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PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS AGRÁRIAS - AGRONOMIA

PHYSIOLOGICAL AND PRODUCTIVITY ATTRIBUTES OF AMAZONIAN ROBUSTAS (C*offea canephora*) CULTIVATED IN RONDÔNIA, BRAZIL

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Rio Verde – GO
julho - 2022

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APROVADO em, 29 de julho de 2022.

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BIOGRAFIA DO AUTOR

Aldo Max Custodio, born in Costa Rica, Mato Grosso do Sul, Brazil, son of Ceila Furtado Custodio and Adelipe Joaquim Custodio. Agronomist graduated from the State University of Mato Grosso (UNEMAT) in 2011. Science Master in Agroecology from the Federal University of Viçosa (UFV) in 2014.

In 2016, he joined to effective teaching staff at the Federal Institute of Education, Science and Technology of Rondônia (IFRO), *Campus* Colorado, as a professor of Agronomy. He teaches mainly in the Agronomy and Agricultural Technician courses, especially in the areas of Agroecology and Plant Production. He has dedicated himself to research and extension activities with a view to the development and improvement of technologies for tropical agroecosystems management, mainly coffee plantations. In 2018, he joined as a regular student in the Doctoral course, in the Postgraduate Program in Agrarian Sciences - Agronomy, of the Federal Institute of Education, Science and Technology Goiano (IF Goiano), Campus Rio Verde, obtaining the title of Doctor of Science in the year 2022.

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LIST OF SYMBOLS, ABBREVIATIONS, AND UNITS

ABBREVIATION	DESCRIPTION	UNIT
A	net carbon assimilation	μmol m^{-2} s^{-1}
ABS/RC	absorbed energy by primary quinone reduced (Q_a^-) by reaction center (RC) (t = 0)	-
Area	area under the fluorescence curve	-
*c*a	ambient (external) CO_2 concentration	μmol/mol
C_{ft}	leaf bulk capacitance	mol m^{-2} MPa
Chl a	chlorophyll a	-
Chl b	chlorophyll b	-
Chlt	total chlorophyll	-
c_i	internal CO_2 concentration	μmol/mol
DIo/RC	dissipated energy by Q_a^- by RC (t = 0)	-
E	transpiration rate	mol m^{-2} s^{-1}
ETo/RC	transported energy by Q_a^- by RC (t = 0)	-
F_0	Initial fluorescence	-
F_{300}	fluorescence intensity at = 300μs	-
f_{gc}	fraction of epidermis allocated to stomata	%
F_i	fluorescence at t=60 ms	-
Fix Area	area under the fluorescence curve between F at 40μs and F at 1s	-
F_j	fluorescence at t=2 ms	-
F_m	maximum fluorescence	-
F_v	variable fluorescence	-
$g_{canpopy\text{-}res}$	canopy minimum conductance	mmol s^{-1}
$g_{leaf\text{-}res}$	leaf minimum conductance	mmol m^{-2} s^{-1}
g_s	stomatal conductance	mmol m^{-2} s^{-1}
g_{wmax}	theoretical maximum stomatal conductance	
HSM_{50}	hydraulic safety margin at P50	MPa
HSM_{88}	hydraulic safety margin at P88	MPa
iWUE	intrinsic water use efficiency	
K_{leaf}	leaf hydraulic conductance	mmol m^{-2} s^{-1} MPa
LWC	leaf water content	%
M_o	approximate initial slope of the fluorescence transient normalized with Fv	-
N	turnover number of Q_a	-
P_{50}	Ψ leaf inducing 50% loss of leaf hydraulic conductance	MPa

P_{88}	Ψ leaf inducing 88% loss of leaf hydraulic conductance	MPa
Phi_{Do}	quantum yield of energy dissipation	-
Phi_{Eo}	quantum yield of electron transport	-
Phi_{Pav}	effective (average) quantum yield of the primary photochemical reactions of photosystem II (t=0)	-
Phi_{Po}	maximum quantum yield of the primary photochemical reactions of photosystem II (t=0)	-
PI_{ABS}	performance index on the ab-sorption base	-
Psi_{o}	probability of an exciton moving an electron in the electron transport chain beyond Q_a.	-
RWC	relative water content	%
SD	stomatal density	Estômato cm^{-2}
S_{m}	normalize area	-
SS	stomata size	μm^2
S_{s}	smallest possible Sm	-
Te	temperatura ambiente	ºC
T_{Fm}	time to maximum fluorescence	-
THF	time to hydraulic failure	dia
T_{L}	leaf temperature	ºC
TRo/RC	trapped energy by Q_{a}^{-} by RC (t = 0)	-
*V*d	vein density	mm mm^{-2}
V_{i}	proportion of Fv until Fi	-
V_{j}	proportion of Fv until Fj	-
VPD	vapour pressure déficit	kPa
VPD_{L}	VPD between the leaf and the air of the IRGA chamber	kPa
WUE	instant water use efficiency	μmol CO$_2$/mol H$_2$O m^{-2} s^{-1}
Ψ_{md}	midday water potential	MPa
Ψ_{tlp}	turgor loss point	MPa

ABSTRACT

CUSTODIO, ALDO MAX. Instituto Federal Goiano – Campus Rio Verde – GO, July 2022. **Physiological and productivity attributes of Amazonian Robustas (C*offea canephora*) cultivated in Rondônia, Brazil.** Advisors: Fabiano Guimarães Silva (Program) and Jairo Rafael Machado Dias (External).

The coffee (*Coffea canephora*) cultivation is one of the main economic activities in the state of Rondônia, in Brazilian Amazon, however, there is a lack of studies about physiology and ecophysiology aspects of the cultivated genotypes. The objective of the research in this thesis was to study physiological parameters of coffee trees cultivated in Rondônia and their implications for management and production. Two different works were carried out. In one of them, we explored how variations in morphoanatomical and physiological leaf traits can explain differences in yield and vulnerability to embolism in eleven *Coffea canephora* genotypes cultivated in Rondônia state. It was observed that higher efficiency in water transport (*K*leaf) was strongly related to an increase in *A*, revealing a diffusive limitation to photosynthesis associated with hydraulic restrictions. All genotypes showed a narrow or negative hydraulic safety margin (HSM), suggesting a high leaf vulnerability to drought-induced mortality (DIM) under non-irrigated conditions, especially on the most productive genotypes. Modeling exercises revealed that variations in DIM across genotypes were associated with differences in leaf and canopy water leaks, as a reflex of their contrasting growth strategies. In the other study the seasonal variation of parameters associated with photosynthesis was studied. The study was carried out under field conditions, in the municipality of Rolim de Moura. Variables of gas exchange, chlorophyll a fluorescence and pigment index of three genotypes (clones 03, 08 and 25), cultivated in three cropping systems (fertigated, irrigated and dryland farming), were evaluate at four times throughout the production cycle (peak drought, onset of rain, rainy season, onset of drought). It was observed that there is a reduction in the net photosynthetic rate (*A*) in the dry period, associated with a drop in stomatal conductance (gs). Use of irrigation reduces the effect of the dry period, but it is not enough to maintain the levels of *A*, observed in the other seasons. In this case, the air vapor pressure deficit (DPVar) is associated with lower gs. Higher values of anthocyanin were observed in coffee leaves during the dry season. The yields obtained could not be explained by the variables obtained at the leaf level. Overall, our results provide a new perspective on the challenges of sustaining coffee production in the Amazon region under a drier and warmer climate. Amazonian Robustas reduce A with the increase in VPD, this decrease is associated with a decrease in gs. The use of localized irrigation, widely adopted in the region, mitigates, but does not prevent, the negative effect of the elevation of VPD on A. The most productive materials present higher $g_{\text{leaf-res}}$, significantly reducing the time to hydraulic failure (THF) of the leaves. The selection of genotypes with greater drought tolerance is essential to anticipate the deleterious impact of water restriction predicted for the region.

Keywords: Coffee, Amazon, photosynthesis, drought, hydraulic failure.

