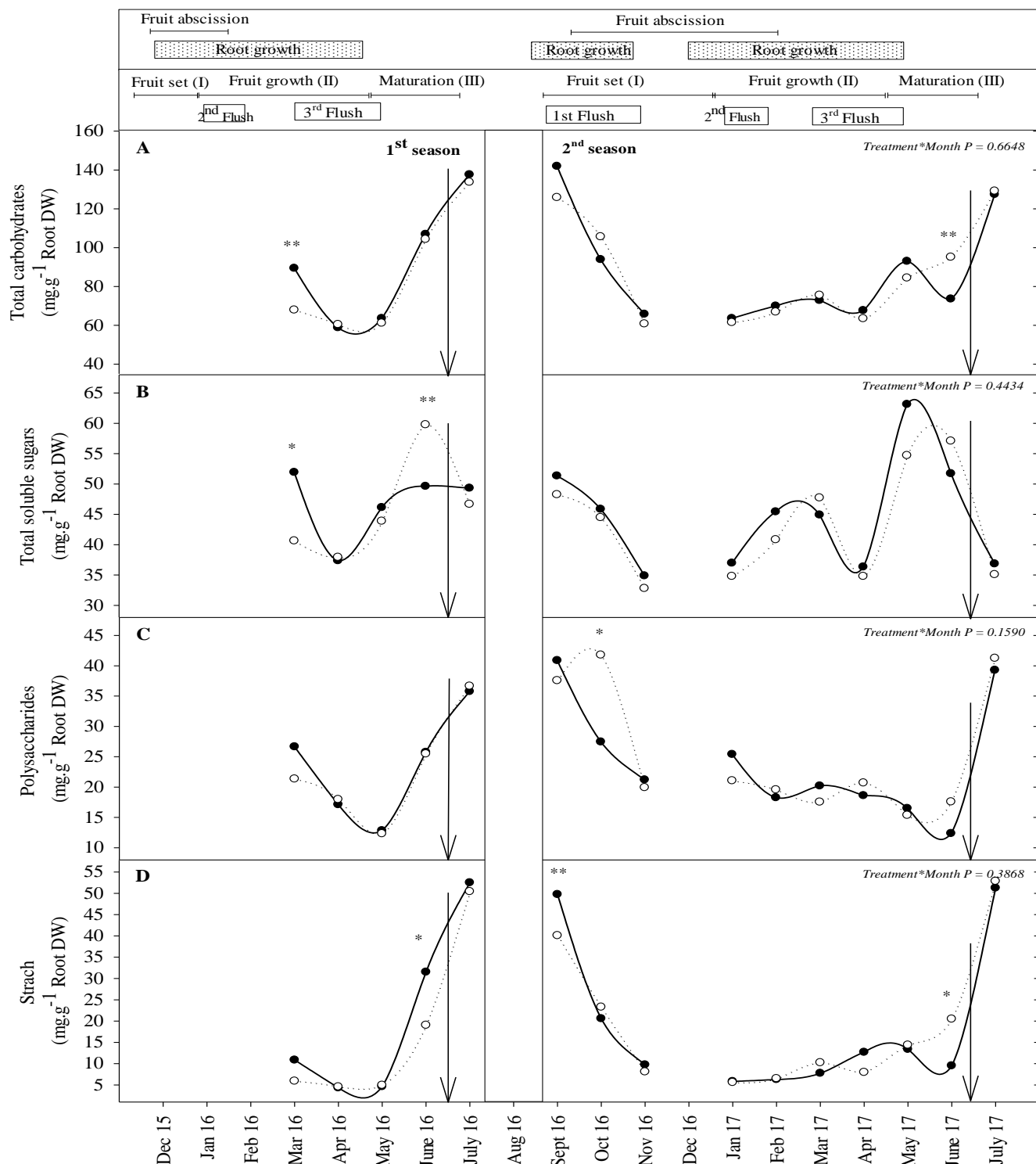Bevington, K.B., and W.S. Castle. 1985. Annual root growth pattern of young citrus trees in relation to shoot growth, soil temperature, and soil water content. J. Amer. Soc. Hort. Sci. 110:840–845.

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Chapter 6: The effect of shade netting and different irrigation regimes on 'Nadorcott' mandarin

Abstract

Shade netting is used to alter the light quantity entering an orchard to potentially alleviate the negative impact of high solar radiation causing physiological processes to be impaired and causing sunburn on fruit, and in return increase potential income. Permanent shade netting over a 'Nadorcott' mandarin orchard reduces the solar radiation and affects the temperature, relative humidity and VPD. In turn these factors affect the evaporative demand and potentially change the soil water content and plant water status of a citrus tree. This study aimed to evaluate how the changes in microclimate due to the 20% white shade netting at three irrigation volumes would affect the tree water status of 'Nadorcott' mandarin during stage II of fruit development. Pre-dawn water potential was measured after 0300_{HR}, from the physiological fruit drop period (Nov. 2016) throughout the second phase of fruit growth (Jan. 2017 to Mar. 2017). During this period, tree water potential increased from -0.58 in the control to -0.52 MPa ($P = 0.0012$) in the netted trees receiving the control irrigation volume. A similar increase in tree water potential under the shade netting was evident for trees that received half the irrigation amount, with an increase from -0.62 MPa in the control to -0.55 MPa. This reduced water stress for trees under the shade netting increased fruit diameter by 3.7 mm ($P = 0.0567$), and shifted the commercial fruit size to larger fruit counts. It was concluded that under the shade netting there is a potential to reduce irrigation without affecting yield as a result of improved growing conditions.

Keywords: Shade net, microclimate, irrigation, tree water potential

Introduction

In agriculture producers need to use technologies to optimise water usage without compromising production and income. Plant water status is the paramount factor affecting yield due to its impact on the regulation of turgor pressure within the plant structure, affecting biochemical

processes driving stomatal conductance and enzymatic activity (Jones et al., 1985; Taiz et al., 2015). Plant water status is firstly affected by the rate of water loss via transpiration and secondly by evaporation from the soil, collectively termed evapotranspiration, which is an indication of soil water potential and the plant's roots ability to take up water (Klepper, 1968). Furthermore, a tree's water status relates to the capability to control transpiration and is, therefore, related to the evaporative demand of the atmosphere (Schulze et al., 1974). The evapotranspiration demand is closely correlated with the ambient environment, i.e. solar radiation, relative humidity and the leaf boundary layer resistance, which determines stomatal conductance directly or indirectly, and affects the rate of CO_2 assimilation (Jones et al., 1985; Syvertsen, 1984). In a 20% white shade netted citrus orchard the microclimate was affected resulting in a reduction in vapour pressure deficit (VPD) (Chapter 3, Fig. 7), possibly affecting the water balance in the citrus trees.

