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STUDIES ON APPLE PHYSIOLOGY BY MANAGING LIGHT QUALITY WITH PHOTOSELECTIVE NETS

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SCIENCE NEVER SOLVES A PROBLEM WITHOUT CREATING TEN MORE.

GEORGE BERNARD SHAW

CHAPTER I
INTRODUCTION and AIM OF THE STUDY

Light is the driving force for plant growth. Plants have to adapt their development to the quality, quantity, direction and duration of light. The effects can be observed throughout the plant life cycle, from seed germination, to leaf expansion, from the synthesis of photosynthetic and protective pigments, to stomatal regulation, lateral branching, from bud dormancy, to flowering, not to mention the sensing of neighbouring plants. Already in the first half of the XX century, studies were showing how timing, quantity and quality of application was affecting plant development (Boysen Jensen, 1928; Du Buy and Nuernbergk, 1929; Duggar, 1936; Johnston, 1937; Avery, Burkholder and Creighton, 1937; Hamner and Bonner, 1938; Oppenhoorth, 1939; Withrow and Withrow, 1939; Went, 1941). Not to mention how Charles Darwin had a strong interest in plant behaviour on the basis of light, in his *The Power of Movement in Plants*, dating back to 1881. An enormous amount of research has been carried out and today it is an indisputable fact that most of these effects are related to photoreceptors, sensors by which the plant is informed about its surroundings and regulated to develop appropriately. Cells contains photoreceptors, so they can sense the presence of certain wavebands and, also, communicate the signal to their neighbours (Bischoff et al., 1997). As they are soluble proteins, they are capable of entering the cell nucleus, constitutively, or through

light stimulation (Lin, 2000) and interact with proteins to affect the expression of light-regulated genes (Smith, 1999).

Research is focusing on light-regulated responses, but little is yet known about signal transduction components. Probably, a very complicated network is to be expected, consisting of a great number of signalling molecules, not necessarily affected by light but also by additional environmental stimuli or endogenous signals like phytohormones (Chory et al., 1996). The study of light signalling in plants is a challenge, as more and more interactions between the signalling channels have been discovered (Mohr 1994; Neff and Chory 1998; Parks et al., 2001).

LIGHT IN PLANTS

As solar radiation passes through the Earth's atmosphere and reaches the ground, it is partially absorbed, reflected, refracted and scattered, considerably changing its amount as well as its spectral composition. Absorption reduces light scattering, due to gases in the atmosphere, causing both light loss and diffusion (i.e. light not directly shining from the sun). Diffuse light includes that reflected from the clouds and from the ground as well. Light may also be absorbed, reflected, transmitted and scattered from plant canopies. Specifically, they absorb ultraviolet radiation and visible light, whereas they reflect and transmit far-red and near-infrared radiation.

The active spectrum of light includes the ultraviolet (UV) region, 280-400 nm, the photosynthetically active radiation (PAR), 400 to 700 nm, also known as visible light, and the near infrared region, 700-1000 nm.

Ultraviolet light is divided in two parts: UV-A, 325 to 400 nm, and UV-B, 280-315 nm. There is also a third category of ultraviolet spectrum, UV-C, which is below 280 nm, but it is considered of less interest, as these waves are trapped in the ozonosphere. UVs and blue light are sensed in plants by a category of photoreceptors, the photolyase-related cryptochromes, the phototropins and the kinase and LOV (*light oxygen volt* regulated) domain proteins. Their chromophores are known as flavins, pterins and carotenoids (Galland and Senger, 1991). When detecting UV and blue light, they trigger different processes, from circadian clock entraining, to anthocyanin formation, to phototropism, to apical hook opening, to flower induction, to stomatal opening, while inhibiting extension growth (Liscum and Hangarter 1994; Short and Briggs 1994; Jenkins et al., 1995; Ninnemann 1995; Liscum and Briggs 1996; Briggs and Liscum 1997a,b; Ahmad et al., 1998; Lin et al., 1998; Rapparini et al., 1999). Usually, high irradiances of ultraviolet light have a general inhibitory effect, or will cause DNA damage, but at low irradiances photomorphogenic effects are also observed (Kim et al., 1998). Blue light depletion especially triggers responses to the shade-avoidance syndrome in many plant species, best known as phototropism (Briggs and Christie, 2002), the ability of the plant to modify the direction of growth. Positive phototropism to blue light, along with negative phototropic responses to reflected FR radiation (Ballaré et al., 1992), can help plants to direct their growth toward canopy gaps in patchy canopies (Ballaré et al., 1995b; Ballaré, 1999). Another very important response to blue light detection is stomatal regulation. Stomata are designed to sense the internal CO_2 concentration and to let in a sufficient amount from the external air, without causing plant dehydration. They sense light indirectly, via photosynthesis, and

directly, through blue light, not only via cryptochromes and phototropins, but also through the xanthophylls zeaxanthin, or violaxanthin.

Photosynthetically active radiation (PAR) of 400 to 700 nm is the most important source of energy for plants, ensuring photochemical reactions used in the process of CO_2 assimilation (Demmig-Adams et al., 1995; Ruban and Horton, 1995; Horton et al., 1996). The principal photoreceptors converting CO_2 into energy are the chlorophylls. They are the key pigments in oxygenic photosynthetic organisms and react as antenna pigments, in the reaction centres I and II of chloroplasts. Their physicochemical properties are affected by their protein environment, allowing them to generate radical cations or anions, or remaining completely redox silent. On a larger scale, chloroplasts have the ability to acclimate for short-term (within 2 hours) altering light levels, inside the mesophyll tissues. High irradiances will move chloroplasts towards anticlinal cell walls so to reduce the amount of intercepted light, whereas under low irradiances they will gather along the periclinal walls to maximise light absorption.

For plant biologists, another interesting waveband range is that above 700 nm, which is sensed by the best known phytochrome photoreceptors. Phytochromes are produced by the plant in the red absorbing form, called P_r, and can be converted to the far-red-absorbing form, P_{fr}, by red light or direct daylight. It is P_{fr} that is considered the active form, or signalling state, but in some cases one, or several intermediates, may be at work. The reverse conversion can take place under far-red light, under daylight filtered through vegetation or soil, or in darkness. Responsible for detecting red and far-red light, they are known to trigger the, previously mentioned, shade-avoidance

syndrome: as leaves re-emit far-red, but absorb blue and red, the spectral quality of the surrounding microenvironment will be enriched with far-red, creating a sense of vegetational shading in neighbouring plants. In response to shading, stem elongation increases, development of lateral organs, such as leaves and branches, is suppressed, apical dominance is strengthened and flowering is accelerated (Smith and Whitelam, 1997; Casal, 2013). In other words, the emission of such wavelengths will create competition for light and sources, between two or more neighbouring vegetative organisms.

LIGHT IN ORCHARDS

The productivity of an orchard is directly influenced by the amount of intercepted sunlight (Palmer, 1980; Barritt et al., 1991; Palmer, 1989; Palmer and Adams,1997; Robinson and Lakso, 1991; Wagenmakers and Wertheim, 1991). Light will not only influence the amount of synthesized carbohydrates for fruits, but also for other reproductive structures and vegetative organs of the tree. Flower bud differentiation can be influenced by different red:far-red ratios (Rossi et al., 1990; Baraldi et al., 1994). Light drives anatomical changes at the palisade tissue level for the formation of different leaf types, like in peach, apple and olive crops, where leaves growing under shade conditions possess lower specific leaf area, larger leaf area and lower thickness, compared to those growing under sunny conditions (Nii and Kuroiwa, 1988; Tustin et al., 1992; Gregoriou et al., 2007). These anatomical changes lead to different leaf photosynthetic potential: sun leaves accumulate more starch in chloroplasts compared to shade leaves, thus their photosynthetic capacity will be greater (Kappel and Flore, 1983; Nii and Kuroiwa, 1988). There is evidence of increased

spongy intercellular spaces in shade leaves, which could positively alter CO_2 conductance from substomatal cavities to carboxylation sites in the chloroplasts (Gregoriou et al., 2007). Light will then have impacts on the type of produced carbohydrates (soluble, to be transported, or not soluble, for reserves) and their translocation. This leads to the capacity of sustaining fruit growth, another light dependant process (Tustin et al., 1992; Corelli-Grappadelli et al., 1994; Bepete and Lakso, 1998), where lack of sunlight will limit photosynthates to the fruit, prioritizing shoot development. All these light driven processes, when working optimally, are fundamental for having a *fruitful* orchard in the long period. Light also impacts the final quality of fruits, from the aesthetic and organoleptic points of view, and on their storage. Excess of solar radiation generally causes fruit sunburn (Racsko and Schrader, 2012), whereas less light reaching the fruit will decrease its pigmentation (Espley et al., 2007; Merzlyak and Chivkunova, 2000; Saure, 1990) and soluble solid content (Iglesias and Alegre, 2006). It is possible that temperature modifications at fruit level may be the main cause triggering these quality disorders (Iglesias and Alegre, 2006).

For the last six decades, orchards have been designed to intercept increasingly higher amounts of radiation, and a good goal was set by researchers at 70% light interception (Heinicke, 1966; Jackson, 1972.). This trend was accompanied by a shift from lower plant densities (bigger trees), to high density plantings of considerably smaller, thinner trees. These new training systems intercepted even higher percentages of light. The Tall Spindle, the V-shaped canopy and, since the mid ‘90s, the use of reflective films have enhanced light exposure (Robinson, 2017). Despite the importance of light in piloting the orchard performances, it is very well known that plants will use no more than 5-

10% of the total absorbed energy during photosynthesis (Long et al., 1994). The RuBisCO enzyme originated in environmental conditions that were different from the present ones, when CO_2 was much present, whereas oxygen was almost absent. It binds carbon dioxide only weakly, as it has a low affinity and a high Michaelis constant for carbon dioxide. This may explain why the properties of this very ancient enzyme are not optimal for today's circumstances. Such inefficiency causes the plant to be frequently exposed to high amounts of incoming energy. In this case, light is a double-edged sword, as too high intensities can be counterproductive (Corelli Grappadelli and Lakso, 2007). In high radiation conditions, the light-harvesting complexes are supplied with an excessive number of photons, causing an excess of excited states in the PSII reaction centres, leading to photoinhibition of the light-dependent reactions of photosynthesis. Plants possess ways to dissipate light excess, via thermal dissipation (i.e. non photochemical quenching) and photochemical pathways: cyclic transport on PSII and PSI, the water-water cycle, photorespiration and the glutathion-ascorbate pathway. When the incoming energy is not totally dissipated by the plant protective systems, another strategy will be used: the plant will "sacrifice" the D1 protein, leading to a deficiency. This is an effective and conservative mechanism that replaces new D1 proteins at a very fast pace. This tactic has been developed to avoid further and worse damage (Krieger-Liszkay, 2005), at the cost of photosynthates withdrawal, potentially used for fruit development, or growth. Peach studies (Losciale, 2008) revealed a 7-11% loss of daily produced carbohydrates, used to mend the damage in PSII centres. Where high solar intensities occur for most of the growing season, photoinhibition is a menace to orchard efficiency and productivity, thus having to be managed.

Shading is a solution to control excessive incoming sunlight and some plants do possess the ability to orient their leaves in order to reduce light interception. The shading power will create a less stressful environment for the plant, in terms of irradiation pressure and theoretically would help against photodamage and photoinhibition processes. Less incoming radiation leads to lower temperatures, therefore the plant would need less water for thermoregulation process. In orchards, cropping can also benefit from the water uptake and evapotranspiration point of view (Nicolas et al., 2005; Lopez et al., 2018; Boini et al., in press), without having negative repercussions on marketable yield. However, there are concerns about having less incoming light during certain phenological stages, for example the fruit cell division stage. Shading has been shown to slow down partitioning of carbohydrates to the fruit, in particular in the early season, to the advantage of vegetative shoot growth (Hansen, 1967; Corelli-Grappadelli et al., 1994; Bepete and Lakso, 1998; Lakso and Goffinet, 2013). Thus, lack of sunlight early in the growing season would potentially be reflected in smaller, i.e. lower quality, fruit. This is why thinner canopies are preferred to enhance light penetration inside the tree. Photosynthetic efficiency would not be lost, as long as shading is maintaining solar intensity no higher than the P_n saturation point (the light intensity above which the response becomes flat). In apple, this threshold has been stated to be approximately between 800 and 1200 μmol m^{-2} s^{-1} (Campbell et al., 1992; Husen and Dequan, 2002; Tartachnyk and Blanke, 2004; Cheng et al., 2000). So, the amount of shading would widely vary, depending on the location of the orchard, more specifically on its latitude and altitude. Nevertheless, in several parts of the world, PPFD (photosynthetic photon flux density) will reach

usually 2000 μmol m^{-2} s^{-1}, or more (Nobel, 1983), and about 50% is enough for reaching the saturating point (Lakso, 1994). Shading in orchards is already applied, indirectly, with anti-hail nets. These protective systems are mainly against environmental hazards, biotic and abiotic, and implicate applying a physical barrier, which automatically eliminates part of the solar pressure. These systems are becoming more and more common, especially against hail storms, and some are evolving towards a complete isolation of the orchards, for example the so-called *Alt'Carpo*. *Keep in touch* is another netting, single-row cover system, very common among sweet and sour cherry orchards, for pest management (*Drosophyla suzukii*) and also for protecting the crop against rain, in an effort to contain fruit cracking. Usually these systems are white, grey, or black nets, shading no more than 20%. On a larger scale, orchard protection is assured against wind storms, thus preventing excessive loss of relative humidity inside the orchard, so to maintain a more favourable Vapour Pressure Deficit (VPD), a common practice in very dry areas (i.e. Israel). Shading impacts temperature, reducing fluctuations inside the orchard. Temperatures underneath such protective systems may then be reduced, due to less incoming solar radiation, or increased, as a consequence of reduced air circulation (triggering the greenhouse effect) (Iglesias and Alegre, 2006; Arthurs et al., 2013). The air, canopy and soil temperature gradients can be influenced, simply by modifying the amount of incoming energy.

LIGHT SPECTRUM IN ORCHARDS

The effect of different light spectra has been widely tested in many crops, from laboratory to field conditions, from simpler plants (Arabidopsis), to the more complex annual and perennial crops, and it is

demonstrated that their physiology is affected differently, in most phenological phases. Arabidopsis studies have extensively covered plant physiological aspects and responses in a laboratory environment, compared to the more complex plant organisms. Regarding the latter, manipulating light spectrum in an open field environment has not occurred until around the beginning of the 2000's, when anti-hail coloured nets began to be used as a research tool, to be tested for commercial purposes. These systems are characterized by higher transmissions of one, or more, specific wavelengths. They were applied above the orchard, or nursery, and differences were discovered when relating to crop responses. Flowering, vegetative growth and final yield quality were influenced, depending on the colour of the filter that was used. Many trials and surveys have taken place, and are ongoing, especially in the Mediterranean area, but also in North and South America, to evaluate their applicability in different parts of the globe, to overcome climate change threats. Light quality modification has demonstrated to be effective on ornamental crops (Nissim-Levi et al., 2008; Oren-Shamir et al., 2001; Ovadia et al., 2009) and horticultural crops (Elad et al., 2007; Shahak et al., 2004; Retamales et al., 2008; Shahak et al., 2008; Basile et al., 2012; Wachsmann et al., 2013). The environmental benefits of using protective netting can include yield increases, reduced or no sunburn symptoms (Kalcsits et al., 2017), reduction in irrigation costs from reduced soil water loss (McCaskill et al., 2016). Red light has long been known to increase shoot length and to increase flower bud burst, due to the shade avoidance syndrome (Vandenbussche et al., 2005; Donohue et al., 2009; Levin, 2009; Pierik et al., 2009). White light behaves in a similar way, tending to increase final yield, due to increased dry matter, and it can influence post-harvest

quality (Basile et al., 2008; 2012; Kong et al., 2013), not to mention higher fruit pigmentation, all possible consequences of increased amount of scattered light, under this particular filter. Blue light can shorten shoots (Nissim-Levi et al., 2008; Oren-Shamir et al., 2001; Ovadia et al., 2009; Bastías et al., 2012; Basile et al., 2014) and tends to increase gas exchanges (Bastías et al., 2012). Lately, yellow light is starting to gain interest, but little is still known about the causes of its effects (Shahak et al., 2016), although it shows to have an even stronger stimulant effect compared to red light, in terms of agricultural applications.

From the environmental point of view, the spectral modification inside the orchard gives different results. Air temperature can decrease, thanks to the shading effect (less radiation income), or increase, due to the greenhouse effect (less air circulation). These variations may be a consequence of different net porosities required to achieve the same shading factor (Iglesias and Alegre, 2006). No differences in temperature above and inside the canopy were found, but a pearl shading net decreased temperatures inside the canopy (Kalcsits et al., 2017). Relative humidity is also influenced, although it is related to parameters outside the orchard, such as the growing environment, its relative humidity and wind speed, and inside the orchard, and plant density. Researchers from very dry environments reported an increase of relative humidity inside the canopy, among different spectra, when air temperature was below 35°C, but no differences were found when air temperature went above (Kalcsits et al., 2017). Soil temperature decreases, with red-black and green-black nets, and increases, with red-white and white nets, at 5 cm depth (Solomakhin and Blanke, 2010). At

20 cm depth soil temperature will decrease, whether the net is red, white or blue, with an increase of moisture (Kalcsits et al., 2017).

These variations in the microenvironment under a photoselective net are the main cause of the wide variability of the physiological responses of orchards. Categorizing different filters for crop responses is still something that is far beyond happening. To be able to predict such responses, more data are needed.

LIGHT SPECTRUM IN APPLE ORCHARDS

Research identifying the effects of photoselective netting on fruit tree species has mainly taken place in apple production systems (Mupambi et al., 2018).

Studies have analysed tree physiology, from gas exchanges (Shahak et al., 2004; Ebert and Casierra, 2000; Bastías et al., 2012; Solomakhin and Blanke, 2008; Smit, 2007), to its reaction to light intensity, to water relations (Shahak et al., 2004; Boini et al., in press; Lopez et al., 2018), vegetative growth and leaf morphology (Do Amarante, et al., 2011; Solomakhin and Blanke 2008; Shahak et al., 2016; Bastías et al., 2012), fruit set (Shahak et a., 2004; Do Amarante et al., 2011), flower induction and return bloom (Solomakhin and Blanke, 2008). Results showed increases and decreases of the studied responses, but also no differences. Research has been carried out on the final quality of fruit (Solomakhin and Blanke, 2008; Kalcsits et al., 2017; Do Amarante et al., 2011), showing improvements or no effects, depending on the filtered spectrum.

As Mupambi outlines in the 2018 review, more research is needed to provide reliable information. Different cultivars in different regions of the world create variable results, thus it is confusing to identify a

general overview of the impacts of photoselective netting in (not only) apple orchards.

GENERAL OVERVIEW

From the previous discussion, it is clear that modifying the quantity of light can give benefits to an orchard for its final production. From the physiological point of view, a lot has been discovered and analysed to further improve orchard performances. However, modifying the quality of light may add further benefits to an orchard *fruitfulness*. Not all fruit crops may gain improvements, therefore research has to identify what species, and/or varieties are more suitable, where in the world would they improve their production and when, in the crops' different phenological stages, would this technique be a further enhancement.

As frequently mentioned, results demonstrate how crop responses are extremely challenging to draw general conclusions from. When using photoselective nets, this wide variability is caused by the interaction between the crop, the cultivar, orchard age and type of management, its altitude and latitude (thus, its surrounding ecosystem), that arise consequently when modifying the quantity and *quality* of the incoming radiation. Plus, having nets of the same colour (same quality), but with a different shading percentage (different quantity), may give very different responses, when considering the influence on the microclimate below.

Plants are poikilothermic organisms, i.e. they closely follow the temperature of their immediate surroundings, and as a result, their internal temperature varies considerably. As plant physical and chemical properties are related to temperature, changes in the

environment have impacts on their physiology. The Mediterranean basin is facing progressive increase of environmental threats for agriculture. Milder winters, followed by abnormal springs, sometimes characterized by unpredicted hail storms, heat waves in summer, accompanied by scarce, but very intense rain or hail events, and shorter autumns that still resemble summer, are creating extreme variability in short periods of time (Field et al., 2014; Mennone, 2018). Orchards reactions, metabolism, thus performances, are taken at a limit. Solutions that can mitigate such extreme changes and, beyond control, fluctuations, are required for optimal functionality.

Photoselective technology might solve this and other issues, as production of fruit and vegetables will have to efficiently use resources, in the scenario of increasing world population.

Based on the discussion of these topics, the productivity of an orchard can be considered not only influenced by the amount of intercepted light, but also on its ability to efficiently exploit it. Modern fruit production (and the rest of agricultural practices) should take into account that *efficient production* has a different impact, rather than just *production* itself. Renewable resources must be used in ever decreasing amounts, and thus require improved production systems, that can preserve quality and quantity of production while increasing their efficiency. Water is the single most important renewable resource, and commands wide research efforts worldwide. Fertilization is another fundamental pillar for successful production. When looking at light as a resource, it too must be considered as a precious ingredient to be dosed during the various phenological stages of an orchard, as to avoid periods of deficiency or excess.

AIM OF THE STUDY

Apple has an extended world production area, with an increasing harvest tendency (FAOSTAT). Moreover, it is apt when testing photoselective netting, as it showed to take profit from shade applications, in Mediterranean (Iglesias and Alegre, 2006) and hot dry climates (for example, Washington State, USA, or Israel) (Kalcsits et al., 2017; Shahak et al., 2004), and in areas where high solar and UV intensities occur (such as Chile) (Olivares-Soto and Bastías, 2018).

The objective of this thesis was to evaluate apple physiological performance by affecting the light quality of the orchard, while focusing on:

- fruit development;
- sap flow and fruit cell expansion;
- carbohydrate dynamics during dormancy and bud break.

To date, research about the effect of light spectrum on these three phenological phases is absent in apple. Light quantity has been tested during fruit development, or cell division stage (Corelli et al., 1994; Bepete and Lakso, 1998). Sap flow during fruit cell expansion and carbohydrates translocation during ecodormancy have been evaluated, however neither under the influence of light manipulation (Liu et al., 2012, 2016; Yoshioka et al., 1988; McQueen et al., 2004). Such topics resemble among the most important processes occurring inside a fruit tree: fruit development, water uptake for evapotranspiration and, last, carbohydrate translocation during the dormant phase anticipating bud break.

The results would add value and knowledge to the responses of these specific processes of apple crop, on whether they may be enhanced or reduced, and on whether a specific filter, or more than one,

is more suitable than others to improve apple crop physiology. Also, the findings would be a further supplement to the pool of information concerning apple behaviour influenced by light quality.

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CHAPTER II

LIGHT QUALITY IMPACTS ON FRUIT DEVELOPMENT. A PRELIMINARY STUDY.

List of figures

top of each column represent the average diameter of fruit, for each light treatment (a). Class size distribution values for each net treatment, of fruit diameters ranging from <65 mm to >85 mm (b). Cross tabulation of light treatments by class size (Table 1). Correspondence analysis map of light treatments by class size, explaining 90.47% of the inertia (c). Tree structure depicting hierarchical clustering of the light treatments. The clustering is in terms of χ^2. The critical level of 17 indicates the significance at 5% between light treatments (d).

INTRODUCTION

Once bloom is complete, carbohydrate support of apple fruit growth is initially dependent on fruiting spur leaves (Hansen, 1971; Quinlan and Preston, 1971; Corelli Grappadelli et al., 1994). Fruit development will also be supported by the lateral bourse shoot tip, however initially this structure is a competing sink within the spur early after bloom (Tustin et al., 1992). About two weeks after bloom, or when 6-8 leaves have unfolded, the bourse shoot can begin exporting to developing fruits. When more than about 12 leaves have unfolded on extension shoots, these leaves too will begin to support the fruit (Corelli Grappadelli, 2003). As shoots stop growing, all leaves will export photosynthates to fruit (Lakso and Goffinet, 2013). This general overview of carbohydrate relations during fruit development will occur in optimal conditions of canopy light penetration. In shaded parts of the tree, during four to five weeks after full bloom, extension shoots show reduced export towards the fruit. Regardless of optimum light penetration, primary spur leaves may even export carbohydrates to extension shoot tips, at one week after full bloom (Corelli Grappadelli et al., 1994). If the carbohydrate demand of a developing fruit exceeds the

supply from the spurs during this time, carbohydrate deficits may be expected. Hence, during fruit development, i.e. cell division stage, there is a very high competition for carbohydrate export, where non-optimal light conditions may have substantial effects on final fruit size.

This is a reason that explains why hail protecting systems are installed at least one month after full bloom. The indirect generated shading is considered a possible threat for fruit development, to the advantage of vegetative shoot growth. In conclusion, light quantity is of great importance during the first part of the growing season.

Conditioning orchard light spectrum is desirable, to obtain beneficial effects for horticultural crops. Apple is receiving especially attention when it comes to use of photoselective netting (Mupambi et al., 2018), although there is a lack of information regarding the effect of the light spectrum on fruit development, in the early stages. The fact that light quality is influencing leaf anatomy (Bastías, 2011; Kong et al., 2012) and plant performance (Shahak et al., 2008) along the season suggests there could be an influence at earlier stages, if light is manipulated in the first part of the growing season. Depending on how the light environment is influencing both spur and extension shoot growth, their carbon export may be affected. Thus, the fruit could receive higher or lower concentrations of carbohydrates, which in turn would affect final fruit size.

In this preliminary study, apple fruit growth was monitored and compared with both bourse and extension shoot growth, in order to see if differences occurred under five qualitatively different light environments, from one month to three months after full bloom. The results were compared with final harvest data.

MATERIALS AND METHODS

The trial took place in an experimental *Rosy Glow* apple orchard, at the Bologna University Experiment Research Station, in season 2017. Trees were 4 years old, trained as solaxe system (Lauri and Lespinasse, 1998), and spacings were 3.3 x 1.0 m. Full bloom occurred on 30/03. On 21/04 (22 DAFB) four differently coloured nets were installed to cover six trees each (2 serving as guard trees) and a classic anti-hail system was deployed above the rest of the orchard, serving as a control. Five different light environments were obtained, each with two replications, in a complete randomized block design with replicates, where each treatment had a total of 8 monitored trees. Each replication was distant 11 meters from the others. The photoselective nets were red (R), blue (B), white (W) and yellow (Y) (ChromatiNet Polysack Plastic Industries, D.N. Negev, Israel), while a standard black (C) hail net served as control. All five netting systems were in polyethylene and had equal shading power, 18-21% (cell size of each net was 2x5 mm). Irradiance under each net had been assessed by a LI-1800 spectroradiometer (LI-COR, Lincoln, NE, USA), on a cloudless day, at midday, outside the orchard, in winter. A reference measurement was taken in full light (wavelength interval between 400 and 1100 nm) and portions of each net were placed over the sensor. Wm^{-2} outputs were converted to μmol m^{-2} s^{-1}; transmittance was then calculated as the difference between the external light input and the light intensity under the net and expressed as a percentage. PAR and blue light regions were extrapolated and analysed separately.

From the end of April to the end of June, the growth of fruit, bourse and extension shoots was monitored, thus including the cell division and part of the cell expansion stages. For each colour treatment,

32 fruits, their related bourse shoots, and 32 extension shoots were selected. Measurements were taken twice a week until the end of May, then once a week during June. Fruit growth was traced with a digital caliper, attached to an external memory (www.hkconsulting.it). Each fruit diameter value (D) was converted to fresh weight (FW), expressed in grammes, using the following conversion equation:

$$FW(g) = a * D(mm)^b$$

where *a* and *b* were 0.0006 (±SE 0.00005) and 2.924 (±SE 0.0194). This equation was obtained by regressing diameter and weight data of about 300 fruit picked from various Pink Lady apple orchards. The R^2 of the relationship was >0.99. The use of this conversion is justified by the expolinear behaviour of apple fruit on a weight basis (Lakso et al., 1995), explaining the linear growth during all its phenological stages. Absolute growth rate (AGR) could then be obtained. Regarding bourse and extension shoots growth, patterns were obtained in terms of absolute extension rate (AER) (cm day^{-1}). During this period, weather data was collected from a meteorological station installed outside the orchard. Air temperature and relative humidity under the nets was not measured.

Physiological harvest occurred in the second half of October, for fruit grown under white and yellow nets, and one week later for red, blue and ctrl nets. Fruits were divided for light treatment repetition. Size class distribution was obtained with a digital caliper and classes were divided from <65 mm to >85 mm, at 5 mm intervals.

STATISTICAL ANALYSIS

PAR and blue light region means were separated with one-way ANOVA, then SNK test was used to evaluate differences, among light treatments. Two-way ANOVA was used to separate averages among light treatments of fruit AGR and of both bourse and extension shoots AER, for each date of measurement. SNK test was used to rank the different treatments ($P<0.05$). Average fruit size was tested for the effect of nets, by using crop load as a covariate. As this analysis revealed no significant effect of crop load, the one-way ANOVA for nets is presented here. SNK test was used to rank the different treatments ($P<0.05$). For evaluating variance among class size distribution in the different light environments, a correspondence multivariate analysis was performed, followed by a cluster analysis, with a chi-square test (Greenacre, 2007).

RESULTS

PAR (400-700 nm) values (Figure 1b) showed higher transmittance for W (76 %), followed by Y, C and B, leaving R with significantly lower values (64 %). In the blue region (450-495 nm), W had higher transmission (75 %), followed by B, Y and C, leaving R with the lower values (57 %) (Figure 1c).

From the second half of April (24 DAFB) to the end of June (92 DAFB) temperatures rose gradually up to a maximum average of 30°C. Accumulated rainfall was 106.4 mm, concentrating mainly during the first half of May (33-47 DAFB) (Figure 2a). The second half of April and most of May (24-63 DAFB), had 20 days where peaks of solar radiation went above 2000 μmol m^{-2} s^{-1} (Figure 2b), while mean PAR, measured between 9 and 18 hours, was around 1100 μmol m^{-2} s^{-1}, but exceeded 1200 μmol m^{-2} s^{-1} 26 times. June (64-92 DAFB) was characterized by

slightly lower peaks of solar intensities, where maximum solar radiation went above 2000 μmol m^{-2} s^{-1} only 10 times and mean PAR, measured between 9 and 18 hours, was around 1280 μmol m^{-2} s^{-1} (Figure 2b), exceeding 1200 μmol m^{-2} s^{-1} 22 times.

