ULTRAVIOLET RADIATION IN *Coffea sp.*: MORPHOLOGICAL, PHYSIOLOGICAL AND BIOENERGETIC ATTRIBUTES

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"Thesis presented to Centro de Ciências e Tecnologias Agropecuárias of Universidade Estadual do Norte Fluminense Darcy Ribeiro, as part of the requirements for obtaining a Master Degree in Plant Production**"**

Advisor: Prof. Eliemar Campostrini

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To my parents, Helena Bernardo, Maria Cristina, Helio Bernardo and Zenite de Paula, for your passion and faith in my dreams. To my friends, for support and teachings for all these years.

"Whoever you are, whatever the social position you have in life, the highest or the lowest, always have as your goal a lot of strength, a lot of determination and always do everything with a lot of love and a lot of faith in God, that one day you get there. Somehow you get there."
Ayrton Senna.

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SUMMARY

ABSTRACT

Bernado, Wallace de Paula; M. Sc.; Universidade Estadual do Norte Fluminense Darcy Ribeiro. October, 2020. Ultraviolet radiation in *Coffea* sp.: morphological, physiological and bioenergetic attributes: Prof. Eliemar Campostrini, Ph. D. Co-advisor: Weverton Pereira Rodrigues, Ph. D. Co-advisor: Miroslava Rakocevic, Ph.D.

Despite the growing concern with increased ultraviolet radiation intensity on plants, these organisms continue to grow and produce under the actual UV condition. We hypothesized that ambient UV intensity can generate responses at plant growth, leaf morphology and photosynthetic functioning in *Coffea arabica* cv. Catuaí Amarelo IAC 62, and *C. canephora* cv. lB1. Coffee plants were cultivated for *ca*. six months in a mini greenhouse under either near ambient (UVam) or reduced (UVre) ultraviolet regimes. At the plant scale, *C. canephora* was substantially more impacted by UVam as compared to *C. arabica*, investing more carbon in all juvenile plant components than under UVre. When subjected to UVam, both species showed anatomic adjustments at the leaf scale, such as increases in stomatal density in *C. canephora*, abaxial and adaxial cuticles in both species and abaxial epidermal thickening in *C. arabica*, although without impact in the thickness of palisade and spongy parenchyma. Additionally, *C. arabica* showed more efficient mechanism of energy dissipation under UVam than *C. canephora*. UVam promoted elevated protective carotenoid content and a greater use of energy through photochemistry in both species, as reflected in the photochemical quenching increase. This was associated to an altered chlorophyll *a/b* ratio (significantly only in *C. arabica*) which

likely promoted a greater capability to light energy capture. Therefore, UV levels can promote important modifications regarding plant biomass production, leaf morphology, and photosynthetic functioning levels, with these changes acting as acclimation responses associated with UV intensity.
Keywords: fluorescence; leaf anatomy; leaf pigments; plant growth; UV-A; UV-B.

RESUMO

Bernado, Wallace de Paula; M. Sc.; Universidade Estadual do Norte Fluminense Darcy Ribeiro. Outubro de 2020. Radiação ultravioleta em *Coffea* sp.: atributos morfológicos, fisiológicos e bioenergéticos: Prof. Eliemar Campostrini, D. Sc. Coorientador: Weverton Pereira Rodrigues, D. Sc. Coorientador: Miroslava Rakocevic, D. Sc.

Apesar da crescente preocupação com o aumento da intensidade da radiação ultravioleta nas plantas, esses organismos continuam a crescer e produzir sob a condição UV real. Nossa hipótese é que a intensidade de UV ambiente pode gerar respostas no crescimento da planta, morfologia foliar e funcionamento fotossintético em *Coffea arabica* cv. Catuaí Amarelo IAC 62 e *C. canephora* cv. Ib1. As plantas de café foram cultivadas por *ca.* seis meses em uma miniestufa sob regime ultravioleta próximo ao ambiente (UVam) ou reduzido (UVre). Na escala da planta, *C. canephora* foi substancialmente mais impactado por UVam em comparação com *C. arabica*, investindo mais carbono em todos os componentes da planta juvenil do que sob UVre. Quando submetidas a UVam, ambas as espécies apresentaram ajustes anatômicos na escala foliar, como aumento da densidade estomática em *C. canephora*, cutículas abaxial e adaxial em ambas as espécies e espessamento epidérmico abaxial em *C. arabica*, porém sem impacto na espessura da paliçada e parênquima esponjoso. Além disso, *C. arabica* apresentou mecanismo de dissipação de energia mais eficiente sob UVam do que *C. canephora*. UVam promoveu elevado teor de carotenoides protetores e maior aproveitamento de energia por meio da fotoquímica em ambas as espécies,

refletindo no aumento da têmpera fotoquímica. Isso foi associado a uma alteração na relação da clorofila *a/b* (significativamente apenas em *C. arabica*) que provavelmente promoveu uma maior capacidade de captura de energia luminosa. Portanto, os níveis de UV podem promover modificações importantes quanto a produção de biomassa vegetal, morfologia foliar e níveis de funcionamento fotossintético, com essas mudanças atuando como respostas de aclimatação associadas à intensidade de UV.

Palavras-chave: fluorescência; anatomia foliar; pigmentos foliares; crescimento vegetal; UV-A; UV-B.

1. INTRODUCTION

Solar radiation is composed of a complex mixture of ultraviolet (UV) (200 to 400 nm), visible light (400 to 700 nm) and wave components in the infrared region (greater than 700 nm) (Verdaguer et al., 2017). The ultraviolet radiation (UV) is separated into three bands, classified as UV-A (315-400 nm), UV-B (280-315 nm) and UV-C (200-280 nm) (Kataria et al., 2014). However, much of the UV radiation does not reach the Earth's surface, due to the interaction with the ozone layer constituent of the stratosphere. In fact, UV-C radiation is completely absorbed by the atmospheric gases present in this layer, while a small part of UV-B radiation (less than 5%), as well as UV-A radiation (between 10 to 100 times more than that UV-B radiation) can reach the Earth's surface, triggering responses at molecular, cellular and whole plant scales in photosynthetic organisms (Lidon et al., 2011; Kataria et al., 2014).

The UV radiation intensity is easily modified by factors such as latitude, time of day, amount of clouds, surface reflectance and plant canopy thickness (Jenkins et al., 2009). However, an increase in the incidence of UV-B spectral radiation on the earth's surface is predicted, because of changes in the chemical composition of the atmosphere, especially the reduction of the ozone protective layer (Kataria et al., 2014).

In addition to the cited climate pattern changes, the anthropogenic emissions have been causing depletion of the ozone layer. These depletions lead to the inefficiency of the main filter of UV-B rays, mainly in the Southern hemisphere (Fahey et al., 2018), causing individual and interactive changes in biological

systems (Ballaré et al., 2011). UV radiation are also characterized as an auxiliary agent of global warming in particular way, since it stimulates the release of volatile organic compounds from plants, litter and soils and it can have its effects increased with disordered drought and temperature events (Bornman et al., 1989; Barnes et al., 2019).

As photoautotrophic organisms, plants are exposed to environmental variations with constant necessity to adapt to external variations during their growth and development. The effects of UV-A and UV-B includes various morphological changes, such as stimulatory effect on biomass accumulation, reduction on plant height, increased auxiliaries branching and changes in resources allocation (Meijkamp et al., 2001; Kataria et al., 2013; Zhang et al., 2014). In addition, the exposure to UV rays generates changes on stem length, leaf size and leaf anatomy (Robson et al., 2015). Also, UV ray exposure enhances the thickness of the palisade parenchyma, abaxial epidermis and shows negative effects on the spongy mesophyll and adaxial epidermis (Victorio et al., 2011). UV rays can reduce the photosynthetic activity through the degradation of PSII proteins, destruction of chlorophyll and carotenoids, and consequently would impact on stomatal functions and bioenergetic effects in inactivation of the plasma membrane-bound ATP-ases (Imbrie et al., 1982; Sullivan et al., 2003; Surabhi et al., 2009).

The UV rays act as damaging agent destroying biomolecules by generating reactive oxygen species (ROSs), a major cause of oxidation of lipids, and proteins, and DNA damaging (Hollosy, 2002). Though, to reduce the ROSs impacts generated by UV exposure, plants produces antioxidants such as ascorbic acid and alfa tocopherol (Kliebenstein et al., 2002; Jain et al., 2003) and synthesizes antioxidant enzymes, like superoxide dismutase, peroxides, glutathione reductase and guaiacol peroxides (Jain et al., 2003; Kataria et al., 2007; Hassan et al., 2013). However, this complex antioxidant defense system can generate additional costs to the organism, in detriment in growth and development.

Genus *Coffea* belongs to the Rubiaceae family (Charrier and Berthaud, 1985). Consisting of 134 species (Davis et al., 2011b). However, only two species, *Coffea arabica* L. (Arabic coffee) and *Coffea canephora* Pierre ex A. Froehner (Robusta coffee) have been expanded as crops over the world, representing approximately 99% of the world's coffee production (DaMatta et al., 2019). Those two species differ essentially in the environmental conditions where they

evolutionarily developed. *C. arabica* is one endemic species, native from tropical forests of Ethiopia, Kenya and Sudan, at altitude of 1600-2800 m (Anthony et al., 2011), with average annual temperature between 18 and 22 ºC, and the annual precipitation varying from 1600 to 2000 mm (Anthony et al., 2011). *C. canephora* has the origin in the lowland forests of the Congo River, expanding from the central to the western Africa, at altitudes up to 1200 m, with average temperatures between 24 and 26ºC, and annual precipitation greater than 2000 mm (DaMatta et al., 2003; Anthony et al., 2011).

In addition, changes in climate pattern can drastically generate deficits in their adaptation, yield and quality in coffee plants (Ovalle-Rivera et al., 2015; Läderach et al., 2017). As a result, the adaptation of Arabic coffee to climate variations is expected to decrease significantly in the Americas and East Africa, and in a future scenario the decrease in the cultivable areas of Arabica coffee can be rewarded by the increase in areas suitable for Robusta coffee, which will migrate mainly to areas of high altitude and elevations, mainly in South and Central America, Indonesia, East and West Africa where the development temperature will be adequate (Magrach e Ghazoul, 2015; Ovalle-Rivera et al., 2015).