Citrus root hydraulic conductivity is relatively low and is described as a water-limiting factor when transpiration demand of leaves is high and water supply through roots cannot be met and reduces turgor pressure (Syvertsen, 1985). Hydraulic conductivity of citrus roots is controlled by soil temperature and with a decrease in soil temperature (ranging from 35 to 5 °C) the root membranes become less permeable to water and restrict water uptake (Kriedemann and Barrs, 1981; Ramos and Kaufmann, 1978; Syvertsen et al., 1983). Syvertsen et al. (1983) showed how two different citrus rootstock's hydraulic conductivity differed at different soil temperatures, and showed that temperatures above 34 °C restricted water uptake due to a reduction in root hydraulic conductivity. Under 20% white shade netting, maximum soil temperature (30cm depth) was reduced by up to 8 °C, and in addition a 17% increase in volumetric soil water content was measured (Chapter 3, Fig. 9 and 10). The shade netting, therefore, resulted in a more favourable root environment.

Deficit irrigation led to a lower soil water potential and/or availability and decreased the stem water potential and affected the vegetative and reproductive phenology of citrus trees (Carr, 2012; Ginestar and Castle, 1996; Kriedemann and Barrs, 1981). The same effect has been reported for apricot trees with an increased stem water potential with irrigated trees compared to water stress

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treatments (Nicolás et al., 2005). Furthermore, water stress in citrus during critical phenological growth phases, especially stage II of fruit growth, could have a significant effect on yield (Ginestar and Castel, 1996).

A reduction in turgor pressure reduces stomatal conductance and reduces CO_2 assimilation during the day especially during midday depression (Brakke and Allen, 1995; Syvertsen and Albrigo, 1980). This decrease in stomatal conductance is possible due to the synthesis of abscisic acid in the roots and leaves which causes stomatal closure (Taiz et al., 2015). It is suggested that citrus stomata do not adjust osmotically as other fruit trees in order to assimilate CO_2 throughout the day and that the stomatal turgor pressure loss is due to a reduction in leaf water potential, from which it can be concluded that water potential of a citrus tree is a good indicator of plant water status (Syvertsen and Albrigo, 1980). Therefore, a change in microclimate or evaporative demand, as seen under shade netting, could potentially alter the water status of a plant.

A reduction in solar radiation, i.e. shaded leaves vs. sun-exposed leaves, decreased increased the water potential in citrus due to reduced transpiration rate and increased tree water status (Jones et al., 1980; Syvertsen and Albrigo, 1980). By using shade netting as a technology to reduce solar radiation, the potential exists to improve the microclimate to increase the water use efficiency of a tree. Nicolás et al. (2005) showed how high irradiation levels and water stress affected stomatal conductance, photosynthesis and leaf water potential in apricot leaves under shade netting compared to the control. Under the shade net of irrigated and water stressed trees, the leaves had higher stomatal conductance, photosynthesis, as well as an increased water potential, i.e. less stressed. This increase in leaf water potential was also found for different apple cultivars under 20% black shade netting due to a reduction in evaporative demand under the netting (Smit, 2007). It was reported that citrus leaf water potential remained unaffected under moderate shade in the midday (Jifon and Syvertsen, 2003) in contrast to a lemon tree where 40% shade netting resulted in higher leaf water potentials due to the reduction in VPD and solar radiation (Nicolás et al., 2008).

Due to the microclimatic changes under the shade netting a positive impact on the evaporative demand of the atmosphere and as a result the soil water content and tree water status could be realised. However, the effect of shade netting on the water status of a 'Nadorcott' mandarin tree during fruit growth has not been investigated. Therefore, the aim of this study was to quantify the affect of 20% white shade netting on tree water potential during critical phenological growth stages. In addition the potential to reduce irrigation volumes under the shade netting was evaluated in an exploratory trial. It was hypothesised that 20% shade netting would affect the tree water potential at different irrigation volumes.

Materials and Methods

Site, plant material and shade net properties

The experiment was conducted in Citrusdal (32° 35′ 22″ S, 19° 0′ 53″ E), Western Cape Province, South Africa, in a commercial orchard of 'Nadorcott' mandarin (*Citrus reticulata* Blanco.) budded onto 'Carrizo' citrange rootstock. 'Nadorcott' mandarin is late maturing and harvested from July until August under Citrusdal conditions. Trees were planted in 2012 at a spacing of 5.5 x 2.5 m in uniform soil. All trees received the same commercial cultural practices, i.e. nutrition and irrigation, unless otherwise specified. A permanent netting structure according to commercial standards was constructed over the orchard as follows. The area was divided into eight equal size blocks of 25×75 m. Thereafter the two treatments, i.e. open (control) and netting, were randomly allocated to four blocks each (Fig. 1). A 20% white shade net (Plusnet, Randfontein, Gauteng, South Africa) with a shade factor of 12 to 17% was used and constructed horizontally at 5.5 m height over four uniform treatment blocks (25×75 m) a month before full bloom in September 2015. To accommodate the randomised block layout within an existing commercial and allow for statistical comparisons orchard, the nets separating the blocks only extended from the roof to the 1.5 m above soil level into the tree canopy of the affected row. This practical solution, which allow for a randomised block layout, was decided on even if it was known the effect on the climate would have been less compared to a fully covered commercial block.

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Irrigation treatments

To evaluate the effect of different irrigation treatments on tree water status as influenced by shade netting during fruit growth, the application of irrigation volumes was changed a month before full bloom on 3 Sept. 2016. Water was supplied through double row drip irrigation with each tree having four drippers supplying a high (3.2 L/h/dripper), low (0.8 L/h/dripper) and control (1.6 L/h/dripper) volume of water and designated 2X, 0.5X and X. The frequency of irrigation was not changed. This technique is commercially used to monitor the impact of irrigation scheduling on fruit size in orchards and would create contrasting effects of under- and over-irrigation to be compared with standard irrigation volumes applied.