RESUMO

CUSTODIO, ALDO MAX. Instituto Federal Goiano – Câmpus Rio Verde – GO, julho de 2022. **Atributos fisiológicos e produtividade de Robustas Amazônicos (*Coffea Canephora*) cultivados em Rondônia, Brasil**. Orientadores: Fabiano Guimarães Silva (Programa); Jairo Rafael Machado Dias (Externo).

O cultivo de café (*Coffea canephora*) é uma das principais atividades econômicas do estado de Rondônia, na Amazônia Brasileira, contudo, há carência de estudos sobre aspectos da fisiologia e ecofisiologia dos genótipos cultivados. O objetivo das pesquisas desta tese foi estudar parâmetros fisiológicos dos cafeeiros cultivados em Rondônia e suas implicações no manejo e produção. Foram realizados dois trabalhos distintos. Em um deles foi avaliado a possibilidade de explicar a produtividade e vulnerabilidade a embolia de 11 genótipos de *C. canephora* cultivados em Rondônia a partir das características morfoanatomicas e fisiológicas das folhas. Foi observado que maior eficiência no transporte de água (K_{leaf}) esteve fortemente relacionado com aumento na assimilação de carbono (*A*), revelando uma limitação difusiva à fotossíntese associada a restrições hidráulicas. Todos os genótipos apresentaram margem de segurança hidráulica (HSM) estreita ou negativa, sugerindo uma alta vulnerabilidade foliar à mortalidade induzida por seca (DIM) em condições não irrigadas, principalmente nos genótipos mais produtivos. Modelagens efetuadas revelaram que as variações na DIM entre os genótipos estavam associadas a diferenças nos vazamentos de água nas folhas e no dossel, como reflexo de suas estratégias de crescimento contrastantes. No outro, foi estudado a variação sazonal de parâmetros associados a fotossíntese. O estudo foi realizado em condições de campo, no município de Rolim de Moura. Foram avaliadas variáveis de trocas gasosas, fluorescência da clorofila *a* e índice de pigmentos de três genótipos (clones 03, 08 e 25), cultivados em três sistemas de cultivos (fertirrigado, irrigado e sequeiro), em quatro épocas ao longo do ciclo produtivo (pico da seca, início da chuva, período chuvoso, início da seca). Foi observado que ocorre uma redução da taxa fotossintética líquida (*A*) no período da seca, associada a queda na condutância estomática (*gs*). O uso da irrigação reduz o efeito do período seco, mas não é suficiente para manter os níveis de *A*, observados nas outras épocas. Neste caso, o déficit de pressão de vapor do ar (DPVar) está associado a menor *gs*. Foi observado maiores valores de antocianina nas folhas dos cafeeiros no período da seca. As produtividades obtidas não puderam ser explicadas pelas variáveis obtidas em nível de folha. Os resultados obtidos a partir dos dois trabalhos fornecem uma nova perspectiva sobre os desafios preocupantes para a produção de café na região amazônica sob um clima mais seco e quente. Os Robustas Amazônicos reduzem a A com o aumento no VPD, esse decréscimo está associado a queda da g_s. O uso de irrigação localizada, amplamente adotada na região, mitiga, mas não impede o efeito negativo da elevação do VPD sobre A. Os materiais mais produtivos apresentam maior $g_{\text{leaf-res}}$ reduzindo significativamente o tempo para falha hidráulica (THF) das folhas. A seleção de genótipos com maior tolerância à seca é fundamental para antecipar o impacto deletério da restrição hídrica prevista para região.

PALAVRAS-CHAVES: Café, Amazônia, fotossíntese, seca, falha hidráulica.

GENERAL INTRODUCTION

The species *Coffea canephora* Pierre ex. Floehner is originally from Africa and has five genetic groups (CUBRY et al., 2013; MONTAGNON et al., 2012). It is known commercially as “Conilon” coffee in Brazil or “Robusta” coffee in other parts of the world (GARAVITO et al., 2016). Vietnam, Brazil, and Indonesia are the biggest producers, with around 38, 27 and 13%, respectively, of a total of around 74 million bags (60 kg) produced in the 2020/2021 harvest (USDA, 2022).

In Brazil the gross value of coffee production (VBP) was estimated at around R$ 41 billion in the year 2021, with more than R$ 11 billion from the cultivation of *C. canephora* (MAPA, 2022). The state of Rondônia accounts for about 15% of the national production of conilon coffee, that is approximately 2.4 million bags in 2021 (CONAB, 2022). Coffee cultivation in this part of the Amazon dates to the beginning of its colonization process, in the 1970s. However, in the last 20 years the coffee culture developed there has undergone significant transformations, especially from using clonal seedlings, obtained from plants selected in the field, mainly by farmers (DALAZEN et al., 2020). As reported in the literature, the use of superior clones provides a qualitative increase in fruit uniformity, yield, and quality (CARVALHO et al., 2019).

The coffee tree currently cultivated in Rondônia have their genetic origin in the natural hybridization of genotypes from Espírito Santo state (eastern coastal region of Brazil), belonging to the SG1 or “Conilon” Group (GARAVITO et al., 2016; MONTAGNON et al., 2012), with some genotypes of unknown genetic group, with characteristics of “Robustas” (SOUZA et al., 2013; TEIXEIRA et al., 2017), introduced from the Germplasm Bank of the Instituto Agronomic of Campinas – IAC, Brazil (SILVESTRINI et al., 2008). The selected and cultivated “hybrid” genotypes have distinct characteristics from the 'Conilon' coffee grown in Espírito Santo state and, as they present characteristics found in plants of the “Robusta” group (SG2) (GARAVITO et al., 2016) they are known as Robustas Amazonians.

The Southwest region of the Brazilian Amazon is characterized by two well-defined seasons throughout the year: a rainy season, from October to May, and a dry season, from June to September. Both, with high average temperature, small thermal amplitude, and high availability of light (DUBREUIL et al., 2018; FRANCA, 2015). In the rainy season, the high-water availability in the soil, high relative humidity, and the low values of vapor pressure deficit in the air (DPV) favor the opening of coffee stomata (RODRIGUES et al., 2016; THIOUNE et al., 2017, 2020). The greater opening of the stomata results in an increase in stomatal conductance (g_s) and, consequently, facilitates of CO_2 diffusion into the leaves (MACHADO FILHO et al., 2021). The increase in the internal concentration of CO_2 (c_i) allows an increase in the net photosynthetic rate (A). Under these conditions, the risks of irreversible damage to the photosynthetic apparatus - photodamage or photooxidation - even with high temperatures and/or days with high light intensity are reduced, due to the high demand for energy - ATP and NADPH (SHARMA et al., 2020).

In turn, during the dry season, water restriction can lead to partial or total closure of the stomata more quickly, throughout the day, causing a drop in g_s and, consequently, in c_i, compromising A (GROSSIORD et al., 2020). However, the decrease in the CO_2 reduction rate, in the biochemical step of photosynthesis, reduces the demand for energy and triggers a cascade effect, which can result in photodamage and compromise of the photosynthetic apparatus due to the formation of reactive oxygen species (ROS) (SHARMA et al., 2020). High light intensity and high temperatures can worsen this situation (SILVA, et al., 2019b; VENANCIO et al., 2020).

Compared to other tropical species, coffee plants have relatively low A rates (about 4 to 11 µmol m^{-2} s^{-1}) (DAMATTA et al., 2018). However, under high CO2 concentration, these A values can increase substantially (for example, 30 to 50 µmol m^{-2} s^{-1}), suggesting that the low photosynthetic capacity of coffee plants is mainly associated with diffusive limitation (DAMATTA et al., 2016, 2018; MARTINS. et al., 2014a). Differences in the diffusive capacity of plants are largely associated with their potential to replace water loss through transpiration (E), which requires an efficient hydraulic transport system (K_{plant}) (MARTINS, et al., 2014b; MENEZES-SILVA et al., 2015).