Fruit growth along the season showed significant differences. AGR (Figure 3a, Table 1), at 26 DAFB, was higher for C which grew 0.18 g day^{-1}, compared to B (0.11 g day^{-1}). C, W and Y values were all higher than B ones, at 44 DAFB, gaining an average of 0.5 g day^{-1}. At 55 DAFB, Y was higher than W, gaining extra 0.26 g day^{-1} and at 92 DAFB both C and Y were growing around 1.4 g day^{-1}, whereas R was significantly lower (1.0 g day^{-1}). At the end of June (92 DAFB), only B fruits were below 7 g of accumulated AGR (Figure 3a).

Bourse shoot growth was the same for all light environments, in terms of AER, except at the very beginning of the monitored season (26 DAFB), where W bourse shoots elongated 0.1 cm and were significantly different compared to B and C, which elongated on average 0.03 cm (Figure 3b, Table 2). However, by the end of the monitoring season, the shoots accumulated more than 2 cm of growth under the yellow net and less than 1.6 cm under the control net (Figure 1b).

Extension shoot growth among light treatments was significantly different three times. AER (Figure 3c, Table 3) at 30 DAFB was higher for C, increasing 0.7 cm day^{-1}, compared to B, W and Y shoots, which grew at an average of 0.4 cm day^{-1}. At 44 DAFB, B had the lowest growth (0.14 cm day^{-1}) and at 70 DAFB Y had the highest value (0.28 cm day^{-1}) compared to the rest of treatments. At 92 DAFB, B shoots had accumulated less than 2.3 cm of growth, whereas R, C and Y more than 3 cm.

At harvest (Figure 4a), higher diameters were found in W treatment (77.5 mm, translated into 202 g of fresh weight). C fruit was lower by 1 mm. Y and R fruits were the same, having reached an average diameter of 76 mm. B treatment had significantly smaller fruits (74.8 mm, which equals to 182 g of fresh weight). Fruit size distribution (Figure 4b) showed a higher presence of fruit ranging 75-80 mm in diameter. Blue fruit had higher percentages of classes 65-70 and 70-75 (Figure 4b, d), whereas R, Y and C were mostly present in 75-80 mm class (Figure 4b, d). W fruit were in higher percentages above 80 mm (Figure 4b, d). Row clustering analysis and χ^2 test (Figure 4d) showed no significant differences between R, Y, C and B, whereas a significant difference occurred between the group R-Y-C-B and W.

DISCUSSION

Photoselective netting had an impact on fruit development.

Among the coloured filters, the yellow one was especially able to enhance fruit growth, although not throughout the whole season (Figure 3, Table 1a). Y bourse shoots (Figure 1b) showed more cumulative growth, whereas extension shoots of this colour reached lower total cumulative growth, compared to R and C (Figure 1c). However, when looking at harvest values, Y net did not reach as high values as the white one did (Figure 4a). Fruit and extension shoot growths were negatively affected by B (Figure 3, Table 1, 3), this could explain the lowest average fruit weight and tendency to have smaller diameter (Figure 4). The W net did not follow any particular trend in both fruit and extension shoot growth, alternating significantly higher, to intermediate, to lower values, during the whole season (Figure 3, Table 1, 2, 3), although bourse shoot extension rate was significantly higher at the beginning of the

monitored period (Figure 3, Table 2). This light treatment did not show particularly high performance in terms of fruit AGR, nonetheless, its fruit were larger and heavier, compared to the other light treatments (Figure 4b). A possible effect of W net, on both higher transmittance in PAR (Figure 1b) and blue light (Figure 1c) regions, may have modified leaf anatomy (Bastías, 2011; Kong et al., 2012). A highly probable increase of photosynthetic performance, due to larger stomata, as reported by Kong et al. (2012), could have taken place. Therefore, thicker palisade layers and higher photosynthetic efficiency may have improved cell expansion during the season, leading to higher diameters at harvest (Figure 4b, d).

It is worthy pointing out that, while fruits were growing at different rates, bourse shoots were elongating at the same pace. As the latter is considered the feeding source for fruitlets (Hansen, 1967; Lakso and Goffinet, 2013), it can be assumed that these shoots were behaving differently in terms of efficiency. The higher amount of scattered light under the yellow net (Shahak et al., 2016) may have altered the bourse shoot photosynthetic capacity, enhancing it. However, Kong et al. (2012) reported that Y nets negatively affected leaf photosynthesis, due to a lower amount of transmitted blue light, which decreases the thickness of palisade mesophyll tissues (Pushnik et al., 1987; Saebo et al., 1995). This may partially explain why at the end of the growing season, yield for Y net was not as high as the W one (Figure 4a). Another possible explanation for higher Y fruit AGRs, may be the high transmittance of the PAR region (400-700 nm) (Figure 1b). High scattering properties added to high transmission of PAR may have enriched the light microenvironment (Hemming et al., 2016) enough to justify higher growth in certain moments of the monitored period. This did not occur

under the white net, even though this filter demonstrated the highest transmittance of PAR (Figure 1b) and even though literature states both white and yellow photoselective nets have equal scattering power (Rajapakse and Shahak, 2007). Higher fruit AGRs under the C net could be a consequence of possibly higher radiation income. As the C net was installed above the orchard, whereas the rest of the treatments covered individual rows, unlike a single-row *Keep-in-touch* installation, higher values of growth would be due to a higher amount of total light filtering below the C treatment. Therefore, light quantity was more effective than light quality. The red net was mostly intermediate and negative, in terms of fruit growth (Figure 3, Table 1). This response is probably due to altered partitioning in favour of shoot growth. As it transmitted the lowest amount of PAR, compared to the rest of the light treatments (Figure 1b), it is expectable that photosynthetic export might have been unbalanced towards shoot extension. In fact, by the end of the monitored period, extension shoots had accumulated more growth (Figure 3c) along with C, probably in search of richer PAR environments. It followed that R extension shoots were experiencing shade avoidance syndrome (Smith and Whitelam, 1997), as a consequence of altered R:FR ratio (Morgan and Smith 1978). Internode elongation towards higher, more light exposed, areas of the canopy would therefore be explained by this inner phytochrome activity (Hendricks and Borthwick, 1959).

Higher fruit growth occurring under certain coloured nets (Figure 3, Table 1) suggests that the crop may have not been suffering from a lack of sunlight, a critical event for satisfactory yields. A contributing factor may have been the training system. The solaxe system (Lauri and Lespinasse, 1998) was developed to improve growth of the fruiting

spur. The structure of the tree aims to form a more open and thinner shape, as competing vegetative branches should be removed, to allow light to penetrate in the inner parts of the canopy, improving the regularity of fruiting. As a matter of fact, the orchard trial does have an open, thin, canopy, with good sunlight penetration. During the season, many days (around 69%) recorded mean solar intensities, between 9:00 and 18:00 hours, above 1200 μmol $m^{-2}s^{-1}$ (Figure 2b), i.e. above the saturating point for apple photosynthesis (Cheng et al., 2000). Moreover, as other research sets this threshold even lower, down to 800 μmol $m^{-2}s^{-1}$ (Campbell et al., 1992; Husen and Dequan, 2002; Tartachnyk and Blanke, 2004), it can be hypothesized that, during the season, there have been prolonged periods of excessive solar radiation. Therefore, photodamage at PSII level is not to be excluded. The yellow net, in addition to decreasing high incoming solar intensity of certain moments of the day, may have allowed an optimal fruit and extension shoot growth due to enough scattered light (Shahak et al., 2016). The possible improvement of the light distribution inside the canopy could have reduced competitive demand in the shoots, making them exporters, rather than importers, contributing more carbon to fruit growth. Air temperature under the nets may have also been modified (Arthurs et al., 2013), creating a more, or less, suitable environment for maximizing photosynthesis.

Another possible explanation might lay with an effect of the netting on the fruitlet sink strength, which could affect photosynthates partitioning (Hansen, 1967). Physical and physiological constraints are thought to determine the potential sink strength. Sink size is determined primarily by the number of cells, and so one of the physical constraints could derive from limitations to cell division processes, reducing the

number (Ho, 1988). Sink activity is related to photoassimilate unloading, post-phloem transport and retrieval by sink cells, utilization, mainly by respiration and novel synthesis of cellular components, and storage of imported carbohydrates (Herbers and Sonnewald, 1998). Enzymatic activity could have been altered (Klages et al., 2001), due to possible differences in air temperature under the different filters (Arthurs et al., 2013). Even though temperatures under the nets were not monitored, it is unlikely that possible differences were capable of impacting metabolic activity. However, it is reported that apple fruit sink strength may be only related to a greater supply of assimilates from source leaves and not from fruit metabolic activity (Klages et al., 2001). Photoassimilates translocation would be influenced by the net assimilation of source leaves, which largely depend on environmental conditions, such as temperature, PPFD (photosynthetic photon flux density) and leaf traits (Kumashiro et al., 1990; Lambers et al., 1998; Corelli Grappadelli, 2003). Unfortunately, no gas exchange measurements nor light-treatment air temperature data were taken during the experiment to confirm these hypotheses.

As the monitored period included part of fruit cell division stage, one could assume that, where higher rates of fruit AGR occurred, there might have been a higher rate of mitosis. Strong positive correlations between cytokinin levels and cell division have been discovered in developing fruits and seeds (Letham, 1963; Letham and Williams, 1969; Bohner and Bangerth, 1988; Lewis et al., 1996). The accelerated fruit development under the Y net may have been a direct consequence of fruitlet and seed enlargement, therefore determining higher sink strength (Ho, 1988).

Since the trial was a pilot study, it would be interesting and explanatory to repeat the experimentation, adding more targeted analysis. Bourse shoot, primarily, added to extension shoot gas exchanges would partially clarify if there is a link to fruit development and growth, due to light manipulation in the orchard. Primary spur leaves photosynthetic activity needs to be evaluated under different light environments, as they are the crucial source of energy for early fruitlet development, although they will be active until around 30 DAFB, or slightly more. Nevertheless, catching a glimpse of their functioning rate under the effect of light manipulation may help understanding if it is possible to intervene at the very early stage of fruit development to further enhance fruit cell division in apple. Fruiting spur and bourse leaf histology developing under different light environments could give more information regarding the photosynthetic potential during cell division stage. Fruit cell demography surveys, at harvest, would also possibly explain if higher growth rates during cytokinesis stage were indeed translated into higher amounts of cells.

CONCLUSIONS

Photoselective netting influenced fruit and extension shoot growth during the developmental stage. As the yellow net seemed capable of improving fruit growth, in this specific phenological stage it could be potentially used in apple orchards for enriching the inner canopy environment with diffuse light. The presence of high solar intensities during the early stages of fruit development can then be exploited by applying 20% shading anti-hail nets with scattering properties. As the blue and red treatments did not give satisfactory results when related to fruit growth, these two nets would not be

suitable in an apple orchard during fruit development. As for the white net, this light treatment appeared less convenient during fruit developmental stage rather than fruit cell expansion, as it gave more satisfying results at final harvest. More targeted and detailed studies would further confirm these hypotheses.

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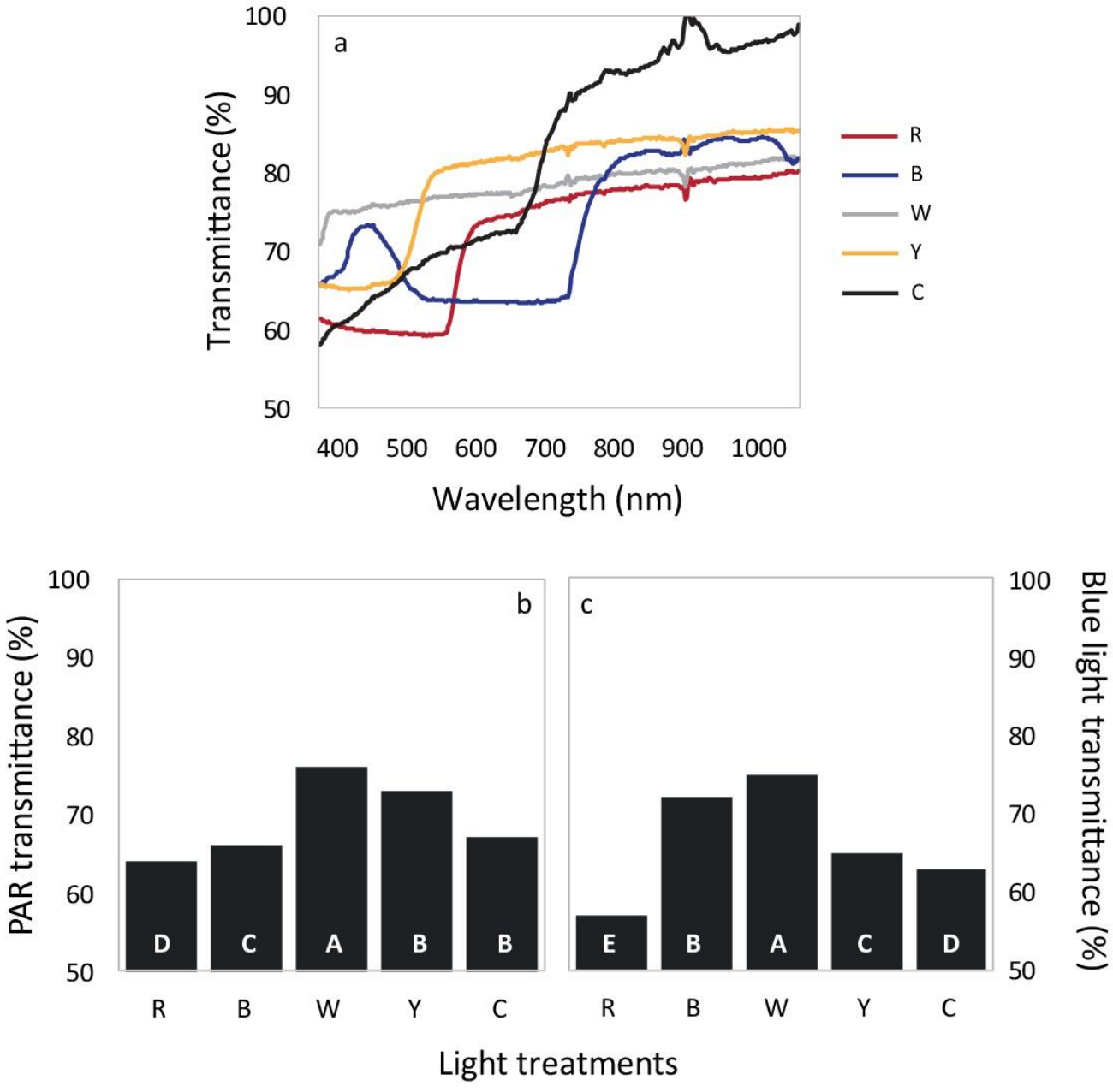

a
Transmittance (%)
100
90
80
70
60
50
400
500
600
700
800
900
1000
Wavelength (nm)
R
B
W
Y
C
b
c
PAR transmittance (%)
Blue light transmittance (%)
D
C
A
B
B
E
B
A
C
D
R
B
W
Y
C
Light treatments

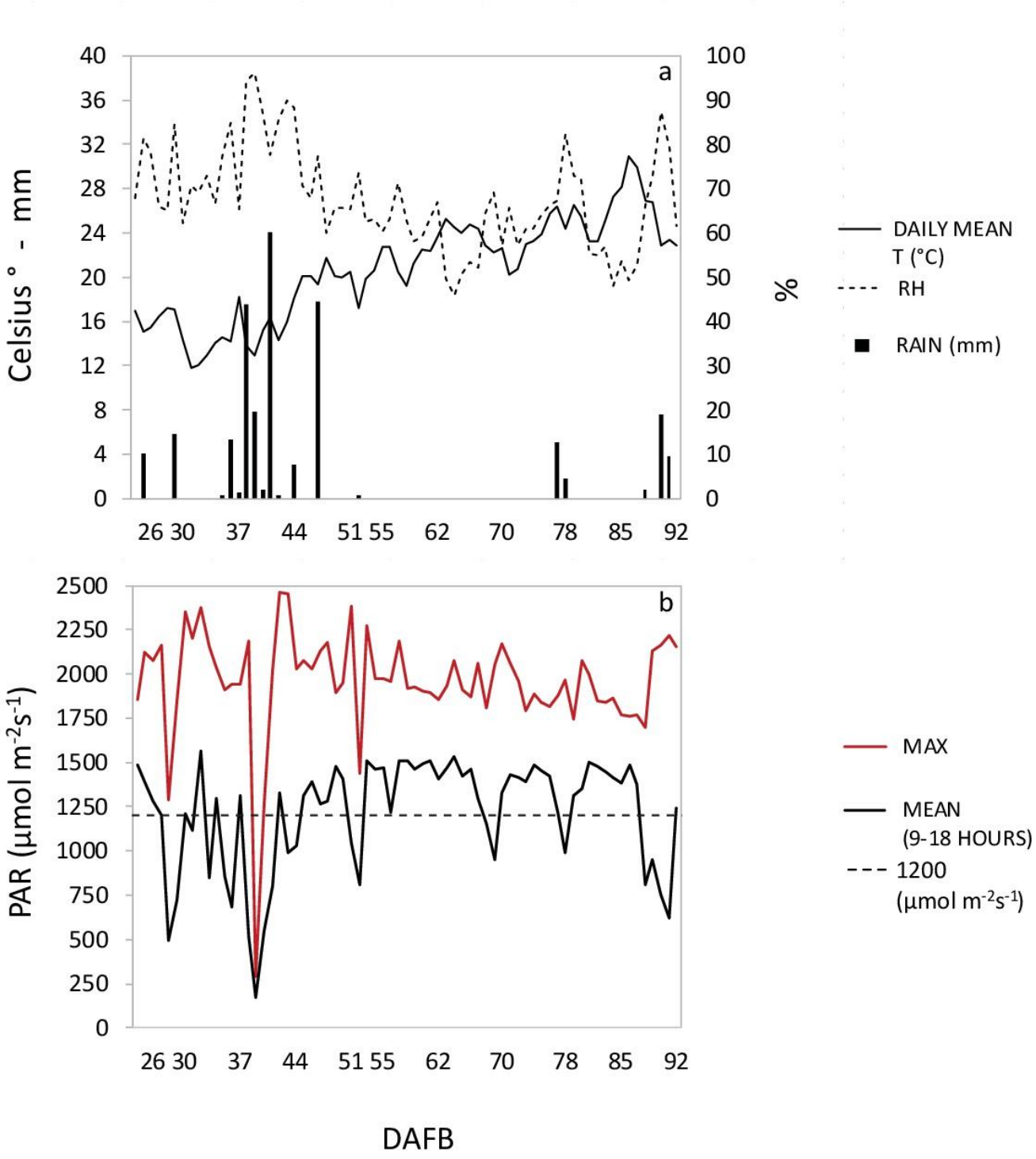

a
b
Celsius ° - mm
%
PAR (µmol m-2s-1)
DAFB
DAILY MEAN T (°C)
RH
RAIN (mm)
MAX
MEAN (9-18 HOURS)
1200 (µmol m-2s-1)
0
4
8
12
16
20
24
28
32
36
40
0
10
20
30
40
50
60
70
80
90
100
0
250
500
750
1000
1250
1500
1750
2000
2250
2500
26 30 37 44 51 55 62 70 78 85 92

TABLE 1

FRUIT ABSOLUTE GROWTH RATE (g d^{-1})

DAFB	R	B	W	Y	C
26	0.137 AB	0.118 B	0.141 AB	0.142 AB	0.181 A
30	0.218	0.194	0.264	0.257	0.259
37	0.401	0.369	0.370	0.391	0.485
44	0.468 AB	0.400 B	0.511 A	0.506 A	0.500 A
51	0.548	0.518	0.569	0.577	0.593
55	0.540 AB	0.674 AB	0.478 B	0.743 A	0.521 AB
62	0.586	0.572	0.669	0.591	0.681
70	0.978	0.887	1.038	0.877	0.931
78	1.142	1.140	1.204	1.173	1.033
85	1.075	0.829	0.999	1.092	1.098
92	1.056 B	1.123 AB	1.267 AB	1.473 A	1.449 A

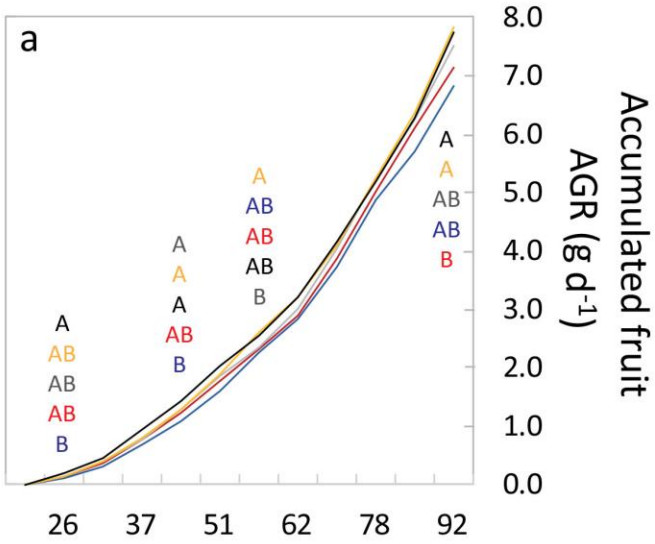


TABLE 2

BOURSE SHOOT ABOSLUTE EXTENSION RATE (cm d^{-1})

DAFB	R	B	W	Y	C
26	0.070 AB	0.031 B	0.174 A	0.073 AB	0.028 B
30	0.299	0.306	0.302	0.355	0.425
37	0.295	0.225	0.172	0.323	0.217
44	0.240	0.207	0.236	0.308	0.184
51	0.302	0.330	0.348	0.288	0.174
55	0.279	0.190	0.185	0.258	0.185
62	0.164	0.179	0.166	0.165	0.213
70	0.073	0.112	0.012	0.057	0.068
78	0.012	0.064	0.039	0.018	0.025
85	0.000	0.000	0.000	0.000	0.002
92	0.058	0.042	0.031	0.028	0.049

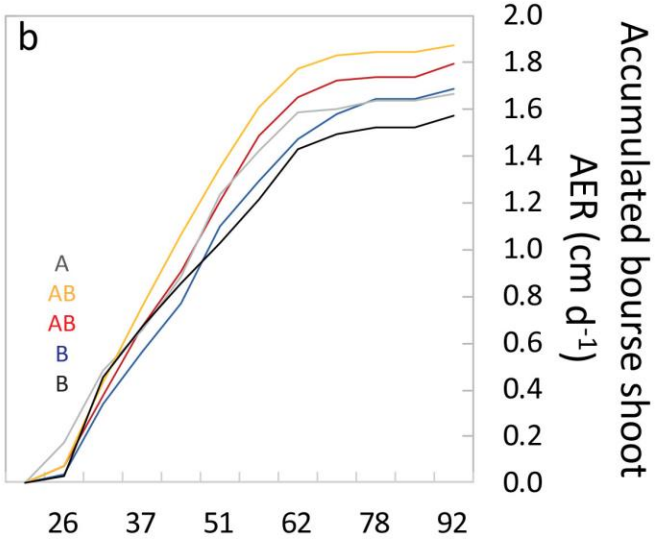
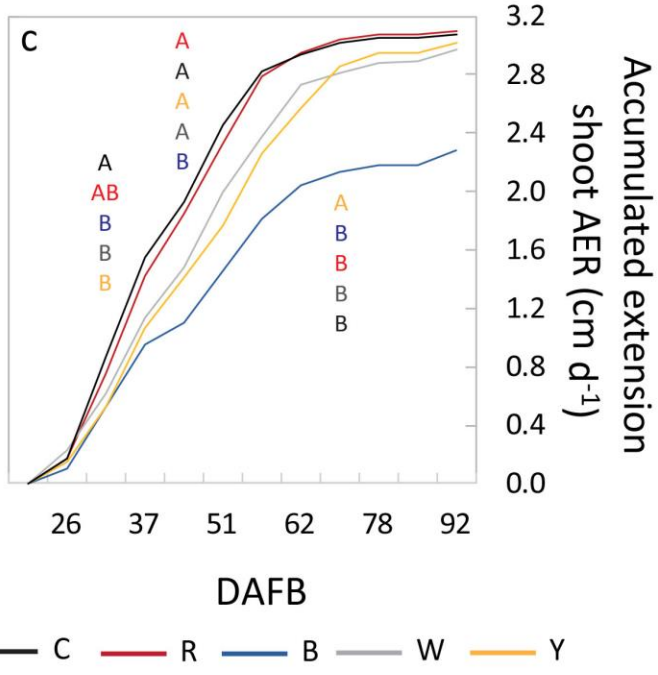
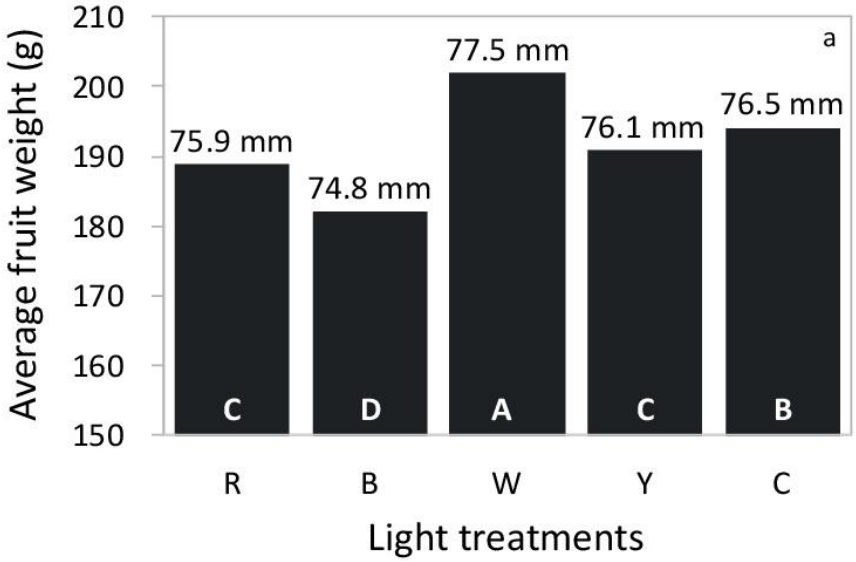


TABLE 3

EXTENSION SHOOT ABSOLUTE EXTENSION RATE (cm d^{-1})

DAFB	R	B	W	Y	C
26	0.167	0.099	0.224	0.144	0.167
30	0.590 AB	0.422 B	0.391 B	0.384 B	0.701 A
37	0.663	0.434	0.519	0.539	0.685
44	0.429 A	0.143 B	0.346 A	0.348 A	0.373 A
51	0.484	0.355	0.513	0.353	0.525
55	0.455	0.363	0.379	0.496	0.375
62	0.163	0.224	0.359	0.306	0.111
70	0.085 B	0.090 B	0.081 B	0.283 A	0.075 B
78	0.038	0.045	0.065	0.092	0.038
85	0.004	0.000	0.009	0.000	0.000
92	0.016	0.109	0.083	0.074	0.023





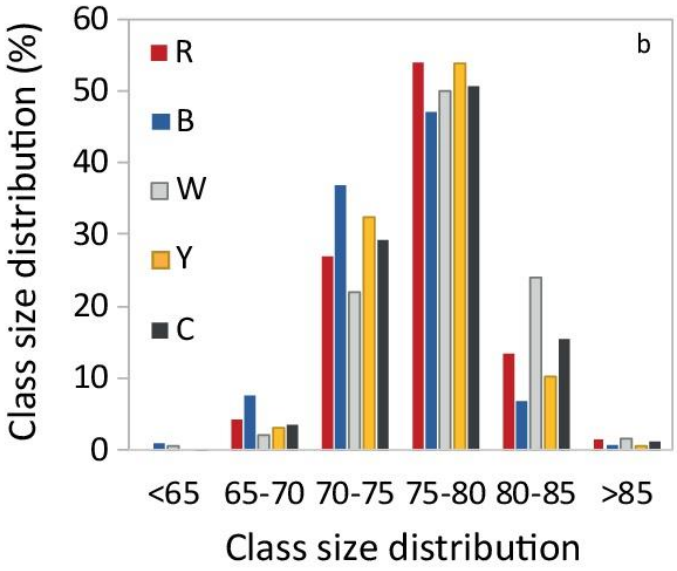
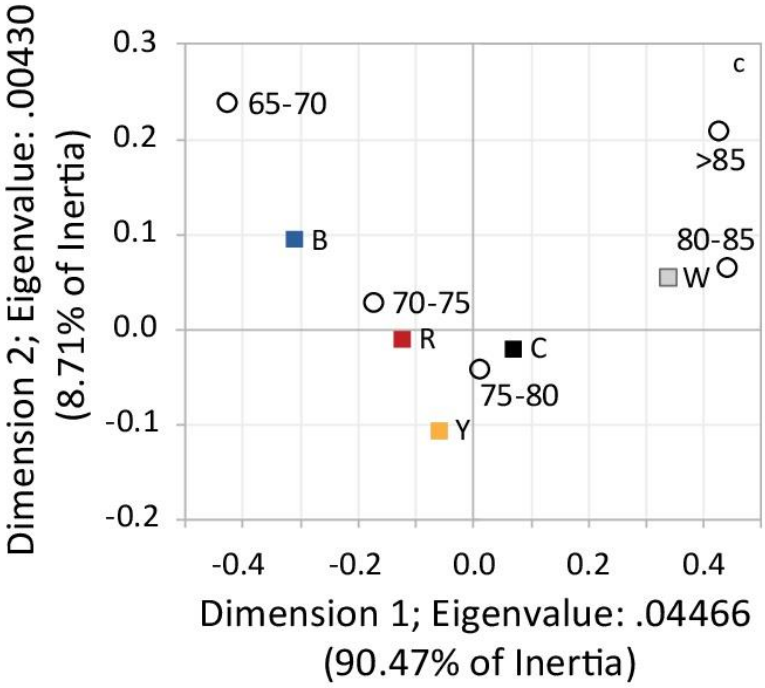
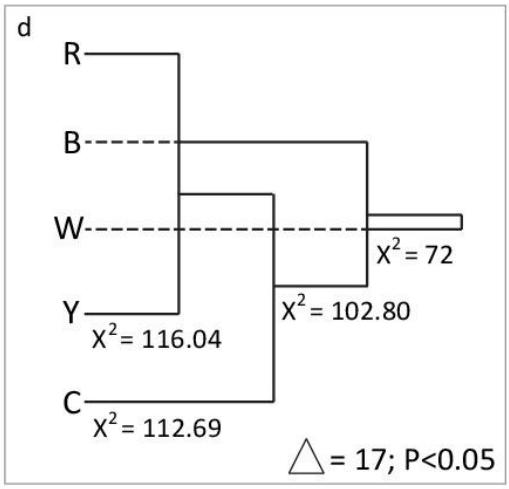


TABLE 1

Light treatments	Class size				
	65-70	70-75	75-80	80-85	>85
R	25	148	242	45	4
B	32	146	177	24	2
W	10	102	235	114	8
Y	14	142	240	47	2
C	20	170	305	92	5





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CHAPTER III

SAP FLOW AS AFFECTED BY DIFFERENT LIGHT QUALITY.