Data and measurements:

Pressure chamber readings

Pre-dawn water potential was measured with a PMS 615 pressure chamber (PMS Instruments, Albany, OR, USA) after 0300_{HR} in order to determine tree water potential. Pre-dawn pressure measurements were used as these readings closely reflect water status of the soil (Germana and Sardo, 1988). The average value of measurements on three mature and similar sized leaves located close to the trunk was used on each tree per replicate. Monthly measurements started during physiological fruit drop in Nov. 2016 (phase I) and continued until the of end phase II to coincide with the end of fruit growth.

Tree canopy volume and fruit development

Fruit size: For the different irrigation treatments, i.e. 0.5X, X and 2X, in each of the two treatments (control vs. shade net), the final fruit diameter (mm) at harvest was measured using a fruit size logger (GÜSS Manufacturing (Pty) Ltd, Strand, South Africa). Five fruit per tree were randomly selected on the outside of the canopy with a single fruit per shoot to eliminate the possible effect of between-fruit competition.

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Tree canopy volume: Initial tree canopy volumes were determined in winter (before spring flush), and final tree canopy volume after harvest (14 July 2017) for each sub-treatment replicate under shade netting and control. The tree canopy volume was calculated with the formula proposed by Burger et al. (1970) by measuring canopy height, width, in-row and across-row dimensions.

$$V = R^2(h - 1046 R^2) = m^3 \qquad \text{[Eq. 1]}$$

$$R = \text{Canopy radius [(In-row width + across-row width / 2) / 2)} \qquad \text{[Eq. 2]}$$

$$h = \text{Canopy height}$$

Fruit size distribution: At commercial maturity the entire tree for each treatment combination, i.e. irrigation and shade netting, was harvested to determine the yield per tree (kg/tree). From these trees 50 fruit per tree were randomly selected and the fruit diameter was measured using a fruit size logger (GÜSS Manufacturing (Pty) Ltd, Strand, South Africa) to determine the fruit size distribution of each treatment. The average weight of the different fruit size calibers, ranging from 5 (small) to 1XXX (large) in increasing order in size, was also calculated and used to determine total fruit per tree by using the fruit size distribution percentage and total yield per tree.

Internal and external fruit quality: Ten fruit per tree were randomly selected at harvest to evaluate the effect of main treatments and sub-treatment (irrigation) on internal and external fruit quality parameters. Rind thickness and fruit diameter were measured with an electronic caliper (Digital data logger, CD-"6CX, Mitutoyo Corp, Kawasaki, Japan). Rind thickness was measured on both sides after cutting the fruit (from the stylar-end to the calyx). The incidence of core gumming was scored and severity (index) was calculated using Figure 3 as a reference scale with 0 as no gumming and 4 with the highest occurrence of gumming. The fruit were juiced using a citrus juice extractor (Sunkist®, Chicago, USA), and the juice percentage was determined by dividing the total juice mass with the total fruit mass. The total soluble solids were determined by a refractometer (PR-32 Palette, Atago Co, Tokyo, Japan) and expressed as °Brix. Thereafter, 50 ml of the juice was used to determine the titratable acid content, expressed as percentage citric acid, with a potentiometric

titrator (888 Tirando, Metrohm, Switzerland) using Tiamo^{TM} software. The soluble solids-to-acid ratio is therefore expressed as the ratio of °Brix to citric acid%.

Fruit mineral nutrients: Ten additional fruit per replicate were selected at harvest for full mineral nutrient concentration analysis for the main (shade netting vs. control) and sub-treatment (irrigation). The mineral nutrient content of the fruit was analysed by a commercial analytical laboratory (Bemlab (Pty) Ltd, Strand, South Africa). The procedure briefly is as follows: 50 ml of the solution was analysed on Nitric/Hydrochloric total acid digestion, ICP-OES (Inductively Coupled Plasma–Optical Emission spectrometer) (Varian PRX–OEX, Varian, Inc. Corporate, Palo Alto, CA, USA) and concentrations were calculated using standards for each nutrient. Macro-nutrients (N, P, K, Ca and Mg) concentrations are expressed as $100 \text{ mg} \cdot \text{g}^{-1}$ fresh weight and micro-nutrients (Na, Mn, Fe, Cu, Zn and B) as $\text{mg} \cdot \text{kg}^{-1}$ fresh weight.

Statistical analysis

STATISTICA data analysis software version 13 (Dell Inc. 2015, Round Rock, TX, USA) was used to analyse the data. Analysis of variance (ANOVA) or repeated-measures ANOVA were performed when responses were repeated on the same respondent. Mean separations were carried out using Fisher's least significant difference test, where applicable, at *P ≤ 0.05* and *0.1*. The experimental design was a split-plot randomised complete block design (RCBD) with four blocks per treatment (n = 4), i.e. control and shade netting (main treatment), and with irrigation volume being the sub-factor.

Results

Pre-dawn pressure chamber readings of tree water potential (Ψ_{tree}) showed a significant interaction between the main treatments (shade netting and control) and the sub-treatments (irrigation volume) indicating that differences between the treatments existed (Fig. 2). For the control irrigation volume (X), the shade netting had an 11% higher Ψ_{tree} (*P = 0.0012*) (-0.58 vs. -0.52 MPa) compared to control trees. A similar significant response was seen at the low irrigation volumes (0.5X), with Ψ_{tree} of shade netted trees being 10% less negative (-0.55 vs. -0.62 MPa) compared to the control trees

($P = 0.0006$). The same trend, however not significant, was measured for the double irrigation volumes (2X). The increase in irrigation volumes from 0.5X to 2X resulted in the Ψ_{tree} becoming less negative, indicating less water stress, of which the effect was enhanced under netting.

The shade netting had a 40% larger canopy volume (m^3) after the second season ($P = 0.0508$; 2.31 vs. 3.98 m^3 [Table 1]) compared to the control, whereas the different irrigation treatments did not affect canopy volume; however, an increased trend of larger volume can be seen as irrigation volume increased.

No differences in fruit per tree and yield (kg·tree^{-1}) were recorded between the shade netting and control (Table 1); however, 0.5X irrigation had lower yield compared to the control and the 2X irrigation volumes ($P = 0.1049$). In addition, there were indications of an increase in yield in trees from low to high irrigation volumes ($P = 0.1298$; Table 2). The shade netting increased final fruit size ($P = 0.0567$) with a 3.7 mm larger fruit diameter (Table 1).