At the leaf level, high hydraulic conductance (K_{leaf}) can be achieved by reducing the average path length for water flow through the mesophyll cells to the stomata

(MCADAM et al., 2017), which usually involves the coordinated investment in a dense network of veins with small and numerous stomatal cells (BRODRIBB et al., 2007; BRODRIBB; JORDAN, 2011; SCOFFONI et al., 2016). Despite the potential of selection based on hydraulic characteristics to reduce diffusive limitations (DAMATTA et al., 2019; MARTINS, et al., 2014b), and thus to improve plant productivity, until now there is no information on variability in hydraulic properties for *C. canephora* genotypes cultivated in the Amazon region, nor how such variability could be related to its carbon assimilation and yield potential.

The close coordination between water carrying capacity and carbon assimilation and coffee productivity highlights the sensitivity of this species to reductions in water availability during cultivation. Drought is considered the main limiting factor for coffee production in the world (DAMATTA et al., 2018). This fact is worrying in a scenario of climatic anomalies with increasingly frequent and intense drought events in the Amazon (PANISSET et al., 2018). Currently, Amazonian Robusta are predominantly cultivated in irrigated systems in Rondônia. In dryland farming system low yields or even no production has been reported in the state. On the other hand, growing coffee in irrigated systems enables high yields, greater than 6 Mg/ha/year of green coffee (SILVA, et al., 2019a; TEIXEIRA et al., 2020).

The genotypes selection with high yield potential commonly results in more vulnerable plants. That is, productivity gains are often accompanied by a reduction in the amplitude of tolerance bands to environmental factors and conditions, such as temperature, radiation, water availability, soil pH, etc. (trade-off) (DWIVEDI et al., 2021). In *C. canephora*, for example, it was observed that plants with higher growth rates are more sensitive to drought (KIWUKA et al., 2022).

Although coffee growing in Rondônia has been successful in recent years, there is a gap in the field of research on the physiology and ecophysiology of the genotypes grown in the state. The knowledge obtained may subsidize other research, including incorporating new perspectives to genetic improvement and plant science. Subsidize the management of coffee plantations and the optimization of the resources use, such as water. At the same time, provide other criteria for the selection or production of genotypes more adapted to adverse growing conditions, such as drought events.

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OBJECTIVES

GENERAL OBJECTIVE

To study physiological traits associated with yield of coffee trees (*Coffea canephora*) cultivated in Rondônia state, Brazil.

SPECIFIC OBJECTIVES

- To analyze morphoanatomical and physiological characteristics associated to the hydraulics of the leaves of *C. canephora* genotypes and their relationship with yield.
- To evaluate the seasonal variation of physiological parameters associated with photosynthesis throughout a productive cycle (year) and its association with coffee tree yield in irrigated, fertigated and dryland farming cultivation system.

CHAPTER 1

(PLANT, CELL AND ENVIRONMENT – ISNN-1365-3040)

A BITTER FUTURE FOR COFFEE PRODUCTION? EXPLORING PHYSIOLOGICAL TRAITS ASSOCIATED WITH YIELD REVEALS HIGH VULNERABILITY TO HYDRAULIC FAILURE IN *Coffea canephora* CULTIVATED IN THE WESTERN AMAZON

Abstract: The increase in frequency and intensity of drought events have hampered coffee production in the already threatened Amazon region, yet little is known about key aspects underlying the variability in yield potential across genotypes, nor to what extent higher productivity is linked to reduced drought tolerance. Here we explored how variations in morphoanatomical and physiological leaf traits can explain differences in yield and vulnerability to embolism in 11 *Coffea canephora* genotypes cultivated in the Western Amazon. The remarkable variation in coffee yield across genotypes was tightly related to differences in their carbon assimilation and water transport capacities, revealing a diffusive limitation to photosynthesis linked by hydraulic constraints. Although a clear trade-off between water transport efficiency and safety was not detected, all the studied genotypes operated in a narrow and/or negative hydraulic safety margin, suggesting a high leaf vulnerability to drought-induced mortality (DIM) under non-irrigated conditions, especially on the most productive genotypes. Modeling exercises revealed that variations in DIM across genotypes were associated with differences in leaf and canopy water leaks, as a reflex of their contrasting growth strategies. Overall, our results provide a new perspective on the challenges of sustaining coffee production in the Amazon region under a drier and warmer climate.

Keywords: Amazon, vulnerability to embolism, drought-induced mortality, leaf minimum conductance, carbon assimilation, leaf morfoanatomy

1.1. INTRODUCTION

Coffee is one of the most important global crops, which supports a multibillion industry that provides the livelihood of millions of people, especially small farmers from developing countries (DaMatta et al., 2018; Davis et al., 2020). From the 126 described species of the genus *Coffea*, only *Coffea arabica* and *Coffea canephora* are economically relevant, accounting for 99% of global production (Davis et al., 2019). In this regard, although the production of *C. arabica* still represents the greatest share of the coffee trade, the economic importance of *C. canephora* has increased markedly in the last decades, especially in Brazil, one of its largest producers. Thus, considering the continuous rise in global demand for coffee, there is an urgent need to improve the *C. canephora* production. However, this increase in coffee production must be coordinated with strategies that minimize the environmental impacts associated with the expansion in land cultivation (Ruiz et al., 2021), especially in areas already threatened by anthropogenic actions, such as the Amazon (Silva Junior et al., 2021), a traditional region for coffee production (Junior et al., 2021; Moraes et al., 2021). Although challenging, an alternative to improve the sustainability of coffee cultivation would involve the increase in production per given land, which may be achieved through a better characterization of the physiological traits associated with coffee yield to allow the selection/generation of improved cultivars.

Compared to other tropical tree species, coffee plants have particularly low rates of carbon assimilation (A; typically ranging from 4 to 11 µmol m^{-2} s^{-1}) (DaMatta et al., 2018). However, under high CO_2 concentration, these A values can increase substantially (e.g., 30 to 50 µmol m^{-2} s^{-1}), suggesting that the low photosynthetic capacity of coffee plants is mainly associated with diffusive constraints (Martins et al., 2014a; Damatta et al., 2016; DaMatta et al., 2018). As for other species, differences in diffusive capacity within coffee plants are largely coupled to their potential to replace the water loss through transpiration (E), which requires an efficient transport system (Martins et al., 2014b; Menezes-Silva et al., 2015).

At the leaf level, high hydraulic conductance (K_{leaf}) can be achieved through the reduction in the mean path length for water flow through the mesophyll cells to the stomata (McAdam et al., 2017), which usually involves the coordinated investment in a dense vein network with small and numerous stomata cells, thus resulting in both higher stomatal conductance (g_{s}) and A rates (Brodribb et al., 2007; Brodribb and Jordan, 2011; Scoffoni et al., 2016). Surprisingly, despite the potential of the hydraulic-based selection to reduce diffusive limitations (DaMatta, 2004; Martins et al., 2014a; DaMatta et al., 2019), and thus improve plant productivity, to date there is no information regarding the variability in hydraulic properties for the *C. canephora* genotypes cultivated in the Amazon region, and as a result, the relationship between *C. canephora* hydraulic traits and their carbon assimilation and yield potential remains entirely unknown.

The tight coordination between water transport capacity with carbon assimilation and coffee yield highlight the sensitivity of this crop species to reductions in water availability (DaMatta et al., 2018). In fact, expressive losses associated with drought episodes have been consecutively reported in several regions of Brazil (CONAB, 2021; 2022). This dramatic scenario is projected to worsen in the coming decades as increasing drought frequency and/or intensity is predicted for almost all Brazilian coffee-producing regions (IPCC, 2021), which likely will result in further losses in productivity (Bunn et al., 2015; Gomes et al., 2020).

The strong impact of drought on coffee growth and production is directly related to impairments in the water transport network of these plants (DaMatta et al., 2018; Martins et al., 2019). In fact, as the soil dries, the tension of water within xylem vessels intensifies until the water column breaks, and air enters (termed cavitation) (Tyree and Sperry, 1989; Brodribb et al., 2020). The gas bubbles generated from cavitation events can rapidly expand, creating large embolisms inside xylem vessels that can further spread to neighboring vessels through pit membranes, resulting in a catastrophic loss in water transport through the plant body culminating in hydraulic failure (HF) and mortality (Tyree and Sperry, 1989; Brodribb and Cochard, 2009; Choat et al., 2018).