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Figure 4 (pp. 77) – Period 3: July, 18-20. Daily solar radiation pattern (a) and daily environmental air temperature increase (°C) (b). Table 1: significance of normalized sap flow, between light treatments, during the day; different letters represent significant difference at P<0.05. No letters indicate no significant difference. Normalized sap flow for each light treatment (c), during the day, from 5:00 to 22:00 hour; every point represents the hourly average of 15 minutes records. Accumulated normalized sap flow for each light treatment (d), from 5:00 to 22:00 hour with a 15 minute interval. Table 2: significance of fruit absolute growth rate, between light treatments, during the day, from 0:00 to 23:00 hour; positive symbols (+) represents positive values; negative symbols (-) represents negative values; different letters represent significant difference at P<0.05. No letters indicate no significant difference. Accumulated fruit absolute growth rate for each light treatment (e), during the day, 0:00 to 23:00 hour; every point represents the hourly average of 15 minutes records.

Figure 5 (pp. 78) – Period 5: July, 25-27. Daily solar radiation pattern (a) and daily environmental air temperature increase (°C) (b). Table 1: significance of normalized sap flow, between light treatments, during the day; different letters represent significant difference at P<0.05. No letters indicate no significant difference. Normalized sap flow for each light treatment (c), during the day, from 5:00 to 22:00 hour; every point represents the hourly average of 15 minutes records. Accumulated normalized sap flow for each light treatment (d), from 5:00 to 22:00 hour with a 15 minute interval. Table 2: significance of fruit absolute growth rate, between light treatments, during the day, from 0:00 to 23:00 hour; positive symbols (+) represents positive values; negative symbols (-) represents negative values; different letters represent significant difference at P<0.05. No letters indicate no significant difference. Accumulated fruit absolute growth rate for each light

treatment (e), during the day, 0:00 to 23:00 hour; every point represents the hourly average of 15 minutes records.

Figure 6 (pp. 79) – Period 5: August 01-02. Daily solar radiation pattern (a) and daily environmental air temperature increase (°C) (b). Table 1: significance of normalized sap flow, between light treatments, during the day; different letters represent significant difference at $P<0.05$. No letters indicate no significant difference. Normalized sap flow for each light treatment (c), during the day, from 5:00 to 22:00 hour; every point represents the hourly average of 15 minutes records. Accumulated normalized sap flow for each light treatment (d), from 5:00 to 22:00 hour with a 15 minute interval. Table 2: significance of fruit absolute growth rate, between light treatments, during the day, from 0:00 to 23:00 hour; positive symbols (+) represents positive values; negative symbols (-) represents negative values; different letters represent significant difference at $P<0.05$. No letters indicate no significant difference. Accumulated fruit absolute growth rate for each light treatment (e), during the day, 0:00 to 23:00 hour; every point represents the hourly average of 15 minutes records.

Figure 7 (pp. 80) – Period 6: August 03-04. Daily solar radiation pattern (a) and daily environmental air temperature increase (°C) (b). Table 1: significance of normalized sap flow, between light treatments, during the day; different letters represent significant difference at $P<0.05$. No letters indicate no significant difference. Normalized sap flow for each light treatment (c), during the day, from 5:00 to 22:00 hour; every point represents the hourly average of 15 minutes records. Accumulated normalized sap flow for each light treatment (d), from 5:00 to 22:00 hour with a 15 minute interval. Table 2: significance of fruit absolute growth rate, between light treatments, during the day, from 0:00 to 23:00 hour; positive symbols (+) represents positive values; negative symbols (-) represents negative values; different letters represent significant difference at $P<0.05$. No letters indicate no significant difference. Accumulated fruit absolute growth rate for each light treatment (e), during the day, 0:00 to 23:00 hour; every point represents the hourly average of 15 minutes records.

Figure 8 (pp. 81) – Period 7: August 23-24. Daily solar radiation pattern (a) and daily environmental air temperature increase (°C) (b). Table 1: significance of normalized sap flow, between light treatments, during the day; different letters

represent significant difference at P<0.05. No letters indicate no significant difference. Normalized sap flow for each light treatment (c), during the day, from 5:00 to 22:00 hour; every point represents the hourly average of 15 minutes records. Accumulated normalized sap flow for each light treatment (d), from 5:00 to 22:00 hour with a 15 minute interval. Table 2: significance of fruit absolute growth rate, between light treatments, during the day, from 0:00 to 23:00 hour; positive symbols (+) represents positive values; negative symbols (-) represents negative values; different letters represent significant difference at P<0.05. No letters indicate no significant difference. Accumulated fruit absolute growth rate for each light treatment (e), during the day, 0:00 to 23:00 hour; every point represents the hourly average of 15 minutes records.

Figure 9 (pp. 82) – Period 8: September 05, and 09-11. Daily solar radiation pattern (a) and daily environmental air temperature increase (°C) (b). Table 1: significance of normalized sap flow, between light treatments, during the day; different letters represent significant difference at P<0.05. No letters indicate no significant difference. Normalized sap flow for each light treatment (c), during the day, from 5:00 to 22:00 hour; every point represents the hourly average of 15 minutes records. Accumulated normalized sap flow for each light treatment (d), from 5:00 to 22:00 hour with a 15 minute interval. Table 2: significance of fruit absolute growth rate, between light treatments, during the day, from 0:00 to 23:00 hour; positive symbols (+) represents positive values; negative symbols (-) represents negative values; different letters represent significant difference at P<0.05. No letters indicate no significant difference. Accumulated fruit absolute growth rate for each light treatment (e), during the day, 0:00 to 23:00 hour; every point represents the hourly average of 15 minutes records.

Figure 10 (pp. 83) – Period 9: September 21-23. Daily solar radiation pattern (a) and daily environmental air temperature increase (°C) (b). Table 1: significance of normalized sap flow, between light treatments, during the day; different letters represent significant difference at P<0.05. No letters indicate no significant difference. Normalized sap flow for each light treatment (c), during the day, from 5:00 to 22:00 hour; every point represents the hourly average of 15 minutes records. Accumulated normalized sap flow for each light treatment (d), from 5:00 to 22:00 hour with a 15 minute interval. Table 2: significance of fruit absolute growth

rate, between light treatments, during the day, from 0:00 to 23:00 hour; positive symbols (+) represents positive values; negative symbols (-) represents negative values; different letters represent significant difference at P<0.05. No letters indicate no significant difference. Accumulated fruit absolute growth rate for each light treatment (e), during the day, 0:00 to 23:00 hour; every point represents the hourly average of 15 minutes records.

Figure 11 (pp. 84) – Period 10: October 06,08,09. Daily solar radiation pattern (a) and daily environmental air temperature increase (°C) (b). Table 1: significance of normalized sap flow, between light treatments, during the day; different letters represent significant difference at P<0.05. No letters indicate no significant difference. Normalized sap flow for each light treatment (c), during the day, from 5:00 to 22:00 hour; every point represents the hourly average of 15 minutes records. Accumulated normalized sap flow for each light treatment (d), from 5:00 to 22:00 hour with a 15 minute interval. Table 2: significance of fruit absolute growth rate, between light treatments, during the day, from 0:00 to 23:00 hour; positive symbols (+) represents positive values; negative symbols (-) represents negative values; different letters represent significant difference at P<0.05. No letters indicate no significant difference. Accumulated fruit absolute growth rate for each light treatment (e), during the day, 0:00 to 23:00 hour; every point represents the hourly average of 15 minutes records.

INTRODUCTION

Water transport in plants occurs mainly through the xylem. When the water potential inside a cell differs from the external, there will no longer be an equilibrium and a net water movement will occur, from the region of higher water potential towards the region of lower water potential (Nobel, 2005). Sap flow depends on a series of factors, from morphological, to physiological, to environmental (Schuepp, 1993). Among the factors influencing the movement of water inside a plant,

light is considered an important one, as it controls stomatal conductance (Jarvis, 1976), but also the relation between light, air temperature and vapour pressure deficit will modify the dynamics of sap flow (Nobel, 2005). Water flux inside plants linearly follows solar radiation patterns (Iwanami et al., 2011) and it has been shown that under shading nets there is a lower evaporative demand, due to less incoming radiation, thus there will be a decrease in sap flow (Nicolás et al., 2005). Monitoring sap flow can be considered as a useful and reliable method to track plant water status, as it determines continuous and direct measurements of plant water flow (Fernández et al., 2008), in other words plant water consumption. However, when modifying the quality of incoming light, photomorphogenic and other physiological processes are impacted, thus affecting plant behaviour (Shahak et al., 2004). Crop water uptake may be subjected to different dynamics, as a consequence of the different performance arising under different light environments. To date, knowledge on how light quality impacts sap flow is missing.

In this study, the effect of coloured nets on the amount and daily patterns of xylem sap, flowing through apple branches, at different phenological stages was investigated. In parallel, fruit growth was monitored to evaluate potential secondary impacts of xylem sap flow rate variations. The results may add new information on how the modification of the light environment can affect tree water use in a commercial orchard.

MATERIALS AND METHODS

The trial was set on a 3-year old *Rosy Glow* apple orchard, located in the experimental farm of Bologna University, from June to October 2016. Trees were grafted on M9 and were spaced 1 m along the row and 3.3 m

between rows, trained as the solaxe system (Lauri and Lespinasse, 2000). Around the end of May, nets were placed on four individual rows, covering six trees each, to obtain four different light environments: a red net (RED), a pearl net (WHITE), a blue net (BLUE) (ChromatiNet Polysack Plastic Industries, D.N. Negev, Israel), which shaded 50%, and a control, neutral shading, black net (CTRL) which shaded 20%. The four central trees were tested in each treatment. Plants were irrigated supplying 100% of the estimated ET_c. Environmental weather data was collected from a central weather station, set just outside the orchard.

Light spectra under each net were assessed by a LI-1800 spectroradiometer (LI-COR, Lincoln, NE, USA) outside the orchard, at midday, on a cloudless day, in winter; a reference measurement was taken in full light and portions of each net were placed over the sensor. Wm^{-2} outputs were converted to μmol m^{-2} s^{-1}; transmittance was estimated as the difference between the external light input and the light intensity under the net and expressed as a percentage.

Xylem flow was determined through the heat balance method (Sakuratani, 1981). Measurements were carried out in ten periods during the season and consecutive days were used as replicates when meteorological conditions, outside the orchard, were statistically the same. The analysed periods were:

-1. June, 22-23,

-2. June, 27-30,

-3. July, 18-20,

-4. July, 25-27,

-5. August, 01-02,

-6. August, 03-04,[1]

-7. August, 23-24,

-8. September, 05,09-11,

-9. September, 21-23,

-10. October, 06,08-09.

In each period, under each net, two custom-built sap flow sensors were placed on 11 to 15 mm diameter branches (the closest to the ground), which were selected to have at least one fruit. The sensors were interchanged between trees during data collection so that at the end of each monitoring period at least 4 trees per treatment were monitored. Data were recorded by a CR10X data logger (Campbell Scientific Inc., Logan, UT, USA) each minute and automatically averaged every 15 minutes, from 3:30 to 22:30 h. During each monitoring period, the system worked continuously. Sap flow was then normalized per unit leaf area and expressed as gm^{-2} h^{-1}. The leaf area of each monitored branch was determined by assessing the area of 20 randomly picked leaves per light treatment, through the use of a LI-3100C Area Meter (LI-COR, Lincoln, NE, USA), and multiplying the average leaf area by the total number of leaves counted on the branch.

During the sap flow monitoring periods, fruit growth was traced. Custom-built fruit gauges (Morandi et al., 2007) were installed at the beginning of June on four fruit in each net treatment. The chosen fruit were growing on the branches where the sap flow sensors were set. These sensors were interfaced with a wireless data logger system (Wi-Net s.r.l. Cesena, Italy) (Giorgetti et al. 2014), composed of four nodes,

[1] Periods 5 and 6 were initially considered as a single period. Data collection had been planned to be taken in a four-day interval (01-04/08). Unfortunately, environmental conditions between the two periods were different and the measurement had to be split.

communicating with a central coordinator. The network coordinator acted as a gateway towards the internet, through a general packet radio service (GPRS) modem. Fruit were monitored continuously, at 15 minutes intervals, until harvest. At each recording time, diameter data (D) from all fruit monitored were converted to fresh weight (FW) using the following conversion equation:

$$FW(g) = a * D(mm)b$$

where *a* and *b* were 0.0006 (±SE 0.00005) and 2.924 (±SE 0.0194). This equation was obtained by regressing diameter and weight data of about 300 fruit picked from various Pink Lady apple orchards. The R^2 of the relationship was >0.99. The use of this conversion is justified by the expolinear behaviour of apple fruit on a weight basis (Lakso et al., 1995), explaining the linear growth during all its phenological stages. Absolute growth rate (AGR) could then be obtained.

For each monitored period, daily mean solar radiation and temperature, from outside the orchard, and accumulated sap flow were traced.

Physiological harvest occurred in the first week of November. Unfortunately, it was not possible to evaluate yield, due to the very crop load that originated from too heavy thinning.

STATISTICAL ANALYSIS

Two-way ANOVA was used to separate averages among net treatments of sap flow and fruit AGR, while SNK test was used to rank the different treatments ($P<0.05$). A one-way ANOVA was used for testing differences in air temperature and relative humidity of consecutive days in which sap flow measurements were taken ($P<0.05$).

The analysed data were collected from a central weather station set outside the orchard (see *Materials and Methods*). When meteorological conditions of consecutive days were the same, sap flow measurements could be grouped in periods.

RESULTS

Light quantity and quality under the nets (Figure 1). In the blue region, the BLUE net had the higher transmittance (70%), while it decreased in the red (40%). The CTRL net had higher transmittance in all wavelengths, being a neutral shade net, except the blue one. The RED net had the lowest transmittance in the blue region (40%), recovering at around 570 nm and reaching 80-90% in the infrared (IR) region. The WHITE net had an intermediate linear increase in all wavebands.

Period 1. June 22-23 (Figure 2). During the day, solar radiation reached a peak of nearly 2000 μmol m^{-2} s^{-1}, (Figure 2a) and temperatures ranged from 17 to 31 °C (Figure 2b). Environmental max VPD was 2.56 kPa. Sap flow patterns showed a general rise in the first half of the day, then a decrease followed by a second increase (Figure 2c). Significant differences occurred only at 6:00 h, where CTRL had higher flow, and once in the afternoon, where RED treatments showed higher sap flow. Significantly lower fluxes occurred in the BLUE and WHITE ones, leaving CTRL as an intermediate (Table 1). By the end of the day, CTRL trees had accumulated nearly 3000 g of sap, whereas the WHITE ones around 1000 (Figure 2d). Fruit growth during the night was significantly lower under the WHITE net, but higher during the day, compared to BLUE and CTRL fruits. However, growth recovery was less pronounced for WHITE fruits, whereas RED fruits gained around 1 g at

the end of the day, and the other treatments stayed below 0.6 g (Table 2, Figure 2e).

Period 2. June 27-30 (Figure 3). Solar radiation reached a maximum of 1800 μmol m^{-2} s^{-1} (Figure 3a). Minimum temperature was 17.4 and the maximum was nearly 30 °C (Figure 3b). Environmental max VPD was 2.49 kPa. Before midday, flows were higher under the CTRL net. After midday, RED trees had the highest values. WHITE trees had always the lowest flow, never exceeding 70 gm^{-2} h^{-1} (Table 1, Figure 3c). The treatments with highest accumulated sap, at the end of the day, were the RED and CTRL one, going above 4000 g, leaving the WHITE one below 2000 g (Figure 3d). Fruit growth during the night was significantly lower under the WHITE net and higher for BLUE fruits. During the day, WHITE agr showed less shrinkage, compared to the other treatments. Like in Period 1, by the end of the day, WHITE fruit slowed down their weight increase, compared to BLUE fruit, leaving RED and CTRL as intermediates (Table 2, Figure 3e).

Period 3. July 18-20 (Figure 4). Solar radiation peaked over 1800 μmol m^{-2} s^{-1} (Figure 4a) and temperatures went from 18.5 to 33.2 °C (Figure 4b). Environmental max VPD was 3.23 kPa. During most of the day, RED trees experienced the highest sap flow, exceeding 240 gm^{-2} h^{-1}. WHITE trees had the lowest flow for most of the day, going no higher than 70 gm^{-2} h^{-1} (Figure 4c). Each net was different from midday to mid-afternoon (Table 1). RED trees had the highest accumulated sap, going above 8000 g, at the end of the day, whereas the WHITE trees remained below 3000 g (Figure 4d). Fruit growth was different only during the night and early morning, where BLUE had higher AGR values, compared to the other treatments, in the first case, but lower in the second one, compared to CTRL fruits, which had higher recovery values (Table 2).

BLUE fruits nevertheless gained around 1.2 g at the end of the day, leaving the other treatments below 1 g (Figure 4e).

Period 4. July 25-27 (Figure 5). Maximum solar radiation was around 1700 µmol m^{-2} s^{-1} (Figure 5a), while air temperatures were between 20 and 31.5 °C (Figure 5b). Environmental max VPD was 2.42 kPa. Before midday, sap flow was higher under RED and CTRL nets and lower under the WHITE net. After midday, only the RED trees were higher (Table 1, Figure 5c). RED and CTRL treatments had the highest accumulated sap, above 3000 g, whereas the WHITE one stayed below 2000 g, by the end of the day (Figure 5d). Fruit growth was different only during the night and early morning, where BLUE had higher AGR values, compared to the other treatments, gaining more than 1.6 g at the end of the day (Table 2, Figure 5e), whereas the other treatments did not reach 1.2 g.

Period 5. August 01-02 (Figure 6). Solar radiation did not go above 1800 µmol m^{-2} s^{-1} (Figure 6a) and temperature ranged from 20 to 29.3 °C (Figure 6b). Environmental VPD was 2.52 kPa. CTRL trees had highest flows for most of the day, whereas the RED ones mostly during the morning. WHITE trees were always significantly lower (Table 1). All four nets did not go beyond 125 gm^{-2} h^{-1} of accumulated sap flow (Figure 6c). CTRL trees accumulated the highest sap, going above 5000 g, leaving the WHITE below 3000 g (Figure 6d). Fruit growth was not different among treatments, although by the end of the day the WHITE treatment had gained nearly 1.2 g of fresh weight, whereas the CTRL one had accumulated around 0.8 g, leaving the RED and BLUE slightly below 1 g (Figure 6e).

Period 6. August 03-04 (Figure 7). Solar radiation went above 1800 μmol m^{-2} s^{-1} (Figure 7a) and temperatures ranged from 18.9 to 32.8 °C (Figure 7b). Environmental VPD was 3.28 kPa. During all day, the RED net had higher sap flows, going up to 200 gm^{-2} h^{-1} (Table 1, Figure 7c) and accumulated the highest amount of sap, over 7000 g (Figure 7d). The other treatments tended to stay below 100 gm^{-2} h^{-1}, accumulating between 3000 and 4000 g, by the end of the day. Fruit growth during the night was higher under the BLUE net and lower in the afternoon, compared to the rest of the treatments (Table 2). At the end of the day, WHITE and CTRL fruits had gained around 1 g of fresh weight, leaving RED fruits at slightly more than 0.6 g (Figure 7e).

Period 7. August 23-24 (Figure 8). Maximum solar radiation did not reach 1800 μmol m^{-2} s^{-1} (Figure 8a) and temperatures ranged from 14.9 to 29 °C (Figure 8b). Environmental max VPD was 2.70 kPa. For most of the day, under the RED net, flows were higher, reaching a maximum of more than 150 gm^{-2} h^{-1} (Figure 8c). The BLUE trees tended to be intermediate in the second half of the day. The CTRL and WHITE ones had a similar trend for most of the day (Table 1, Figure 8c). RED trees accumulated the highest amount of sap, reaching nearly 6000 g, whereas CTRL trees remained below 3000 g (Figure 8d). Fruit growth was different in the early morning and in the late afternoon, where CTRL showed higher AGR values, compared to both RED and BLUE fruits (Table 2). Due to technical problems, WHITE fruit growth data were not collected. Accumulated AGR was higher for CTRL fruits, by the end of the day, where fruits gained more than 1.2 g, leaving the RED and BLUE ones below 0.8 g (Figure 8e).

Period 8. September 05, 09-11 (Figure 9). Maximum solar radiation went slightly above 1300 μmol m^{-2} s^{-1} (Figure 9a) and temperatures were between 19.8 and 29.7 °C (Figure 9b). Environmental VPD was 2.31 kPa. Sap flow was different only once, before midday, where RED was higher than WHITE (Table 1). Due to technical problems, BLUE trees sap flow data were not collected. RED trees accumulated nearly 4000 g of sap, leaving the WHITE treatment at around 2500 g (Figure 9d). Fruit growth was higher for CTRL fruits and lower for the BLUE ones in the morning, and vice versa in the late afternoon and at night (Table 2). RED and CTRL fruits accumulated 1 g of fresh weight, whereas BLUE and WHITE gained around 0.6 g (Figure 9e).

Period 9. September 21-23 (Figure 10). Solar radiation did not exceed 1400 μmol m^{-2} s^{-1} (Figure 10a) and temperatures ranged from 14.1 and 22.1 °C (Figure 10b). Environmental VPD was max 1.78 kPa. For most of the day WHITE trees had significantly lower sap flow rates, reaching around 50 gm^{-2} h^{-1}, compared to other three treatments. BLUE trees were higher in the morning, reaching around 120 gm^{-2} h^{-1} at midday, leaving RED and CTRL as intermediates, whereas in the late afternoon RED trees were significantly higher, as well (Table 1, Figure 10c). The BLUE treatment was highest in accumulating more than 4000 g of sap, whereas the WHITE gained less than 2000 g (Figure 10d), and was the one to have higher fruit AGR values, during the night and the evening, gaining around 1.2 g of fresh weight (Table 2, Figure 10e).

Period 10. October 06, 08-09 (Figure 11). Maximum solar radiation was below 1000 μmol m^{-2} s^{-1} (Figure 11a) and temperatures were between 9.1 and 18.2 °C (Figure 11b). Environmental VPD was 1.14 kPa. Sap flow was not different among light treatments, although RED trees accumulated 1300 g of sap, leaving the WHITE ones at around 750 g

(Figure 11d). As for fruit growth, the BLUE treatment had higher AGR values, compared to RED and WHITE, twice during the day (Table 2) and gained more than 1.2 g of weight (Figure 11e). Due to technical problems, CTRL fruit growth data were not collected.

DISCUSSION

Sap flow differences among treatments were recorded for most of the season. Within coloured nets, RED showed the highest sap flow rates, compared to BLUE and WHITE. CTRL was in some cases similar to RED, whereas in other cases to BLUE. For most of the season, WHITE had the lowest velocity of sap flow.

Light quality appeared more important than light quantity under the RED net. RED trees were subjected to a higher absorbance of IR, compared to the BLUE and WHITE (Figure 1). Although no meteorological data for each treatment is available, a possible explanation may be given by the alteration of the microclimate inside the nets, due to the different spectra. Higher transmission of IR under RED could have increased the temperature of the inner part of the canopy (Arthurs et al., 2013), doubling RED sap flow in certain moments of the day compared to other nets, even though its trees were shaded at 50%. This occurred during most of the monitored periods and was particularly evident when temperature increases (Figures *x*b) were especially steep and environmental maximum VPD was higher (Periods 3, 6, 7). RED trees might have been experiencing higher evaporative cooling rates, due to an additional effect of high increase in temperature and IR. CTRL trees transpired more in the morning, until midday, then RED would overtake. Here, light quantity may have been playing a more important role (Syvertsen, 1985) than light quality. Under CTRL, the

higher latent heat flow generated by higher radiation (Figure 1) may have led to a higher heat dissipation (Green et al., 2003). The more shaded trees were under lower evaporative demands, thus their sap flows were lower, compared to CTRL. Although RED trees were not subjected to such high heat dissipation, their IR absorbance placed them, nevertheless, above BLUE and WHITE, in the first part of the day. From midday onwards, RED trees would leave behind also the CTRL ones. CTRL trees, in this case, may have reached and exceeded the "threshold" at which plant transpiration was higher than root water uptake, caused by increases of both temperature and radiation. As CTRL trees were more exposed, they may have had to close earlier their stomata, and in a higher proportion, in order to prevent dehydration (Nicolás et al., 2005). This is particularly evident in Period 7, where CTRL sap flow rate was as low as the WHITE one, compared to RED and BLUE. A cumulative effect of both high solar radiation and temperature during time may have caused CTRL trees to lower their fluxes, as to avoid excessive water loss. BLUE and WHITE trees showed lower sap flows, probably thanks to the higher shading of the nets, compared to CTRL, and to less IR absorbance compared to RED (Figure 1). WHITE trees maintained very low rates, resulting in the lowest accumulated sap flow, during most of the season. In nearly all the monitored periods, by the end of the day, WHITE trees had accumulated half the amount of sap compared to RED trees, or even less than half (Figures 2d, 3d, 4d, 10d), even though both these light treatments had equal shading power (50%). In a commercial apple orchard, a 50% shading white net appears to be more efficient in saving water, particularly under high VPD conditions.

From the fruit growth point of view, the situation does not necessarily correspond linearly to that of sap flow. In the first part of the season (Periods 1, 2, 3), fruit still shrink, due to xylem backflow drawing water towards the leaves, a consequence of their more negative water potential (Lang, 1990; Morandi et al. 2011a, 2011b). CTRL and BLUE fruit show higher shrinkage during the central parts of the day, especially in Periods 1 and 2, although having more pronounced recovery during the night (Table 2 in Figures 2, 3). An explanation may be given by the higher amount of incoming radiation, under the CTRL net (shading 20%) (Figure 1), thus creating a less advantageous environment for the trees, which had to cope with more evaporative cooling demand (Green et al., 2003). This is not the case for BLUE fruits, as they were growing in a more shaded environment, hence the quality of the light may have been more relevant in influencing shrinkage. Probably, as literature states a higher effect of blue light in keeping stomata open (Bastías, 2011a; Bastías et al., 2011b, 2012; Farquhar and Sharkey, 1982), leaves may have been transpiring more in the hottest moments of the day, resulting in a more pronounced back flow of water from fruit to leaf. Sap flow should therefore reflect such condition and be higher, in fact sap fluxes for BLUE and CTRL trees were higher compared to WHITE trees, in the monitored periods, but not compared to the RED ones (Table 1 in Figures 2, 3). Both CTRL and BLUE behaviour leads to think that the hydraulic functioning of these two treatments is more susceptible to the surrounding environment, with noticeable effects on fruit growth. In the first periods of the monitored season, fruit cannot avoid water back flow due to higher evaporative cooling rate, caused by higher irradiance, under the CTRL net, and possibly by higher stomatal conductance caused by greater amounts of

blue light, under the BLUE net. As the season continues and fruit xylem functionality progressively decreases (Figures 4, 5), BLUE fruit grow significantly more during the night, than all the other treatments, gaining by the end of the day higher amounts of fresh weight (Figures 4e, 5e), although they still experience slight, but not significant, negative growth in the central parts of the day. In Period 7 (Figure 8), CTRL fruit are growing more than RED and BLUE ones (data for WHITE fruit growth is absent) (Table 2, Figure 8e). Sap flow patterns reflect inversely fruit behaviour, showing significantly lower sap velocity for CTRL trees, compared to RED and BLUE. It is possible that CTRL trees had to close earlier their stomata, to prevent dehydration, as a consequence of higher incoming radiation. This may have been a benefit, as it could have made more water available for fruit growth. On the other hand, RED and BLUE trees would have kept higher transpiratory losses, inducing higher sap flows, thus reducing available water for fruit. Fruit growing under the WHITE net have a different behaviour. They do not experience pronounced shrinkage, as CTRL and BLUE do, in fact, their growth is slowed down, rather than becoming negative, resulting in higher AGR in the central parts of the day. However, they do not have the same growth velocity as the other treatments during the night (Table 2, Figures 2,3,4,5,7,9), thus they are not accumulating as much fresh weight, in most part of the season. RED fruit often have intermediate behaviour, or significantly lower AGR values, for most of the monitored periods. The higher transmittance of IR, under the net, is probably affecting sap fluxes excessively, limiting water to be transported for evaporative cooling rates, rather than allowing the trees to be able to distribute water also towards the fruit. A 50% shading red net is not advisable to be placed over a commercial apple orchard.

The vpd values reported here are well within normal conditions, which indicate that nets might have affected vascular flows by a mechanism not necessarily related to changes in relative humidity and, or, temperature. As such, this effect has not been reported and warrants further investigation.