Furthermore, the main treatment affected the fruit size distribution (% fruit per size class) (Table 2). Trees under the shade netting produced 4.4% more fruit in 1XXX class ($P = 0.0709$) and 7.1% more in 1XX ($P = 0.0502$), and 3.1% less fruit of the smaller class 3. Therefore, under the shade netting the fruit size distribution was shifted towards larger fruit sizes compared to the control trees.

In addition to the main treatments, the irrigation volumes also effected fruit size distribution. The highest irrigation volume (2X) had significantly less small fruit (classes 2 and 3) and resulted in larger fruit (1XXX and 1XX) compared to the other two irrigation treatments. In contrast the 0.5X irrigation volumes had less fruit in larger calibres and peaked at size count 1 with fruit distributed evenly over the smaller count fruit.

In terms of fruit dimensions, the fruit height was increased by 2.6 mm under the shade netting ($P = 0.0671$), without affecting fruit diameter. In addition, a new physiological disorder, core gumming, was reduced in terms of incidence ($P = 0.0958$) and gumming index ($P = 0.0876$). Internal fruit quality parameters, i.e. juice percentage, soluble solids content (SSC), citric acid percentage

(CA) and ratio of SSC-to-CA were not affected by the shade netting (Table 3), neither were total fruit weight and rind thickness (Table 3).

The 2X compared to the control and 0.5X irrigation treatments increased the total fruit weight as well as fruit diameter and height (Table 3). The rind thickness was reduced by the 0.5X irrigation treatment (*P = 0.0627*) without influencing the juice percentage. The internal quality parameters, i.e., SSC, CA and SSC:CA, were influenced by the different irrigation treatments, with half the irrigation volume having the highest SCC, CA, and lowest ratio. At the *0.1* significant level both the control irrigation (X) (*P = 0.07*) reduced the percentage incidence of the internal disorder, core gumming.

The shade netting treatment did not affect the majority of the fruit mineral nutrients at harvest, except for the reduction of manganese (*P = 0.0468*) and higher magnesium (*P = 0.06*) contents. Irrigation volumes had a positive effect on calcium, magnesium, manganese, zinc and boron. All of these elements had higher concentrations in the fruit from the 0.5X irrigation treatment compared to the control and 2X treatments (Table 4).

Discussion

The 20% white netting created a more shaded environment, and as a result altered the soil and tree water status of the 'Nadorcott' mandarin orchard. A higher Ψ_{tree} under the shade netting indicates that the trees were in a positively modified microclimate resulting in reduced water demand. In addition, the shade netted trees receiving the control irrigation volume had an 11% higher tree water potential (less negative) compared to the open trees during the measurement period, indicating a less water stressed environment. Furthermore, those trees under the shade netting that received half the normal irrigation, had a similar Ψ_{tree} compared to the open trees receiving the control irrigation volume.

In general, it is known that the driving force for plant water uptake and transpiration is the water potential gradient between the soil and atmosphere surround the leaf, and a reduction in transpiration normally coincides with reduced water uptake (Taiz et al., 2015). The reduction in transpiration normally occurs in citrus trees during periods of high temperatures and low humidity as part of a

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seasonal cycle as well as within a day. The result was a decreased stomatal conductance and transpiration due to an increased water vapour deficit (VPD) and eventual reduction in water uptake (Brakke and Allen, 1995; Cohen et al., 1985a). Therefore, a change in the microclimate as seen under the shade netted orchard in this study (Chapter 3) can potentially have an effect on the trees' response in terms of water uptake due to changes above and below ground as illustrated by the higher Ψ_{tree}.

It was established that under the shade netting the soil water content ($m^3 \cdot m^{-3}$) was increased by 17% compared to the open treatment (Chapter 3, Fig. 9 and 10). Furthermore in this part of the study the tree water potential, measured at pre-dawn, provides a suitable reflection of soil water status (Germanà and Sardo, 1988) and tree water uptake, when VPD was lower, resulting in a possible reduced water stressed condition during the day. This concurred with previous research linking the positive correlation between soil water matrix potential and tree water potential of citrus (Levy et al., 1978; Ginestar and Castle, 1996). Soil water availability has a direct effect on hydraulic resistance between the roots and soil (Jones et al., 1985) and as a result during deficit irrigation, water uptake by roots can be reduced by up to 50% due to the higher hydraulic resistance (Cohen et al., 1983b). In the shade netting treatment more soil water was available and could potentially reduce the hydraulic resistance between the soil and root interface and thereby increased water uptake and increased the tree water potential.

An additional positive effect of the shade netting was the increase in optimal hours for root growth and function (Chapter 3, Table 3) as characterised by Bevington and Castle (1985). This was not quantified in the study, but it could be construed that these more favourable conditions could lead to more roots being initiated under the shade netting and, therefore, in addition to more available water, it is possible that a greater rooting density could lead to a reduction in hydraulic resistance and have a positive effect on tree water balance. Furthermore the shade netting treatment resulted in the minimum daily soil temperature being higher and the maximum soil temperature lower compared to the control (Chapter 3; Fig. 8), which could have a positive effect on root permeability (Ramos and Kaufmann, 1978) and thereby increasing water uptake, especially at night. This aspect of improved

root conditions should be further evaluated in shade netting studies to quantify to what extent changes in soil temperature influence root metabolism and not only water uptake but also nutrient uptake and, therefore, potentially influence fertigation regimes.

Less solar radiation on shaded leaves reduces the leaf temperature and vapour pressure (Cohen et al., 1997; Cohen and Naor, 2002; Syvertsen and Albrigo, 1980), which lowers the evaporative demand and in return can reduce transpiration and increase water potential in a citrus leaf (Jones et al., 1985). In this study, shade netting reduced both solar radiation ($Mj·m^{-2}$) as well as VPD, however, without affecting the transpiration rate in the mornings from 0900_{HR} to 1200_{HR} (Chapter 3; Fig. 2 A, C and chapter 4; Fig. 6 C). However, during the warmest part of the day, i.e., 1200 to 1500_{HR}, the reduction in solar radiation and VPD (Chapter 3, Fig. 2 and 7) under the shade netting could result in increased stomatal conductance for this part of the day (Jifon and Syvertsen, 2003). If water loss through stomatal openings exceeds that of the water uptake by roots, due to a high evaporative demand during midday, stem water potential can become more negative (Dzikiti et al., 2007). The lower VPD during the midday period could decrease the transpiration demand of a tree and prevent further moisture loss (Syvertsen, 1982), as reported in apricot and apple orchards (Nicolás et al., 2005; Smit, 2007). It could, therefore, be possible that the shade netting treatment decreased leaf transpiration demand during the period known as midday depression, in order for roots to maintain water uptake for a longer period. This could have prevented moisture stress of the trees and increased the pre-dawn tree water potential as measured.