However, with increasing altitude, the effect of solar radiation is more pronounced and consequently, exposures to UV rays, because of the reduced to the sun. This scenario can generate multiple abiotic stresses and possible inefficiency of the photosynthetic machinery, consequently impacting growth and productivity (Barnes et al., 2019). Therefore, it is extremely important to characterize the effects of UV rays on crops of economic importance such as coffee at the morphological, physiological and biochemical levels, as well to identify the possible strategies developed by plants to mitigate the harmful effects of this radiation.

It was hypothesized that reduced levels of ultraviolet rays would increase a growth and development of coffee plants, due to reduced production of defense complexes at cell scale. Also, it was hypothesized that *C. canephora* would have greater adaptability to nocive effects of UV rays than *C. arabica*, due to original habitat of species. The aim of this study was to detect the impacts of the current levels of ultraviolet rays, compared to reduced ones, considering morphology and physiology of two coffee species at cell, leaf and plant scales.

2. LITERATURE REVIEW

2.1 Botanical aspects of *Coffea* sp. and impacts of climatic variables on its growth and development

Genus *Coffea* belongs to Rubiaceae family (Charrier and Berthaud, 1985). Consisting of 134 species (Davis et al., 2011b). However, only two species, *C. arabica* L. (*Arabic coffee*) and *C. canephora* Pierre ex A. Froehner (Robusta coffee), have been expanded as crops over in the world, representing approximately 99% of the world's coffee production (DaMatta et al., 2019).

C. arabica and *C. canephora* are segregated into two groups, according to their chromosomal number. *C. arabica* is a tetraploid, with 2n = 4x = 44 chromosomes, predominantly self-pollinated, since only ca. 10% of cross-pollination can occur. Differently, *C. canephora* is a diploid, with 2n = 22 chromosomes, self-incompatible, requiring cross-pollination (Conagin and Mendes, 1961; Berthaud, 1980).

Additionally, difference between *C. arabica* and *C. canephora* is also justified by the contrast of the environmental conditions from which they evolutionarily originated. *C. arabica* is native from the African tropical rainforests, of Ethiopia, Kenya and Sudan, from high altitudes of 1600-2800 m, average annual temperature between 18º and 22 ºC, and the annual precipitation varying from 1600 to 2000 mm (Anthony et al., 2011).

However, *C. canephora* is native from the lowland forest of the Congo River, which extending to central to the western Africa, from altitudes up to 1200 m,

average annual temperatures between 24º and 26º C, and annual precipitation greater than 2000 mm (Coste, 1992; Davis et al., 2006).

Coffee production is the important economic and social basis in many countries. Around 12 million of bags benefited beans of two important coffee species are exported from Asia and South America (ICO, 2020).

Brazil is the largest producer and exporter of coffee of the world, with around 64.875 thousands of 60 kg bags in 2020 (ICO, 2020). The coffee production chain consists on 2 million ha including about 300.000 producers, predominantly small, distributed in approximately 1.900 municipalities in the states of Minas Gerais, Espírito Santo, São Paulo, Bahia, Paraná and Rondônia, which are responsible for about 95 % of Brazilian production, and is estimated that the chain of coffee generates an income of *ca.* US$ 5.2 billion per year in Brazil (CONAB, 2020).

Many reports have predicted that global changes will act on temperature, water, and solar radiation patterns, increasing climate variability, which can impact on plant levels and consequently on coffee production areas (Ovalle-Rivera et al., 2015; Läderach et al., 2017). Regarding the coffee production, those changes still caused significant impacts in agroclimatic zoning of coffee production, with loss of adequate areas in Brazil, up to 75% in Paraná and 95% in Góias, Minas Gerais and São Paulo (Assad et al., 2004). In the world, the climate changes will reduce the global area suitable for coffee by about 50% in future scenario (Bunn et al., 2014), with likely negative impacts and consequences for the entire coffee production chain.

Regarding Ultraviolet effects in plants, most of studies of exclusion of these rays has been conducted in grown chamber and laboratory conditions. In addition, a small amount of species as soybean, wheat and sorghum were characterized (Guruprasad et al., 2008; Kataria et al., 2012a; Kataria et al., 2012b). In fact, indoor experiments are important for understanding the effects of current levels of UV on physiological parameters (Kataria et al., 2013). However, studies of natural exclusion of UV is scarce. Furthermore, nothing is known about the effects of exclusion or reduction of these rays for an economically important crop like coffee.

2.2. UV-A and UV-B: Morphological effects

Ultraviolet rays have a potential to trigger changes in morphology, physiology, biochemistry and bioenergetics in plant tissue, generating impacts in photosynthetic and productive processes (Kataria et al., 2014).

The ultraviolet-A radiation (315-400 nm) is one of the main components of solar radiation that exerts a series of morphological changes (Verdaguer et al., 2017). The exposure of high levels of UV was reported to cause alterations in plant morphology in sorghum (*Sorghum bicolor)* and amaranthus (*Amaranthus tricolor*), such as reduction in plant height, increased axillary branching in soybean, negative effect on biomass accumulation, and changes in resources allocation in *Vicia faba* (Meijkamp et al., 2001; Kataria et al., 2013; Zhang et al., 2014). However, UV-A increases was also found to mediate plant growth, with biomass accumulation increase and differential partitioning of biomass between shoots and roots in cucumber (Krizek et al., 1997).

The ultraviolet-B radiation (280-315 nm) is another component of solar radiation that acts directly on plant morphology. The UV-B may affect on plant stem length, leaf size and anatomy in corn (*Zea mays* L.) (Reddy, et al., 2013). However, many studies related changes in plant morphology by increasing of UV B rays. Among the changes, they lead to reduction in size of stem, increases in branching and, chlorosis and necrotic spots at leaf scale (Meijkamp et al., 2001; Kakani et al., 2003; Reddy et al., 2013). The increase in UV-B rays leads to reduction in biomass accumulation and an increase in the thickness of the leaf abaxial and adaxial epidermis (Kakani et al., 2003; Ruhland et al., 2005).

Additionally, elevated UV intensity generates changes in leaf size and leaf anatomy in *Arabidopsis* (Robson et al., 2015), enhances the thickness of the palisade parenchyma and the abaxial epidermis, with negative effects on the spongy mesophyll and adaxial epidermis thickness in *Phyllanthus tenellus* (Victorio et al., 2011). Increases in UV radiation can also lead to chlorosis and necrotic pots in the leaves (Meijkamp et al., 2001; Kakani et al., 2003; Reddy et al., 2013). In addition, physiological modifications under the elevated UV radiation are associated with stomatal density reduction and/or regulation of stomatal opening, the latter regarding the specific impact of high levels of UV-B radiation on guard cells control mechanisms (Nogués et al., 1999). On other hand, the exclusion of UV rays leads to an increase, leaf area, and plant height, reflecting in the accumulation of biomass (Guruprasad et al., 2008; Kataria et al., 2012b).

2.3. Effects of UV on photosynthesis

Photosynthetic activity is a sensitive metabolic process extremely dependent on light. UV rays also affect the photosynthesis, directly and indirectly. One of its primary effects is the degradation of carotenoid and chlorophyll molecules, and inhibition of *de novo* synthesis of pigments (Xing-Chun et al., 2011; Ranjbarfordoei et al., 2011). The damaging effects of UV rays generate direct and indirect impacts on photosynthetic activity, causing damage mainly to photosystem II (Sullivan et al., 2003). In this protein-pigment complex, the main target of UV rays is the Mn binding site in the water oxidation complex, associated with the oxygen evolution complex (Zuk-Golaszewska et al., 2003; Zinser, et al., 2007). These rays also act in degradation of the D1 and D2 polypeptide subunits of photosystem II (PSII), and cause damage to the binding sites of quinone A (Qa) and quinone B (Qb). These effects of UV can generate 68% loss in the photochemical action of PS II (Swarna et al., 2012).

Regarding the other membrane proteins of the electron transport chain, studies suggest that both cytochrome b6f and PS I, are the last to be affected by UV-B rays, which may be related to the presence of two quinones (Qa and Qb), where reduction occurs, avoiding severe damage to cytochrome b6f (Hope et al.,1993; Sang et al., 2010). Indirectly, the effects of UV rays can cause a decrease in photosynthetic activity due to a reduction in the content and / or activity of Rubisco or PEP-carboxylase (Prado et al., 2012). Additionally, UV radiation can promote the accumulation of ROSs, which degrade lipids and proteins, and can act by inhibiting *de novo* synthesis of proteins of PS ll, and / or its repair mechanism (Takahashi et al., 2011). Considering that, solar radiation contains more UV-A than UV-B rays, may suggest that despite lower quantum efficiency of UV-A rays, they are more harmful on plants (Sicora et al., 2006.)

UV rays can affect ATP-synthase, an important membrane protein complex, responsible for transformation of electrical into chemistry energy. Those rays reduce the phosphorylation rate inducing decrease in photochemical capacity (Zhang et al., 1994; Yu et al., 2013). Among the indirect effects, UV-B rays can affect stomatal regulation, by reducing stomatal density, or by reducing stomatal opening, since

high UV-B irradiance can affect the control of opening of guard cells (Nogués et al., 1999).

On other hand, the exclusion of UV rays on photosynthesis increases the liquid photosynthetic rate, the chlorophyll *a* and *b* contents, the efficiency in reducing Qa by electrons, the transport of electrons between photosystems, the maximum efficiency of PSII and the photosynthetic index (PI) and Rubisco's activity in C3 and C4 species are observed (Kataria et al., 2012a; Kataria et al., 2013).

2.4 Biochemical responses to UV-A and UV-B

Some wavelengths of solar radiation impact on plant growth and development in different ways, such as inhibition/elevation of photosynthesis, activation of specific photoreceptors, causing or not damage through the photomodification of molecules (Verdaguer et al., 2017). Plants are organisms able to synthesize and accumulate various metabolites, including phenols, alkaloids and terpenoids. These components play an important role in plant acclimations to light, as well as in reduction of pests and diseases attacks (Kliebenstein, 2004).

Plants exposed to high UV-A levels, tend to regulate the pool of phenolic compounds as a strategy, which can result in the increase of these compounds in leaves, described in *Mentha piperita* (Maffei et al., 1999), *Lactuca sativa* (Lee et al., 2014) and *Ixeris dentate* (Lee et al., 2013). However, this effect is equally observed after the UV-B exposure, which suggests that the increase in the pool of phenolic compounds is dependent on both UV-A and UV-B (Lee et al., 2013). UV-A rays also act as regulators of the content of flavonoids, which, in turn, play a fundamental role in the cell protection against UV rays, by their characterization of antioxidant activity (Agati et al., 2010).