These statements arise in the presence of very low crop load. Further studies are needed to assess the influence of different light spectrum on apple performances, with the occurrence of commercial crop loads. Differences are to be expected, as different crop loads are known to impact plant physiology. Nevertheless, this trial aims to the potential usefulness of photoselective netting for lowering water consumption in apple.

CONCLUSIONS

Photoselective nets are capable of influencing sap flow in apple. A 50% shading red net is certainly going to increase sap flow, even more than a 20% shading black classic anti hail net. On the contrary, a 50% shading pearl net is going to maintain lower sap flow rates. A 50% shading blue net will maintain intermediate sap fluxes.

Fruit growth under photoselective nets was also influenced. Under a 50% shading red net, absolute growth rates were not as high as under a 50% shading blue, or pearl, net. In most of the monitored periods, a 50% shading net increased fruit growth compared to a 20% shading net.

When considering both sap flow rates and fruit growth, in a commercial apple orchard, a 50% shading red net is not suitable, as it unbalances water transportation towards leaves, rather than towards the fruit. Moreover, this specific net would not be suitable from a water saving point of view, as it showed to have the highest amounts of

accumulated sap flow, therefore it demonstrated a very high inefficiency in water use. A 50% shading pearl net appears to be the most suitable, when it comes to water saving. However, apple production under such a net may not give as high results as a 50% shading blue net. Although sap flow rates under the 50% shading blue net were not as low as in the case of the 50% shading pearl net, fruit absolute growth rates were most of the times higher.

In conclusion, management of the light environment in the orchard, both in terms of intensity and quality, obtained with coloured anti hail or shading nets, can effectively be used to modify tree performance. However, future studies should focus on how limitations of water from irrigation can modify plant water consumption, while modifying the intensities of the light spectrum and still maintaining high performance in terms of yield.

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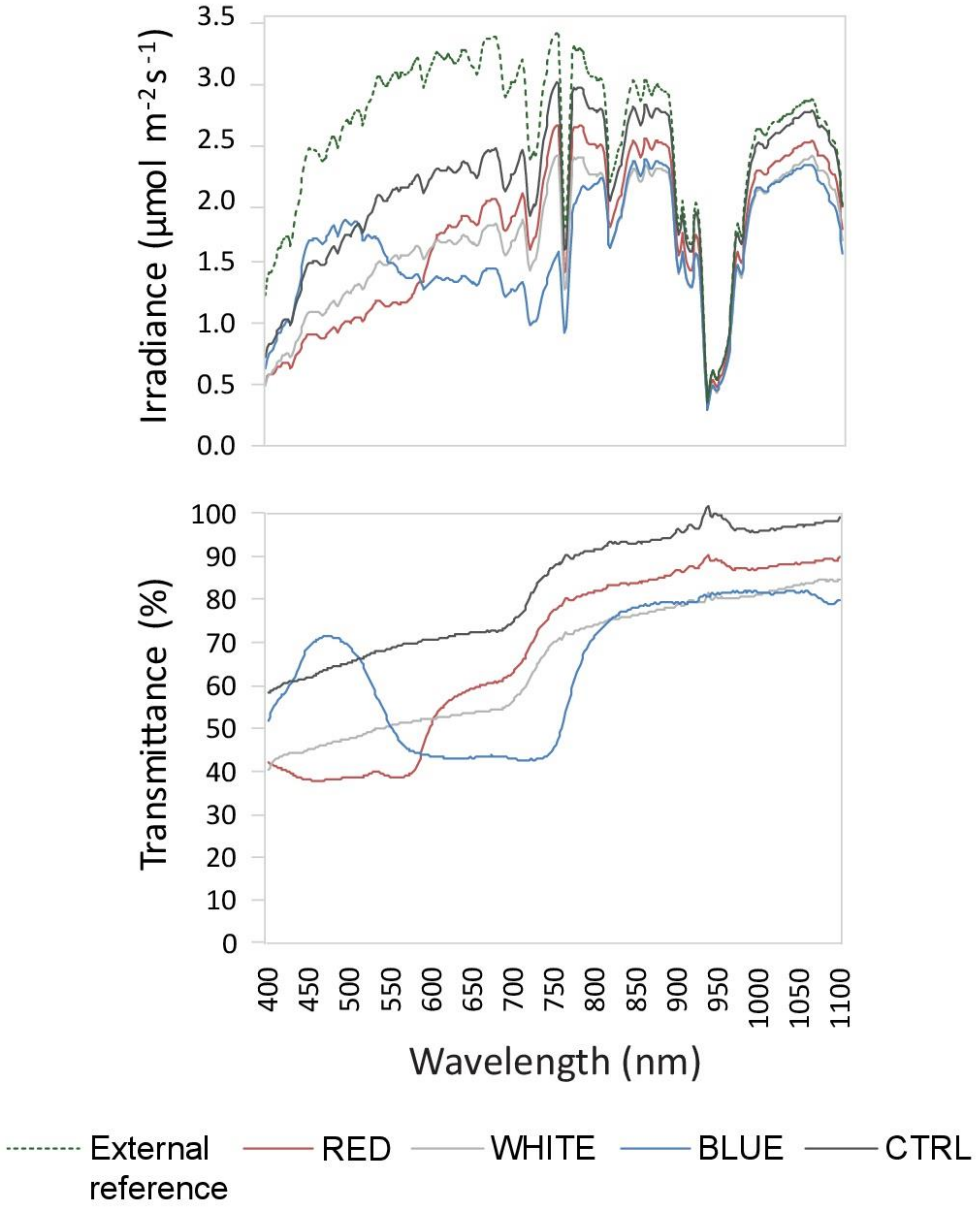

Irradiance (µmol m-2s-1)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Transmittance (%)
100
90
80
70
60
50
40
30
20
10
0
400
450
500
550
600
650
700
750
800
850
900
950
1000
1050
1100
Wavelength (nm)
External reference
RED
WHITE
BLUE
CTRL

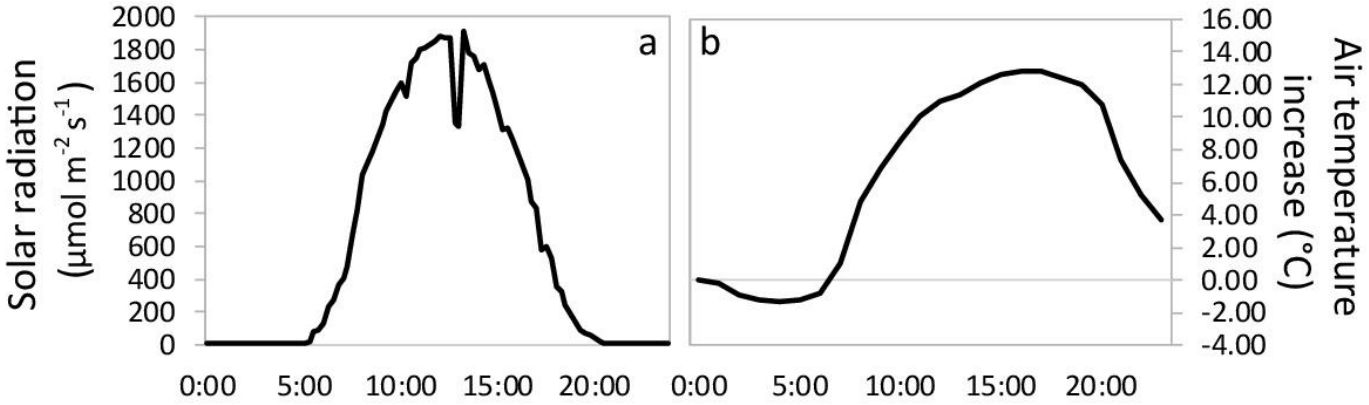


Table 1

time	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
RED							AB									A								
WHITE							B									B								
BLUE							B									B								
CTRL							A									AB								

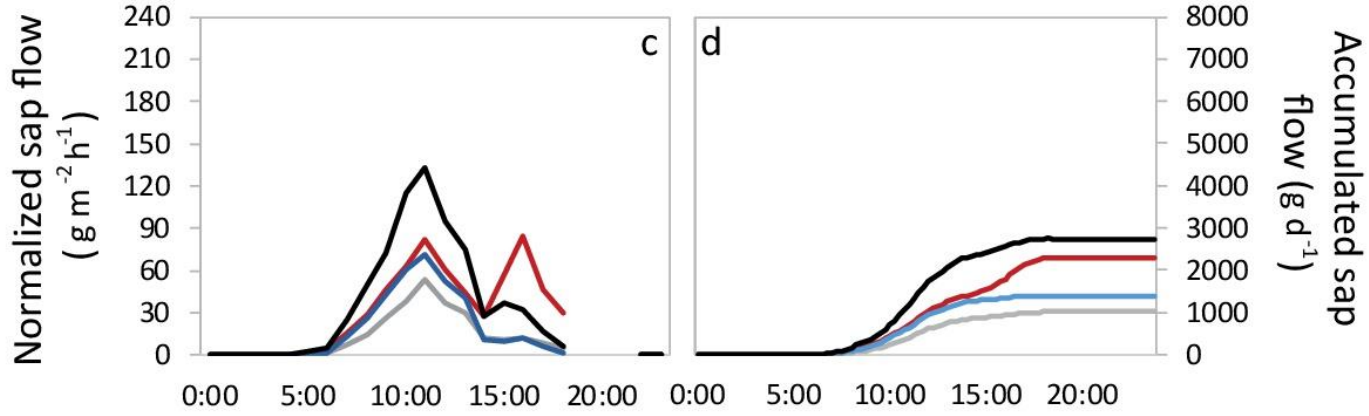
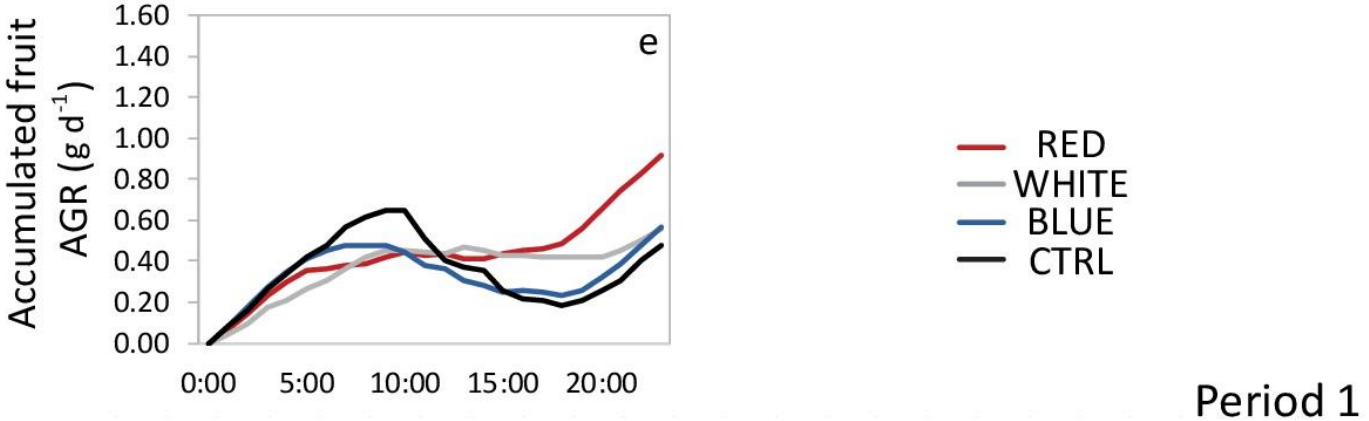


Table 2

time	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
RED	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+
		AB			A							A	A				A				A			
WHITE	+	+	+	+	+	+	+	+	+	+	-	-	-	+	-	-	-	-	+	-	+	+	+	+
		B			B							A	A				AB				B			
BLUE	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	+	-	-	+	+	+	+	+
		A			A							AB	A				A				AB			
CTRL	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	+
		A			A							B	B				B				AB			



Period 1

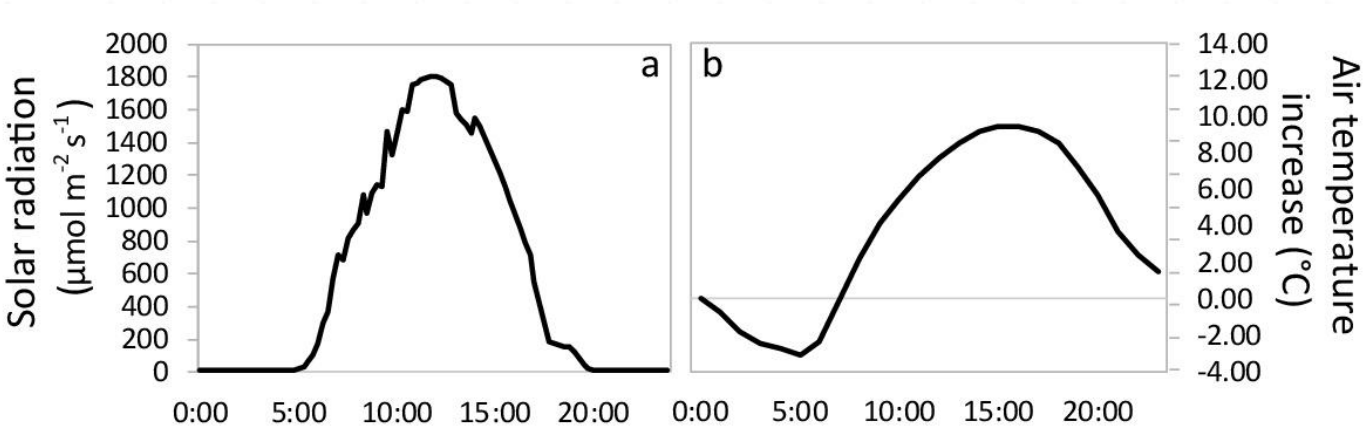


Table 1

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RED								B	B	AB	B	A	A	A	A	A	A	A	A	A	A	A	A	
WHITE								C	C	C	C	B	C	B	B	C	C	C	B	B	B	B	B	
BLUE								BC	B	B	B	B	BC	AB	AB	BC	BC	BC	AB	B	AB	B	B	
CTRL								A	A	A	A	A	B	A	A	AB	AB	AB	AB	B	A	B	B	

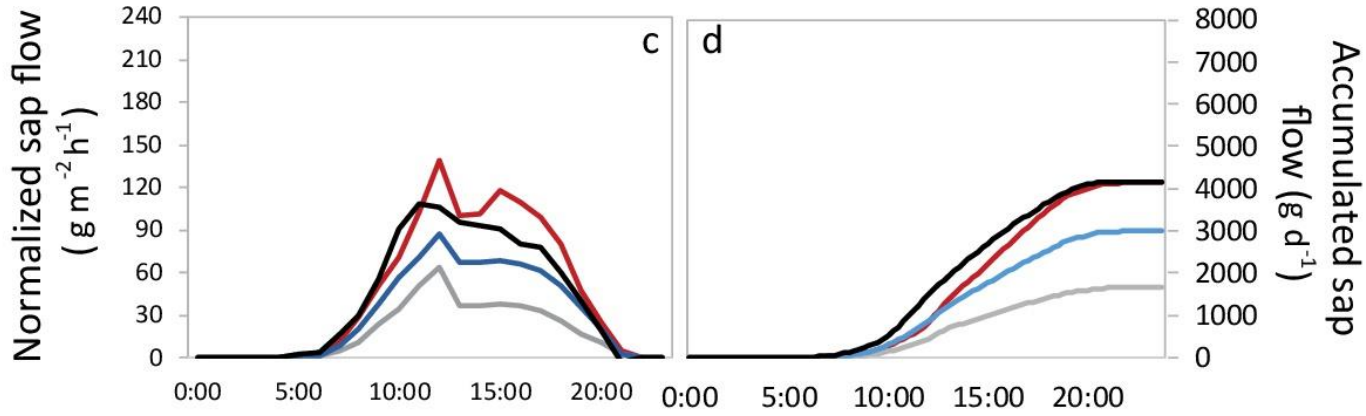
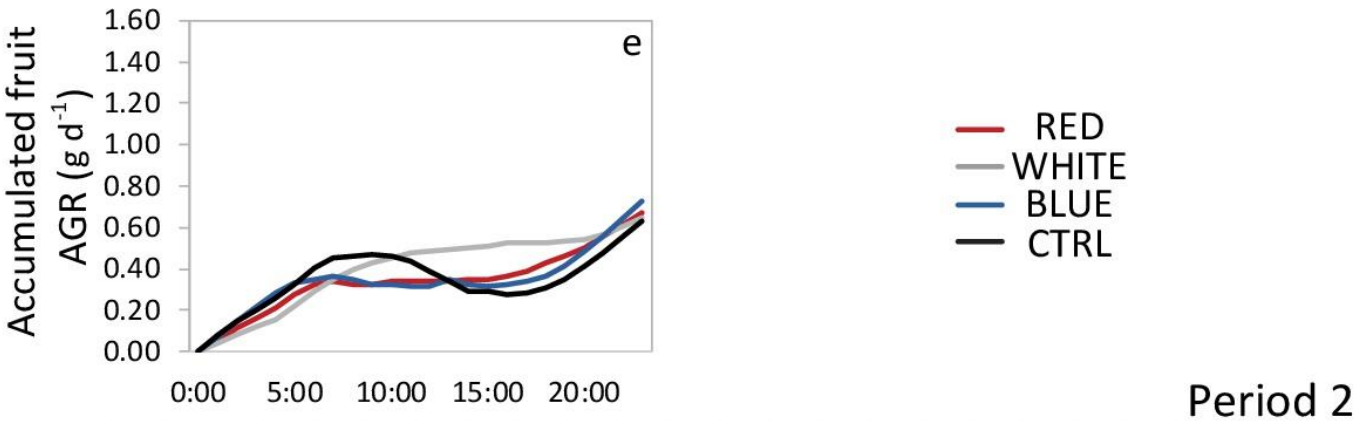


Table 2

time	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
RED	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+
	AB	B	AB	AB	AB	B	AB	B	B	B	AB	AB	A	B	A		A		A	AB	A	AB	AB	BC
WHITE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
	B	B	B	B	B	AB	A	A	A	A	A	A	A	AB	A		A		A	B	B	B	B	C
BLUE	+	+	+	+	+	+	+	+	-	-	+	-	-	+	-	-	+	+	+	+	+	+	+	+
	A	A	A	A	A	AB	B	B	B	C	B	B	A	A	AB		AB		B	A	A	A	A	A
CTRL	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	+	+	+
	A	A	AB	AB	AB	A	A	A	AB	B	B	B	B	C	B		B		A	AB	A	A	A	AB



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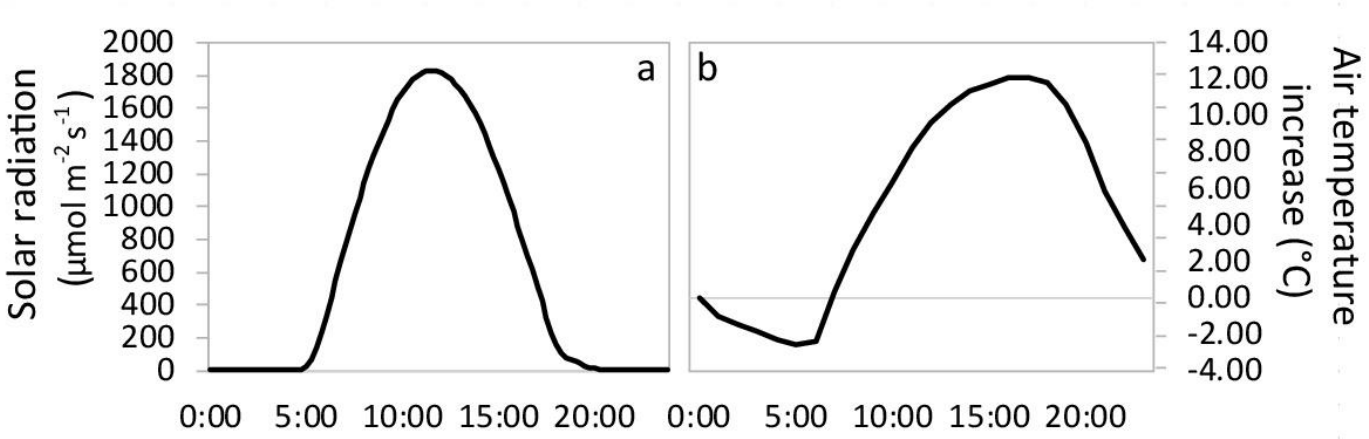


Table 1

time	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
RED								A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	
WHITE								B	C	C	C	C	D	D	D	D	C	C	C	C	C	C	C	
BLUE								B	B	C	C	C	C	C	C	C	B	B	B	B	B	B	B	
CTRL								B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	

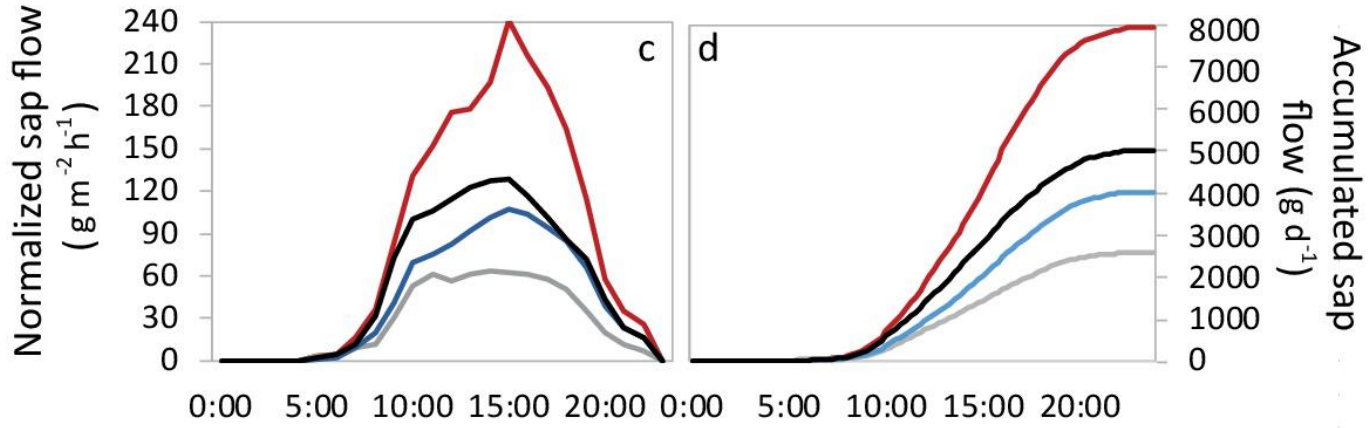
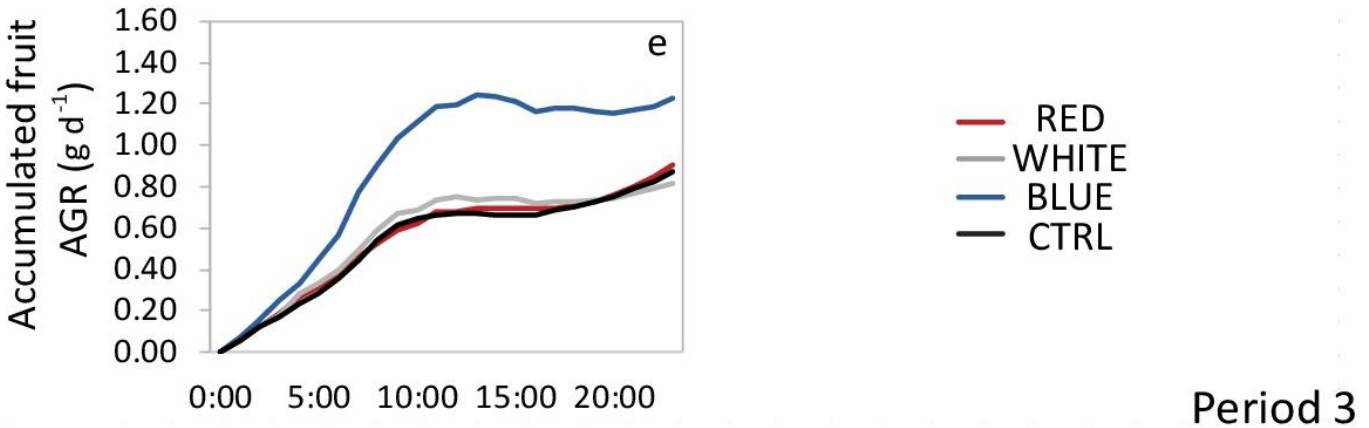


Table 2

time	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
RED	+	+	+	+ AB	+	+ B	+ B	+ B	+	+	+	+	-	+	+	-	+	-	+	+ AB	+ AB	+	+	+
WHITE	+	+	+	+ B	+	+ B	+ B	+ B	+	+	+	+	+	-	+	-	-	+	+	+ AB	+ AB	+	+	+
BLUE	+	+	+	+ A	+	+ A	+ A	+ A	+	+	+	+	+	+	-	-	-	+	-	- B	- B	+	+	+
CTRL	+	+	+	+ B	+	+ B	+ B	+ B	+	+	+	+	+	+	-	-	-	+	+	+ A	+ A	+	+	+



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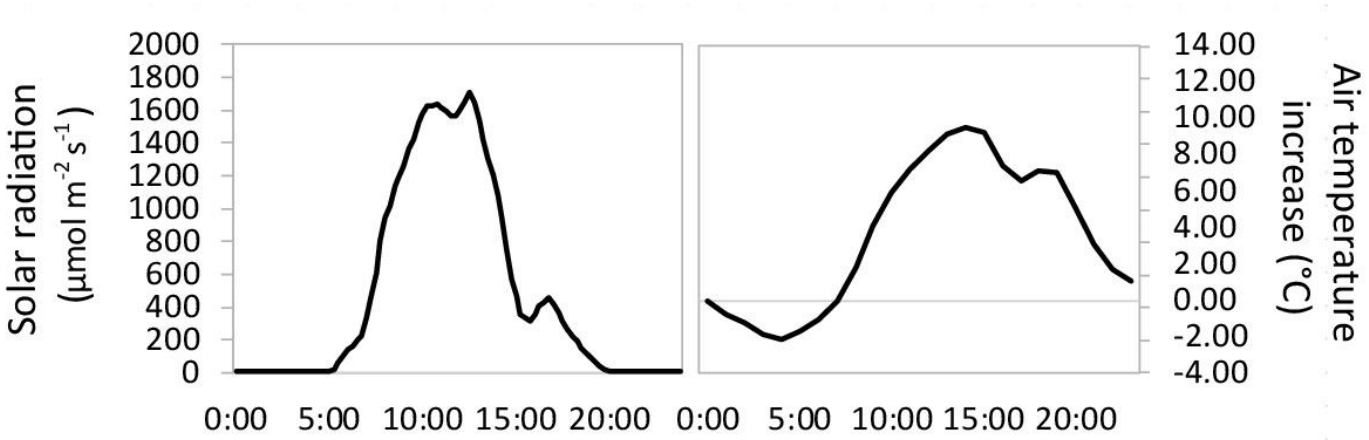
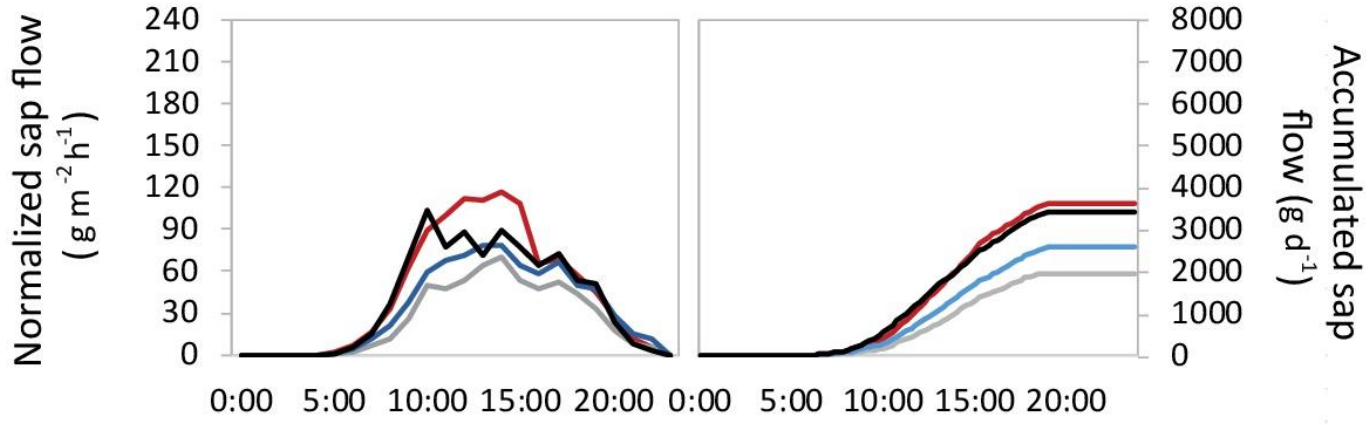
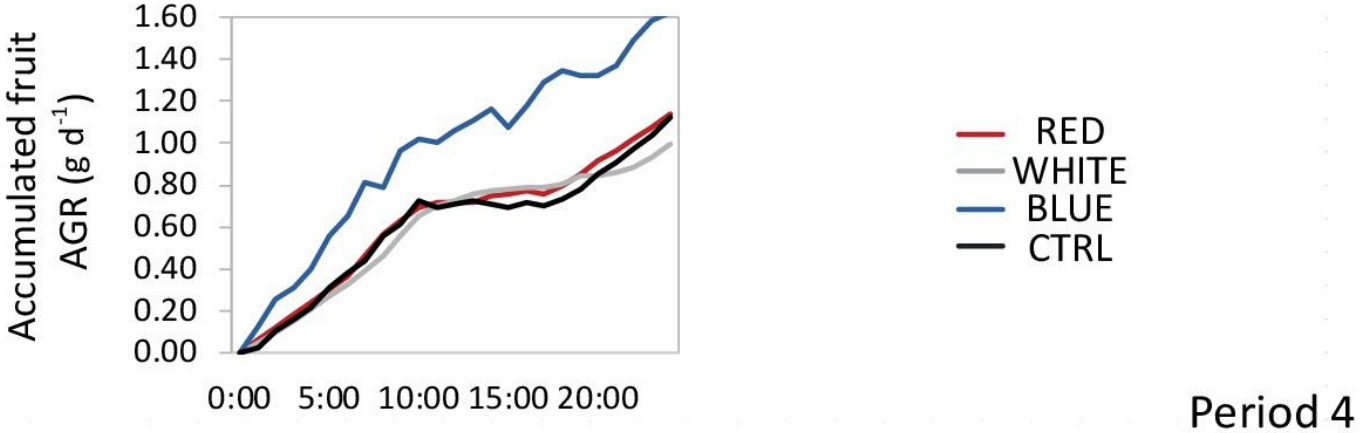


time	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
RED							AB	A	A	A	AB	A	A	A	A	A				A		A	A	
WHITE							C	C	C	B	B	C	B	B	B	B				B		B	B	
BLUE							BC	B	B	B	AB	BC	B	B	B	B				AB		B	AB	
CTRL							AB	A	A	A	A	BC	AB	B	B	B				AB		AB	AB	



time	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
RED	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+
	B	B					B		B													B		
WHITE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+
	B	B					B		AB													B		
BLUE	+	+	+	+	+	+	+	-	+	+	-	+	+	+	-	+	+	+	-	-	+	+	+	+
	A	A					A		A													A		
CTRL	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	-	+	+	+	+	+	+	+
	B	AB					B		B													B		