In citriculture a high pre-dawn water potential is important as it effects stomatal conductance throughout the day (Fereres et al., 1979) thereby directly impacting on carbon assimilation. This study has indicated that under the shade netting there exists the possibility that adequate stomatal conductance can be maintained for longer periods during the day, increasing gas exchange and carbon assimilation (Chapter 4, Fig. 7).

In addition to a citrus's tree water status being critical to enable carbon assimilation, it is important to allow all the physiological processes critical to realise a commercially valuable crop

such as flowering, fruit set, as well as directly influencing the final fruit size, quality, and yield (Ginestar and Castle, 1996). In the current study the increased water potential under the shade netting did not increase the number of fruit per tree, which concurs with results obtained under 50% continuous shade (Jifon and Syvertsen, 2001). In addition the control irrigation volume (X) had the highest fruit number per tree, with 21% more fruit compared to the 0.5X and 12% to the 2X irrigation treatments. This could be indicative of a possible under- and over-irrigation scenario.

Even though fruit number (set) was not increased by the shade netting, the evident shift towards larger fruit size could be due to greater water availability during the second phase of fruit growth, responsible for cell enlargement (Ginestar and Castle, 1996; Gonzà Lez-Altozano and Castle, 1999). In addition, the increased photosynthesis measured during the cell enlargement stage under the shade netting treatment (Chapter 4, Fig. 7) could also have positively impacted on the fruit size distribution (Goldschmidt and Koch, 1996). The 2X irrigation treatment indicates how more water available could increase fruit size by shifting the fruit size to larger fruit.

Tree canopy volume was not affected by irrigation treatments; however, canopy volume growth tended to be smaller with less water (0.5X). It is, therefore, possible that by continuing these treatments over two or more seasons, a cumulative effect of reduced irrigation would negatively affect canopy volume due to decreased shoot elongation of vegetative flushes (Gonzà Lez-Altozano and Castle, 2000).

Internal and external fruit quality was not negatively affected by shade netting which is critical from a commercial point-of-view. The irrigation treatments did affect the internal quality to some extent with differences seen in citric acid (%), °Brix, and the ratio thereof. However, the irrigation did not affect the eating quality of the fruit in terms of commercial guidelines as indicated by Botes (2018). Core gumming is a new undocumented internal physiological fruit disorder of 'Nadorcott' mandarin. It was found that the shade netting resulted in lower gumming incidence ($p < 0.1$), which is the first indication that a possible reduced lowered heat and water stress could reduce the incidence thereof and should be further investigated.

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The mineral nutrient content of the fruit at harvest was analysed to document any possible changes due to the treatments. The mineral uptake of the fruit did not change significantly under the shade netting; however, the irrigation volume affected Ca, Mg, Mn and B concentrations and all of these nutrients were increased by the half irrigation volume treatment. As nutrient studies in fruit trees are a complex topic and with different nutrients impacting the tree physiology and phenology differently throughout a growing season, more in-depth research for citrus trees under shade netting is suggested.

To conclude, 20% permanent white shade netting over a 'Nadorcott' mandarin orchard increased the soil water content and in return improved tree water potential. This decrease in water stress experienced under the shade netting could lead to improved stomatal conductance for parts of the day, thereby, increasing the potential to assimilate CO_2 and improve carbohydrate status of 'Nadorcott' mandarin trees. Under the shade netting the higher soil water availability, as well as higher tree water potential could have led to the increased fruit growth and increased the production of larger fruit ($P < 0.1$). It can be hypothesised that the trees under shade netting could use the available water more efficiently during the day due to a reduction in evapotranspiration from the soil and plant tissues. On a practical level, the data indicate the possibility to reduce irrigation volumes under shade netting in citrus orchards without reducing yield or fruit quality. Therefore, during a restrictive water period, in a season or between seasons, shade netting of trees could receive less water and still maintain a commercially valuable crop.

Tables and Figures

Table 1. The effect of 20% white shade netting and different irrigation treatments (0.5X, X and 2X) on change in canopy volume, yield, fruit per tree and final fruit size of 'Nadorcott' mandarin in Citrusdal, South Africa.

Effect	Δ^{v}Tree canopy volume (m^3)	Yield (kg·tree^{-1})	Fruit·tree^{-1}	Fruit size (mm)
Treatment				
Control	2.31 ns^w	52.6 ns	496 ns	64.1 ns
Shade netting	3.98	53.4	477	67.8
Irrigation				
0.5X^z	2.55 ns	46.7 ns	432 ns	64.7 ns
X^y	3.72	55.5	548	65.6
2X^x	3.16	57.0	478	67.7
P-value				
Treatment	*0.0583*	*0.9182*	*0.7933*	*0.0567*
Irrigation	*0.2227*	*0.1114*	*0.1049*	*0.1298*
*TMT * Irrigation*	*0.7404*	*0.6927*	*0.1583*	*0.6537*

^{z} Half the irrigation volume
^{y} Control irrigation volume
^{x} Twice the normal irrigation volume
^{w} Indicates non-significant differences within a column at $p < 0.05$ (n = 4)
^{v} Difference between initial and final tree canopy volume measurements

Table 2. The effect of 20% white shade netting and different irrigation treatments (0.5X, X and 2X) on the commercial fruit size distribution of 'Nadorcott' mandarin in Citrusdal, South Africa

Effect	Caliber Diameter (mm)	Fruit size distribution (%)							
		5	4	3	2	1	1X	1XX	1XXX
		48 - 50	51 - 54	55 -58	59 - 63	64 - 67	68 - 71	72-77	78-86
Treatment									
Control		0.7ns[v]	1.7ns	6.4a[w]	18.0ns	25.9ns	22.3ns	18.5ns	5.7ns
Shade netting		0.3	1.1	3.3b	13.0	22.2	23.8	25.7	10.1
Irrigation									
0.5X[z]		0.9ns	1.4ab[w]	5.4a	15.5b	26.4ns	24.8ns	21.8b	3.1c
X[y]		0.4	2.4a	7.1a	20.4a	25.1	20.1	16.8b	7.4b
2X[x]		0.1	0.4b	2.1b	10.6c	20.6	24.4	27.8a	13.1a
P-value									
Treatment		*0.4639*	*0.3557*	***0.0406***	*0.6691*	*0.2031*	*0.5502*	***0.05015***	***0.0709***
Irrigation		*0.2634*	***0.0160***	***0.0002***	***0.0003***	*0.1165*	*0.0657*	***0.0013***	***<0.0001***
*TMT*Irrigation*		*0.2634*	*0.1733*	*0.3853*	*0.1727*	*0.1757*	*0.1187*	*0.4001*	*0.3413*

[z] Half the control irrigation volume

[y] Control irrigation volume

[x] Twice the control irrigation volume

[w] Mean values within a column with different letters indicate significant difference at either $P \leq 0.05$ or $P \leq 0.1$ levels as tested by Fisher's LSD test (n = 4).