PS I and PS II are the main sites of production of singlet oxygen and superoxide radical, which are harmful agents but also may be involved in signaling (Gill et al., 2010). Exposure to high levels of UV-B can lead to increases in the production of these ROS, which can damage cell membranes and alter the lipid composition of chloroplast membranes (Lidon et al., 2012; Kataria et al., 2014).

Plants can develop strategies to decrease the damage induced by UV-B. Among these strategies, the protection of organelles by phenolic compounds that absorb UV (Jansen et al., 1998), the DNA repair mechanism, and the *de novo*

synthesis of proteins associated with UV radiation, especially D1 and D2 of the PS II, and finally, enzymatic and non-enzymatic defense mechanisms are observed (Bornman et al., 1989).

The enzymatic and non-enzymatic defense mechanisms include different enzymes and metabolites synthetized to reduce ROS, produced because of excess UV, especially UV-B in order. The enzyme complex includes catalase, superoxide dismutase, glutathione reductase, ascorbate peroxidase, among others, and the non-enzymatic antioxidant complex includes, for example, α-tocopherol, ascorbic acid, carotenoids, etc. (Jansen et al., 1998; Jain et al., 2003; Kumari et al., 2009). However, despite being efficient, these action mechanisms represent an additional cost for the plants, since the organism stops investing in growth and development, and is pressured to invest in defense.

The exclusion of UV-A and UV-B rays generate changes in the structure of chloroplasts (Amudha et al., 2005). The reduction in the formation of ROS, oxidative enzymatic activity, and the ascorbic acid content is also verified under exclusion of UV radiation (Xu et al., 2008; Shine et al., 2012). The changes in the primary metabolism pattern result in decrease of UV-absorbing substances; such as phenolic compounds, which is observed in plants cultivated in environments with exclusion of UV (Kataria et al., 2012a; Kataria et al., 2013).

3. CHAPTER

3.1 PLANT BIOMASS AND LEAF RESPONSES TO ULTRAVIOLET SOLAR RADIATION IN JUVENILE *COFFEA* SP. PLANTS

INTRODUCTION

Climate changes have important potential impacts on the structure, function, and diversity of terrestrial ecosystems and consequently, national economies. Estimates of stratospheric ozone depletion and associated changes in ultraviolet radiation (200-400 nm) suggest that solar radiation can be one of the most damaging stress factors for many crops. Current estimates of the ultraviolet index (UVI), thirty years after the considerations proposed by the Montreal Protocol, show that the prohibition of substances that deplete the ozone layer is highly efficient in the recovery of stratospheric ozone (Banerjee et al., 2020). However, without the Protocol, UVI values at northern and southern latitudes less than 50º could be 10 to 20% higher in all seasons, similar to what happened in 2018, compared to those observed UVIs in the 90s (Bernhard et al., 2020).

Coffea arabica L. and *Coffea canephora* Pierre ex A. Froehner have been cultivated in the tropical region under somewhat different conditions. These two species, which dominates the coffee trade worldwide, differ in their evolutionarily environmental conditions. *C. arabica* is originally native from the African tropical

rainforests of Ethiopia, Kenya, and Sudan, and is found at high altitudes of 1600-2800 m, with an average annual temperature between 18 and 22 ºC, and annual precipitation varying from 1600 to 2000 mm (Davis et al., 2006). On the other hand, *C. canephora* is native from the lowland forest of the Congo River, with extensions to central and western Africa, at altitudes lower than 1200 m, average annual temperatures between 24 and 26 ºC, and annual precipitation greater than 2000 mm (Coste, 1992; DaMatta and Ramalho, 2006).

In Brazil, most elite coffee plants have been selected under high irradiance of full sunlight conditions (DaMatta et al., 2019). Solar UV is characterized by high energy levels, with significant impacts on the biosphere, namely on morphological, physiological, and biochemical processes of plant species (Kakani et al., 2003; Hectors et al., 2007; Lidon and Ramalho, 2011; Lidon et al., 2012a). Although some studies reported that coffee plants show physiological and metabolic plasticity as regards altered availability of light quantity and quality at the leaf (Ramalho et al., 2000; 2002), plant (Rakocevic et al., 2018), and canopy scale (Rakocevic et al., 2021) nothing is known about the effects of UV on this important crop.

Solar radiation includes ultraviolet (UV) (200 to 400 nm), visible (400 to 700 nm) and infrared radiation (Verdaguer et al., 2017). The UV can be sub-divided in three bands, classified as UV-A (315-400 nm), UV-B (280-315 nm) and UV-C (200-280 nm), which significantly differ regarding energy and their interaction with biological processes (Kataria et al., 2014). UV-C radiation is completely absorbed by the atmospheric gases present in ozone layer. One small part of UV-B (less than 5%), together with UV-A radiation (between 10 to 100 times more than that UV-B radiation), reaches the Earth's surface, triggering responses at molecular, cellular, and whole plant scales in photosynthetic organisms (Lidon et al., 2011; Kataria et al., 2014).

Exposure to high levels of UV was reported to cause alterations in plant morphology, such as reduction in plant height, increased axillary branching, negative effect on biomass accumulation, and changes in resources allocation in Vicia faba, Sorghum bicolor, Amaranthus tricolor and soybean (Meijkamp et al., 2001; Kataria et al., 2013; Zhang et al., 2014). High UV-A mediates plant growth, for example, decreases biomass accumulation and increases biomass partitioning to shoots and leaves in some plants species such as cucumber (Krizek et al., 1997). Elevated UV-A intensity generates changes in leaf size and leaf anatomy (Robson

et. al., 2015), enhances the thickness of the palisade parenchyma and the abaxial epidermis, and reduces the spongy mesophyll and adaxial epidermis thickness (Victorio et al., 2011). Increases in UV-B radiation can lead to chlorosis and necrotic spots in the leaves (Meijkamp et al., 2001; Kakani et al., 2003; Reddy et al., 2013). Additionally, physiological modifications under the elevated UV-B radiation are associated with stomatal density reduction and/or regulation of stomatal opening, the latter regarding the specific impact of high levels of UV-B radiation on guard cells control mechanisms (Nogués et al., 1999). High UV-B levels can promote deleterious impacts on the photosynthetic performance, by promoting oxidative stress conditions that will affect photosynthetic pigments (Lidon and Ramalho, 2011; Ranjbarfordoei et al., 2011), proteins and lipids, while significantly increase grana disorganization, followed by decreases in thylakoid membrane functions (Hollósy, 2002; Vass et al., 2005; Lidon et al., 2012a). Although both photosystems are affected by UV-B, the efficiency of photosystem II (PSII) is particularly impaired, mainly in the reactions coupled to the Mn-binding site of the water splitting complex, and in polypeptides D1 and D2 (Lidon et al., 2012a,b). This induces an inefficient electron transfer (Kataria and Guruprasad, 2012a), with losses of PSII functioning up to 68% under elevated UV-B (Swarna et al., 2012).

Notably, to increase the coffee crop resilience and mitigate the impacts of global warming and increasingly water shortage, coffee growers have implemented agroforestry management systems, which, among others, provide better micro-environmental conditions (e.g., lower temperature and greater air humidity) to the coffee plants (Oliosi et al., 2016; Dubberstein et al., 2018; Gomes et al., 2020), while likely reduce UV irradiance. Despite the relevant information concerning the effects and responses of elevated UV intensities in various plant species, no information is available regarding the coffee species. Considering the origin of coffee species from the deep forest understory, the hypothesis was that the current UV levels can already impacted those species, related to the possible investment in protection mechanisms, which demands a significant amount of metabolic energy. In this sense, we suppose that the current UV intensities provoke alterations in responses at plant/leaf scale, possibly different among the two main cropped coffee species. The work aimed to study the responses of two plant species grown under two UV solar radiation regimes during the juvenile stage, addressing the following key questions: (1) Is near ambient UV radiation intensity already causing different

acclimations in the two coffee species when compared to reduced UV? (2) Can a reduced UV radiation intensity enhance photochemical efficiency, change leaf anatomical traits, and affect coffee plant biomass partitioning?

MATERIAL AND METHODS

Experimental site, species description and light microclimate

The experiment was conducted at the State University of Northern Rio de Janeiro, Campos dos Goytacazes (21º 44' 47" S and 41º 18' 24" W, at 10 m altitude), Southeastern Brazil, using important cropped genotypes in Brazil, from the two most important coffee species: *Coffea arabica* L. cv. Catuaí Amarelo IAC 62, and *C. canephora* Pierre ex. A. Froehner cv. lB1. On 2nd June 2018 (tropical cold period), 120-day-old *C. arabica* seedlings and *C. canephora* cuttings were transplanted to 32 L pots (containing a substrate composed of Oxisol and cattle manure, 2:1), which was considered the beginning of the experiment and first day after transplanting (DAT). At this moment, plants had three pairs of leaves and a similar average height of 45 mm and 43 mm in *C. arabica* and *C. canephora*, respectively.

During the experiment, all plants were regularly watered, maintaining well-watered conditions. Agricultural practices of coffee plant cultivation, including fertilization and disease control were used, according to the species demands.

Eight plants of each species were randomly distributed and were grown under distinct UV solar radiation conditions: 1) near ambient UV environment (UVam) inside the mini-greenhouse, with lateral walls and roof of corrugated glass, where were excluded low levels of solar UV (16% UV-A and 0% UV-B, and 2) reduced UV levels (UVre), with a cut of ca. 70% of UV-A and 90% UV-B of the solar radiation, with lateral walls and roof of a transparent polycarbonate screen. Plants were maintained for six months under these conditions before starting with measurements.

Photosynthetic active radiation (PAR, W m^{-2}) in two light environments was recorded using a data logger (model 2000 Weather Stations, Spectrum Technologies, Plainfield, Illinois, USA). The UV radiation (W m^{-2}) in the incident light was monitored with a spectroradiometer (OceanOptics model USB2000+, USA), distinguishing UV-A (315-400 nm) from UV-B (280-315 nm). Measurements of PAR and UV-A and UV-B were performed daily in nine points in each UV environment. All data were collected every 15 min from sensors positioned at the top of coffee canopies within each UV environment. The average diurnal and maximum values were averaged for each month from June to December 2018.