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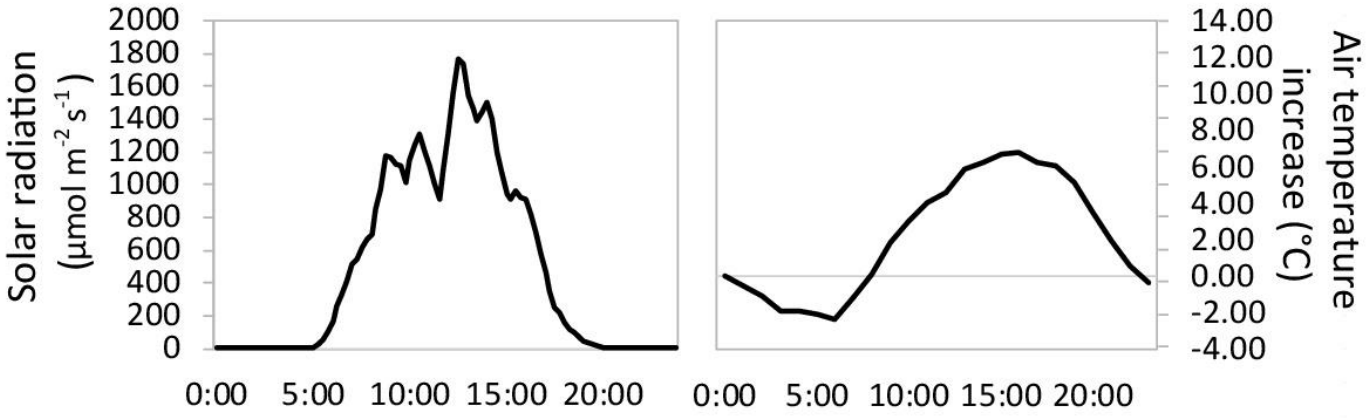
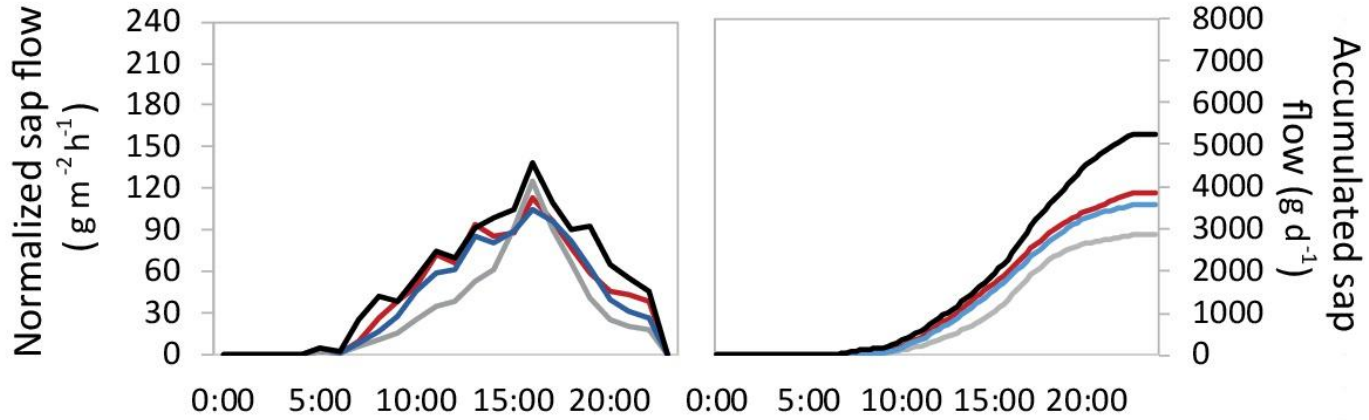


time	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
RED						A		B	B	A	A	A	A	A	AB					B	B	AB	AB	
WHITE						B		B	C	B	B	B	B	B	B					C	C	C	C	
BLUE						B		B	C	AB	A	A	A	A	AB					B	B	BC	BC	
CTRL						A		A	A	A	A	A	A	A	A					A	A	A	A	



time	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
RED	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
WHITE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
BLUE	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	-	+	-	+	+	-	+
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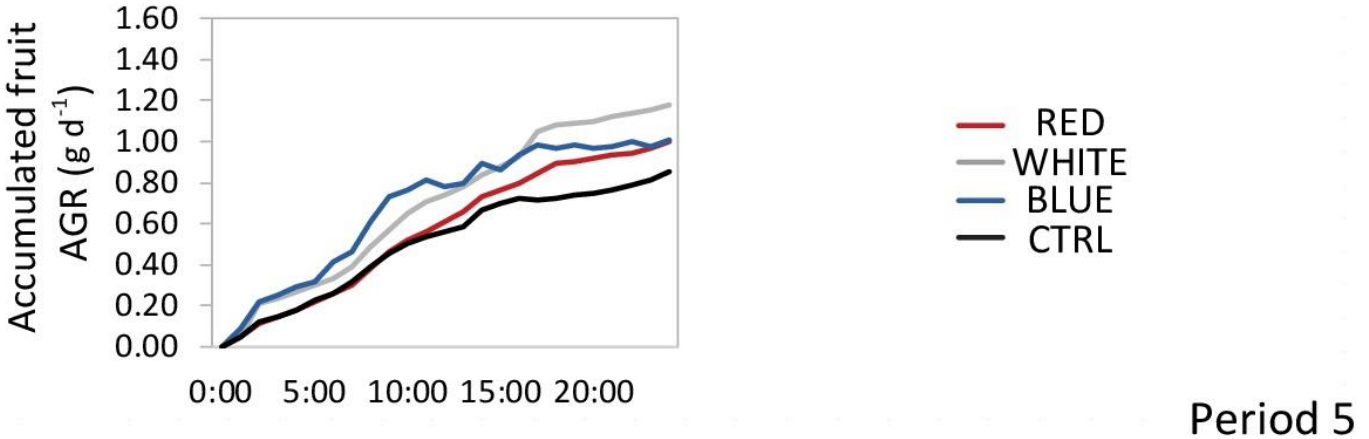

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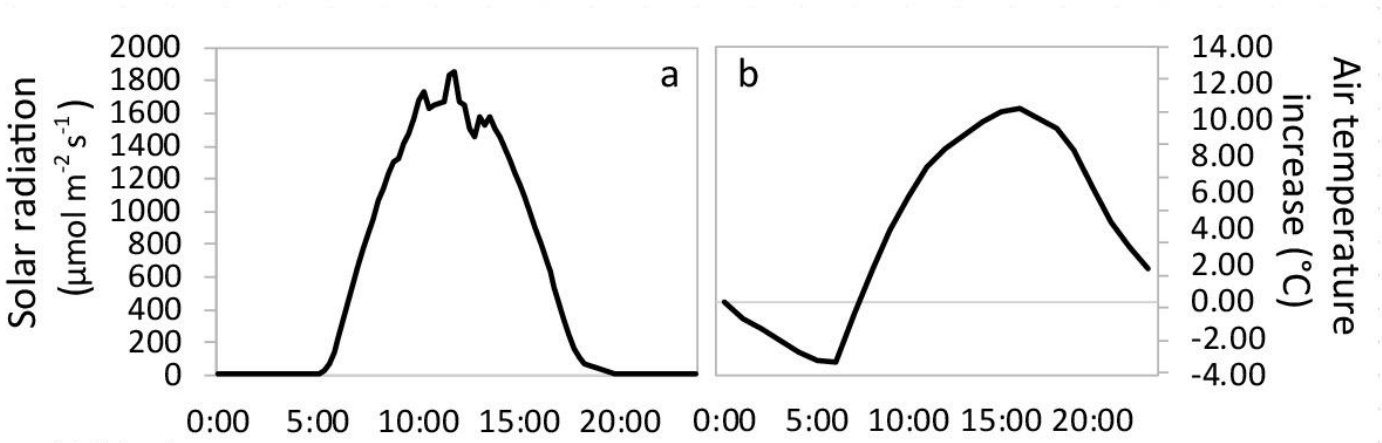


Table 1

time	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
RED								A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	
WHITE								B	B	B	B	C	C	C	B	B	B	B	B	B	C	C	C	
BLUE								B	B	B	B	C	C	C	B	B	B	B	B	B	C	C	C	
CTRL								B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	

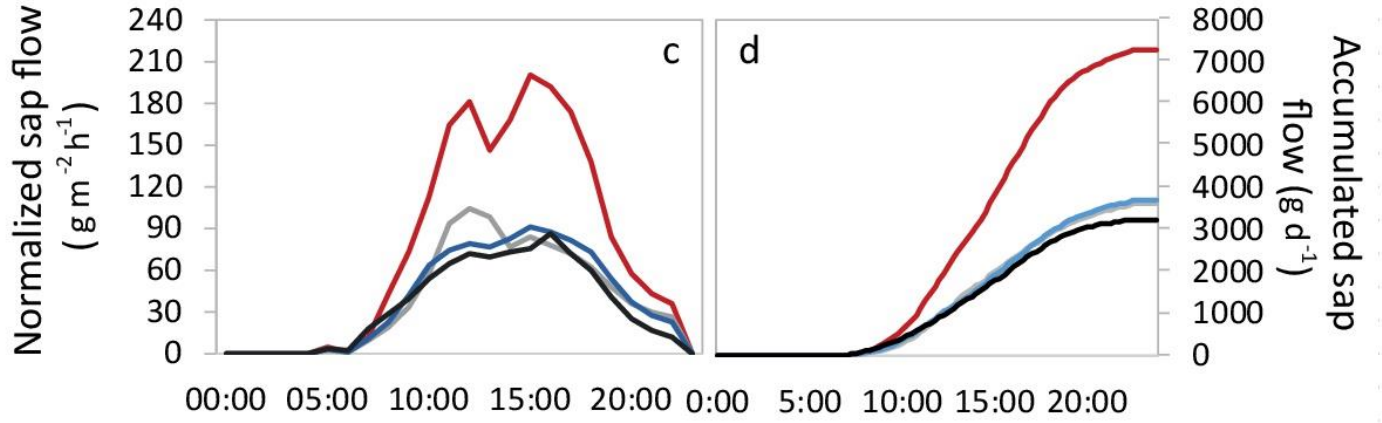
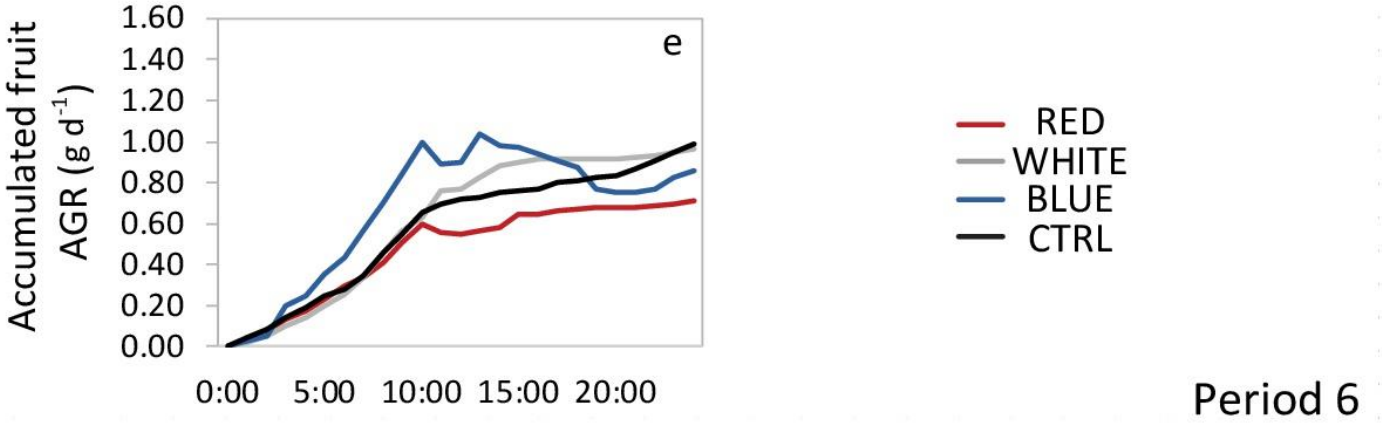


Table 2

time	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
RED	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+
			B				B				AB		B			A		A	A				B	
WHITE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+
			B				AB				A		AB			A		A	A				B	
BLUE	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	-	-	-	-	-	-	+	+	+
			A				A				B		A			B		B	B				A	
CTRL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
			B				AB				AB		B			A		A	A				AB	



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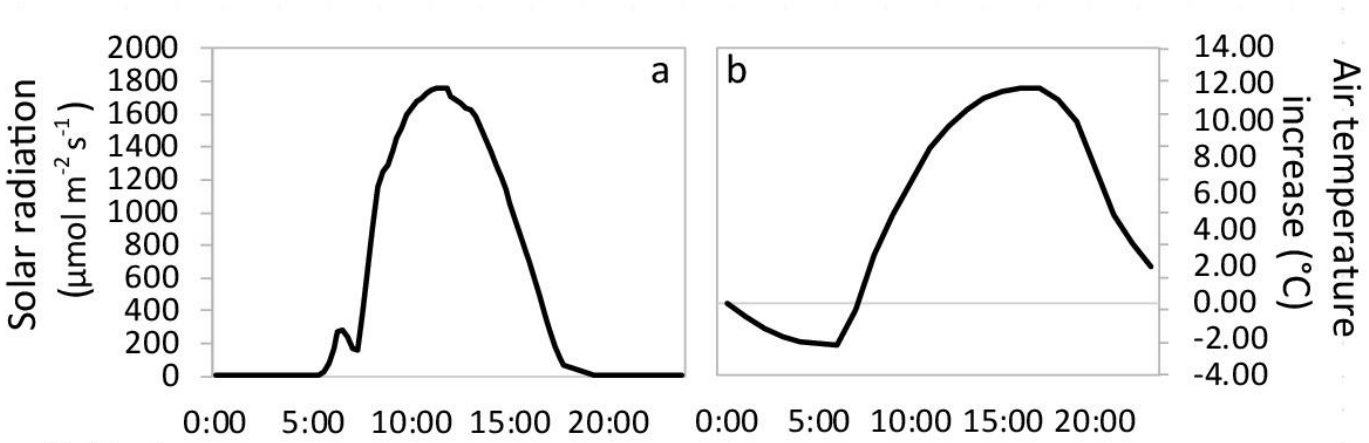


Table 1

time	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
RED						B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	
WHITE						B	B	B	B	B	BC	B	B	AB	C	C	C	C	C	C	BC	B	B	
BLUE						A	A	A	B	C	BC	B	B	AB	AB	B	B	B	B	B	B	B	A	
CTRL						B	B	B	B	C	C	B	C	B	BC	C	C	C	C	C	C	C	C	

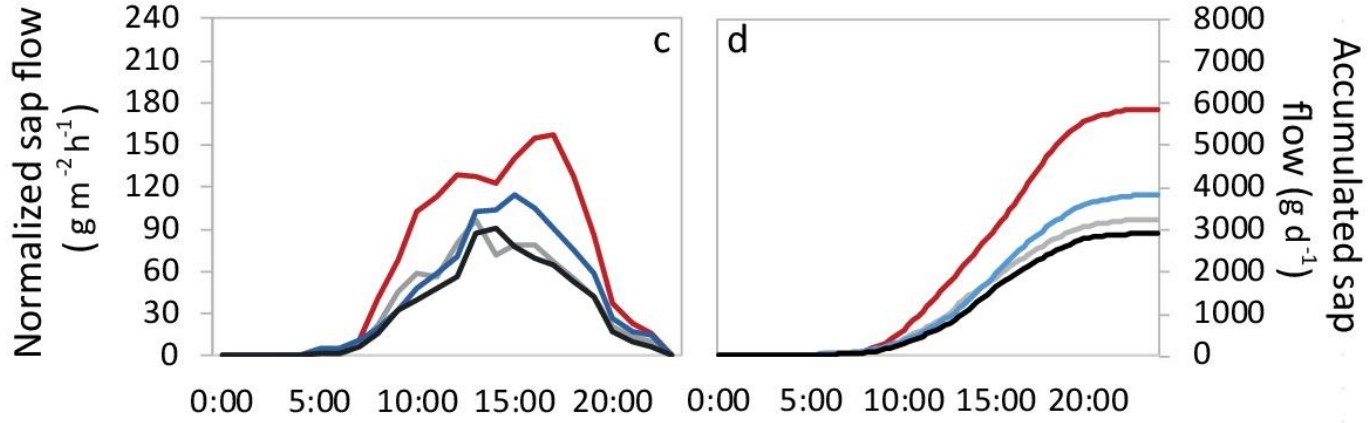
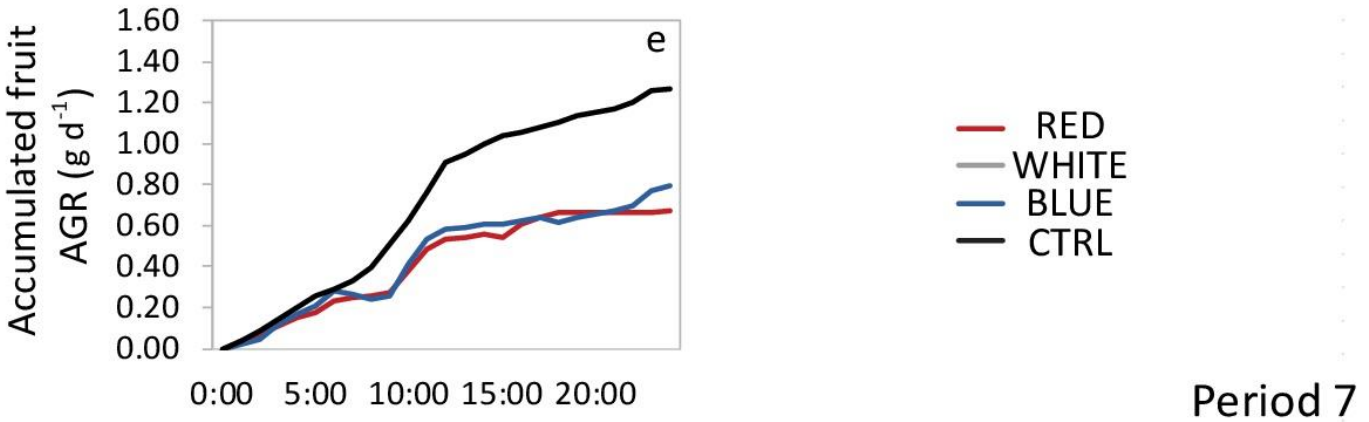
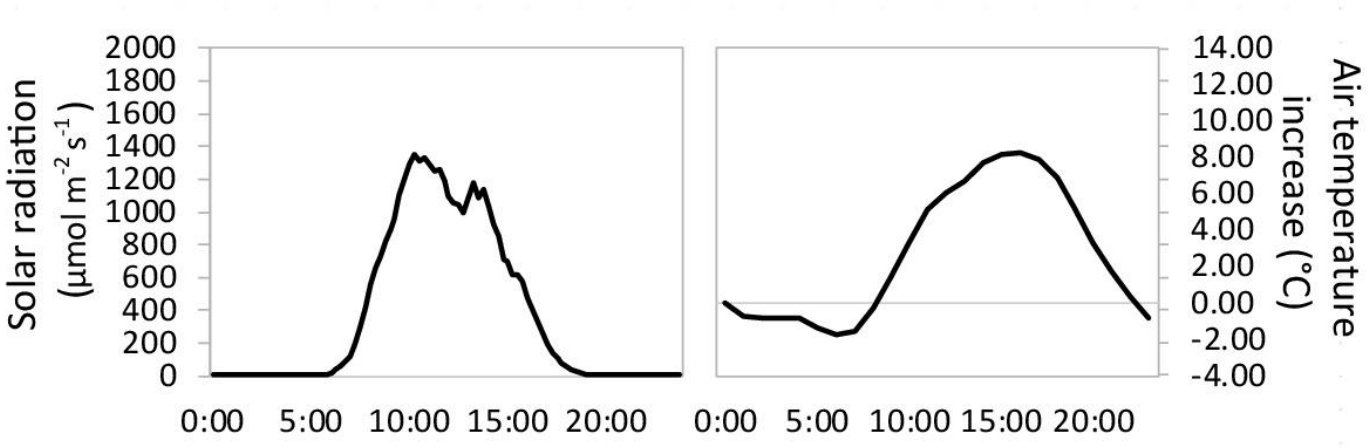


Table 2

time	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
RED	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	-	+	+
							AB		B										B					
WHITE																								
BLUE	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
							B		B										AB					
CTRL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
							A		A										A					



Period 7



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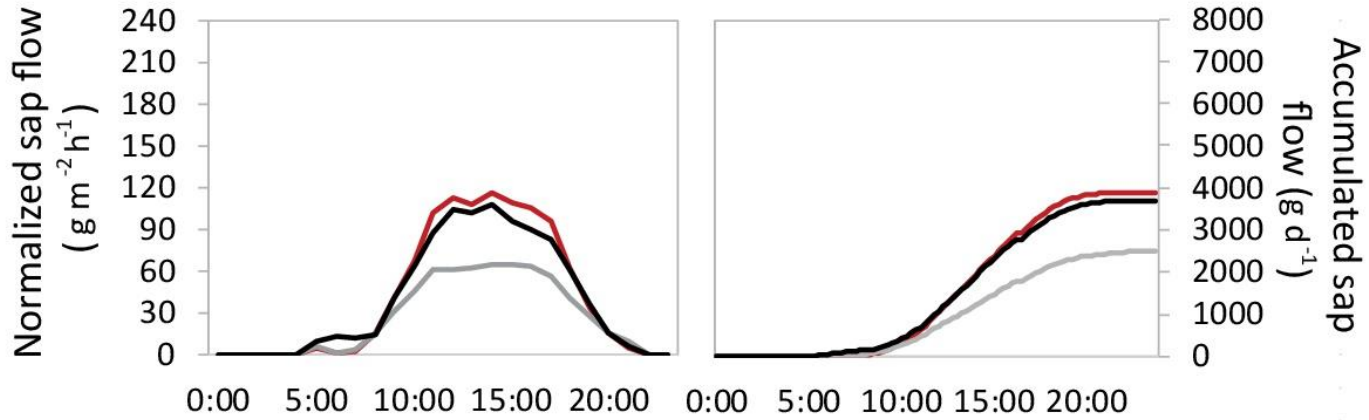

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					AB		A					AB							B					
WHITE	+	-	+	-	+	+	-	-	-	+	+	+	-	-	+	+	-	+	+	+	+	+	+	+
					C		AB					AB							AB					
BLUE	+	+	+	+	+	+	-	-	-	-	+	+	+	-	+	-	+	+	+	+	+	+	+	+
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CTRL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
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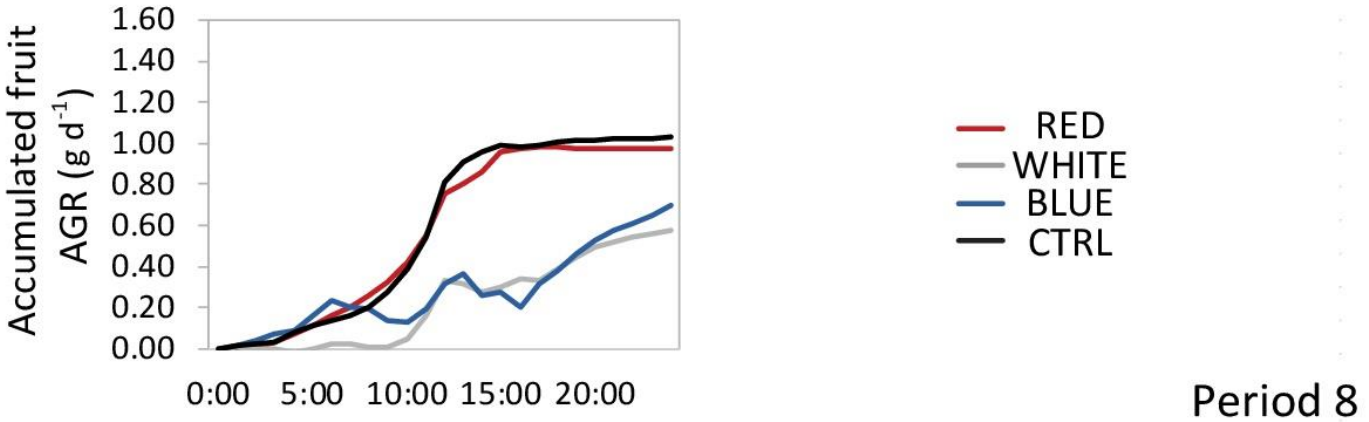

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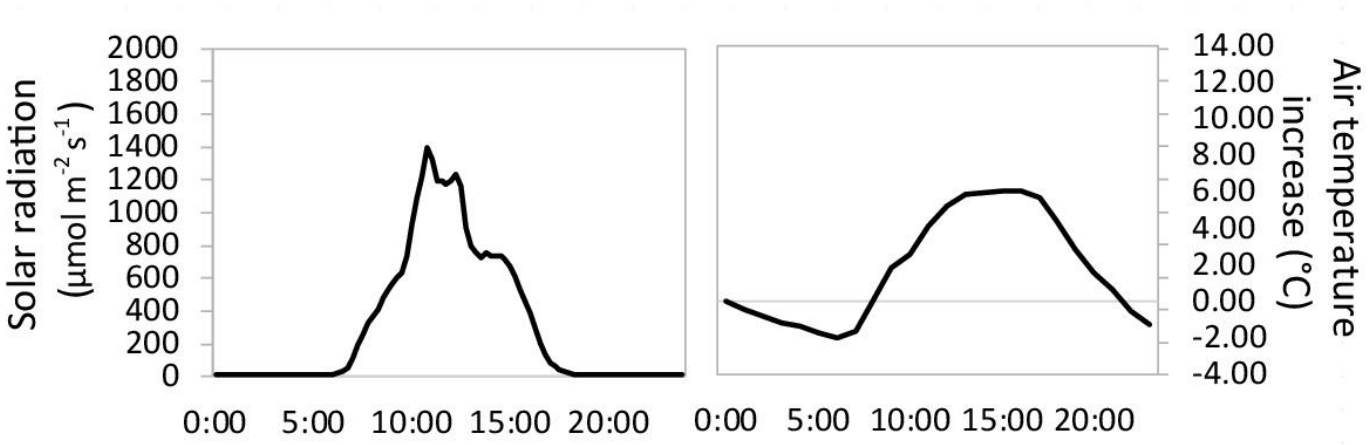

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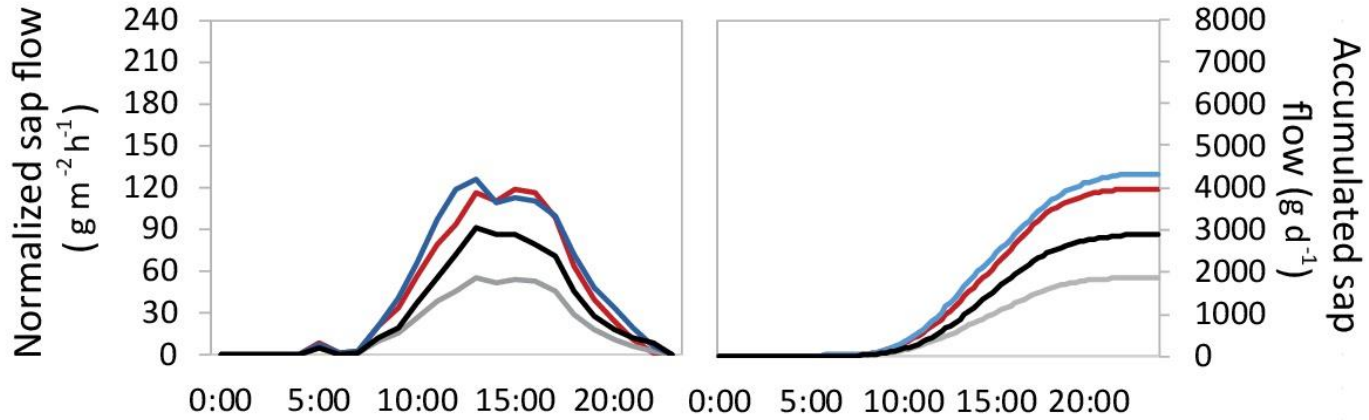