[v] Indicates non-significance within a column

Table 3 The effect of 20 % white shade netting and different irrigation treatments (0.5X, X, 2X) on the internal and external fruit quality parameters 'Nadorcott' mandarin in Citrusdal, South Africa

Effect	Tot. Weight (g)	Diameter (mm)	Height (mm)	Rind Thickness (mm)	Juice %	SSC (°Brix)	Citric acid %	SSC/CA	Gumming %	Gumming index
Treatment										
Control	1127.5ns[v]	65.9ns	49.55ns	3.65ns	38.40ns	11.43ns	1.14ns	10.20ns	46.67ns	6.41ns
Shade netting	1232.00	67.75	52.13	3.57	39.20	11.40	1.06	10.87	31.54	3.75
Irrigation										
0.5X[z]	1036.44b[w]	63.70b	47.91b	3.35ns	38.95ns	11.75a	1.24a	9.58b	45.82ns	6.00ns
X[y]	1305.06a	69.17a	53.15a	3.70	38.07	11.03b	0.98b	11.32a	27.64	3.50
2X[x]	1197.63a	67.57a	51.46a	3.77	39.37	11.48a	1.08b	10.71a	43.85	5.75
P-value										
TMT	*0.1682*	*0.2291*	***0.0671***	*0.7248*	*0.6991*	*0.8858*	*0.1999*	*0.1651*	***0.0958***	***0.0876***
Irrigation	***0.0230***	***0.0060***	***0.0017***	***0.0627***	*0.8491*	***0.0073***	***0.0015***	***0.0066***	***0.0700***	*0.1509*
TMT*Irrigation	*0.1473*	*0.1726*	*0.6803*	*0.4774*	*0.4447*	*0.5566*	*0.7067*	*0.9941*	*0.1304*	*0.4901*

[z] Half the control irrigation volume

[y] Control irrigation volume

[x] Twice the control irrigation volume

[w] Mean values within a column with different letters indicate significant difference at either *P ≤ 0.05* or *P ≤ 0.1* levels as tested by Fisher's LSD test (n = 4).

[v] Indicates non-significance within a column

Table 4 The effect of 20 % white shade netting and different irrigation volumes (0.5X, X, 2X) on fruit mineral content at the day of commercial harvest

2017 a 'Nadorcott' mandarin orchard in Citrusdal, South Africa

Effect	Fruit mineral nutrient content										
	N	P	K	Ca	Mg	Na	Mn	Fe	Cu	Zn	B
	(100 mg·g^{-1} FW^Z)					(mg·kg^{-1} FW)					
Treatment (TRT)											
Control	214.9 ns^y	19.1 ns	207.4 ns	42.4 ns	16.0 ns	35.2 ns	1.83 a^x	5.58 ns	0.38 ns	1.57 ns	2.58 ns
Shade netting	208.1	20.0	208.0	40.3	17.1	33.1	1.00 b	3.76	0.34	1.48	2.78
Irrigation											
0.5X^w	218.9 ns	19.5 ns	211.4 ns	45.1 a	17.8 a	33.8 ns	1.94 a	5.49 ns	0.35 ns	1.61 a	2.89 a
X^v	208.0	19.8	204.0	40.3 b	16.2 b	32.5	1.23 b	4.26	0.40	1.49 b	2.63 b
2X^u	207.6	19.3	207.6	38.7 b	15.1 b	36.2	1.08 b	4.25	0.34	1.46 b	2.54 b
P-value											
Treatment	*0.3704*	*0.2425*	*0.9045*	*0.3733*	*0.0600*	*0.4052*	***0.0468***	*0.2098*	*0.3120*	*0.4720*	*0.1356*
Irrigation	*0.1869*	*0.8634*	*0.1433*	***0.0092***	***0.0006***	*0.3799*	***0.0358***	*0.4489*	*0.2314*	***0.0320***	***0.0338***
TMT X Irrigation	*0.7746*	*0.0816*	*0.7642*	*0.6715*	*0.4673*	*0.4810*	*0.1879*	*0.9697*	*0.5894*	*0.3799*	*0.7616*

[z] Fresh weight
[y] no significant difference
[x] Different letters in the same column denote significant differences between values [$P<0.05$; Fisher's least significant difference (LSD) test]; n=4.
[w] Half the control irrigation volume
[v] Control irrigation volume
[u] Twice the control irrigation volume