Plant growth traits

Leaf anatomy

On 203 DAT (21st December), leaf imprints from the abaxial leaf surface (from the tagged leaves used for some of the previously mentioned measurements) were taken and observed under a light microscope. Three samples (0.050 mm^2 each) per plant and treatment (n=8) were observed from one field of view. Stomatal density (SD) was determined exactly as previously described by Ramalho et al. (2013).

On 204 DAT (22nd December), leaf blade fragments were obtained from the tagged leaves (n=5) fixed in a 2.5% aqueous solution of glutaraldehyde formal at 4.0% with 50 mM sodium buffer, pH 7.2, washed in the same buffer and post-fixed in 1% aqueous osmium tetroxide solution, in the same buffer for 2 h. After washing again in the same buffer, the fragments were dehydrated in an increasing series of acetone. After dehydration, the fragments were infiltrated with epoxy resin (Epon®). Finally, the samples were soaked in pure resin, placed in molds, and incubated in an oven at 60ºC for 48 h, for polymerization and block formation. In an ultra-microtome (Reicheit Ultracut S), semi-thin cuts, with section thickness of between 0.60 and 0.70 µm, were obtained using a diamond knife (Diatome®). The sections were stained with 1% Toluidine blue for 1 min. Sections were mounted using Entellan® (Merck) and observed under bright field microscopy (Axioplan ZEISS, Germany).

Leaf tissue anatomical values were calculated from cross sections of the middle third of the leaf blade. Using 40x objective were measured the thickness of abaxial cuticle, adaxial cuticle, and epithelia. Under 20x objective were observed

the thickness of palisade and spongy parenchyma. Leaves at five plants per treatment were analyzed (n=5), where 25 fields of view were examined for each repetition. The images obtained were processed and analyzed using Image Pro-Plus digital image processing software (Media Cybernetics, Inc., USA).

Photosynthetic pigments evaluation

Photosynthetic pigment content was evaluated on the 200 DAT (18th December), by collecting one leaf (located in the previously emitted metamer than the one used for leaf expansion measurements) at 13h. Five leaf discs (each of 28.26 mm^2) were cut into fine strips and placed in a test tube containing 5 mL of dimethyl sulfoxide (DMSO) and incubated at 70 °C for 30 min in the dark (Hiscox and Israelstam, 1979). After cooling the extract in the dark, the absorbance of a 3 mL aliquot was analyzed spectrophotometrically (700PlUs Femto, São Paulo, Brazil) at 480, 649 and 665 nm. Chlorophyll (Chl) *a* and *b*, as well as total carotenoid concentrations (µmol g^{-1} of dry mass) were determined according to Wellburn (1994).

Anthocyanin content was determined according to Huang et al. (2014) with a methodology adapted for *Coffea* sp. From the same leaves referred above for Chl, five leaf discs (each of 28 mm^2) were cut into fine strips and placed in a test tube containing 3 mL of methanol + hydrochloric acid (1%) and incubated at 8ºC for 24 h. The content of anthocyanins (µmol g^{-1}) was calculated according to Mancinelli (1975).

Chlorophyll *a* fluorescence

Fluorescence measurements were performed on light-adapted leaves, on the 201 DAT (19th December), in four diurnal periods (08 h, 13 h, 15 h and 17 h). The third pair of leaves counted from the top of branches was used, localized at the plagiotropic axes emitted from the fourth orthotropic metamer that formed plagiotropic branches counting down from the top of the plant. Fluorescence yield changes were estimated using pulse amplitude modulation (PAM) fluorometer MultispeQ V1.0 (PhotosynQ LLC, MI, USA). From these measurements, the various estimations were performed: fraction of PSII centers that were ’open‘ (qL), a

parameter estimating the fraction of PSII centers in open states based on a lake model from the photosynthetic unit), and the estimate of the yield of energy dissipated through non-photochemical photoprotective processes (YNPQ) (Kramer et al., 2004). Linear electron transport (LEF) was estimated from the equation: LEF = f(PAR).YϕII, where f = 0.45, the factor that relates the absorption of PAR and the fraction of absorbed light that is transferred to PSII centers, and ϕII represents the effective quantum yield (Kuhlgert et al., 2016). The series of transmission measurements were performed over a range of progressively increasing light intensities, to increase the dynamic range of results (Kuhlgert et al., 2016).

Statistical analyses

A linear mixed-effects model (lme) was used to perform two- and three-way analysis of variance (ANOVA) to estimate the effects of UV regime, genotype, and their interactions, including effects of diurnal periods of measurements, when present, followed by a Tukey test for mean comparison of treatments. Models were compared by the likelihood ratio test and, when appropriate, reduced models were used. Linear regressions of leaf elongation over time were compared by covariance analyses, up to the end of linear responses. Covariance analyses permitted comparation either among species or among environments for each species. All statistical analyses were performed using R software (R Core Team, 2020), employing the ‘nlme’, ‘emmeans’ (Lenth, 2018) and ‘reshape2 packages, and’ and ‘lm’ function.

RESULTS

Light microclimate

At the beginning of the experiment (June 2018), the average maximum diurnal values of irradiance were 520 and 470 W m^{-2}, decreasing to the lowest recorded values of 410 and 390 W m^{-2} in August, and increasing afterwards to a peak of ca. 900 and 790 W m^{-2} in December (200 DAT), always for UVam and UVre

conditions, respectively (Figures 1A, B). The similar monthly variation pattern along the experimental period was observed in average diurnal monthly PAR (Fig. 1) and UV radiation values (Fig. 2).

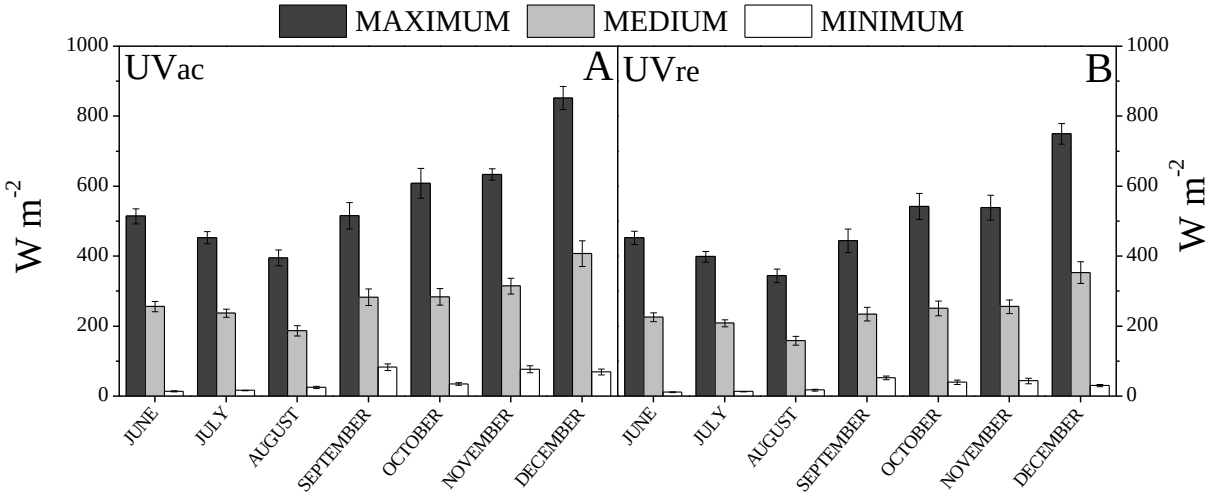

Figure 1: Average monthly values for maximum, medium and minimum solar radiation (W m^{-2}) in either **A**) ambient (UVam) or **B**) reduced (UVre) UV conditions. The values represent the monthly averages based on diurnal averages (± 12 hours) registered during the second semester of 2018.

The monthly average diurnal maximum UV-A values ranged between 14 and 20 W m^{-2} for UVam, and between 4 and 6 W m^{-2} for UVre, what represented ca. 70% reduction in the latter (Figures 2A and 2B). Regarding the monthly average diurnal maximum UV-B radiation, the values ranged between 0.6 and 1.4 W m^{-2} for UVam, and between 0.2 and 0.4 W m^{-2} for UVre, in the same period, what represented ca. 90% reduction in the latter (Figures 2C and 2D).

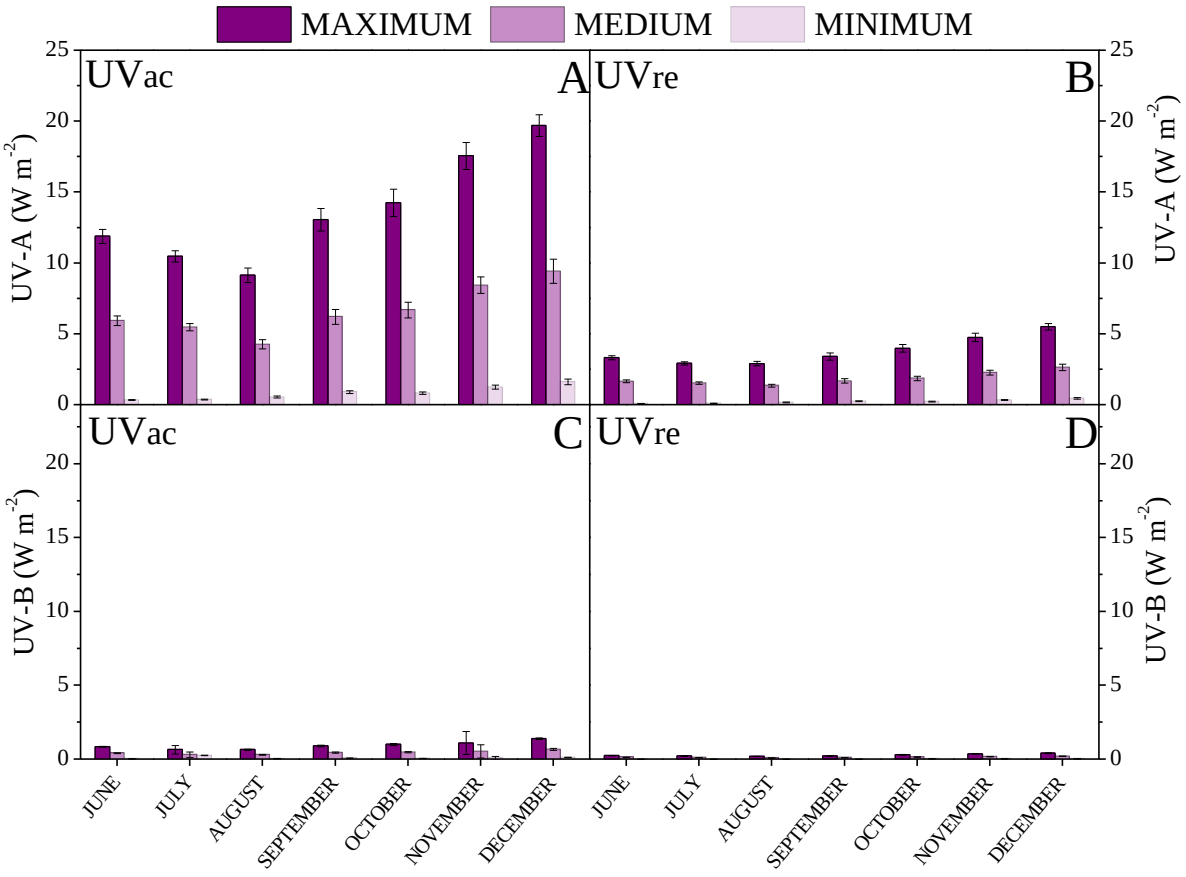

Figure 2: Average values for maximum, medium and minimum of: **A**) UV-A levels in ambient (UVam) environment, **B**) UV-A levels in reduced (UVre) environment, **C**) UV-B in the UVam environment and **D**) UV-B in the UVre environment. The values represent the monthly averages based on diurnal averages (± 12 hours) registered during the second semester of 2018.