To protect the integrity of their vascular system, plants typically reduce g_s in early drought stages to avoid excessive water loss through E (Martin-StPaul et al., 2017). This strategy minimizes plant water depletion in the soil and plant body, thus postponing the drop in xylem water potential (Ψ_x), but it also limits CO_2 uptake necessary for photosynthesis, compromising productivity. This protective role of stomatal closure against the collapse of the hydraulic system has been evidenced by different studies which show that xylem embolism usually occurs after complete stomatal closure (Hochberg et al., 2017; Creek et al., 2020), typically after leaf turgor loss point (Ψ_{TLP}; a proxy for the Ψ_w inducing complete stomatal closure) (Farrell et al., 2017; Trueba et al., 2019; Dayer et al., 2020). Therefore, the combination of a timely stomatal closure with a lower vulnerability to embolism (usually expressed as Ψ_x resulting in 50% decrease in K_{leaf} – P_{50}) represents a key strategy to ensure survival under drought conditions (Martin-StPaul et al., 2017), since it determines the hydraulic safety margin (HSM) of a given species, which reflects its natural degree of vulnerability to water shortage (Choat et al., 2012; Delzon and Cochard, 2014).

While observations are sparse, the leaves of Coffea species apparently have a moderate resistance to embolism (Mauri et al., 2020a), even though a recent study has reported *C. arabica* cultivars operating in negative HSM during an extreme drought event in Southwest Brazil (Martins et al., 2019). These observations suggest that, although g_s regulation apparently occurs rapidly upon reductions in water availability (Pinheiro et al., 2005; Silva et al., 2013), complete stomatal closure in coffee plants occurs in Ψ_x closer to, or bellow, P_{50} (Martins et al., 2019), making the leaves of these plants highly vulnerable to drought-induced mortality (DIM). Although these negative HSM might explain the high degree of leaf shedding during the dry season in cultivars of *C. canephora* cultivated in non-irrigated regions of Western Amazon, the lack of information regarding the variability in P_{50} and the point of stomatal closure (e.g., Ψ_{TLP}) of the most cultivated genotypes increase the uncertainties about how vulnerable to climate change the coffee production in that region can be.

A further challenge to supply the growing coffee demand in a scenario of reduction in water availability involves the fact that breeding towards the selection of more productive genotypes might have resulted in plants more vulnerable to drought. For example, several studies already reported a negative association between K_{leaf} with P_{50}, suggesting that species/genotypes with higher water transport capacity are more prone to hydraulic dysfunction (Nardini and Luglio, 2014; Scoffoni and Sack, 2017), even though such trade-off was poorly explored within *C. canephora* (Mauri et al., 2020b).

Regarding the control of water loss, drought-sensitive genotypes of this species were also reported to be less responsive to reductions in Ψ_w and increases in VPD (Pinheiro et al., 2005). Moreover, in addition to a reduced HSM (due to lower P_{50} and delayed stomatal closure), the selection of more productive *C. canephora* genotypes can also result in plants with higher water leaks, which could make them even more vulnerable to hydraulic failure (Martin-StPaul et al., 2017b; Machado et al., 2021). In fact, even after complete stomatal closure, plants keep losing water through their leaves to the atmosphere (Duursma et al., 2019). This residual water loss of leaves ($g_{leaf\text{-}res}$) determines the rate of water depletion of the soil and plant body and has been suggested as one of the main determinants of the time to hydraulic failure (THF) under drought conditions (Choat et al., 2018; Duursma et al., 2019). Importantly, variations in $g_{leaf\text{-}res}$

across species have been attributed to morphoanatomical adjustments associated with contrasting growth strategies. For example, high $g_{\text{leaf-res}}$ have been related to the high investment in stomatal density to improve CO_2 diffusion. These observations suggest that variations in $g_{\text{leaf-res}}$ are associated with trade-offs between carbon assimilation with water loss (Machado et al., 2021). In this regard, although the association between yield potential with $g_{\text{leaf-res}}$ was never tested, it is likely that the more productive coffee genotypes would present higher leaf water leaks. Thus, considering that the physiological adjustments required to sustain high coffee yield can lead to reduced resistance to embolism, poorer stomatal control, and higher water leaks, it is also feasible to suggest that the more productive *C. canephora* genotypes would be more prone to DIM.

Given the above, this study aimed to investigate how variations in leaf morphoanatomical and physiological traits affecting water transport and carbon assimilation capacity can explain the differences in yield across *C. canephora* genotypes cultivated in the Western Amazon. In addition, we also seek to characterize the extent of the vulnerability to hydraulic failure across these genotypes and check if higher productivity potential is ultimately linked to reduced drought tolerance. To achieve these objectives, eleven *C. canephora* genotypes cultivated in the Western Amazon, including some of the most economically relevant for the region, were analyzed to test the following hypothesis: 1) differences in coffee yield across *C. canephora* genotypes are related to variations in leaf morphoanatomical traits affecting their carbon assimilation and water transport capacity; and 2) the set of traits that maximize coffee yield (higher g_s, A, K_{leaf}) would lead to genotypes more prone to hydraulic failure due to higher vulnerability do embolism, lower hydraulic safety margins, and higher leaf water leaks, thus evidencing the existence of a trade-off between yield potential and drought tolerance within *C. canephora* genotypes cultivated in the Amazon region.

1.2. MATERIAL AND METHODS

Plant material and growth conditions

To explore the relative impact of physiological and morphoanatomical traits in determining the variation in yield potential across *C. canephora*, and their possible association with differences in vulnerability to hydraulic dysfunction in this species, 11 genotypes cultivated in the Western Amazon were evaluated. Based on their overall coffee yield, the studied genotypes are empirically classified as high [genotypes 010 (10), 25, 08 (8)] moderate [BRS1216 (16), AS2 (2), 06 (6), BRS3213 (13), BRS3137 (37)], and low [BRS2357 (57), BRS3220 (20), BRS3193 (93)] productivity. Importantly, some of these genotypes (clones 10, 6, 25, 8, 2) rank among the most widely cultivated in the region, thus reflecting their economic importance.

The experiment was conducted under field conditions, with adult plants (3 years old) grown on a Red Yellowish Latosol with sandy clay texture (clay - 40%, sandy – 55%, silt – 5%) in Rolim de Moura, Western Amazon, Brazil. The site is characterized by a tropical with a dry winter climate, with a mean annual temperature of 25.5 ºC and rainfall of 1800 mm, distributed mainly from October to April. Plants of clonal origin were cultivated at a spacing of 2.0 x 0.5 m under full sunlight. Routine agricultural practices for coffee production were performed following the recommendations for the culture. Supplementary irrigation is provided daily (for two hours) throughout the dry season (May to October) using drip emitters at a spacing of 0.5 m and a flow rate of 2.2 l h^{-1} during two hours for day. The applied irrigation is high enough to maintain plants near to the field capacity throughout the growing season, as evidenced by their high predawn leaf water potential, which is always close to -0.1 MPa (data not shown). The physiological and morphoanatomical analyzes described below were conducted on four individuals per genotype, using the youngest, fully expanded leaves, which corresponded to the third or fourth leaf pair from the apex of plagiotropic branches. In addition, for some analysis, sun-exposed plagiotropic branches, collected from the middle of plant canopies, were also sampled.

Coffee yield

The coffee harvest was performed manually throughout May of 2021 year when all the plants (4 individuals per genotype) had at least 70% of the fruits in the “cherry” stage. During the harvest, all the berries of the trees were collected, dried in the open air until reaching 12% of humidity (dry berry), and then weighed to determine the mean yield per genotype (kg dry berry per plant).