Fig. 1. Randomised complete block design of the shade netting experiment in Citrusdal, Western Cape, South Africa. Treatments were allocated randomly in four blocks each (75 m x 25 m). Treatment consisted of the control (open) and 20 % white shade netting.
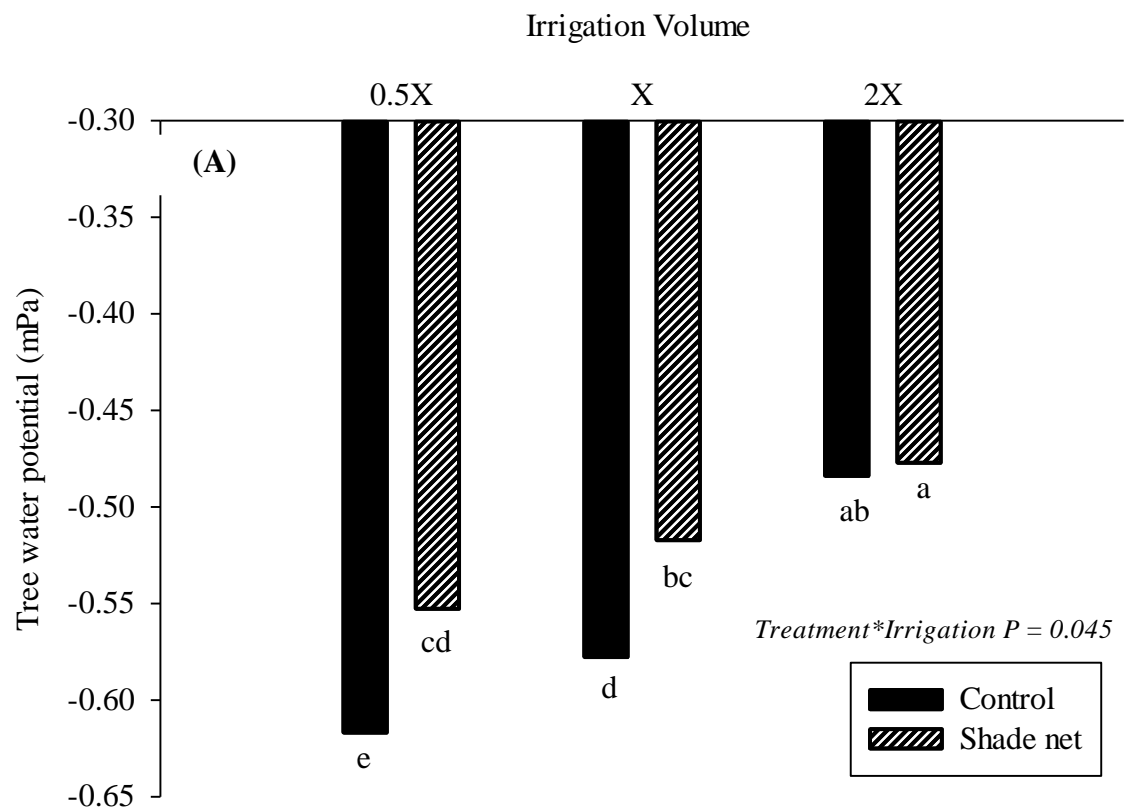
144

Fig. 2. The effect of 20% white shade netting combined with different irrigation treatments on the pre-dawn (after 0300_{HR}) tree water potential (mPa) of 'Nadorcott' mandarin from physiological fruit drop (Nov. 2016) throughout fruit enlargement phase II (Jan. to Mar. 2017) in Citrusdal, South Africa. Different letters within and between irrigation treatments (0.5X, X, 2X) differ significantly at 95% confidence level as tested by Fisher's least significant difference (LSD) test; (n=4).
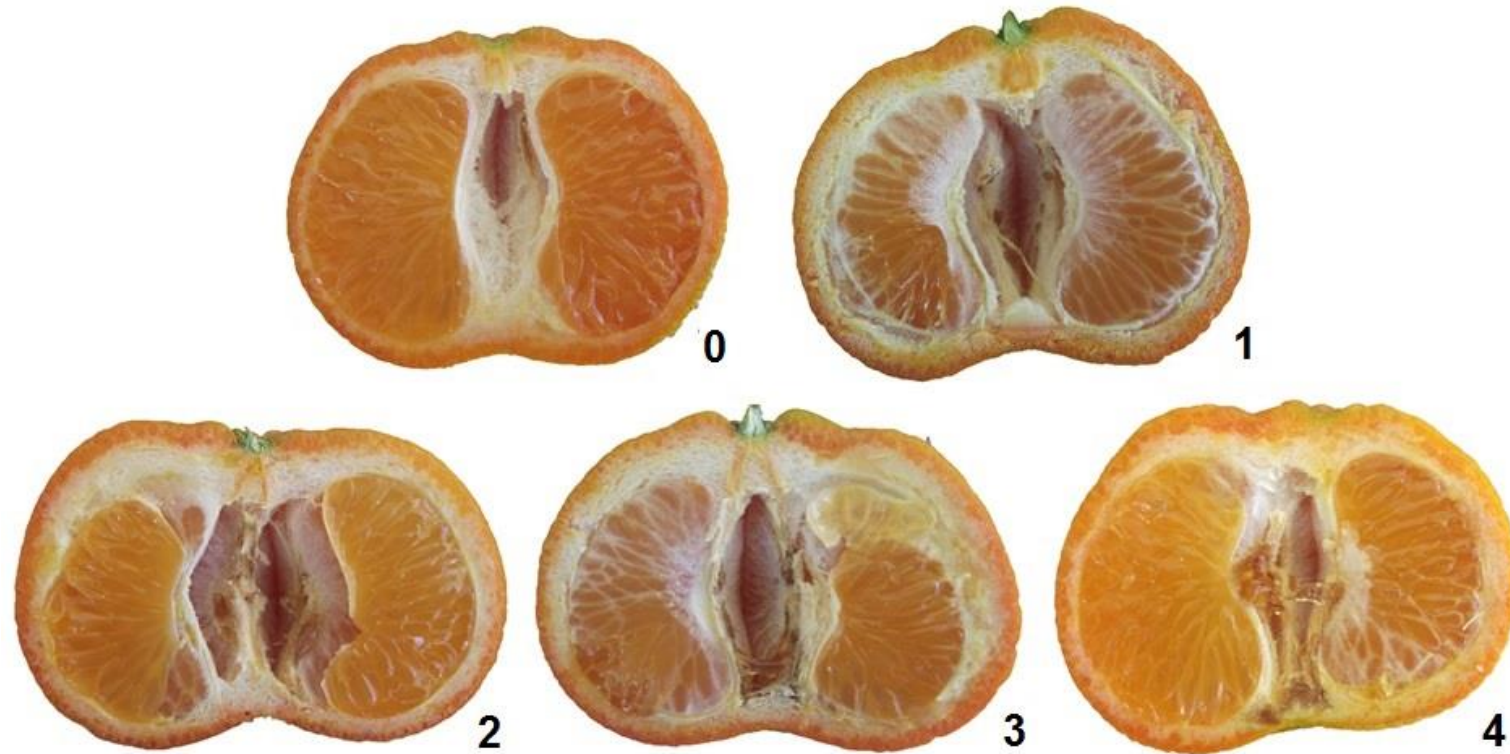
Fig. 3. Visual illustration of how rating was applied to determine the incidence of core gumming in 'Nadorcott' mandarin. Scaling from no gumming (0) to severe (4): 0 = No gumming; 1 = Low gumming (small droplets on juice sacs); 2 = Moderate gumming (small droplets on juice sacs); 3 = Mild gumming (occurrence of droplets on juice sacs with browning of vascular bundles); 4 = Severe gumming (occurrence of dried brown droplets on juice sacs with burst vascular bundles and juice sacs).