Plant scale: morphology and growth traits

The UV regime under which the plants were grown did not show significant impact on plant height and main stem diameter (Figure 3B) but *C. arabica* showed increased height when compared to *C. canephora* in both environments, while the stem diameter in *C. arabica* under UVre was bigger than in *C. canephora* under UVam.

The UVre environment increased the total number of leaves by 21% in *C.*

canephora, and consequently, the total leaf area significantly only in this species (Figures 3C and 3D). Under the UVam, the total number of leaves was significantly lower in *C. canephora* compared to *C. arabica*, while under the UVre two genotypes showed the similar leaf numbers.

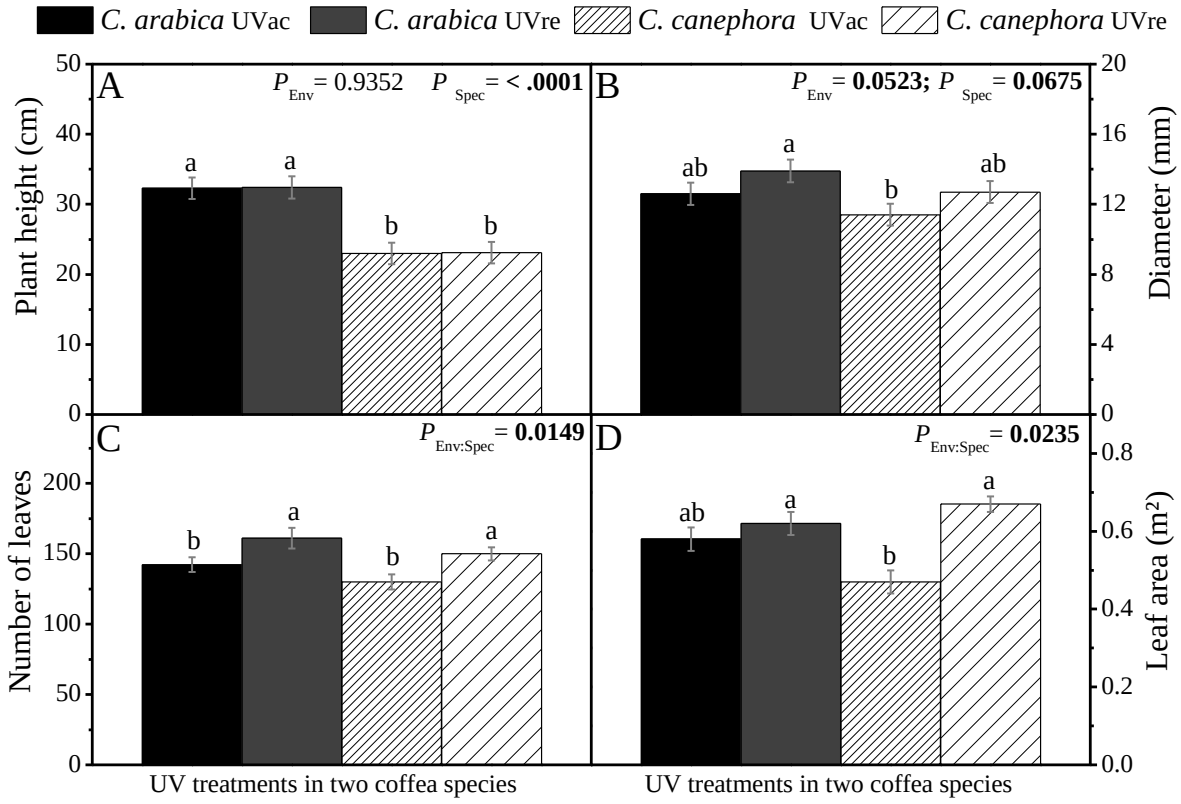

Figure 3: Mean values ± S.E. and ANOVA *P*-values of effects on two species of coffee plants (*C. arabica* and *C. canephora*) cultivated using two UV regimes (UVre and UVam) when considering **A**) plant height, **B**) diameter, **C**) number of leaves and **D**) leaf area (n=8). Different lower-case letters indicate significant differences between species and UV environment.

The UVre condition promoted significant increases of leaf (*ca*. 36%), stem (*ca*. 27%) and root (*ca*. 44%) biomass in *C. canephora*, while in *C. arabica* only of stem (*ca*. 25%) biomass, always as compared with their respective UVam plants (Figures 4A, 4B and C). Interestingly, the UVre condition induced significant reduction in leaf biomass in *C. arabica* (*ca*. 11%). In agreement, total biomass significantly increased 40% in *C. canephora* under UVre compared to UVam, while

C. arabica showed to be irresponsive to UV treatment (Figure 6A). *C. arabica* produced lower total biomass than *C. canephora* under UVre.

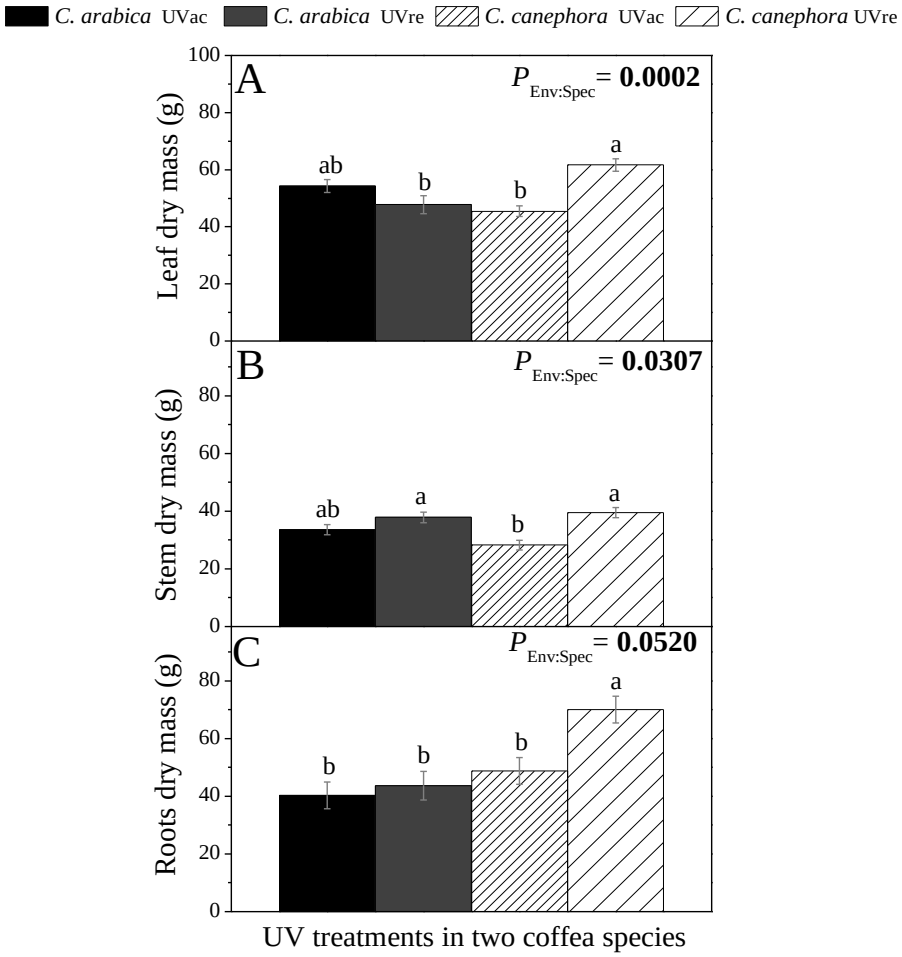

Figure 4: Mean values ± S.E. and ANOVA *P*-values of the effects of two UV regimes (UVre) and ambient (Uvam) for two species of coffee plants (*C. arabica* and *C. canephora*) on **A**) leaf dry mass **B**) stem dry mass and **C**) root dry mass at the end of experiment (204 DAT. n=8). Different lower-case letters indicate significant differences when comparing species and UV levels.