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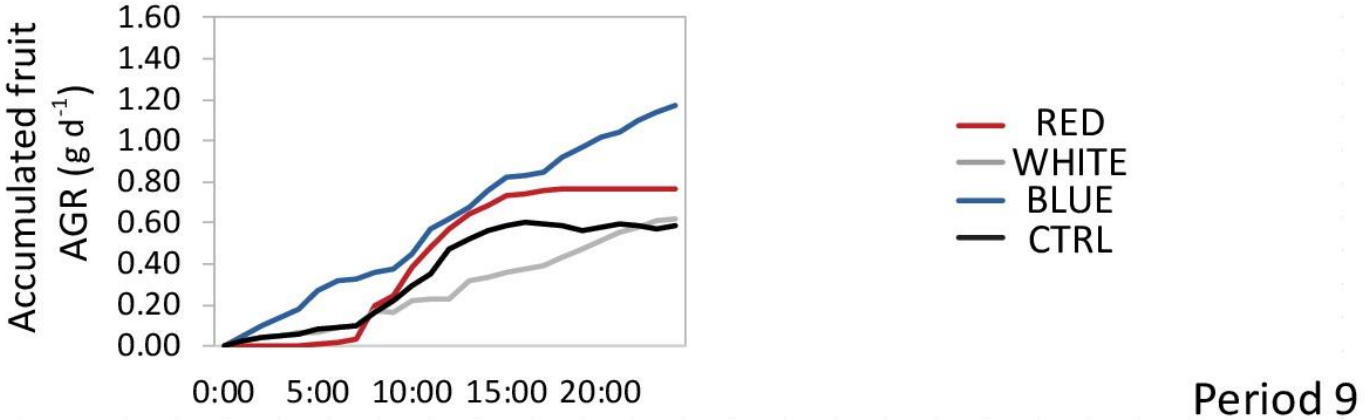

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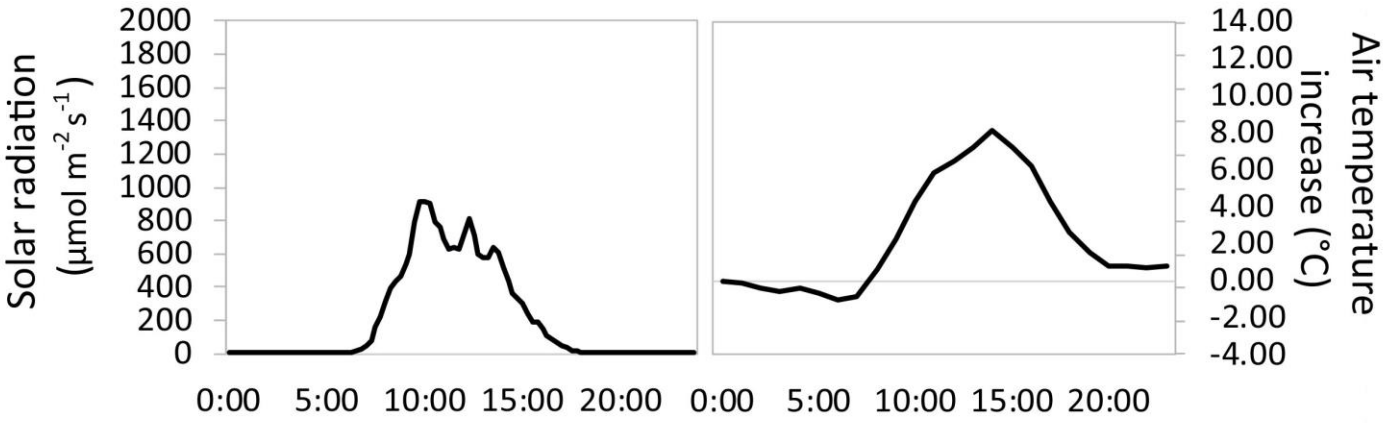

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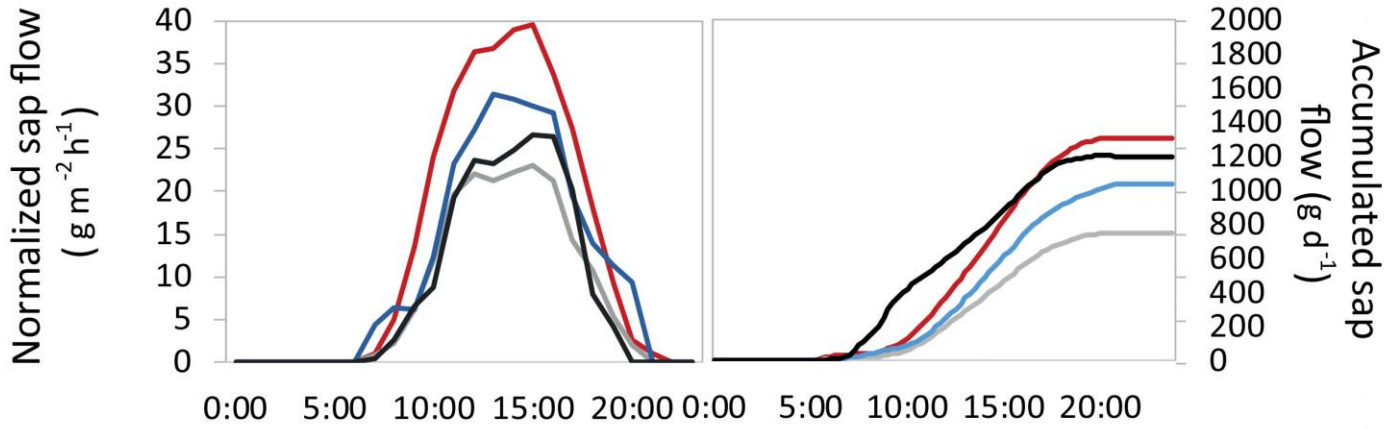

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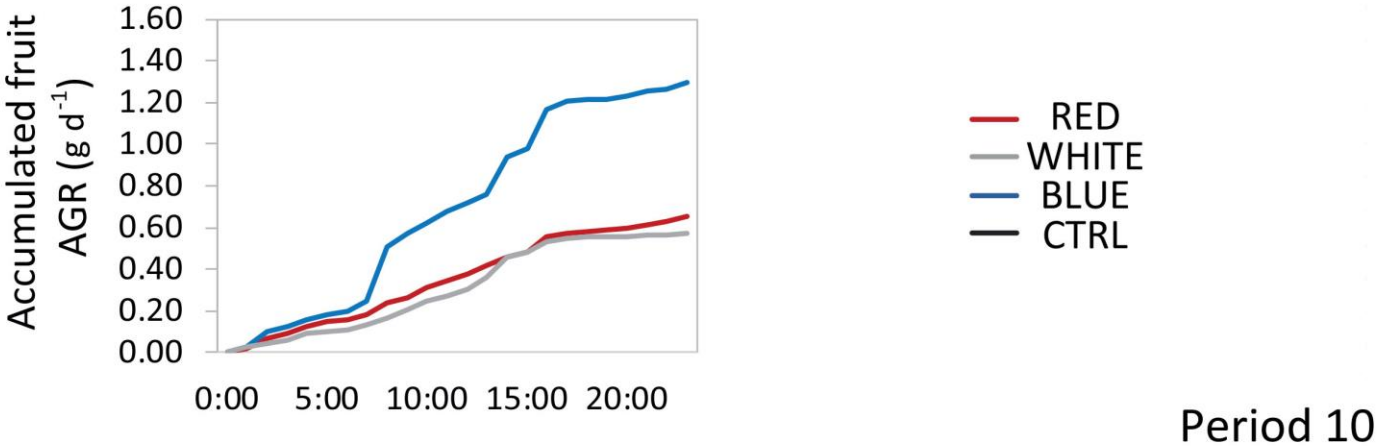

Period 10

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CHAPTER IV
EFFECTS OF LIGHT QUALITY DURING DORMANCY AND BUD BREAK.

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INTRODUCTION

The early development of apple flower clusters after budbreak initially utilizes stored reserves of carbohydrates and nutrients (Hansen, 1971; Hansen and Grauslund, 1973).

Accompanying the fall in temperature and the cessation of photosynthetic activity, starch is converted to soluble carbohydrates, whose concentration in plant organs reaches its highest peak in full winter, playing a strategic role in cold and frost resistance (Yoshioka et al., 1988; Nagao et al., 2005) and providing energy for exiting dormancy, thus bud break and bloom. Although lowering temperatures decrease plant activity at minimums, cells maintain respiration to remain alive, and increase the process to support spring growth (Thorn, 1951; Cole et al., 1982). Hence, before and during breaking of dormancy, conversion of non-soluble to soluble sugars takes place (Priestley, 1969, 1981; Skene, 1971; Wang et al., 1986, 1987) in the shoots and is followed by translocation to the buds. Carbohydrate dynamics and changes in their status during dormancy have been studied in several fruit crops species, such as apple (Yoshioka et al., 1988), to grapevine (Wang et al., 1998), red raspberry (Palonen et al., 2000), almond (Esparza et al., 2001), peach (Maurel et al., 2004; Bonhomme et al., 2005), walnut (Bonhomme et al., 2010; Tixier et al., 2017a,b), Japanese pear (Marafon et al., 2011), pistachio (Sperling et al., 2015), black currant (Pagter et al., 2015) and sweet cherry (Kaufmann and Blanke, 2017).

During dormancy, in non-photoperiodic species, such as apple, pear and other *Rosaceae* species (Garner and Allard 1923; Wareing 1956; Nitsch 1957), bud-break is triggered by the same low-temperature conditions that induce it. Of course, the various species differ in the level and duration of chilling required for an effective

dormancy release (Coville, 1920; Couvillon and Erez, 1985). Thus, temperature is considered the most important factor acting on dormancy (Wang and Faust, 1988) and on the related metabolic activities (Sperling et al., 2017). In fact, trees respond to root-to-canopy temperature gradients by changing their local non-soluble carbohydrate (NSCs) management and within-tree redistribution. Redistribution of carbohydrates is important during periods of low transpiration and for successful periods of intensive biological activity, e.g. bud-break in spring (Gordon and Dejong, 2007). Dormancy release initiates when the soil is colder than the canopy, implying allocation of NSCs from the roots to the warmer canopy (Zwieniecki et al., 2015). Nevertheless, the described allocations would not occur if chilling accumulation did not reach the minimum threshold for breaking dormancy (Marafon et al., 2011).

Research has shown that light can have both promotive or inhibitive effects, depending on the time of application (Samish et al., 1967; Gur, 1985). Light quantity manipulation during dormancy and ecodormancy (Lang et al., 1987) showed promotive effects for peach (Buchanan et al., 1977; Freeman and Martin, 1981) and apricot (Ruiz et al., 2005; Campoy et al., 2010). However, studies focusing on post-blossom growing season, found inhibitory effects in apple, apricot and grapevine (Jackson, 1969; Jackson and Palmer, 1977; Kohlet et al., 1996) and negative impacts of artificial shading on flower bud weight were found in Japanese pear, during flower bud formation (Ito et al., 2003). There appears to be no information about the effect of light spectrum, during dormancy in orchard systems. Light spectrum in orchards can be modified with the use of photoselective nets (Ganelevin, 2008), which alter the transmission of certain wavebands, compared to others. Thus,

the ratios between various wavebands change. Supposedly, no in-field research has taken place during dormancy in orchards due to the low economic interest in installing anti-hail protective systems in the winter period. However, research highlights positive effects of decreased incidence of solar radiation during dormancy and ecodormancy (Ruiz et al., 2005), on orchard productivity in Mediterranean climates. Lower temperatures would decrease specific hormones activity, in this case, gibberellins (Beppu et al., 2001), which are known to have negative impacts on flower bud development (Painter and Stembridge, 1972). Therefore, indirect shading originating from anti-hail nets may have positive effects when exiting ecodormancy, although the previously mentioned studies have been focusing on stone fruit. Also, it has to be pointed out that light quantity will be fundamental during bloom and soon after, during fruit set, in order to avoid low fruit cell division rates. In fact, the application of these screens is usually not occurring before 30 DAFB.

However, as light quality has proven to modify the microenvironment in terms of temperature (Arthurs et al., 2013), there may be effects on the phenological stages during ecodormancy and bud break. It is likely that these differences could modify the accumulation of chilling requirements, thus carbohydrate dynamics and consumption. If the light environment were modified in the “long” period (whole winter), the accumulation of these, even small, variations may anticipate or delay breaking of dormancy. Possible consequences may be related to different hormonal levels, which depend on temperature (Beppu et al., 2001).

This paper aimed at using light spectra manipulation to affect apple dormancy and bud break. The results may give new insights of

apple physiology and carbohydrate translocation while exiting dormancy and bud break, and of possible applications of photoselective nets during these periods.

MATERIALS AND METHODS

Two trials were conducted: one in an orchard (field conditions) and one in the laboratory (controlled conditions). Both experimentations were carried out at the *Plant physiology Z-LAB*, UC Davis, California (USA).

Field trial

Field, treatments and weather. The trial took place in an experimental orchard (38°32'32.7N, 121°47'47.1W), where apple rows (cv. Gibson Golden Delicious) were alternated with almond ones. Each apple row was planted in consecutive years and consisted of 15 trees, spaced at 1.8 m along the row and 4.5 m between rows. In the second half of December 2017, two apple rows, planted in 2011 and 2012, were selected: 8 trees were chosen in the first one and 7 in the second one. Every tree was divided in 4 sectors, facing the 4 cardinal points: 3 sectors were covered with 3 photoselective nets and 1 was left exposed, serving as the control (C). The photoselective nets shaded 20% and were red (R), blue (B) and white (W) (ChromatiNet Polysack Plastic Industries, D.N. Negev, Israel). The latter was stated to possess a UV-filter. The experiment was set-up as a randomized complete block of 4 light treatments (LTs) replicated on all the trees, which served as blocks. Care was taken so that every light treatment (LT) faced all four cardinal points (Figure 1).

Weather data for the trial site (38°32'8N, 121°46'35W) was monitored from the Cimis (California Irrigation Management Information System) database, starting from the beginning of November 2017, until the second half of April 2018. Chilling units (CU) were obtained following the Utah model (Richardson et al., 1974) and degree days (DD) were calculated, with 10°C as the threshold temperature.

Spectrum analysis. In the first half of January, net light spectra were assessed by spectrometer (JAZ-EL200-XR1, Ocean Optics, Largo, FL, USA), covering the band from the UV to the NIR regions (300-900 nm). The quantification was carried out on 4 trees, where all 4 LTs were facing all four cardinal points. For each measurement, 3 outputs were automatically generated. Reference measurements were also taken outside the orchard, in full light. Irradiance values (Wm^{-1}) were converted to μmol m^{-2} s^{-1}; transmittance could be then obtained as the difference between the external light input and the light intensity for each treatment and expressed as percentage.

Variance analysis of the 4 LTs was performed ($P<0.05$) for 4 bands of the spectrum: UV (300-380 nm), PAR (380-700 nm), FR (700-750 nm) and NIR (750-900 nm). Means were separated using SNK test ($P<0.05$).

Air and stem temperature. In the second half of January thermocouples were installed on 3 adjacent trees, in each LT. A needle was used to pierce a hole through the bark, until reaching the stem, and thermocouples were then glued in place. Each sector had 3 thermocouples: i) monitoring air temperature, ii) monitoring stem temperature on the north side of one shoot and iii) on the south side of the same shoot. The sensors were connected to a CR1000 datalogger (Campbell Scientific Inc., Logan, UT, USA) and values were collected

every 15 minutes, until the middle of April. Before placing the system in the field, all thermocouples were calibrated.

Variability of air and stem temperature were analysed with linear mixed effect models, where radiation and environmental air temperature were used as covariates and the tree and the branch were considered as random effects. Differences among least square means (LSMEANS) were considered significant when applying $P<0.05$, using t-Student test.

Phenology. From the beginning of March to the second half of April, bud phenology was monitored, following the BBCH scale (Meier et al., 1994). For each LT, the initial number of monitored buds was 30.

A two-way analysis of variance was performed and means were separated using SNK test ($P<0.05$).

Carbohydrate quantification. Before applying the LTs, 10 1-year shoots, with an apical flower bud, were collected randomly in the field and analysed for the content of starch and soluble sugars. This represented T_0. After the onset of light modification, carbohydrate quantification was carried out 5 times: at the end of January (T_1), at the beginning, mid and end of March (T_2, T_3, T_4), and at the beginning of April (T_5). For each timing, 5 shoots per LT were collected randomly, mixing all cardinal points. The method of Leyva et al. (2008) was used for carbohydrate determination modified as follows. Bark and wood were separated, then dried at 70 °C for 48 h before being ground into a fine and homogeneous powder. Soluble carbohydrates were extracted by incubating 25±4 mg of dry material in 1 mL of acetate buffer (pH 5.5) for 15 minutes at 70 °C, followed by centrifuging for 10 minutes at 15000 rpm. The supernatant was diluted 1:20 and quantified using anthrone as a reagent [0.1% (m/v) in 98% sulfuric acid] by reading absorbance at 620 nm. The remaining pellet was further processed to

determine concentrations of starch. The pellet was exposed to 100 °C for 15 minutes and submitted to enzymatic digestion for 4 h, with 50 µL of amylase and 50 µL of amyloglucosidase, at 37 °C. Once the digestion was completed, the samples were centrifuged for 10 minutes at 15000 rpm, the supernatant was diluted 1:20 and quantified using the method described above.

In total, four carbohydrate classes were quantified: wood soluble carbohydrates (WSC), wood starch (WS), bark soluble carbohydrates (BSC) and bark starch (BS).

To evaluate differences in translocation of carbohydrates among the four LTs, for each sampling time, a two-way analysis of variance was performed and means were separated using SNK test ($P<0.05$).

Temperature gradients and multivariate analysis. To evaluate possible effects of temperature gradients on carbohydrate dynamics, among the four LTs, during the sampling times, a multivariate analysis was performed. Temperature gradients were obtained by averaging the hourly temperature values of the 3 days prior to each sampling date. The selected gradients were between:

- environmental soil and environmental air temperatures (1);
- environmental soil and air (of light treatment) temperatures (2);
- environmental soil and stem (of light treatment) temperatures (3);
- stem (of light treatment) and air (of light treatment) temperatures (4).

The environmental and biological variables, sampling times and carbohydrate classes, respectively, were analysed separately, through a principal component analysis (PCA). A canonical correlation analysis

(CCA) followed, where the single interactions of LTs and sampling times were displayed. To evaluate the single LTs, the effect of temperature gradients and the distribution of carbohydrate classes were analysed with a discriminant correspondence analysis (DCA), separately.

Laboratory trial

Treatments and laboratory conditions. The trial took place in a controlled environment, where temperature and relative humidity were constantly 22-23 °C and 30%, respectively. Three cardboard boxes were deprived of the lid and layered, internally, with aluminium foil. Over the aluminium layers, patches of nets were applied to perfectly enclose the boxes, on all four sides, the bottom and the top. One box was fully white, which served as control (W), one was fully red (R) and one was fully blue (B), all three nets shading 20% (ChromatiNet Polysack Plastic Industries, D.N. Negev, Israel), as to obtain three light treatments (LTs).
A spectrum survey was taken by a CL-500A spectrophotometer (Konica Minolta, Chiyoda, Tokyo, Japan), of the three boxes, and of an empty one (covered only with aluminium foil) which served as reference, under a white neon lamp (F32T8/TL741, 700 series, 32 W, Philips, Amsterdam, The Netherlands). The spectrum band range was 360-780 nm. Irradiance values (Wm^{-1}) were converted to μmol m^{-2} s^{-1}; transmittance could then be obtained as the difference between the empty box readings and those for each coloured box, which were then expressed as percentage. Variance analysis of the 3 LTs was performed ($P<0.05$) for spectrum bands 400-700 nm (PAR) and 700-715 nm (FR).
On Feb-28-2018, apple cuttings were collected randomly from the field (see *field trial*), bearing an apical flower bud. The cuttings were picked from trees which had not been influenced from the LTs set up (see *field*

trial). This period corresponded to around 1280 accumulated CU and around 1030 accumulated GDD. In the laboratory, the cuttings were shortened to 10 cm and placed in plastic tubes with 8 mL of tap water. 10 cuttings were put aside for immediate carbohydrate quantification, serving as T_0. In each box, 20 cuttings were placed. All three boxes were placed under the white neon lamp, which was left on permanently.

Phenology. Every 2-3 days, bud phenology was monitored, following the BBCH scale (Meier et al., 1994). When cuttings reached certain key phenological stages, they were harvested for carbohydrate quantification. Key phenological stages were selected to be the so called "green bud stage" (T_1) and "first flowers open" (T_2) (nr 56 and 60, respectively, Meier et al., 1994). A final sampling (T_F) was also harvested, determining the end of the trial, on the same date for all three LTs. A two-way analysis of variance was performed ($P<0.05$), to detect differences in phenology evolution, among LTs, during time. A simple ANOVA was also performed to evaluate the mean phenological stage, among LTs, using the SNK test to separate means ($P<0.05$).

Carbohydrate quantification. For detecting differences in carbohydrate amounts of both wood and bark, 5 cuttings that had reached the key phenological stages were collected in each box. Each cutting was divided in two parts, so to quantify sugars in the lower and upper parts. Wood and bark were separated, then dried at 70 °C for 48 h. The method used for quantifying the amount of soluble carbohydrates and starch can be found in section *Field trial – Carbohydrate quantification*.

RESULTS

Field trial

Field, treatments and weather (Figure 2). Until the beginning of March soil temperature was mostly higher than the environmental air (Figure 2a), except at the end of January, where an abnormal air temperature increase reversed the difference. From the first half of March, environmental air temperature tended to be higher than the soil. Precipitation events accumulated around 250 mm of rain, concentrating mostly from the end of February. The accumulation of environmental CU reached its maximum at around 1550 at the end of March, then started slowly decreasing, and converged with accumulated DD around the middle of April (Figure 2b).

Spectrum analysis (Figure 3a). Compared to full light measurements taken outside the orchard, C transmits slightly lower amounts across all the spectral waveband range, with no specific variation. Among photoselective nets, in the UV region (300-400 nm) B transmits significantly higher percentages (50%), followed by R (40%) and W (35%). In the PAR region (400-700 nm) transmittance is higher for W (55%), followed by B (50%) and least for R (44%). Far-red wavelengths (700-750 nm) are highly transmitted in W (61%), R (55%) and least in B (50%). In the near-infrared (750-900 nm), higher transmission occurs for both B and W (67%) than for R (58%).

Air and stem temperature (Figure 3b-c). When comparing light treatments for air temperature, there is a significant difference between B and W, the first having a mean seasonal lower temperature than the second, of 0.47 °C (Figure 3b). Light treatments did not influence stem temperature during the season.

Phenology (Figure 3d). No significant difference was found during ecodormancy and bud break. All treatments bloomed together, approximately in the middle of April.

Carbohydrate quantification (Figure 4). WSC (Figure 4a) generally increased until the middle of March (T_3), where there were significant differences for B and R, against C and W. B was significantly lower in T_4, but at T_5 all LTs had equal amounts of WSC (around 100 mg g^{-1}). WS, BSC and BS, tended to decrease and increase again by T_3, after which a second plunge occurred. There was no statistical difference at T_5 (a week before bloom), for WSC, BSC (60-70 mg g^{-1}) and BS (10-20 mg g^{-1}) (Figure 4a-c-d), only for WS, where B and W had higher amounts, compared to C, leaving R as intermediate (Figure 4b). Significantly lower amounts of carbohydrates, in general, are mostly seen for W.

Temperature gradients and multivariate analysis (Figures 5-6-7). Temperature gradients between soil and environmental air (1) (Figure 5a) were positive in T_1, T_2 and T_3, reaching differences of 3 °C, and negative in T_4, going below -3 °C. T_5 had a negative small gradient. The same patterns occurred for temperature gradients between soil and air (LTs) (Figure 5b), or stem (LTs) (Figure 5c), in all timings, except T_5, where gradients were positive. Temperature gradients between stem (LTs) and air (LTs) (Figure 5d) were always narrower compared to the other gradients, except in T_5, where they reached -1°C. In general, this gradient was always negative for W, mostly negative for R and C, while mostly positive for B.

PCA in Figure 6a shows the effect of temperature gradients on LTs in the various sampling times. Temperature gradient 2 is explained at more than 75%, while 3 and 1 are very close to each other and to 2. Temperature gradient 4 is explained by the remaining 24%. CCA shown

in Figure 6b reflects PCA in Figure 6a, adding each LT in each sampling time. B appears always above, and W always below, C and R. The more the clusters are towards the left side of the graph, the more they are influenced by gradients 2, primarily, then 1 and 3, secondarily. The more the clusters are spread upwards, the more they will be influenced by gradient 4. Carbohydrate dynamics does not appear to be influenced by the LTs (Figure 6c), rather than by sampling times, when different temperature gradients seemed to have higher effect (Figure 6d). PCA shown in Figure 7a depicts the general dynamics of carbohydrates in LTs in the various sampling times: factor 1 explains WS at nearly 49%, along with BS, whereas WSC is explained by factor 2 at 27%. In Figure 7b, CCA shows the projection of each LT*sampling time: the more the points are towards the bottom of the graph, the higher amounts of WSC are present, while the more they are distributed on the right side of the graph, the higher are, primarily, WS, and, then, BS. The dynamics of each carbohydrate type, based on the effect of LT, and of sampling time, shows the low effect of the first (Figure 7c), compared to the latter (Figure 7d). Relatively higher amounts of WSC can be found in T_4, whereas lower can be found in T_1. Higher amounts of BS and WS are present in T_2 and T_3, while less in T_5 (Figure 7d).

Laboratory trial

Spectrum analysis (Figure 8a-b-c-d). The irradiances <400 and >715 nm were not analysed, due to highly irregular patterns (data not shown). The outputs ranging 400-700 nm inside the boxes had low irradiances, with a couple of high peaks, in the green and orange regions, never going above 0.6 μmol m^{-2} s^{-1} (Figure 8a). From the transmittance point of view, compared to the reference measurement

(Figure 8b), all three nets are transmitting higher percentages in parts of the violet and green regions, and also in the far-red region. In the areas where no peaks were measured, in the PAR region (400-700 nm) (Figure 8c), W (85.9%) was significantly higher than R (67.7%), which was higher than B (61.1%). As for 700-715 nm range (Figure 8d), both R and W are transmitting higher percentages (85%) against B (54%).

Phenology (Figure 8e-f). In general, the evolution of phenology was quicker in the white box, in four dates, compared to the other two light treatments (Figure 8e). The white cuttings reached T_1 four days earlier (15/3) and T_2 five days earlier (21/3), compared to both red and blue ones. Bud phenology reached an average higher stage in the white box, followed by the red and leaving the blue one with the lower stage (Figure 8f).

Carbohydrate quantification (Tables 1, 2, 3). Soluble sugars and starch showed a decreasing tendency in time (Table 1), with significant less amounts at T_F. At T_1, all LTs had equal amount of all carbohydrate types, in the lower section of the cuttings (Table 2), whereas WSC and WS are higher, in the upper part of W cuttings and lower in the B ones. At T_2, higher amounts of all carbohydrate types, both in lower and upper sections, are mostly in W (Table 2). At T_F, higher amounts of starch in bark can be found in both lower and upper sections of W cuttings (Table 2). Translocation of BSC from the lower to the upper sections of the cuttings was evident at T_1, in all three LTs (Table 3), where the upper parts had significantly higher quantities. Both R and B continued to have this tendency in T_2 and T_F. Translocation of carbohydrates in wood, from the lower to the upper sections, did not show significant differences for all LTs, in both T_1 and T_2. Statistical variations can be seen only in T_F, in W (both WSC and WS) and B (WSC) (Table 3).

DISCUSSIONS

Field trial

The presence of coloured nets influenced air temperature, where clear differences were evident between B and W, the first being significantly colder than the latter, even if the average seasonal difference is less than 0.5 °C (Figure 3b). Such a variation could be related to the spectral properties of the nets (Figure 3a). The two filters have statistically contrasting transmission of UV, PAR and FR, although these wavebands are not responsible for heat radiation. Solar radiation is indeed influencing the thermal properties of the LTs, although these traits should probably be sought in wavelengths above 900-1000 nm. Literature does not state higher temperatures under a white net compared to a blue net, or differences in amount of scattered light (Rajapakse and Shahak, 2007; Kalcsits et al., 2017), however temperature had been measured in fully developed canopies (Kalcsits et al., 2017), i.e. the presence of leaves and transpiratory losses may have played a part in determining the final temperature. Despite LTs influenced temperatures (Figure 3b) and generated different gradients with environmental soil (Figure 5b), they did not appear to have strong impacts on the different dynamics of carbohydrates (Figures 6c, 7c). Plus, no difference was detected when monitoring bud phenology (Figure 3c). This may be explained by the fact that, although the single tree was displaying different spectra, the effect was not strong enough to have an impact in the single sections. Phenology evolution did not seem to be primarily controlled by the buds, rather than by a central system in the tree (hormones, for example) which was not influenced by the light treatments. The main reason may be found below ground (Greer et al., 2006). The temperature gradients including environmental

soil (1, 2, 3, Figure 5a-b-c) were primarily dictating carbohydrate dynamics in the field (Figure 6a-b-d). The gradient deriving from LTs stem and LTs air (4, Figure 5d) is influencing only secondarily (Figure 6a), although it appeared to have had a stronger impact on the translocation of WSC in T_4 (Figure 6b-d, 7b-d). However, as stated in literature, lower soil temperatures, compared to the air above, will promote the degradation of NSC (Zwieniecki et al., 2015; Gordon and Dejong, 2007). Figure 7b-d shows higher amounts of soluble carbohydrates in wood at T_4, meaning a conversion from non-soluble to soluble carbohydrates had probably occurred.