Gas exchange

The net carbon assimilation rate (A), stomatal conductance (g_s), and the transpiration rate (E) were determined in an open system under saturated light conditions (1500 µmol photons m^{-2} s^{-1}), temperature of 25 ºC and a CO_2 partial pressure of 40 Pa using an infrared gas analyzes (Li-6800; Li-Cor, Lincoln, NE, USA) equipped with a blue/red light source. Gas exchange measurements were conducted on two consecutive days (e.g. two leaves of each genotype per day, four leaves in total), between 08:00 and 10:00 h in sun-exposed, fully expanded leaves, still connected to the trees, of the third or fourth nodes from the apex of the plagiotropic branch, chosen of middle third of the tree canopy. Climatic conditions remained stable through the measurement days, with no significant variation in mean air temperature or relative humidity.

Leaf hydraulic conductance and water status

To better integrate the coordination between water transport efficiency with carbon assimilation, the leaf hydraulic conductance (K_{leaf}, mmol s^{-1} m^{-2} MPa) was determined using the evaporative flux method (Brodribb and Holbrook, 2006), in the same leaves used for gas exchange analysis (described above). Before analysis, a neighbor leaf from that one used for gas exchange was covered in aluminum foil to prevent transpiration and used to estimate stem water potential (Ψ_{stem}). After gas exchange reached equilibrium, the transpiration rate (E, mmol m^{-2} s^{-1}) was recorded, and then both leaves were sampled, bagged, and equilibrated for at least 10 min. The pressure potentials of the transpiring and non-transpiring leaves were measured in a pressure chamber and assumed to provide estimates of the leaf (Ψ_{leaf}) and stem (Ψ_{stem}) water potentials, respectively (Tsuda and Tyree, 2000). K_{leaf} was then calculated as:

$$K_{\text{leaf}} = E/(\Psi_{\text{stem}} - \Psi_{\text{leaf}}) \qquad \text{Eqn 1}$$

Given that the temperature from the chamber of the infrared gas analyzer was set at 25 ºC during all measurements, K_{leaf} data were not corrected for temperature (Mauri et al., 2020b).

To evaluate the water status of the plants during peak transpiration, the midday water potential $\Psi_{\text{leaf-md}}$ of a sun-exposed leaf, in the same branch used for K_{leaf} determination, was measured using a pressure chamber. Importantly, before each measurement of $\Psi_{\text{leaf-md}}$, the sampled leaves were equilibrated for 5 min inside zip-lock bags with damp paper towels. The water content of leaves (LWC) was also measured at midday, in a neighbor leaf of that used for the $\Psi_{\text{leaf-md}}$ estimation, according to the following equation (Loram-lourenço et al. 2020):

$$\text{LWC (\%)} = 100\left(\frac{fresh\ mass - dry\ mass}{dry\ mass}\right) \quad \text{Eqn 2}$$

Vulnerability to embolism, pressure-volume traits, and hydraulic safety margins

For each genotype, leaf hydraulic vulnerability curves were constructed by measuring the percentage loss of leaf hydraulic conductance from maximum values (K_{max}) in leaves rehydrated from a range of water potentials (Ψ_{leaf}), following the described by Blackman et al. (2010). To construct these curves, K_{leaf} was measured by assessing the kinetics of Ψ_{leaf} relaxation upon leaf rehydration (Brodribb and Holbrook, 2003). In the predawn, sun-exposed branches from four individuals per genotype were cut, immediately immersed into a bucket of water, wrapped into black plastic bags containing moist paper, and transported to the laboratory. After arriving at the laboratory, the branches were placed on benches and allowed to desiccate for a different amount of time to reach a range of leaf water potentials. Branches were then bagged, placed in the dark, and allowed to equilibrate for at least 1 h. Initial Ψ_{leaf} was determined by measuring leaves neighboring the sample leaf in a pressure chamber. The sample leaf was then cut underwater and allowed to rehydrate for a period of between 30 and 300 s depending on the initial Ψ_{leaf}. Final Ψ_{leaf} was measured with the pressure chamber, and K_{leaf} was calculated from the ratio of the initial to final Ψ_{leaf} and the capacitance of the leaf:

$$K_{\mathrm{leaf}} = C_{\mathrm{leaf}} \ln (\Psi_{\mathrm{o}}/\Psi_{\mathrm{f}})/t \quad \text{Eqn 3}$$

where C_{leaf} is leaf capacitance, derived from pressure-volume curves (described below), Ψ_{o} is leaf water potential before rehydration, and Ψ_{f} is leaf water potential after rehydration for t seconds. For each genotype, leaf vulnerability curves were fitted using a sigmoidal function by plotting K_{leaf} against Ψ_{o} using the fitplc package in R (Duursma and Choat, 2017). From these curves, the vulnerability of leaf hydraulic conductance to decreasing water potential was defined as the Ψ_{leaf} value at which K_{leaf} had declined by 50 (P_{50}) or 88% (P_{88}) from the mean maximum rate (K_{max}) for each genotype (Blackman et al., 2010).

For the construction of pressure-volume curves, four leaves of each genotype (from the same branches used for vulnerability curves) were sampled and rehydrated overnight. Before analysis, each leaf was scanned to calculate leaf area, and then Ψ_{leaf} and leaf mass were repeatedly measured as they dehydrate on the bench (Yao et al., 2021).

Following standard pressure-volume curve analysis, the osmotic pressure at full turgor (π_o), osmotic pressure at turgor loss point (π_{tlp}), modulus of elasticity (ε), turgor loss point (Ψ_{tlp}), and the area-based leaf-specific capacitance (C_{leaf}), before and after Ψ_{tlp}, were calculated.

The leaf hydraulic safety margins (HSM) were calculated as the difference between Ψ_{tlp} (a proxy for drought-induced stomatal closure; (Brodribb et al., 2003; Farrell et al., 2017)) and the water potential inducing 50% (P_{50}) or 88% (P_{88}) loss in leaf hydraulic conductance (HSM_{50} and HSM_{88}, respectively) (Choat et al., 2012; Delzon and Cochard, 2014).

Leaf water leaks: minimum transpiration rates

Leaf minimum conductance to water vapor ($g_{leaf\text{-}res}$) was determined gravimetrically from the consecutive weight loss of desiccating organs (Sack et al., 2003; Machado et al., 2021). Before analysis, high-melting-point (68 ºC) paraffin wax was used to seal the wounds of cut petioles of water-saturated leaves. Leaves were dried on a bench under dark conditions, to induce stomatal closure, and weighted at regular intervals (30-45 min). Leaf minimum transpiration was measured as the slope of the water loss versus time, normalized by the total leaf surface area (sum of the adaxial and abaxial projected areas). For the slope estimation, only the linear part of the regression was used ($R^2 > 0.99$). The value of $g_{leaf\text{-}res}$ was calculated as transpiration/mole fraction gradient in water vapor from the leaf to air, assuming the leaf internal air to be fully saturated (Sack et al., 2003; Schuster et al., 2017). Ambient temperature and relative humidity, measured at 30 min intervals with a digital thermal hygrometer, varied minimally during the measurements, both within and across genotypes. The residual water loss was also expressed on a canopy area basis ($g_{canoy\text{-}res}$), by the product of $g_{leaf\text{-}res}$ and total canopy leaf area.

To estimate the canopy leaf area, for each genotype, in the same four individuals used for the analysis described above, the number of all leaves within the canopy was registered. A subsample of 100 leaves per genotype (25 per individual) was collected around their canopy, photographed, and measured using the ImageJ software (Rasband, 2018). Then, the total canopy leaf area was estimated as the product between leaf number and averaged leaf area.

Stomatal and venation anatomical traits

The epidermis impression technique was used to determine the stomatal properties (Franks et al., 2009). Fully expanded leaves (four leaves per genotype) were sampled in the field, between 08:00 and 10:00 h, re-cut under pure water, and brought to the laboratory. In the detached leaves, a small amount of instant glue was placed on a histological slide, and the vegetable material (adaxial and abaxial leaf sides) was pressed against the slide for a few minutes. Then, the slides were observed at X20 magnification with the aid of a light microscope (model AX70TRF; Olympus, Tokyo, Japan). It is important to note that, after careful inspection of the patterns of stomatal distribution on both leaf sides, it was found that all sampled genotypes were hypostomatous, and thus all stomatal measurements, described below, were performed only on the abaxial leaf side.