Under UVam, *C. arabica* showed a greater leaf mass allocation than *C. canephora* (42.5 vs. 37.4%), but both genotypes showed similar biomass allocation values (ca. 36-37%) under UVre (Table 1, Figure 5). The allocation in stems was more important in *C. arabica* than in *C. canephora*, regardless of UV condition. *C. arabica* allocated more carbon into stems under UVre compared to UVam (29.6 vs. 26.2%). As regards the biomass allocation in roots, similar values were observed

for each genotype (*C. arabica*: 31-34%; *C. canephora*: 40-41%), regardless of UV condition, although *C. canephora* showed greater investment in roots than *C. arabica.*

Table 1**. A)** Mean values ± S.E. and **B)** ANOVA *P*-values of the effects of UV environment (UV exclusion, -UV and without exclusion, +UV) and species (*Coffea arabica* and *C. canephora*) of allocated biomass into leaves, stems and roots (%)(n = 8). When different, lower-case letters indicate significant levels of species and environment effects

A/	Biomass allocation (%)			
Species	UV environment	*Leaf*	*Stem*	*Roots*
C. arabica	UVam	42.52 ab	26.21 ab	31.25 b
	UVre	37.77 ac	29.56 a	32.67 b
C. canephora	UVam	37.42 b	23.34 a	39.23 b
	UVre	35.89 c	22.88 b	41.22 a
B/				
UV environment		0.1314	**0.0008**	**0.1486**
UV species		0.3224	0.5457	**0.0015**
UV environment : species		**0.0001**	**0.0407**	**0.0937**

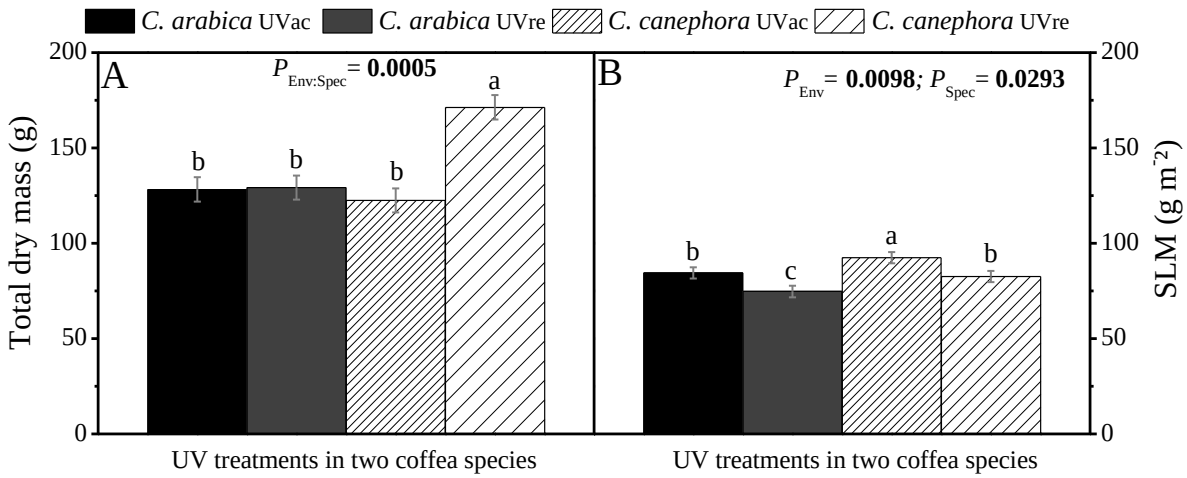

Figure 5: Mean values ± S.E. and ANOVA *P*-values of effects of species (*C. arabica* and *C. canephora*) and UV levels (UVre and UVam) on biomass allocation in the leaves, stems and roots.

The exposure to UVre significantly decreased the SLM (Figure 6B), similarly in both species, although *C. canephora* showed higher values than *C. arabica* for each UV treatment.

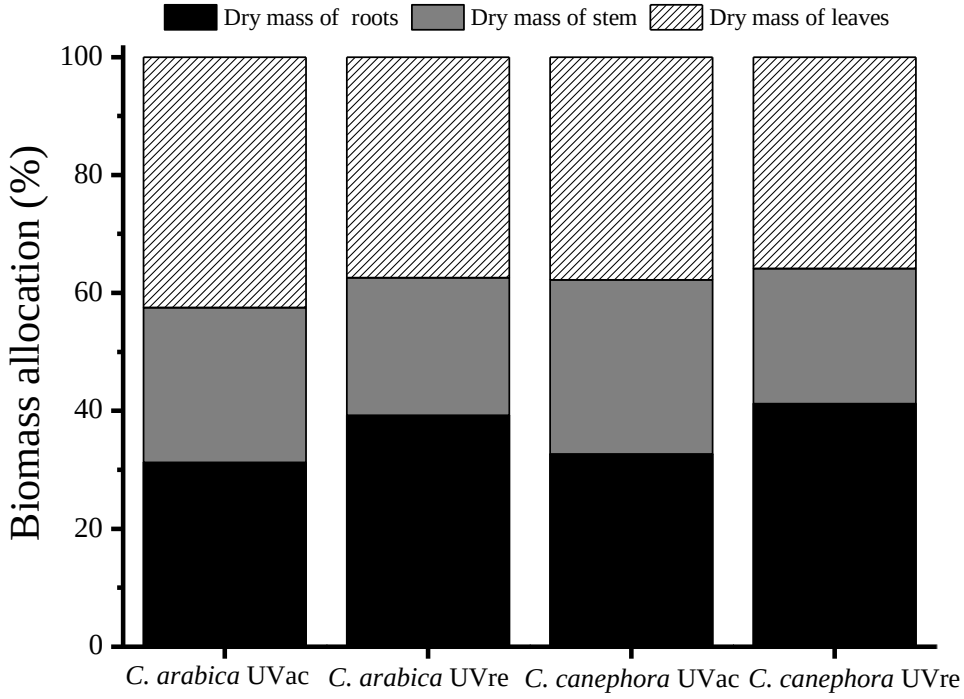

Figure 6: Mean values ± S.E. and ANOVA *P*-values showing the effects of species (*C. arabica* and *C. canephora*) and UV levels (UVre and UVam) on **A**) total plant dry mass and **B**) SLM. Different lower-case letters indicate significant differences when comparing species and UV levels.

Leaf scale: tissue thickness over the vertical cut and leaf elongation

A reduction in the UV level (UVre) significantly decreased the thickness of the abaxial (AbC) and adaxial (AdC) cuticle layer in both species (Table 1). No differences were observed between species for each UV treatment in both AbC and AdC, except for a greater value of AdC in *C. arabica* than in *C. canephora* under UVam. AbC decreased ca. 21% and 22% under UVre compared to UVam for *C. arabica* and *C. canephora*, respectively.

The thickness of abaxial epidermis decreased in *C. arabica* under UVre, while thickness of adaxial epidermis was mostly irresponsive to UV decline, in both species (Table 1). Among the two species, *C. arabica* displayed greater thickness

of abaxial epidermis than *C. canephora* under UVam, and of adaxial epidermis under UVre.

As regards the leaf palisade and spongy parenchyma thickness, no significant changes were promoted by UVre in both species, as compared to their respective UVam values (Table 2, Figure 3). However, it was noteworthy that *C. arabica* showed a lower thickness than *C. canephora* in the palisade parenchyma, whereas the opposite was observed for the spongy parenchyma, always for both UV conditions.

The UVre environment decreased stomatal density in *C. canephora* when compared to the UVam environment (Table 1). The higher stomatal density was observed in *C. canephora* than *C. arabica*, regardless of UV condition.

The main leaf vein elongation rate in *C. arabica* was in average 0.4318 cm and 0.5096 cm for the two days interval, attaining length of 10.68 and 11.64 cm at the end of linear elongation period, always for UVam and UVre respectively (Figure 7). In contrast, *C. canephora* showed a greater leaf elongation under UVam, with the average elongation rate of 0.5148 cm and 0.4373 cm for the two days interval, attaining length of 12.31 cm and 11.28 cm at the end of linear elongation period, always for UVam and UVre respectively.

Table 2**. A)** Mean values ± S.E. and **B)** ANOVA *P*-values of the effects of UV environment (UV exclusion, -UV and without exclusion, +UV) and species (*C. arabica* and *C. canephora*) of anatomy parameters and stomatal density (%)(n = 8). When different, lower-case letters indicate significant levels of species and environment effects

	C. arabica		*C.canephora*				
	UVam	UVre	UVam	UVre	UV env	UV spe	UV env/spe
Abaxial cuticle (µm, 40x)	3.45 (± 0.17) a	2.72 (± 0.17) b	3.29 (± 0.17) a	2.56 (± 0.17) b	**0.0003**	0.2892	
Adaxial cuticle (µm, 40x)	4.30 (± 0.14) a	2.69 (± 0.14) c	3.72 (± 0.14) b	2.90 (± 0.14) c			**0.0149**
Abaxial epidermis (µm, 40x)	17.18 (± 0.87) a	13.88 (± 0.87) ab	13.08 (± 0.87) b	13.04 (±0.87) b	0.0781		
Adaxial epidermis (µm, 40x)	22.35 (± 0.66) a	20.83 (± 1.15) a	19.74 (± 0.85) b	20.27 (± 1.23) b	0.5347	**0.0602**	
Palisade parenchyma (µm)	57.08 (± 2.17) b	55.64 (± 3.22) b	63.44 (± 5.09) a	66.02 (± 3.49) a	0.8489	**0.0135**	
Spongy parenchyma (µm)	164. 0 (± 7.12) a	161.0 (± 7.12) a	138.0 (± 7.12) b	135.0 (± 7.12) b	0.6915	**0.0072**	
Stomatal density (number mm-2)	192.0 (± 11.7) c	200.0 (± 12.5) c	320.0 (± 11.7) a	291.0 (± 11.7) b			**0.00884**

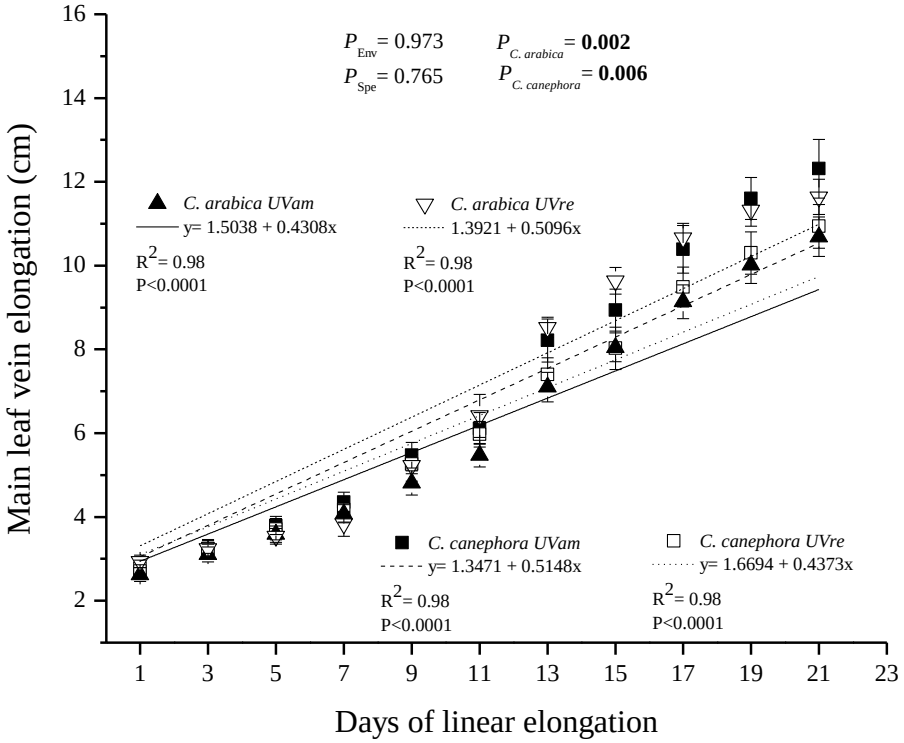

Figure 7: Main leaf vein elongation measured between 172 and 192 DAT (Day 1 and 21) for *C. arabica* and *C. canephora* (Spe) grown under near ambient UV (UVam) and reduced (UVre) levels (Env). Mean values ± S.E. (n=8) and ANOVA *P*-values are shown.