Stronger differences in translocation of carbohydrates were more evident among sampling times, which were characterized by different temperature gradients. T_1, T_2 and T_3 were mostly influenced by temperature gradients 1, 2, 3 (Figure 6b-d), while having less WSC, rather than NSC in general (Figure 7b-d). Higher soil temperature occurring in these three periods characterized the presence of more NSC (Zwieniecki et al., 2015). However, T_5 especially seemed to have lower quantities of all carbohydrates. It is possible that intense metabolic activities were ongoing, as full bloom would have occurred soon after, thus the trees might have been experiencing a depletion of carbohydrates and were in need of sugar remobilization from the lower portions, for bud break (Loescher et al., 1990; Witt and Sauter, 1994; Lacointe et al., 2004; Bazot et al., 2013; Dietze et al., 2014; Hartmann and Trumbore, 2016; Tixier et al., 2017a,b). Such consideration may explain why T_5 was not particularly influenced by any of the temperature gradients (Figure 6b-d). Based on these results, influencing only a section of canopy light microenvironment is not going to impact sugars' remobilization, based on the different treatment. Although there

will be different temperature gradients between soil and air inside the nets, the whole tree will, all the same, behave as a single structure, balancing the general response. Therefore, the single buds do not seem to be solely controlling and determining the translocation activity. To generate clear differences in transport and remobilization, the soil and root apparatus and, or, other parts, such as the trunk, or the canopy, should be subjected to a treatment strong enough to significantly influence stem temperature (Tixier et al., 2017b). As photoselective nets have shown to alter soil temperature (Kalcsits et al., 2017), and as apple phenology is known to be influenced by root-zone temperatures (Greer et al., 2006), the application of these nets during winter and early spring could modify the inner metabolic processes of the tree and modify tree responses (Ruiz et al., 2005; Loescher et al., 1990).

Laboratory trial

Variation and translocation of carbohydrates in the laboratory trial was more in line with the phenology evolution. By completely isolating the cuttings, it was possible to obtain clear differences in phenology. W anticipated both the phenological key stages and tended to have higher amounts of carbohydrates, when there were statistical differences among LTs (Table 2). This LT might have anticipated the other two because of the different light environment. Light spectrum results (Figure 8c-d) showed significantly higher transmission of PAR and FR. Regarding the transmission of far-red (700-715 nm) W has a significantly higher percentage, compared to R, while B is transmitting the lowest. The possible activation of photoreceptors dedicated to bud break and bloom might be influenced by this waveband range. Studies demonstrated how bloom could be promoted by irradiation with far-red light (700–740 nm) (Goto et al., 1991; Bagnall et al., 1995; Lin, 2000;

Björn, 2015). On the other hand, poor far-red light in combination with blue light applications delayed flowering (Halliday et al., 1994; Guo et al., 1998). A highly suitable explanation for anticipated bloom in far-red enriched environments, i.e. the perceiving of shade of a nearby competitor, would allow the plant to fasten its phenology, to achieve as much light as possible (Devlin et al., 1999; Yuan et al., 2017), although this happened in W cuttings and not in the R ones, where far-red was higher in both boxes (Figure 8d). It could be speculated that higher PAR transmission, i.e. higher transmission of light in general, in the white box may have, also, taken part in influencing W bud phenology. Studies on tomato flowering showed how the growth rate of buds and flowers is significantly delayed by weak light applications (Zhu et al., 2017). It has to be pointed out, however, that such statements come from studies focusing on a photoperiodic species, which also bloom after the development of their vegetative organs. It is possible that the results of the present trial may not necessarily be explained by research based on the manipulation of these species photoperiod and light environment.

Even though there was a delay of red and blue boxes in phenology evolution, carbohydrates were nevertheless consumed, very probably due to maintenance respiration. At T_F, nearly half the amount of BS was found in R and B (an average of 4.2 mg DW^{-1}), compared to W cuttings (an average of 8.1 mg DW^{-1}), both in the lower and upper cutting sections (Table 2), thus starch had been consumed more quickly. In wood, W cuttings were significantly moving reserves upwards (Table 3), unlike R and B. This trial demonstrated the higher efficiency of the white treatment in managing carbohydrates while exiting dormancy, given a higher amount of reserves at the end of the experiment (Table 2). In a wider scenery (like the field, for example), those extra reserves could

have been potentially used for future needs. On the other hand, it demonstrated the lower efficiency of the blue net, as this treatment had the lowest amount of carbohydrates in the lower parts of the cuttings, meaning it was using sugars for maintenance respiration and other processes than bud break. This more pronounced depletion leads to think that the blue LT might cause the remobilization of carbohydrates from lower parts of a branch, like the trunk, or roots (Lacointe et al., 2004; Loescher et al., 1990; Hartmann and Trumbore, 2016; Tixier et al., 2017a,b), without dedicating sugars to exiting dormancy. The blue treatment could, on the other hand, be of use, if the target would be to delay bloom and the consequent phenological stages, for commercial purposes, with a possible risk of extra consumption of carbohydrates. It has to be underlined that these are speculations deriving from results of a trial conducted in a controlled environment, where light was not representing the solar spectrum. Different findings may emerge when transferring this set-up in the field.

It would be informative and clarifying to test photoselective netting in orchards, during dormancy, while influencing the whole tree-soil system, to evaluate possible effects on the crops' carbohydrate management. Also, in the view of climate change and warming of temperatures (Field et al., 2014; Luedeling et al., 2013), crops winter biology would be significantly affected. Therefore, if a specific light environment was able to improve carbohydrate translocation's efficiency, whether it would be a matter of temperature gradient modification, or impacts on photoreceptors activity, practical applications in the field should not be excluded.

CONCLUSIONS

Light spectrum manipulation influenced canopy air temperature during dormancy and bud break. However, the experimental set-up did not allow to obtain differences in carbohydrate dynamics among the different light environments. The complexity of the problem made it difficult to carry out an experiment at the same time capable of removing the unwanted effects of tree and soil conditions at the same time. This may be tested in further studies; the solution chosen for this work would appear to be difficult to surpass, under field conditions. Communication signals between the root apparatus and the canopy were not strongly influenced by the different spectra. As temperature gradients including soil had the highest impact on the translocation of sugars in the trees, it followed that flower bud phenology was not influenced by the different light treatments. On the other hand, the results of the laboratory trial suggest the potential of photoselective nets to improve the efficiency, or modify the timings, of carbohydrate management and translocation in apple, during ecodormancy and bud break. Future trials in field conditions should influence the whole tree-soil system to induce differences in temperature gradients and possibly influence the management of carbohydrates.

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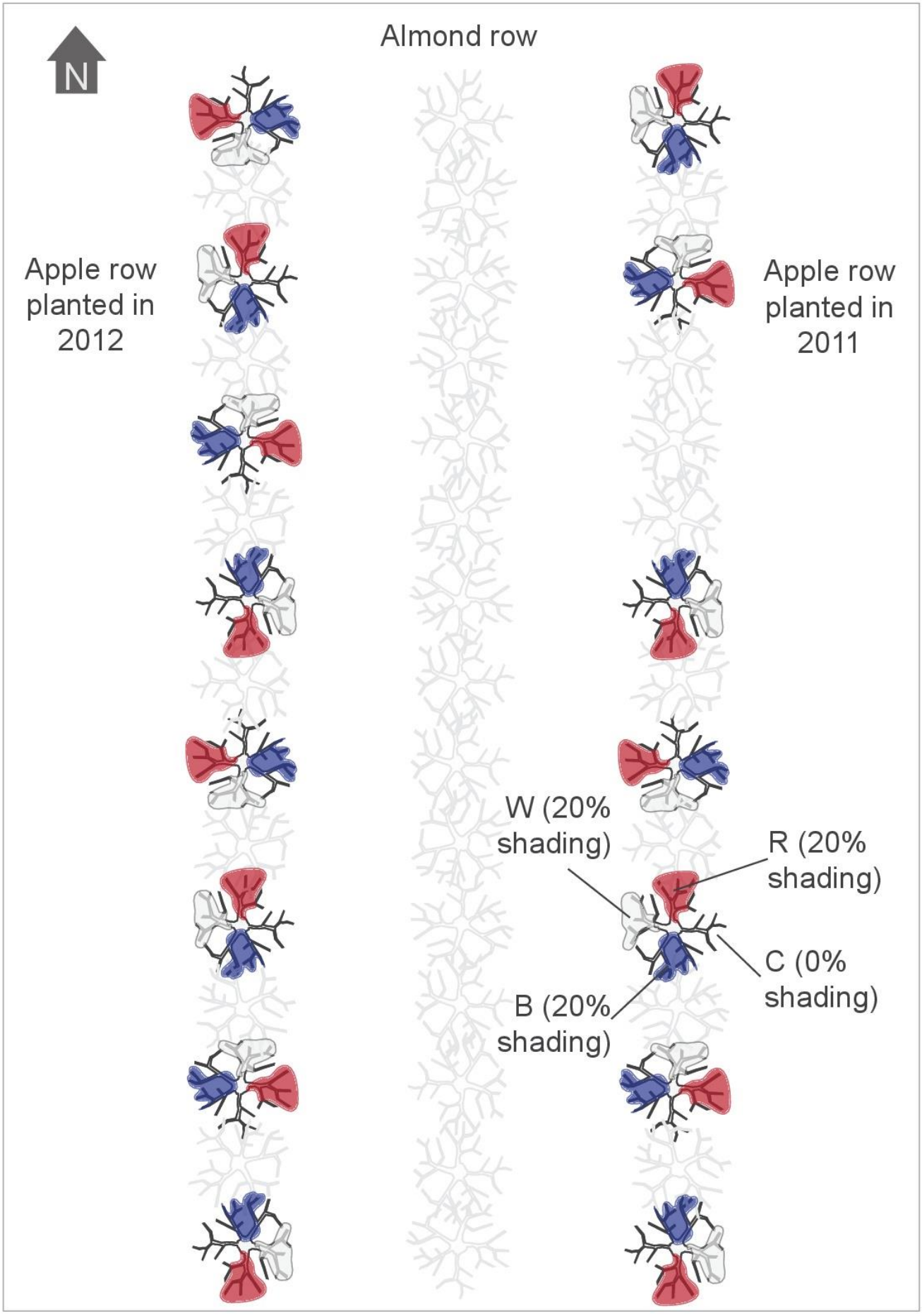
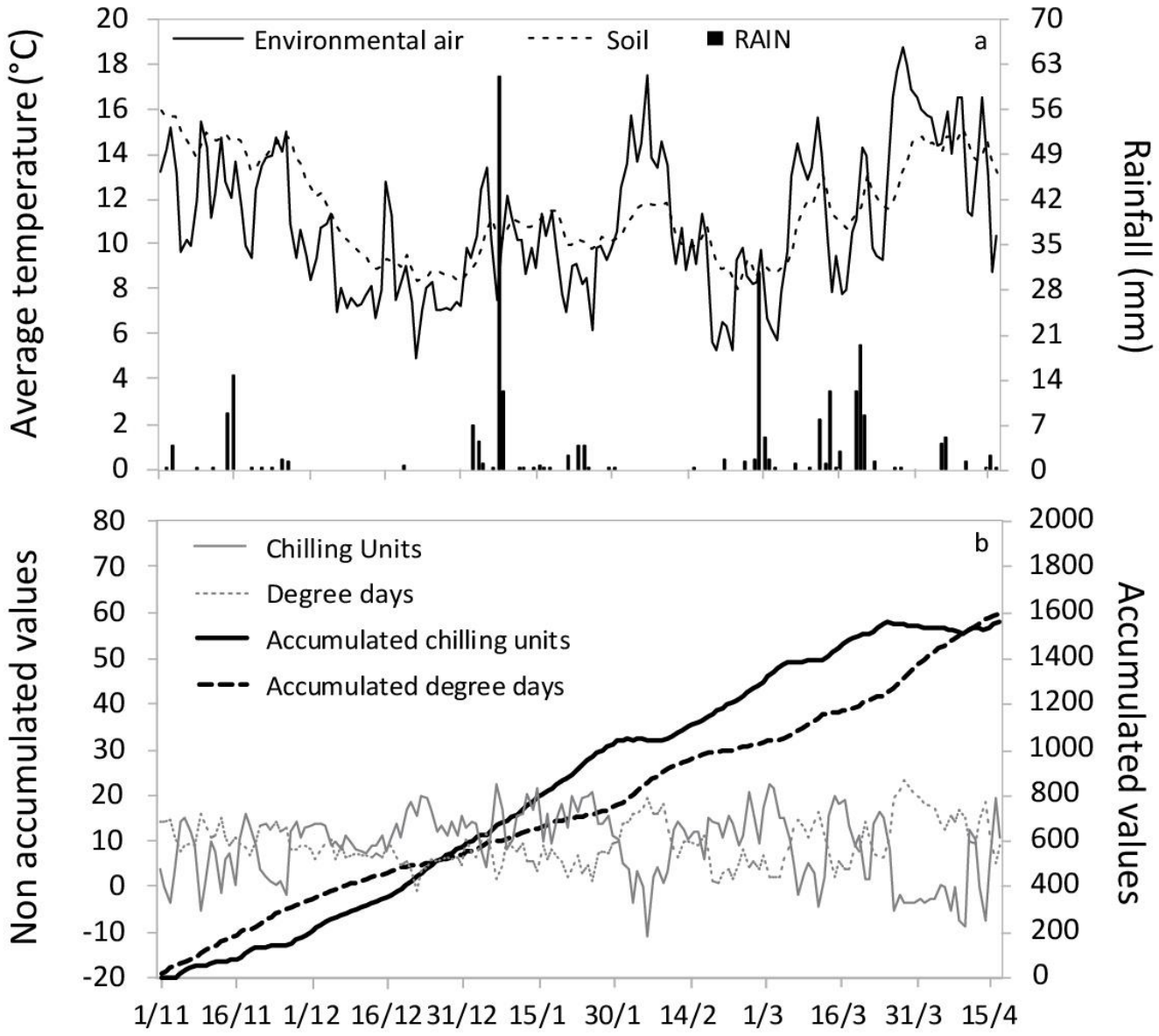

N
Almond row
Apple row planted in 2012
Apple row planted in 2011
W (20% shading)
R (20% shading)
C (0% shading)
B (20% shading)


Environmental air
Soil
RAIN
a
Average temperature (°C)
Rainfall (mm)
20
18
16
14
12
10
8
6
4
2
0
70
63
56
49
42
35
28
21
14
7
0
Chilling Units
Degree days
Accumulated chilling units
Accumulated degree days
b
Non accumulated values
Accumulated values
80
70
60
50
40
30
20
10
0
-10
-20
2000
1800
1600
1400
1200
1000
800
600
400
200
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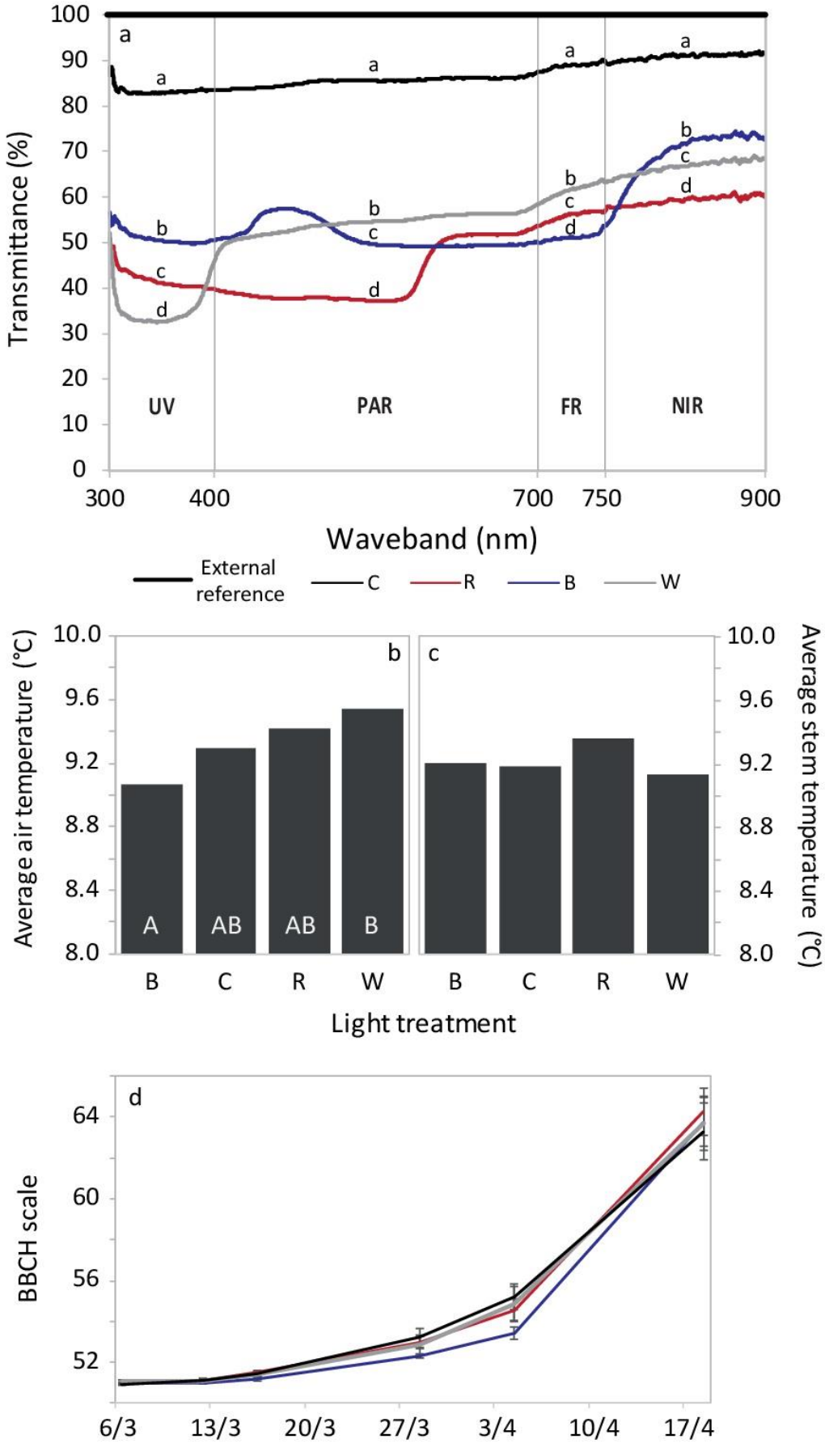
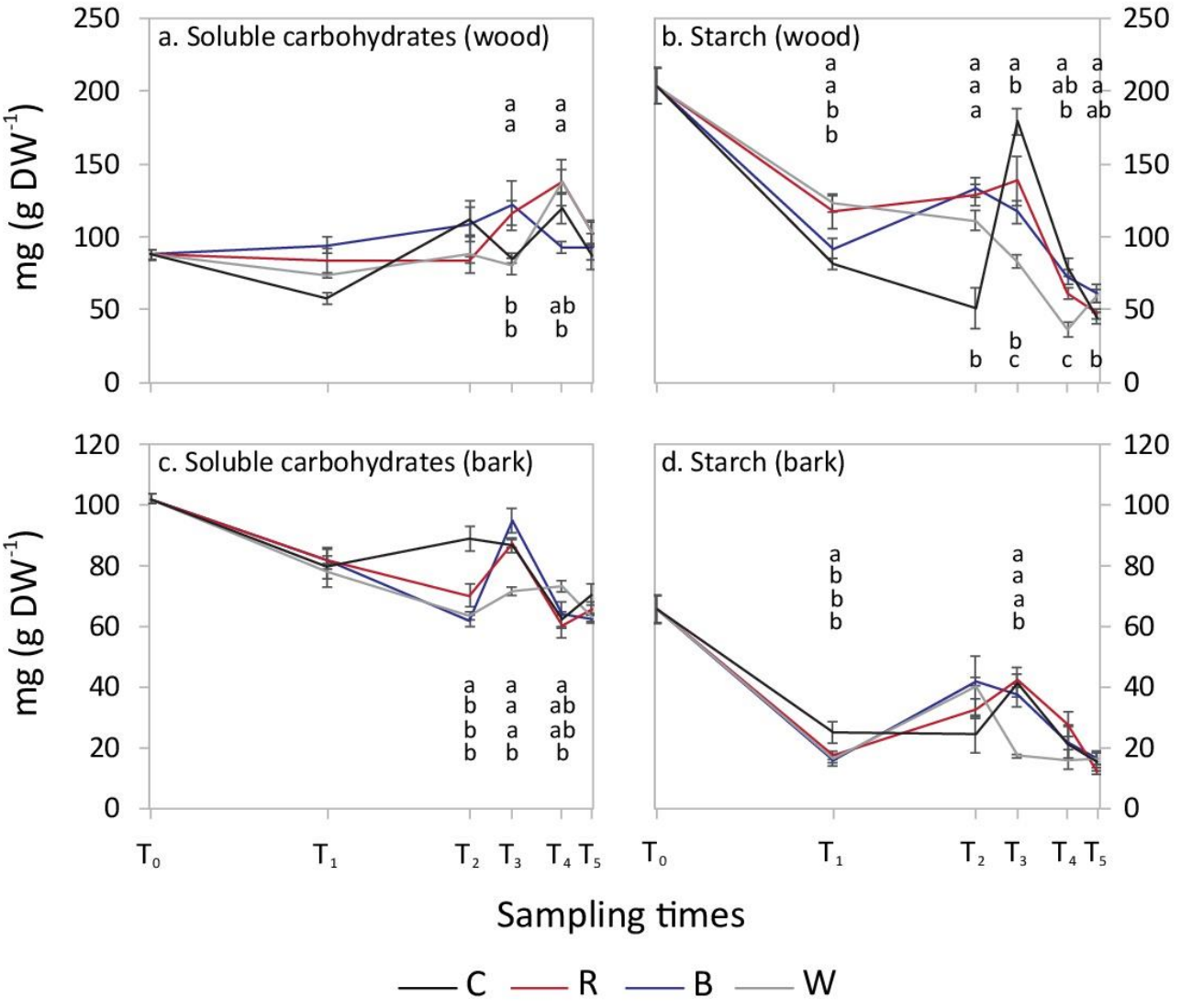
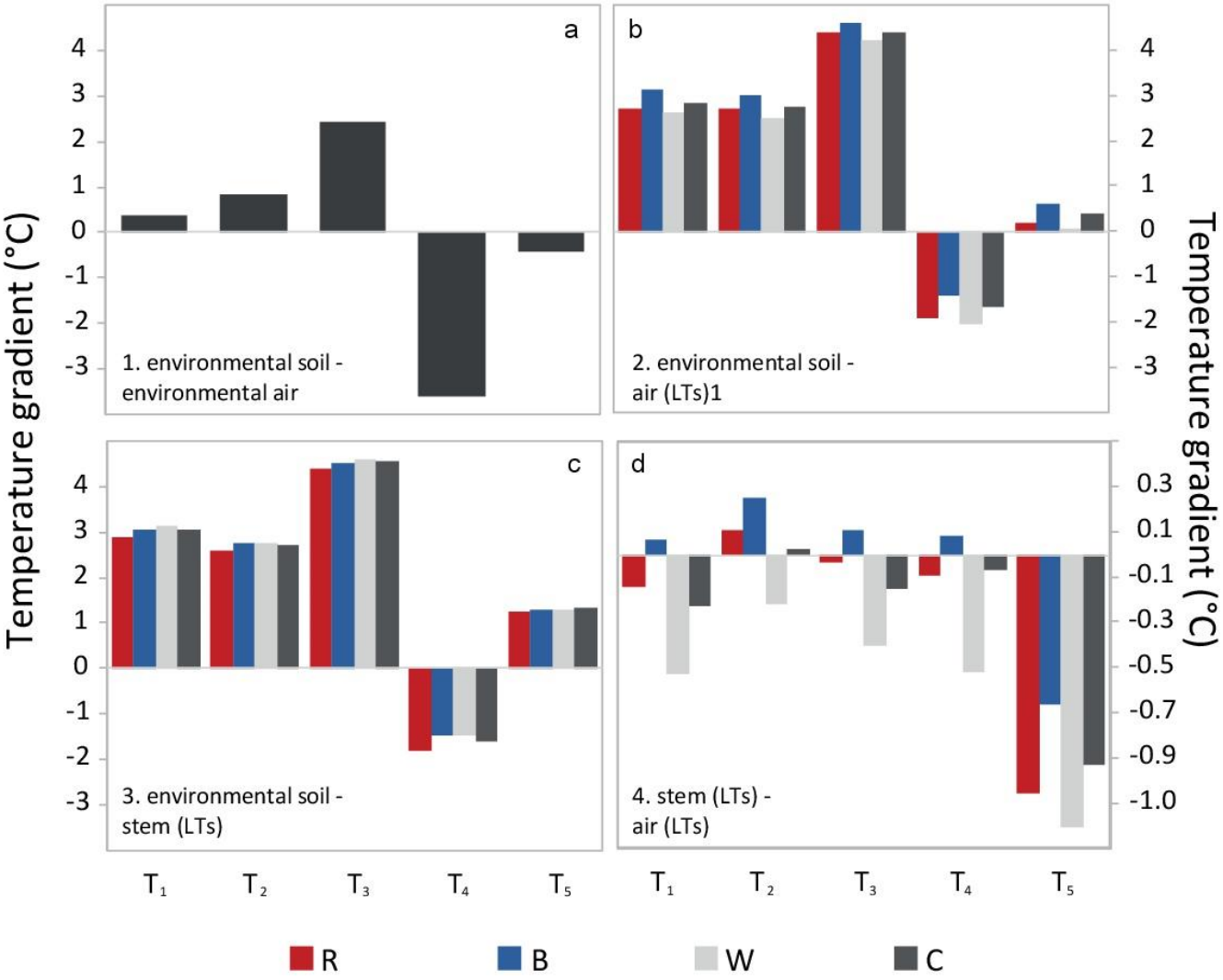
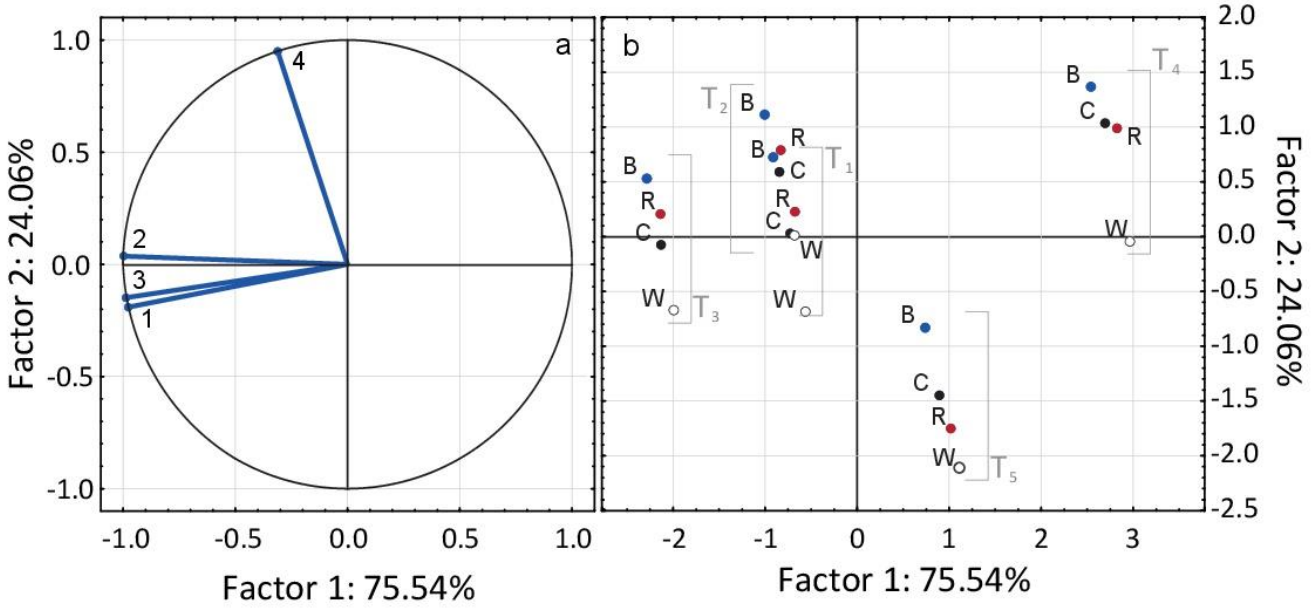
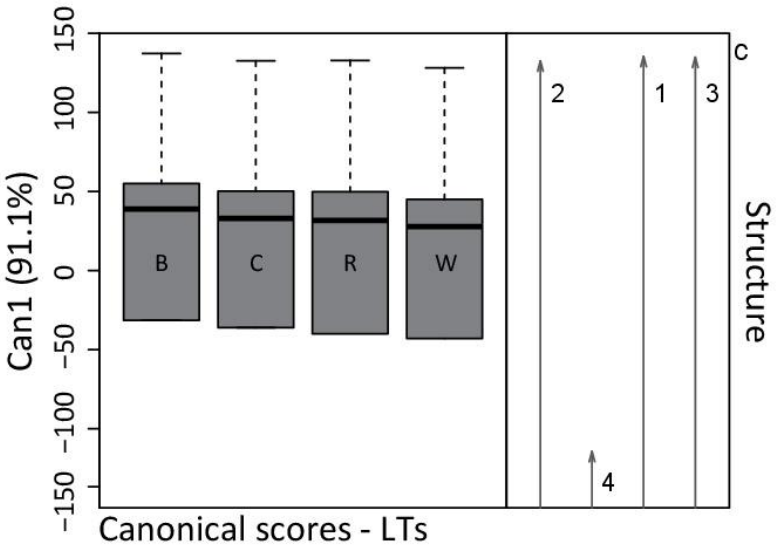
30/1
14/2
1/3
16/3
31/3
15/4


a
Transmittance (%)
100
90
80
70
60
50
40
30
20
10
0
UV
PAR
FR
NIR
300
400
700
750
900
Waveband (nm)
External reference
C
R
B
W
b
c
Average air temperature (°C)
Average stem temperature (°C)
10.0
9.6
9.2
8.8
8.4
8.0
A
AB
AB
B
B
C
R
W
Light treatment
d
BBCH scale
64
60
56
52
6/3
13/3
20/3
27/3
3/4
10/4
17/4


a. Soluble carbohydrates (wood)
b. Starch (wood)
c. Soluble carbohydrates (bark)
d. Starch (bark)
mg (g DW-1)
Sampling times
T0 T1 T2 T3 T4 T5
C R B W


a
b
c
d
1. environmental soil - environmental air
2. environmental soil - air (LTs)1
3. environmental soil - stem (LTs)
4. stem (LTs) - air (LTs)
Temperature gradient (°C)
Temperature gradient (°C)
T1 T2 T3 T4 T5
R B W C