In each slide, a minimum of five images per field was taken at x100 and x400 magnification to determine the stomatal density (SD), guard cell length (L), guard cell pair width (W), and stomatal pore length (p), using the ImageJ software (Rasband, 2018; Franks et al., 2009; Galmés et al., 2013). The stomatal size (SS) was determined using the L and W measurements. The theoretical maximum stomatal conductance (g_{wmax}) was calculated on these data, as proposed by Franks et al. (2009):

$$g_{\mathrm{wmax}} = \frac{SD\ d_w\ a_{max}}{\nu\left(\mathrm{l} + \frac{\pi}{2}\sqrt{\frac{a_{max}}{\pi}}\right)} \qquad \text{Eqn 4}$$

Where d_{w} presents the diffusion coefficient of water vapor in the air, a_{max} is the maximum pore area of fully open stomatal, ν is the molar volume of air, and l represents the pore depth of a fully opened stomata. For normalization of the values, the constants d_{w} and ν represent the values at 25ºC (24.9×10^{-6} m^2 s^{-1} and 24.4×10^{-3} m^3 mol^{-1}, respectively). a_{max} was calculated as $\pi(\rho/2)^2$, according to Franks et al. (2009). l for fully open stomata was taken as $L/4$ assuming guard cells inflate to a circular cross-section (Franks and Beerling, 2009). In addition, the average fraction of the leaf epidermis that is allocated to stomata (f_{gc}) was determined by the average area of the guard cell pair (a_{gc}) and average stomatal density (de Boer et al., 2016);

$$f_{\mathrm{gc}} = a_{\mathrm{gc}}.SD \qquad \text{Eqn 5}$$

where a_{gc} was calculated as:

$$a_{\mathrm{gc}} = \frac{\pi}{2}.W.L \qquad \text{Eqn 6}$$

For determining venation traits, samples of leaf lamina were cut and cleared as described by Scoffoni et al. (2011). Regions of approximately 6 mm^2 were imaged at 30X using the aforementioned microscope, and the venation density (D_{v}) was calculated as the sum of the vein lengths divided by the total image area using the ImageJ program (Rasband, 2018; Martins et al., 2014).

Statistical analysis and modeling approach

Before analysis, traits were log$_{10}$-transformed, if necessary, to improve homoscedasticity and normality. Differences in morphoanatomical and physiological traits across genotypes were assessed by one-way ANOVA, followed by a post-hoc Tukey test ($P < 0.05$). Pearson's linear correlation analyses were used to investigate the overall coordination between morphoanatomical and physiological traits. To reduce the dimensionality of the dataset and to identify the variables that explained most of the total variation, a principal component analysis (PCA) of the mean genotypes values was used to explore multiple associations among traits that might explain the variability in yield and vulnerability to drought-induced mortality across genotypes. All variables were log$_{10}$-transformed before analysis, which is adequate for data with different measurement scales (Díaz et al., 2016). All the statistical analyses were performed in R v.4.0.3 R Core Team, 2020).

To predict how the contrasting growth strategies across genotypes can affect the survival of their leaves under a scenario of water deprivation, we used the SurEau model (Cochard et al., 2021), which simulates water transport in the soil-plant-atmosphere continuum and includes a detailed representation of the capacitance in the stem and leaf tissues. Is this model, tree mortality is assumed to be triggered by hydraulic failure, and a plant is considered dead when it loses 99% of hydraulic conductivity. For the simulations, the time to hydraulic failure of leaves (THF) was computed based on traits related to water storage capacity, structural properties, and drought tolerance. Those traits were measured and/or derived from the same plants used in the analyses described above (Tables 2, 3, 4, and S1) and included in the model as the mean values across species; with all other traits, including those that we not measured, assumed to be equal across them (Table S1).

1.3. RESULTS

Variation in diffusive and hydraulic traits associated with yield potential across *C. canephora*

The studied genotypes had a substantial variation in their yield (3.71-fold), with the higher values observed for genotypes 10, 25, and 08 (~2.12 Kg per plant), and the lowest for genotypes 93, 20, and 57 (~0.70 Kg per plant) (Fig. 1; Table 5). This variation in yield potential was tightly associated with differences in their carbon assimilation capacity (2.37-fold; Tables 3 and 5), as denoted by the high positive correlations between yield and A (Fig. 2; Tables 6). The high and linear association between A and g_s (Fig 2; Table 6) indicates that such variations in carbon assimilation across genotypes (values ranging from 7.42 to 17.6 µmol m^{-2} s^{-1}; Table 3) were strongly associated with differences in their diffusive capacity (g_s), which also varied substantially across them (from ~82.7 to ~457 mmol m^{-2} s^{-1}; Table 3). Differences in g_s, on the other hand, were largely influenced by morphoanatomical determinants of CO_2 diffusion, as denoted by the positive correlations between g_s with g_{wmax} and f_{gc} (Fig. 2; Tables 2, 5, and 6). In this regard, differences in g_{wmax} (1.44-fold) and f_{gc} (2-fold) across genotypes (Tables 2 and 5) were driven by variations in both stomatal density (*SD*) and size (*SS*), as denoted by the high positive correlation between *SD* and *SS* (Fig. 2; Table 6).

The Amazonian *C. canephora* genotypes also differed substantially in their water transport efficiency (K_{leaf}) (4.43-fold; Tables 3 and 5), and such variation was positively associated with the relative investment in veins (V_d) (Fig. 4; Table 6). K_{leaf} and V_d were positively associated with leaf water status (Ψ_w), diffusive capacity (g_s and g_{wmax}), carbon assimilation (A), yield and water loss by transpiration (E), and negatively associated with water use efficiency (A/g_s) and g_{wmax}/g_s ratio (Figs. 3 and 4; Table 6). Finally, in contrast to the high variability in K_{leaf}, a surprising lack of significant variation was found across genotypes regarding their leaf water storage capacity, represented by C_{ft} and LWC (Tables 3 and 5).

Exploring the resistance to embolism reveals low hydraulic safety margins and high vulnerability to drought-induced mortality in leaves from Amazonian *C. canephora* genotypes

The studied genotypes varied significantly in their resistance to embolism (P_{50} and P_{88}) (Figure 9; Table 4). In fact, whereas some genotypes had a very vulnerable hydraulic system (genotypes 02, 10, and 16; ~1.10 MPa), others showed much higher resistance to hydraulic dysfunction, especially the genotype 25 (-1.95 MPa). A similar trend was observed for P_{88}, but with lower values (Table 4). In this regard, although K_{leaf} was positively associated with yield potential, a higher water transport efficiency was not translated into a lower vulnerability to embolism (Table 6). Conversely, in contrast to P_{50} and P_{88}, the studied genotypes had a minimal, but significant, variation in Ψ_{tlp} (1.19-fold) (Tables 4 and 6). Based on the results of P_{50}, P_{88}, and TLP, a high variability in the hydraulic safety margins (TLP – P_{x}) was evidenced in the leaves of *C. canephora* (Fig. 5). Importantly, despite this variation, except for a few genotypes (25, 06, and 20), which showed very narrow HSM, all other genotypes had negative HSM (Fig. 5). A similar trend was observed under more conservative scenarios (TLP – P_{88}) since all genotypes still had narrow (< -1 MPa), and even negative HSM (Fig. 5).