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Chapter 7: General conclusions

Permanent shade netting is becoming a popular technology used in citriculture, not only to produce high-value seedless fruit but also to manipulate the orchard microclimate by reducing the amount of solar radiation entering an orchard. Several studies established that a change in orchard microclimate exists under shade netting. However, it is unknown to what extent the reduction in solar radiation caused by 20% shade netting will influence the microclimate of an orchard based in a Mediterranean-type climate and how that change would impact the physiology of 'Nadorcott' mandarin trees.

In the first research chapter the changes in microclimate were described in a commercial 'Nadorcott' mandarin orchard covered with a permanent 20% white shade net. The shade net reduced solar radiation and acted as a synthetic windbreak and as a result changed the orchard microclimate with regards to the important climatic parameters affecting citrus physiology. However, it is suggested that a follow up in-depth study should be done on individual climatic parameters in relation to specific physiological stages, as this initial study was designed to provide an initial description of climatic changes over a season. Furthermore, the impact of different climates, i.e. semi-tropical, sub-tropical and semi-arid, and the impact on microclimate under shade netting throughout the canopy should be evaluated. Leaves adjust anatomically and physiologically to a more shaded environment, and due to the microclimate changes found it could, therefore, be expected that leaf physiological processes associated with CO_2 assimilation could be altered.

Therefore, the second research chapter quantified how the changed microclimate would influence the leaf physiology over a season. Changes of climatic parameters normally limiting to citrus leaf physiology and physiological measurements associated with CO_2 assimilation were documented throughout a season. The reduction in solar radiation and other changes in orchard microclimate, due to the shade netting did not negatively affect physiological parameters associated with photosynthesis over the season. However, during summer (Jan. to Feb.) when heat stress typically occur, the shade net increased stomatal conductance due to a lower VPD, and in return

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resulted in a higher photosynthesis compared to the open trees. In future studies it could be beneficial to analyse this specific aspect in more depth during heat stress periods as well as to document short term changes throughout an entire day. In mature orchards differences within the canopy, related to photosynthesis, could also be documented to establish pruning practices under shade netting to optimise photosynthesis in a whole tree as shading within the canopy could increase. This was not possible for this study, as the commercial layout of the experiment was large and the limited time available to evaluate the effect of the shade netting during the optimal photosynthetic period in a day.

The next step was to determine if the higher carbon assimilation during the summer months would result in more carbon being accumulated within the tree structure. The changes in microclimate under the shade net influenced carbohydrate levels during various phenological stages of a 'Nadorcott' mandarin tree with emphasis on leaf carbohydrates. Even though the shade net did not consistently alter the assimilation and distribution pattern at all stages throughout the two growing seasons, some seasonal differences were recorded. Trees under shade net had an increased starch concentration especially after harvest until flower/fruit set. The changes in the amount of non-structural carbohydrates in the leaves under the shade net, could result in a more sustainable cropping cycle by possibly influencing flower quality. There was an indication that under the shade net, the trees have the potential to assimilate more soluble sugars in the leaves, especially during the summer months, which can be converted into starch, thereby favouring the production of reserve carbohydrates. This aspect should be included and expanded on in further studies to determine if shade net has a cumulative effect of starch in leaves throughout the day. Increased starch levels can potentially alter the reproductive and vegetative phenology of a tree under the shade net due to a greater reserve carbohydrate level. Shade netting can, therefore, potentially increase the yield due to a less profound physiological fruit drop period, if vegetative growth is contained. In order to further elucidate the period of increased carbohydrates accumulation under the shade netting, the use of molecular techniques is suggested to evaluate the gene expression profile of α-amylase which may

determine the turnover rate of starch within leaves and roots, thereby determining when starch accumulation takes place under shade nets.

In the fourth part of this study the focus was on the second key physiological process of the tree and aimed to evaluate the tree water status of 'Nadorcott' mandarin under shade netting. Not only was the VPD altered by the shade net, but also an increase in average soil temperature and water content over the two seasons was recorded. The known positive relationship between tree water potential and these factors was observed. The higher, less negative, tree water potential under the shade netting indicates the tree experienced less water stress due to a milder environment. Therefore, the trees under shade net could use the available water more efficiently during the day due to a reduction in evapotranspiration from the soil and plant tissue. This increased tree water potential could also be associated with the increased stomatal conductance observed during the summer months which led to increased CO_2 assimilation. Detailed physiological measurements of the tree, leaves and fruit can be done in further studies, simultaneously with pressure chamber readings in order to establish how the reduced water stressed trees under the shade net would influence the tree water balance during the midday. Therefore, an orchard under shade netting could potentially receive less water without negatively impacting on the yield due to the increase in the water use efficiency.

To conclude, the shade netting did alter the microclimate of a 'Nadorcott' mandarin orchard in Citrusdal, Western Province, South Africa as well as the leaf physiology associated with photosynthesis especially during the summer when heat stress may occur. These changes in microclimate and leaf physiology affected the carbohydrate level in a citrus tree especially in the leaves with an increased availability of non-structural carbohydrate starch. However, fewer changes in root carbohydrates were observed over the two growing seasons. The increased soil water content and lower solar radiation experienced under the shade net increased the tree water potential

Recommendations as a result of this study are that shade netting does offer the citrus producer the potential to improve the microclimate of an orchard, especially during the summer months, when conditions considered stressful to a citrus tree physiology arise. Shade netting can alter the potential

to increase carbon assimilation during heat stress periods thereby improving fruit growth in the specific period. This aspect should be further evaluated in different climatic regions to establish whether the shade net has the same effect in winter and summer rainfall areas. Due to reduced evaporative demand in summer, the shade net could offer the potential to reduce irrigation volumes without causing water stress on the trees.

During this study the complexity of using available natural resources to realise a commercial aim was made clear. By altering only one major environmental factor, i.e. solar radiation, by the use of shade netting, resulted in a cascade of changes in the tree physiological response. Shade netting technology offers considerable advantages to citrus producers but will add complexity to the tree management.