Leaf physiological responses: photosynthetic pigments and chlorophyll a fluorescence

The UV conditions did not significantly impact chlorophyll (Chl) a, Chl b, and Total Chl in the two species (Figures 8A, 8B and C). The Chl a/b ratio increased under UVre in *C. arabica* but was not impacted by UV conditions in *C. canephora* (Figure 5D). On the other hand, total carotenoid content significantly decreased under UVre in both species (Figure 8E). This implicated an increased tendency in ratio of Total Chl/Total carotenoids under UVre in *C. arabica* (Figure 8F).

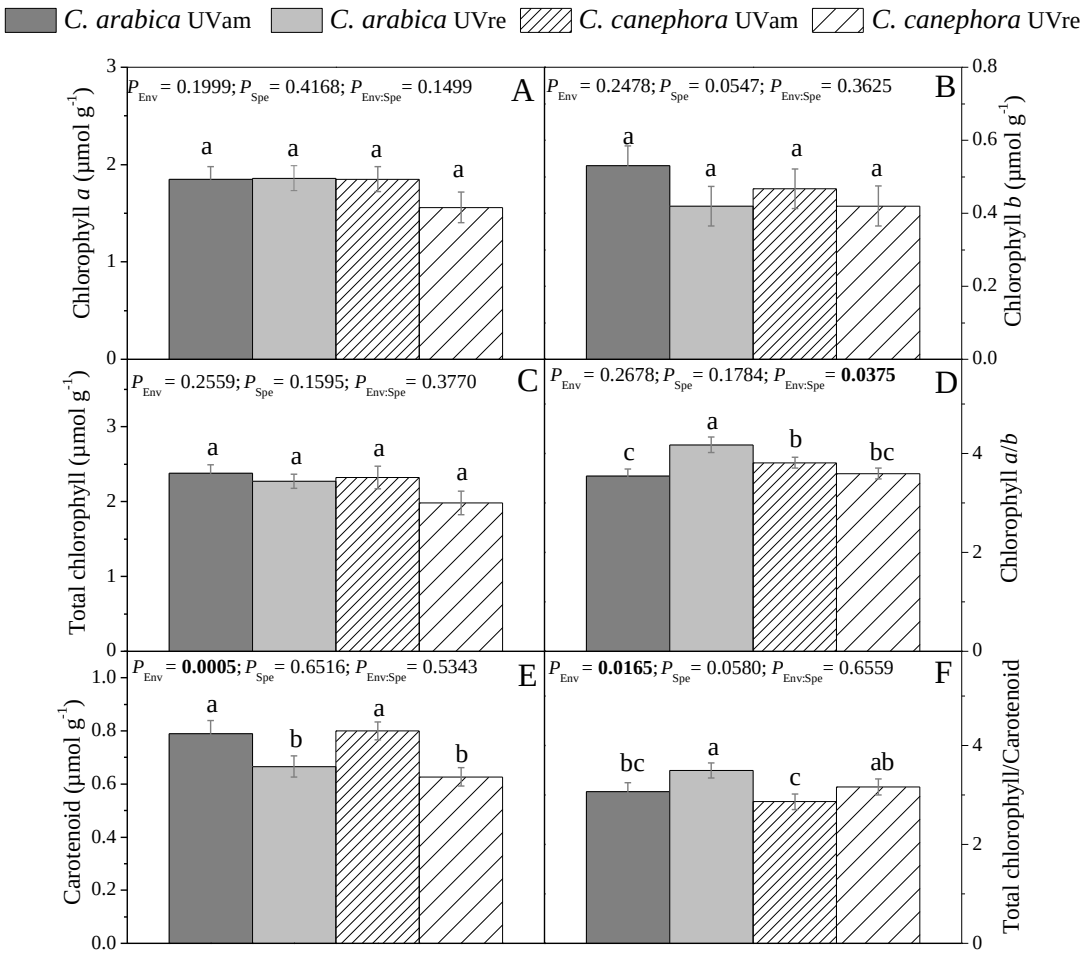

Figure 8: **A**) Variations in contents of chlorophyll *a* (A), b (B), and total (C), chlorophyll *a*/*b* ratio (D), carotenoid content (E) and total chlorophyll /carotenoid ratio for *C. arabica* and *C. canephora* (Spe) grown under UV near ambient (UVam) and reduced (UVre) levels (Env). Different lower-case letters indicate significant differences when comparing species and UV effects. The marginal significance was considered as 0.1. Mean values ± S.E. (n=8) and ANOVA *P*-values for effects of species and UV regimes are shown.

Linear electron transport (LEF), measured in light-adapted leaves, was not altered by UV radiation (Figure 9A). However, higher LEF values were obtained in *C. canephora* maintaining greater values than *C. arabica* in all evaluated diurnal periods. LEF values were higher until 13 h, decreasing afterwards, for both species and both UV conditions.

The fraction of 'open' PSII centers (qL) was reduced under UVre when compared to UVam similarly in both genotypes (Figure 9B). Along the diurnal period,

similar qL values for each treatment were maintained until 15 h, increasing only by 17 h.

The ratio of energy dissipated through non-photochemical processes ($Y_{(NPQ)}$) also decreased under UVre environment in both species, as compared to their respective UVam values, in all evaluated hours (Figure 9C). Notably, *C. arabica* maintained greater $Y_{(NPQ)}$ values than *C. canephora* in both UV conditions and along the diurnal period, reflecting a higher energy dissipation through non-photochemical processes. Regarding the evaluation of the diurnal period, higher $Y_{(NPQ)}$ values were observed in the period of 13 to 15h than at 8h and 17h.

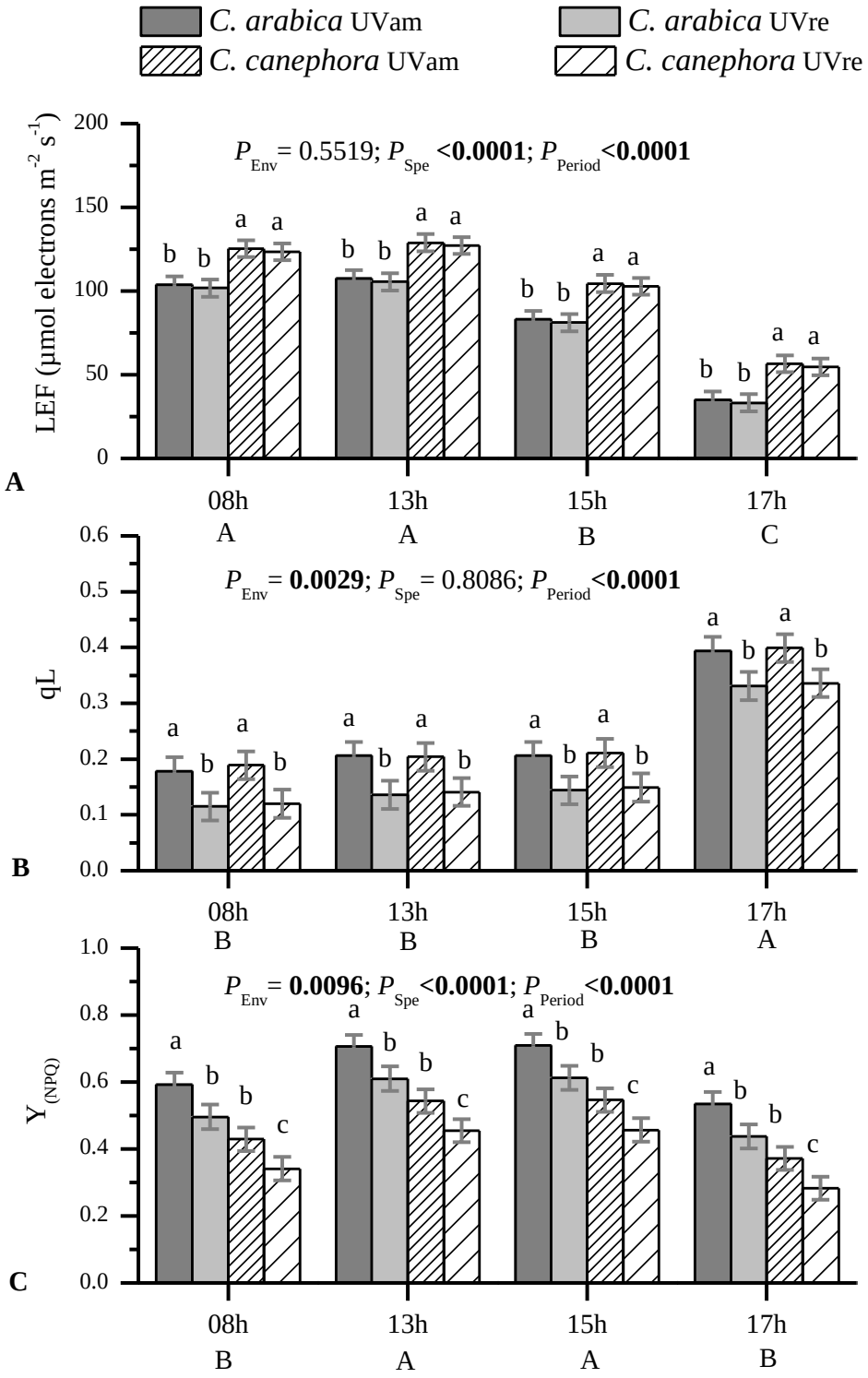

Figure 9: Linear electron transport- LEF (A), the fraction of "open" PSII centers that were – qL (B), and the yield for dissipation by downregulation – Y(NPQ) (C), measured in light-adapted leaves during four diurnal periods (08:00 h, 13:00 h, 15:00 h and 17:00 h) for *C. arabica and C. canephora* (Spe) grown under near ambient (UVam) and reduced (UVre) UV levels (Env). Mean values ± S.E. (n=8)

and ANOVA P-values are shown. Different lower-case letters indicate significant differences when comparing species and UV effects during each diurnal period, whilst different upper-case letters below the x-axis indicate significant differences between diurnal periods.