Trade-offs underlying the variability in leaf minimum conductance explains the lower time to hydraulic failure in the most productive *C. canephora* genotypes

Our simulations evidenced a high variation in the time to hydraulic failure (THF) across the studied genotypes (~2.61-fold; Fig. 5), which was tightly associated with their yield potential, as denoted by the high positive correlations between THF and yield (Fig. 6). Such variations in THF were strongly associated with differences in leaf water leaks, as suggested by the positive association between THF with $g_{\text{leaf-res}}$ (Fig. 6). In this regard, the positive correlations between *A* and g_{s} with $g_{\text{leaf-res}}$ (Fig. 7; Table 6) evidence that differences in $g_{\text{leaf-res}}$ resulted from a trade-off between carbon assimilation and residual water loss linked by leaf morphoanatomical properties. In fact, although allowing for high CO_2 diffusion, and consequently higher *A* rates and yield, genotypes with high g_{wmax} and f_{gc}, due to higher *SD* and *SS*, ultimately had increased $g_{\text{leaf-res}}$ (Figs. 2 and 7; Table 6). Importantly, the significant differences in $g_{\text{leaf-res}}$ across genotypes (1.42-fold; Table 5) were strengthened (2.52-fold; Tables 3 and 5) when those sources of residual water loss were expressed on a total canopy leaf area basis ($g_{\text{canopy-res}}$). Multivariate associations among carbon assimilation, yield, water leaks, and THF are further evidenced by the PCA analysis (Fig. 8). The first two principal components explained 71.4% of the variation. The first axis explained 52.2% of the variation and shows that genotypes with higher yield potential had leaf morphoanatomical and physiological characteristics that maximized water transport (higher K_{leaf} and V_d) and carbon assimilation (higher A, g_s, SS, SD, g_{wmax}, and f_{gc}) efficiency which, in turn, resulted in higher $g_{leaf\text{-}res}$ and lower THF (Fig. 8). The second component explained an additional 19.2% of the variation and shows that, along this axis, the genotypes were primarily separated by differences in their resistance to embolism (P_{50} and P_{88}) and water storage capacity (C_{ft}) (Fig. 8).

1.4. DISCUSSION

Our results highlight the importance of exploring hydraulic traits across *C. canephora* genotypes to the cultivation/selection of genotypes with improved coffee production. However, it is important to note that such observations were based on irrigated conditions and that the higher yield of the most productive genotypes came at expense of higher water loss, and less efficiency in the use of this resource, given that yield was positively associated with E, and negatively with A/g_s (Figure 2; Table 6). Although supplementary irrigation is a widespread practice in coffee-producing areas from the Amazon, water availability may diminish under further climate change, and certainly cannot be unlimited across the region. Climatic projections for the Amazon forecast a significant reduction in pluviosity for the decades to come, which can significantly reduce available water for irrigation, as well as the viable land area for coffee cultivation (IPCC 2021). However, despite the central role of water transport for coffee production, as discussed below, to date there is little information regarding how vulnerable to drought-induced hydraulic dysfunction the leaves of *C. canephora* can be. By combining physiological analysis with modeling exercises, our results suggest a high vulnerability to hydraulic failure, and thus mortality, in leaves of genotypes widely cultivated in the Western Amazon, which includes some of the most economically relevant genotypes for the region. As leaves, through photosynthesis, are the major engines of economic coffee production, their mortality under future climate could have dire human consequences.

Morphoanatomical and physiological leaf traits underlying the variability in yield across *C. canephora* genotypes cultivated in the Amazon region

The remarkable variation in coffee yield across the *C. canephora* genotypes investigated in the present study was tightly related to differences in their carbon assimilation capacity (Figs.1 and 2; Tables 3, 5, and 6). Differences in *A* rates, by its turn, apparently were chiefly governed by variations in CO_2 diffusion, as denoted by the high positive correlations between *A* and gs (Fig 2; Table 6). In this regard, considering that (i) all the measurements were performed on hydrated plants, (ii) stomatal and mesophyll conductances are intrinsically co-regulated in coffee (Martins et al., 2014b), and (iii) that biochemical limitations to photosynthesis are usually triggered by extreme drought events (Flexas et al., 2009; Tosens et al., 2016), our results strongly suggest that the yield of *C. canephora* genotypes cultivated in the Amazon, at least under irrigated conditions, are mainly constrained by diffusive limitations, as already reported for other *C. canephora* and *C. arabica* genotypes cultivated in Southern Brazil (Martins et al., 2014b; Menezes-Silva et al., 2015). Importantly, the strength of such diffusive limitations depended on the coordination between morphoanatomical adjustments to improve CO_2 diffusion. Variations in stomatal morphology, for example, had a significant impact in explaining differences in g_s across genotypes. In fact, the genotypes that invested in more stomatal cells per area (higher *SD*) had higher g_{wmax}, which ultimately allowed for higher g_s (Fig. 2; Tables 2, 3, and 6). Surprisingly, the increase in *SD* was not followed by reductions in *SS*, as commonly already observed for other species (Franks et al., 2009; de Boer et al., 2016; Machado et al., 2021); on the contrary, a positive correlation was found between these two traits (Fig. 2; Table 6). This result, which was also reported for *C. arabica* and *S. lycopersicum* (Galmés et al., 2013; Nardini et al., 2014), indicates that the morphological limits for CO_2 diffusion (g_{wmax}) on Amazonian *C. canephora* genotypes were determined by variations in both *SD* and *SS*, which ultimately affected the variation in f_{gc} (Fig. 2; Table 6). Thus, considering the strong linear increase of *A* in response to g_s (Fig. 2), and that differences in g_s were driven by variations in g_{wmax} and f_{gc} (Fig. 2; Table 6), our results suggest that the selection of genotypes with a higher allocation of stomatal cell into the epidermis could minimize the strength of the diffusive limitations in *C. canephora*, thus resulting in genotypes with improved yield potential.

The higher *A* rates of the most productive genotypes required a more efficient water transport system to sustain their higher transpiration (*E*). The high positive correlations found between *A*, g_s, and *E* with K_{leaf} (Fig. 3) reinforce the strong coordination between carbon assimilation with water demand and transport capacity, a result already reported for several other species (Brodribb et al., 2007; Scoffoni et al., 2016; McAdam et al., 2017), including coffee (Nardini et al., 2014). In this regard, our results evidence the importance of exploring the hydraulic divergence across genotypes to better understand the physiological limits to coffee production, as denoted by the positive correlations between K_{leaf} and yield (Fig. 3; Table 6). However, although the hydraulic-based selection of coffee genotypes has long been suggested (DaMatta, 2004; Martins et al., 2014b), little is known about the variability in their water transport efficiency, especially across *C. canephora*. For example, a high variability in K_{leaf} was found across 11 clones of *C. canephora* cultivated in Southern Brazil (Menezes-Silva et al., 2015), but those determinations were based solely on anatomical measurements (theoretical K_{leaf}), which neglects important resistance along the water path (e.g. pit properties) (Gleason et al., 2016); whereas no differences in K_{leaf} were found in a field study with two hybrids of this species (Mauri et al., 2020b). To the best of our knowledge, this is the first study to explore the variability in K_{leaf}, based on direct measurements, across a large group of *C. canephora* genotypes. In this regard, our results support theoretical observations and evidence a large variability in K_{leaf} within *C. canephora* (~4-fold; Tables 3 and 5).

The remarkable variation in K_{leaf} across genotypes apparently was related to differences in the mean terminal path length for water flow through the mesophyll, which involved a differential investment in veins (McAdam et al., 2017), as denoted by the positive correlations between K_{leaf} and V_{d} (Fig. 4; Table 6). In fact, although other anatomical adjustments may contribute to the reduction in water path (e.g. changes in leaf thickness and bundle sheath extension) and increase in water flow (e.g. investment in wider xylem vessels) (Gleason et al., 2016; McAdam et al., 2017), several studies have evidenced the key role of V_{d} in determining maximum K_{leaf} (Scoffoni et al., 2016; Brodribb et al., 2017). Importantly, our results also suggest that differences in K_{leaf} strongly shaped the morphological and physiological determinants of CO_2 diffusion capacity, and thus A rates, across genotypes. The positive correlations between K_{leaf} and V_{d} with g_{wmax} and g_{s} (Figs. 3 and 4; Table 6), for example, evidence the tight coordination between the morphoanatomical adjustments associated with water delivery and demand, which affected the physical limits to CO_2 diffusion (g_{wmax}). The genotypes with higher K_{leaf}, due to a more intricate vein network, also had a better leaf water status (less negative Ψ_{md}), which allowed for higher g_{s} (Fig3; Table 6). Importantly, variations in water transport efficiency significantly affected the differences between the potential and actual stomatal conductance (g_{wmax} to g_s ratio), as denoted by the negative correlations between K_{leaf} with g_{wmax}/g_s (Table 6), thus evidencing the differential strength of hydraulic limitation to CO_2 diffusion in the studied genotypes (Galmés et al., 2013; Martins et al., 2014b). When taken together, our results reinforce the evidence that carbon assimilation, and thus yield potential, on coffee species are majorly constrained by the leaf hydraulic architecture of these plants, as already demonstrated for *C. arabica* (Martins et al., 2014b; DaMatta et al., 2018), and provide experimental evidence that the selection of genotypes with higher water transport efficiency could reduce the pressures to expand areas for cultivation, especially on threatened areas like the Amazon, by potentially improving the production for a given land area.