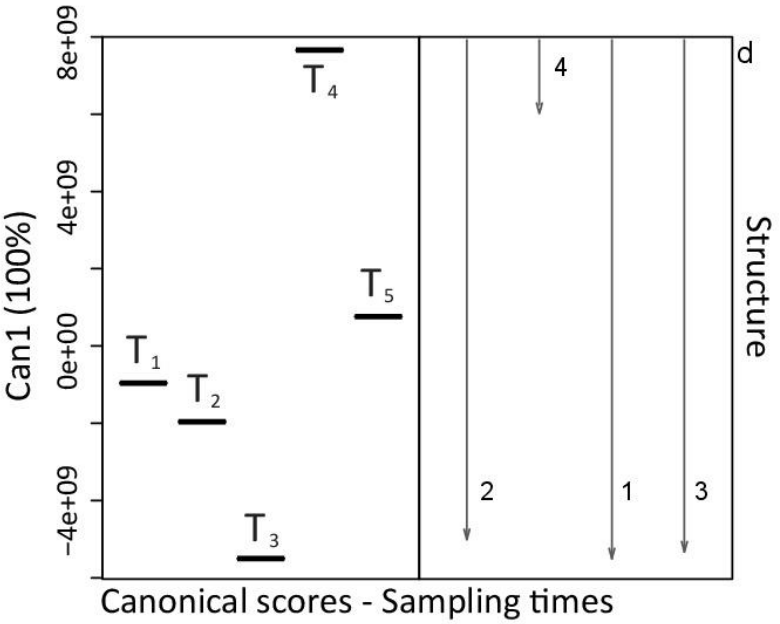
Temperature gradients:

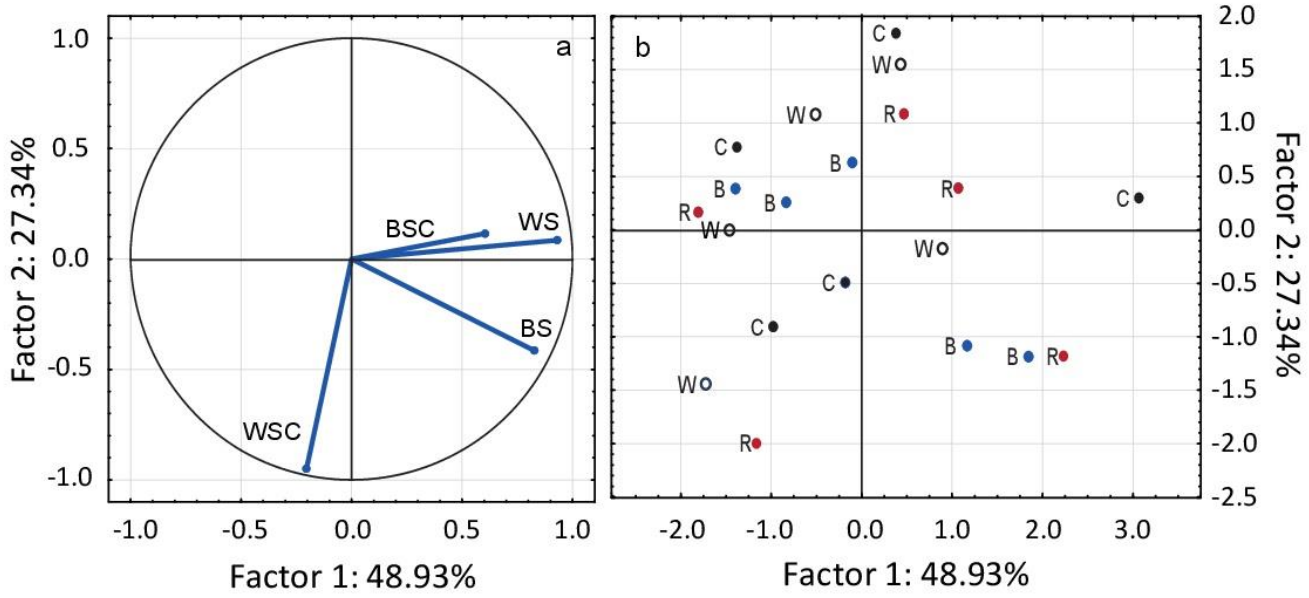
1 environmental soil - environmental air

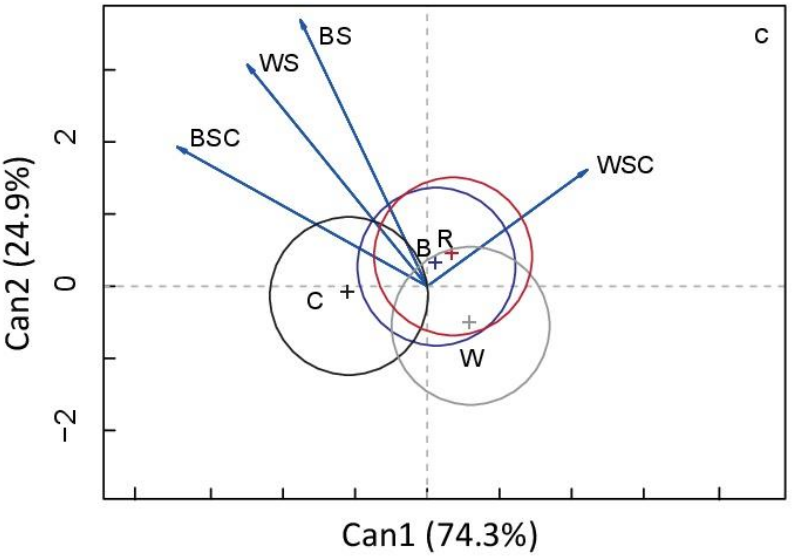
2 environmental soil - air (LTs)

3 environmental soil - stem (LTs)

4 stem (LTs) - air (LTs)





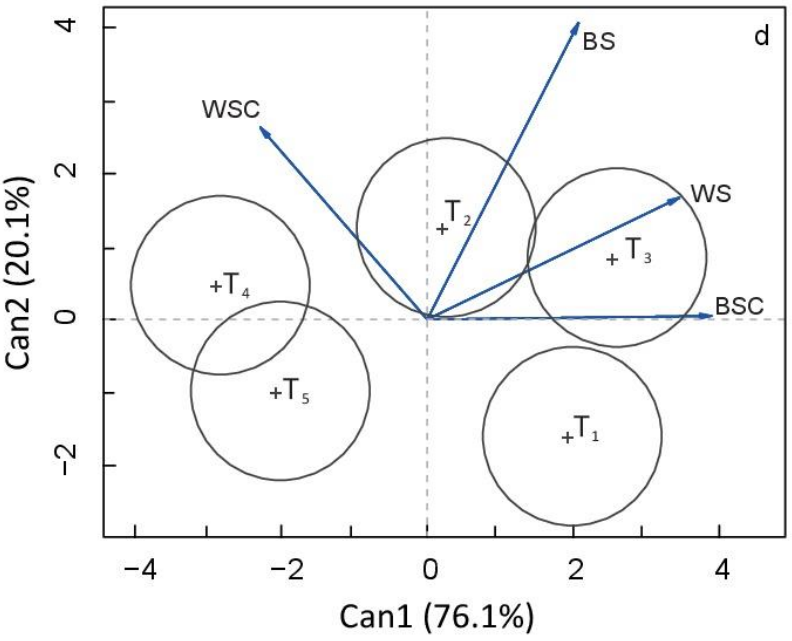


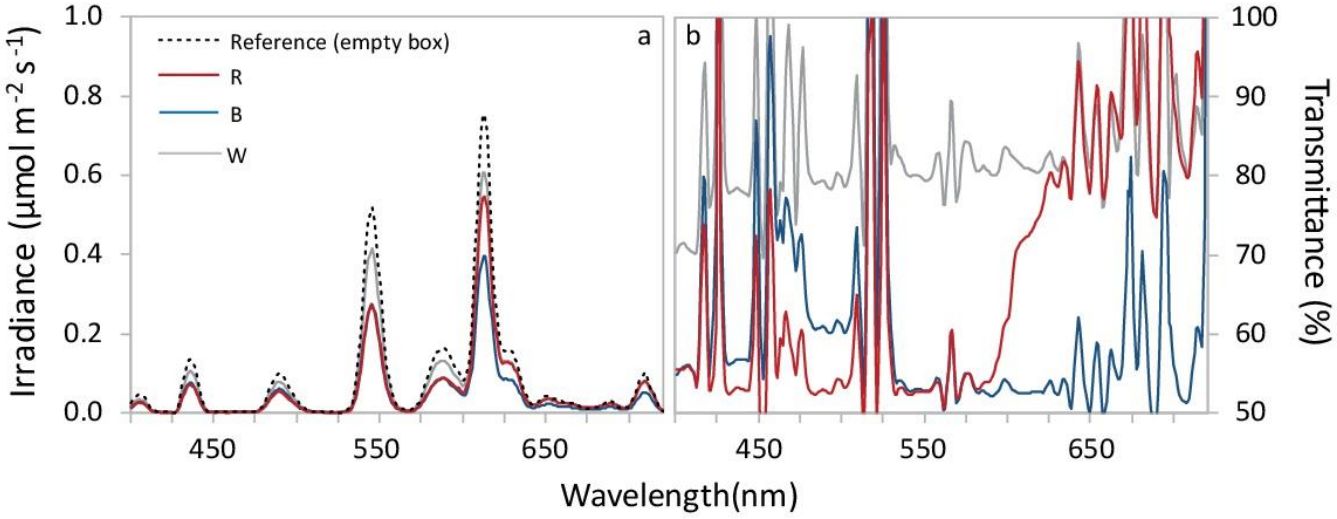
WSC wood soluble carbohydrates

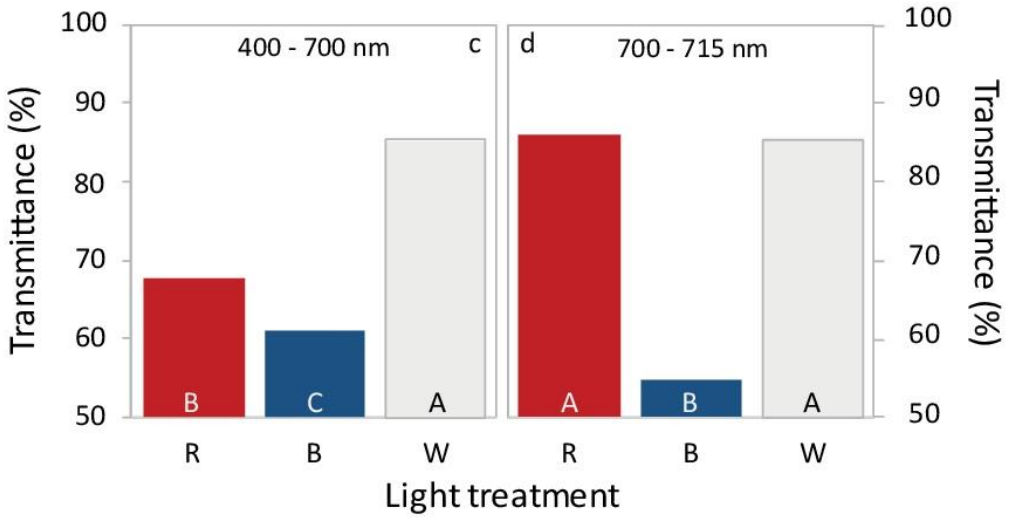
WS wood starch

BSC bark soluble carbohydrates

BS bark starch




Irradiance (µmol m-2 s-1)
Reference (empty box)
R
B
W
a
b
Transmittance (%)
Wavelength(nm)


400 - 700 nm
c
d
700 - 715 nm
Transmittance (%)
Light treatment

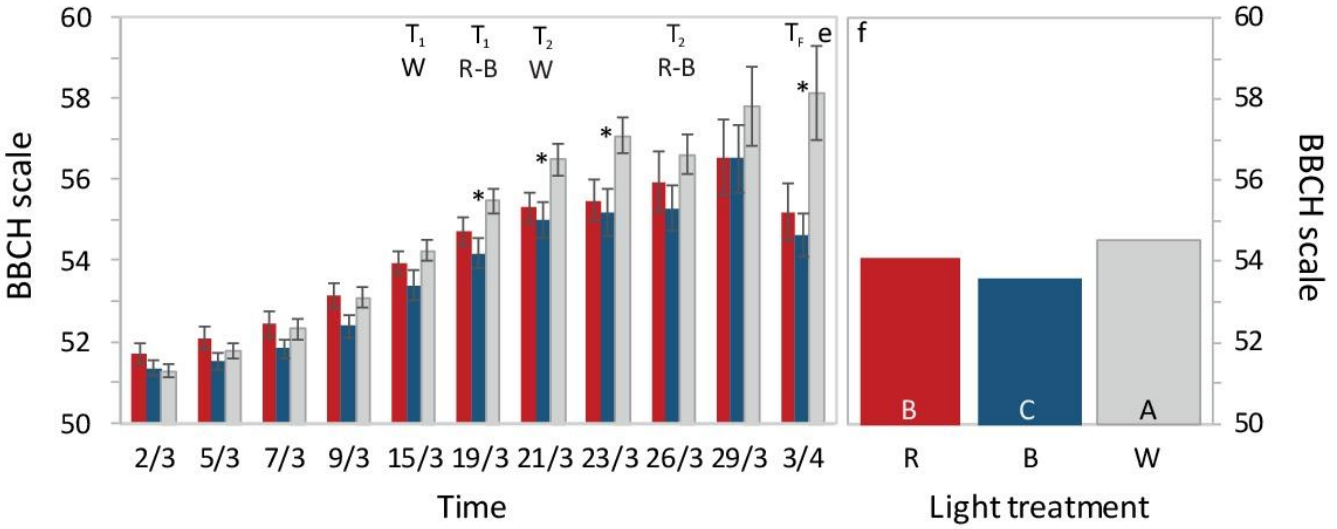

BBCH scale
T1 W
T1 R-B
T2 W
T2 R-B
TF
e
f
Time
Light treatment

Table 1

Light treatment	R			R			R			R		
Location	down			up			down			up		
Sample	BSC			BSC			WSC			WSC		
Timings	T_0	55.35 ab		T_0	66.75 a		T_0	105.26 a		T_0	132.94 a	
	T_1	57.30 a	a	T_1	70.88 a	a	T_1	38.09 b	a	T_1	34.53 b	a
	T_2	49.63 b	b	T_2	58.86 b	b	T_2	24.81 b	b	T_2	22.30 b	b
	T_F	49.12 b	b	T_F	53.50 c	c	T_F	22.57 b	b	T_F	20.91 b	b
Sample	BS			BS			WS			WS		
Timings	T_0	23.29 a		T_0	25.34 a		T_0	124.31 a		T_0	136.84 a	
	T_1	9.06 b	a	T_1	8.51 b	a	T_1	22.06 b	a	T_1	14.11 b	a
	T_2	6.47 bc	ab	T_2	2.29 c	b	T_2	8.26 b	a	T_2	8.87 b	a
	T_F	3.55 c	b	T_F	3.80 c	b	T_F	12.86 b	a	T_F	9.59 b	a

Light treatment	B			B			B			B		
Location	down			up			down			up		
Sample	BSC			BSC			WSC			WSC		
Timings	T_0	55.35 a		T_0	66.75 a		T_0	105.26 a		T_0	132.94 a	
	T_1	54.79 b	a	T_1	65.47 a	a	T_1	31.91 b	a	T_1	29.30 b	a
	T_2	45.69 b	b	T_2	52.79 b	b	T_2	20.91 b	b	T_2	21.94 b	b
	T_F	46.58 b	b	T_F	51.97 b	b	T_F	14.69 b	c	T_F	19.96 b	b
Sample	BS			BS			WS			WS		
Timings	T_0	23.29 a		T_0	25.34 a		T_0	124.31 a		T_0	136.84 a	
	T_1	10.06 b	a	T_1	10.96 b	a	T_1	14.72 b	a	T_1	10.49 b	a
	T_2	7.41 b	a	T_2	5.31 c	b	T_2	13.41 b	a	T_2	13.37 b	a
	T_F	4.46 b	a	T_F	4.39 c	b	T_F	2.97 b	b	T_F	5.92 b	a

Light treatment	W			W			W			W		
Location	down			up			down			up		
Sample	BSC			BSC			WSC			WSC		
Timings	T_0	55.35 b		T_0	66.75 a		T_0	105.26 a		T_0	132.94 a	
	T_1	52.60 b	b	T_1	57.78 b	a	T_1	38.37 b	a	T_1	42.38 b	a
	T_2	64.05 a	a	T_2	63.68 ab	a	T_2	34.87 b	a	T_2	38.77 b	b
	T_F	44.73 c	c	T_F	51.79 c	b	T_F	13.82 c	b	T_F	18.70 b	b
Sample	BS			BS			WS			WS		
Timings	T_0	23.29 a		T_0	25.34 a		T_0	124.31 a		T_0	136.84 a	
	T_1	9.59 b	a	T_1	11.73 b	a	T_1	20.48 b	a	T_1	21.41 b	a
	T_2	8.84 b	a	T_2	14.32 b	a	T_2	15.29 b	a	T_2	22.87 b	a
	T_F	8.60 b	a	T_F	7.14 c	b	T_F	6.16 b	b	T_F	10.45 b	b

Table 2

Time	T_1		T_2		T_F		T_1		T_2		T_F	
Location	down		down		down		up		up		up	
Sample	BSC		BSC		BSC		BSC		BSC		BSC	
Light treatments	R	57.30 a	R	49.12 b	R	49.63 a	R	70.88 a	R	58.86 a	R	53.50 a
	B	54.79 a	B	45.69 b	B	46.58 b	B	65.47 ab	B	52.74 b	B	51.97 a
	W	52.60 a	W	64.05 a	W	44.73 b	W	59.78 b	W	63.68 a	W	51.80 a
Sample	BS		BS		BS		BS		BS		BS	
Light treatments	R	9.06 a	R	6.47 a	R	3.55 b	R	8.51 a	R	2.29 b	R	3.68 b
	B	8.49 a	B	6.31 a	B	3.37 b	B	10.96 a	B	5.31 b	B	3.40 b
	W	9.59 a	W	8.84 a	W	8.60 a	W	11.73 a	W	14.32 a	W	7.14 a

Time	T_1		T_2		T_F		T_1		T_2		T_F	
Location	down		down		down		up		up		up	
Sample	WSC		WSC		WSC		WSC		WSC		WSC	
Light treatments	R	38.09 a	R	24.81 b	R	22.57 a	R	34.53 ab	R	22.30 a	R	18.70 a
	B	31.91 a	B	20.91 b	B	14.69 b	B	29.30 b	B	21.94 a	B	19.96 a
	W	38.37 a	W	34.87 a	W	13.82 b	W	42.38 a	W	38.77 a	W	20.91 a
Sample	WS		WS		WS		WS		WS		WS	
Light treatments	R	22.06 a	R	7.19 b	R	11.01 a	R	12.81 ab	R	8.87 b	R	9.59 a
	B	14.72 a	B	13.41 a	B	2.20 b	B	10.49 b	B	13.37 b	B	4.21 a
	W	20.48 a	W	15.29 a	W	5.28 ab	W	21.41 a	W	22.87 a	W	9.64 a

Table 3

Time	T_1		T_2		T_F		T_1		T_2		T_F	
Light treatment	R		R		R		R		R		R	
Sample	BSC		BSC		BSC		WSC		WSC		WSC	
Locations	up	70.88 a	up	58.86 a	up	53.50 a	up	34.53 a	up	22.30 a	up	20.91 a
	down	57.30 b	down	49.12 b	down	49.63 b	down	38.09 a	down	24.81 a	down	22.57 a
Sample	BS		BS		BS		WS		WS		WS	
Locations	up	10.30 a	up	3.70 a	up	4.36 a	up	14.11 a	up	8.87 a	up	9.59 a
	down	9.06 a	down	6.47 a	down	4.17 a	down	22.06 a	down	8.62 a	down	12.86 a

Time	T_1		T_2		T_F		T_1		T_2		T_F	
Light treatment	B		B		B		B		B		B	
Sample	BSC		BSC		BSC		WSC		WSC		WSC	
Locations	up	65.47 a	up	52.74 a	up	51.97 a	up	29.30 a	up	21.94 a	up	19.96 a
	down	54.79 b	down	45.69 b	down	46.58 b	down	31.91 a	down	20.91 a	down	14.69 b
Sample	BS		BS		BS		WS		WS		WS	
Locations	up	10.96 a	up	5.31 a	up	4.39 a	up	10.49 a	up	13.37 a	up	5.92 a
	down	10.06 a	down	7.41 a	down	4.05 a	down	14.72 a	down	13.41 a	down	2.97 a

Time	T_1		T_2		T_F		T_1		T_2		T_F	
Light treatment	W		W		W		W		W		W	
Sample	BSC		BSC		BSC		WSC		WSC		WSC	
Locations	up	59.78 a	up	63.68 a	up	51.79 a	up	42.38 a	up	38.77 a	up	18.70 a
	down	52.60 b	down	64.05 a	down	44.73 b	down	38.37 a	down	34.87 a	down	13.82 b
Sample	BS		BS		BS		WS		WS		WS	
Locations	up	11.73 a	up	14.32 a	up	7.67 a	up	21.41 a	up	22.87 a	up	10.45 a
	down	9.59 a	down	8.84 b	down	8.60 a	down	20.48 a	down	15.29 a	down	6.16 b

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CHAPTER V
CONCLUSIONS

The objectives of this thesis were to seek possible improvements of physiological performances in apple, when affecting the light quality of the orchard.

During fruit developmental stage (Chapter II), a yellow net seems more prone to give the higher growth rates. On the other hand, red and blue nets slowed growth. The reasons may be found in the different scattered light and in the transmission of PAR, which impacted on photomorphogenic responses. Developing fruit under lower transmitting PAR environments appear to have lower growth rates. It is possible that spur leaves of those fruit were in a less suitable condition to be photosynthetically efficient. Water transport, i.e. sap flow, was influenced by light spectrum modification. Based on the obtained results (Chapter III), the daily patters of sap transport indicated a red photoselective net less suitable and a white net more apt, for commercial purposes, at the orchard level. When considering fruit growth, a blue photoselective net appeared more convenient, as fruit weight gain during the summer was higher, compared to a red and a white net, despite slightly higher water flows compared to a white net. As for carbohydrate dynamics during dormancy and before bud break (Chapter IV), the soil effect was too strong to generate distinctions among different light environments. However, canopy air temperature was influenced, being warmer under a white net and colder under a blue one. The dynamics of carbohydrate translocation was more evident

when completely isolating apple cuttings, under the effect of different spectrum. A blue net will delay flowering, compared to a white one, while consuming more starch, for metabolic processes, leaving a red net with an intermediate behaviour. In this specific case, the results could be due to the influence of spectrum on photoreceptors activity. Future studies could take into account these differences and endeavour to modify soil temperature and test photoselective netting during dormancy, to evaluate how spectrum can influence carbohydrate translocation in the field and repercussions on bloom quality.

The results of this thesis add knowledge to apple crop physiology when modifying the quality of light and further stands photoselective netting as a highly useful technique for apple production. It is worthy considering the scattering properties and mitigating effect of certain coloured nets. It is also worthy underlining the possible applications during certain periods of the year, which until now have been excluding the use of nets (dormancy and fruit cell early stages of development). Still, generalizing, or standardizing, a specific colour is difficult, given the fact that these preliminary findings need to be further elucidated and explained by more targeted analysis.

Hereupon, more research is required to better understand what can be done to further enhance crop performances. Physiological parameters have to be monitored while adding stress and variability, inside the orchard, to identify efficient and sustainable production strategies. Not only physiological parameters have to be monitored from the outside and macroscopic point of view, i.e. field surveys. Detailed and physicochemical analysis, during the growing season, can give further information to comprehend, at a more microscopic level, what is

causing differences and variability when modifying light spectrum, at an orchard level.

It is possible that certain spectrums can be more useful than others, in certain phenological stages, rather than others. Improving fruit development, or saving water, are important tasks for sustainable orchards. They can be the result of different spectrum effects. The possibility of interchanging spectrums during the season, on the basis of the higher potential in a certain phenological stage, can create a more eco-friendly and sustainable production protocol. These assumptions need to be verified.

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(TO BE WRITTEN BY HAND…)

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THESIS ABSTRACT

Research highlights the benefits of light quantity modification, for orchard final production. From the physiological point of view, a lot has been discovered and analysed to further improve orchard performances. However, modifying the quality of light may add further benefits to an orchard fruitfulness.
Many studies demonstrate how crop responses are extremely challenging to draw general conclusions from. The use of photoselective nets generates wide variability, caused by the interaction between the crop, the cultivar, orchard age and type of management and the surrounding ecosystem.

The Mediterranean basin is facing progressive increase of environmental threats for agriculture, thus orchards performances are taken at a limit. Solutions that can mitigate such extreme changes and, beyond control, fluctuations, are required for optimal functionality. Photoselective technology might solve this and other issues, as production of fruit and vegetables will have to efficiently use resources, in ever decreasing amounts. Modern production systems will have to improve plant stress mitigation, while preserving quality and quantity of production, and while increasing their efficiency.
When testing photoselective netting, apple crop has shown to take profit from shade applications, in Mediterranean and hot dry climates and in areas where high solar and UV intensities occur. Many aspects of apple physiology have been tested under the effect of different light environments, quantitively and also qualitatively.

However, to date, light quality impacts on:

- fruit development,
- sap flow and fruit cell expansion,
- carbohydrate dynamics during dormancy and bud break,

is absent in apple. Light quantity has been tested during fruit development, whereas, sap flow and carbohydrates translocation during ecodormancy have been evaluated, though neither under the influence of light manipulation. Such topics resemble among the most important processes occurring inside a fruit tree: fruit development, water uptake for evapotranspiration and, last, carbohydrate translocation during the dormant phase anticipating bud break.

The objective of this thesis was to evaluate these three specific aspects in apple, while affecting the light quality of the orchard.

In Chapter II, a preliminary study was undertaken, where apple fruit (cv. *Rosy Glow*) growth was monitored and compared with both bourse and extension shoot growth, in order to see if differences occurred under five qualitatively different light environments: black, red, blue, white and yellow, all shading at 20%. The study was carried out from one month to three months after full bloom, covering most of fruit cell division stage. The purpose of the work was to seek differences in partitioning between competing sinks (fruit and extension shoots), as affected by different spectra. Results showed that photoselective netting influenced fruit and extension shoot growth during the developmental stage. The presence of high solar intensities during the early stages of fruit development can then be exploited by applying 20% shading anti-hail nets with scattering properties. The yellow net seemed capable of improving fruit growth, during cell division stage, thus it could be potentially used in apple orchards for enriching the inner canopy environment with diffuse light. The blue and red treatments did not give satisfactory results when related to fruit growth, therefore, these two nets would not be suitable in an apple orchard during fruit development. As for the white net, this light treatment appeared less convenient during fruit development, rather than fruit cell expansion, as it gave more satisfying results at final harvest. More targeted and detailed studies would further confirm these hypotheses.

In Chapter III, the effect of coloured nets (black, shading 20%, red, blue and white, all three shading 50%) was investigated on the amount and daily patterns of apple (cv *Rosy Glow*) sap flow, at different phenological stages. In parallel, fruit growth was monitored to evaluate potential secondary impacts of xylem sap flow rate variations. Results showed that photoselective nets are capable of influencing sap flow in apple. A 50% shading red net is certainly going to increase sap flow, even more than a 20% shading black classic anti hail net. The main reason is due to infrared transmission, which increased evaporative cooling rates of

trees growing in this specific light environment. On the contrary, a 50% shading pearl net is going to maintain lower sap flow rates. A 50% shading blue net will maintain intermediate sap fluxes. Fruit growth under photoselective nets was also influenced. Under a 50% shading red net, absolute growth rates were not as high as under a 50% shading blue, or pearl, net. In general, a 50% shading net increased fruit growth compared to a 20% shading net. This study demonstrated how photoselective netting can be potentially used for saving water purposes.

In Chapter IV, different light environments were tested on the dynamics of carbohydrates during dormancy and bud break, in field and laboratory conditions. In the field trial, a red, blue and white nets (all three shading 20%) were compared with a control (no net), while monitoring air temperature, carbohydrate translocation and bud phenology. Although there were significant differences in air temperature, among the nets, communication signals between the root apparatus and the canopy were not strongly influenced by the different spectra. This is mainly due to the chosen statistical design set-up, which did not allow to influence soil temperature. Results showed that the temperature gradients which were including soil had the highest impact on the translocation of sugars in the trees. It followed that flower bud phenology was not influenced by the different light treatments. On the other hand, the results of the laboratory trial suggest the potential of photoselective nets (the same types used in the field) to improve the efficiency, or modify the timings, of carbohydrate management and translocation in apple, during ecodormancy and bud break. In fact, when completely isolating apple cuttings in a given light environment, clear differences arose. The blue net delayed flowering, compared to the white one, while consuming more reserves, for metabolic processes, leaving the red net with an intermediate behaviour. In this specific case, the results could be due to the influence of spectrum on photoreceptors activity. Future studies could take into account these differences and endeavour to modify soil temperature and test photoselective netting during dormancy, to evaluate how spectrum can influence carbohydrate translocation in the field and repercussions on bloom quality.

These results add value and knowledge to the responses of these processes of apple crop. Certain aspects have been enhanced, others reduced, demonstrating that specific filters are more, or less, suitable for improving apple crop physiology. These findings are also a further supplement to the pool of information concerning apple behaviour influenced by light quality.

More research is required to better understand what can be done to further enhance crop performances. Physiological parameters have to be monitored while stressing and adding variability, inside the orchard, in order to find efficient and sustainable production protocols. It is also important to analyse, during the growing season, more detailed physicochemical aspects and responses, deriving from different imposed light quality. Such analysis could give further information to comprehend, at a more microscopic level, what is causing differences and variability when modifying light spectrum, at an orchard level.