DISCUSSION

The findings of this study offer an integrated view at whole plant and leaf scale of morphological, anatomic, and photochemical impacts of a reduction in UV-A and UV-B radiations on young plants of the two economically most important *Coffea* species.

Morphological and anatomical responses to UV radiation

Morphological and anatomical responses supported our initial hypothesis, for example, near ambient UV solar radiation intensity is provoking an impact in *Coffea* sp., differently among coffee species. In fact, increased participation of UV in ambient solar radiation negatively affects biomass accumulation of some other species, such as soybean (Guruprasad et al., 2008), sorghum (Kataria and Guruprasad, 2012a) and wheat (Kataria and Guruprasad, 2012b). Furthermore, leaf expansion is one of the most sensitive growth parameters impacted by UV-B radiation (Kakani et al., 2003). The somewhat lower responsiveness at plant and leaf scale in *C. arabica* was probably related to species sites of origin, for example, high altitude of African tropical rainforests for *C. arabica* e and large forest stands with altitudes lower than 1200 m for *C. canephora* (Ferrão et al., 2019). In fact, high altitudes naturally receive greater levels of UV solar radiation when compared to the low altitude sites of origin of *C. canephora*, which could naturally select adaptations to UV solar radiation intensity in *C. arabica*. In addition, the modern *C. arabica* genotype used in our study has been selected for cultivation as a monoculture in full sunlight, which may also contribute to UV tolerance to some extent (DaMatta et al., 2019), therefore, supporting a greater stability regardless of our UV conditions.

Despite similar main leaf vein elongation rate between environments for both genotypes (Figure 7), UVre increased leaf area in C. canephora associated

with greater number of leaves (Figure 1). Leaf area determines light interception, thus, it is an important trait in determining crop growth and yield (Koester et al., 2014). In fact, *C. canephora* had increased total biomass under UVre (Table 1). Therefore, reducing UV levels on coffee canopy, especially on *C. canephora*, could be potential strategy for increasing coffee yield to some extent.

Both species showed higher SLM under UVam (Figure 6), as also observed in several previously studied species such as soybean and cucumber (Murali et al., 1986; Britz et al., 1994), which could be related to the increased investment in mesophyll cells. Leaf cell number, dimension and mass density determine SLM (John et al., 2017). An increase in SLM related to increased leaf density might cause mesophyll cells to be densely packed (Weraduwage et al., 2016), or to increased accumulation of metabolites (Li et al., 2013), predominantly starch (Britz et al., 1994). The SLM was significantly higher in *C. canephora* than in *C. arabica*. The increasing metabolic mass (leaf thickness) is favored in high altitude vegetation, as a key strategy of high-altitude plants for efficient resource capture and use in harsh environments (Thakur et al., 2019). This is opposite to differential altitude in sites of origin of two *Coffea* species. It is worth noting that a genotype-dependent resource allocation was observed, regardless of the UV regime, with *C. arabica* displaying greater investment in the stem, while *C. canephora* had greater investment in the roots (Figure 3). However, *C. arabica* displayed lower leaves and greater stem investment under UVre, suggesting an acclimation at the leaf level and higher resources allocated greatly more to storage in the stem.

Notably, both coffee genotypes representing two species, showed a greater investment in both abaxial and adaxial cuticles under UVam, which is likely acting as a protective mechanism in coffee leaves in relation to the UV solar radiation intensity. In fact, the increased cuticle thickness provide protection against mechanical injuries and environmental changes (Paoletti, 2005; Domínguez et al., 2010), being considered the first leaf barrier to high UV levels, especially of UV-A radiation (Krauss et al., 2005). This is associated with biochemical defense mechanisms, since cuticle tissue contains phenolic compounds, such as cinnamic acids, flavonoids and flavones (Kolb and Pfündel, 2005). The cuticle also has a screening potential for UV radiation and an antioxidant capacity (Peng et al., 2009). Additionally, as superficial tissues, the cuticle layers in adaxial and abaxial leaf superficies act also as biophysical barriers by light reflectance and scattering,

reducing light absorption by epidermal layers (Rozema et al., 1997). Additionally, *C. arabica* displayed greater epidermis thickness than *C. canephora* under UVam what might promote a better ability of to cope with higher UV radiation levels through epidermal transmittance and screening (Day et al., 1993; Bilger et al., 1997; Nybakken et al., 2004; Barnes et al., 2013).

Our findings suggest that mesophyll thickness differed between the two coffee species, with *C. canephora* having a thicker palisade parenchyma, whereas *C. arabica* displaying a greater thickness a spongy parenchyma, both leaf tissues being irresponsive to altered UV conditions (Table 2). Phenolic synthesis in the leaves occurs in the mesophyll tissue and can have substantial role in UV attenuation by scattering the short electromagnetic wavelengths by those molecules (Caldwell et al., 1983). However, in this present research, modifications of the UV levels did not show significant impact on anthocyanin content (Supplementary material, Figure S1), but greater content in *C. arabica* than in *C. canephora*, supporting the segregation in adaptability of the two species.

Stomata play a crucial role in the control of leaf photosynthesis, regulating the precise balance between CO_2 fixation and water loss to the atmosphere (Jones, 1998). In this way, the balance of stomatal size and density is crucial to determine the diffusion of CO_2 into the leaf. Interestingly, UV levels promoted changes in stomatal density (SD) in a species-dependent manner (Table 2). Under the two UV conditions, *C. arabica* did not show any difference in SD, whilst *C. canephora* reduced SD under UVre. Genotype-dependent manner in responses to UV-B is observed in rice (Dai et al., 1995), and soybean (Gitz III et al., 2005) more reducing SD on the adaxial surface than on the abaxial surface in responding genotypes. Coffee leaves develop stomata only in the abaxial side, which is still an adaptation to excess of light (Morais et al., 2004). Anatomic modifications, such as changes in SD, can modify the stomatal conductance (Dow et al., 2014). The SD increment was associated with an increase of stomatal conductance during the 12 h to 14 h diurnal periods (data not shown). In this way, the anatomic changes at stomatal level, together with those observed for both cuticle and epidermal thickness could, in turn, affect the leaf gas exchange dynamics (Ren et al., 2019).

Physiological responses to UV radiation

Chl *a* and *b* contents were not impacted by UV levels, although the *a/b* chlorophyll ratio declined in *C. arabica* under UVam (Figure 8). This suggested that the near ambient UV levels might affect the organization of LHC, before having an impact in Chl content. In our study, near ambient UV level provided a better adaptive advantage for *C. arabica* than for *C. canephora* leaves, indicated by reduced *a/b* chlorophyll ratio. The synthesis of Chl *b* confers an advantage by stronger absorption of a wider range of light waves (Hoober et al., 2007). Chl *b* is synthesized from Chl *a* and is catabolized after it is reconverted to Chl *a* (Tanaka and Tanaka, 2011). Chl *b* levels are determined by the activity of the three enzymes participating in the chlorophyll cycle, namely, chlorophyllide a oxygenase, chlorophyll b reductase, and 7-hydroxymethyl-chlorophyll reductase, which are being more resistant to proteolysis than those that determine the Chl *a* activity related to photochemistry (Tanaka and Tanaka, 2011).

The Chl *a/b* ratio modifications in *C. arabica* leaves suggested acclimation to ambient UV levels of this species, as occur as a general angiosperm adaptation to various light spectrum ranges (Tanaka et al., 1991). In this context, the reduction of the Chl *a/b* ratio changes under UVam in *C. arabica,* suggested that coffee leaves adaptively developed rearrangement of chlorophylls in the LHCs, to improve the efficiency of photosynthetically active radiation (Tanaka et al., 1991). UVam plants, as acclimation response, probably had fewer PSII polypeptides, preferential loss of chloroplast proteins and a deficiency in the Chl *a/b* LHC, as found in acclimation to high irradiance in coffee (Nunes et al., 1993). Additionally, UVam maintained a greater carotenoid content than UVre, in both genotypes, suggesting a higher need for chlorophyll photoprotection from eventual photo-oxidative conditions triggered by higher UV (Agrawal et al., 2007). This is in line with the greater non-photochemical energy dissipation ($Y_{(NPQ)}$) in the UVam plants of both genotypes, what reflects a stronger photoprotective mechanism for energy dissipation (Müller et al., 2001), as compared to their UVre counterparts. Moreover, such higher $Y_{(NPQ)}$ values in UVam plants were accompanied greater qL values, thus reflecting a more efficient photochemical energy use (qL), despite the absence of differences in LEF between UV conditions (Figure 9). Altogether, these results showed no strong physiological differences between genotypes, which suggested a total acclimation

to UVam (with higher carotenoid content and $Y_{(NPQ)}$ that allow the plants to show even greater photochemical performance (qL) than the plants under UVre.

CONCLUSION

In the evolution of two economically important coffee species, from forest shade in their African centers of origin to the monoculture cultivated under the full sunlight, various acclimations were developed to mitigate the possible damages caused by increased levels of UV solar radiation. Our study showed these acclimations at whole plant and leaf scales. Under UVam, both species increase SLM, carotenoid content, leaf abaxial and adaxial cuticle thickness, q_L and $Y_{(NPQ)}$, while decreased leaf and stem dry mass and Chl/carotenoid ratio. Despite some morphological and anatomical differences among species to UVam, such as: i) reduced root and total biomass, number of leaves and leaf area, with increased leaf elongation rate and SD in *C. canephora*, and ii) reduced biomass allocation in stems, leaf elongation rate and Chl a/b ratio, with increased abaxial epidermis thickness in *C. arabica*, no species difference had been observed at photochemistry. This suggested a total acclimation to UVam (with higher carotenoid content and $Y_{(NPQ)}$) that allow the plants to show even greater photochemical performance (q_L) than the plants under UVre. The interlinked responses demonstrated that: i) the UVam levels can generate significant modifications in plant and leaf morphology in coffee plants, and that ii) these changes act as an acclimation mechanism to near ambient UV level, resulting in protection of the plant and increased efficiency in energy dissipation, leaf functions and biomass production.

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