A bitter future for coffee production in the Amazon region?

One of our working hypotheses was that traits associated with increased yield (e.g. high K_{leaf}, $g_{s,}$ and A) would result in lower resistance to embolism (high P_{50} and P_{88}) in coffee plants. Our results strongly suggested that a higher water transport efficiency was associated with higher carbon assimilation and yield potential (Fig. 3; Table 6), but not necessarily with lower resistance to embolism. In fact, although some of the genotypes with the highest K_{leaf} ranked among the most vulnerable to embolism, other genotypes showed both high K_{leaf} and low P_{50}, as already reported for *C. arabica* (Table 3 and 4) (Nardini et al., 2014). Surprisingly, a third group of genotypes presented both low hydraulic efficiency and safety (Tables 3 and 4). A similar lack of a clear trade-off between K_{leaf} and P_{50} was also observed across other *Coffea* species, including two genotypes of *C. canephora* (Mauri et al., 2020b). These results, when combined with the large variation in K_{leaf} and the relatively small variation in P_{50} across the Amazonian *C. canephora* genotypes, suggest that the morphoanatomical determinants of water transport are likely to be decoupled from those related to embolism resistance in the species from the *Coffea* genus, as already suggested for other species (Gleason et al., 2016). This differential coordination between K_{leaf} with P_{50} within *C. canephora*, and possibly across other species from the Coffee genus, is likely to involve a different combination of traits that can affect, on different levels, both water transport efficiency and safety. Higher resistance to embolism, for example, can be achieved through increases in pit membranes thickness (Gleason et al., 2016), whereas the increase in the resistance to water flow involved in this strategy can be compensated by investing in a dense vein network composed of vessels with high diameter, thus allowing for both high safety and efficiency to water transport (Yao et al., 2021). Although needs to be further investigated, a similar strategy is likely to be used by clone 25, one of the most productive genotypes, which had the highest K_{leaf} and lowest P_{50} values (Tables 3 and 4).

An important finding of the present study was that, despite their variability in embolism resistance, all the investigated genotypes are likely to operate under narrow and even negative hydraulic safety margins (HSM) when exposed to drought (Fig. 5). Since the HSM represents the degree of conservatism in the hydraulic strategy of a given species and considering that plants with low HSM (e.g. < -1 MPa) are more prone to hydraulic dysfunction (Choat et al., 2012; Delzon and Cochard, 2014), our results suggest that the leaves of Amazonian *C. canephora* may experience large amounts of embolism even under moderate drought conditions. The high hydraulic risks taken by *C. canephora* genotypes are evident even when more conservative HSM is considered (Fig. 5). It is important to note that, comparatively to embolism resistance, the Ψ_{TLP} (here used as a proxy for stomatal closure) varied minimally across genotypes (Tables 4 and 5), thus suggesting a convergence to late drought-induced stomatal closure across them, as a possible consequence of breeding under ample water availability. Although it may allow for an extended period of carbon assimilation in the early drought stages (Hochberg et al., 2018), this strategy used by *C. canephora* genotypes can significantly increase their vulnerability to hydraulic failure since the early stomatal closure represents a key mechanism to postpone the generation and propagation of embolism within the plant body (Martin-StPaul et al., 2017a; Machado et al., 2021). This apparent poor ability of coffee plants to completely cut-off stomatal transpiration was already reported by other studies on *C. canephora* and *C. arabica* (DaMatta et al., 2003; Martins et al., 2019). In line with our observations, a field study also reported narrow and even negative HSM on leaves of two *C. arabica* cultivars during an extreme natural drought event (Martins et al., 2019). Importantly, the high degree of K_{leaf} loss due to widespread embolism formation was associated with intense leaf abscission (Martins et al., 2019), a result also reported for other species (Hochberg et al., 2017). When taken together, these results suggest that the massive loss in canopy area of *C. canephora* genotypes cultivated in the Amazon region under non-irrigated conditions is likely to be triggered by leaf hydraulic dysfunction, as a result of their low HSM (Fig. 5). These results also help to explain why the cultivation of the studied genotypes is highly dependent on supplementary irrigation. Field observations in the region support the evidence brought by this study since the cultivation of none of these genotypes, which include some of the most economically important for the region, is no longer suitable without supplementary irrigation due to massive losses in canopy leaf area throughout the dry season. Given the above, a worrying scenario for coffee production in the Amazon region can be expected if the predictions of changes in precipitation for the region are confirmed in the decades to come.

The deadly costs associated with high productivity: coordination between yield potential, residual water loss, and time to hydraulic failure

Despite the overall low HSM observed within *C. canephora*, it is worth noting that some genotypes are likely to operate under safer margins than others. Importantly, some of these genotypes ranked among the most productive, especially the clone 25 (Figs. 1 and 5). Thus, given that impairments to water transport due to embolism is one of the major causes of yield losses on crop species during drought conditions, and considering the existence of highly productive *C. canephora* genotypes that have both high K_{leaf} and low P_{50}, and especially wider HSM, should farmers and breeders favor such genotypes to mitigate the deleterious effects of climate change on coffee production? According to our simulations, the apparent higher safety of some of the most productive genotypes hides important aspects that can potentially make them extremely vulnerable to drought-induced mortality. The tight negative correlation between yield and THF (Fig. 6) seems to confirm this hypothesis, and points to an intriguing question: how highly productive genotypes with an apparent safer hydraulic system (low P_{50} and wider HSM) can be more prone to hydraulic failure than genotypes with lower embolism resistance and narrower HSM? Our results suggest that the large variability in THF within *C. canephora*, and especially the higher vulnerability to foliar drought-induced mortality (DIM) of the most productive genotypes, are largely explained by differences in their leaf water leaks, as denoted by the high positive correlations between THF with $g_{\text{leaf-res}}$ (Fig. 6). In line with our findings, an emerging body of evidence is highlighting $g_{\text{leaf-res}}$ as a central driver of DIM in a diverse group of species since after complete stomatal closure the rate of water depletion within the plant body and the soil is majorly influenced by those leaf water leaks (Martin-StPaul et al., 2017a; Choat et al., 2018; Duursma et al., 2019; Machado et al., 2021). The surprisingly lack of significant variation in leaf and stem water storage capacity (Tables 3, 5, and S1) associated with the small range of Ψ_{TLP} across the genotypes (Table 5), gives support to this hypothesis, and further evidence that differences in $g_{\text{leaf-res}}$ had a central role in dictating the THF within *C. canephora*.

However, although the faster water depletion rate on the most productive genotypes, due to higher $g_{\text{leaf-res}}$, helps to explain their lower THF, even when they had a safer hydraulic system, it does not explain the link between yield potential and residual water loss (Fig. 6).

In line with recent findings in a large group of species (Machado et al., 2021), our results evidence that the variation in $g_{\text{leaf-res}}$ across *C. canephora* is associated with a trade-off between carbon assimilation and water loss through minimum transpiration, as denoted by the positive correlations found between *A*, $g_{s,}$ and yield with $g_{\text{leaf-res}}$ (Figs. 7 and 8; Table 6). In fact, due to a more efficient water transport system, the most productive genotypes covered their leaf epidermis with a higher fraction of stomatal cells to improve CO_2 diffusion (Fig. 8; Table 6). Although this strategy leads to higher *A* rates, thus allowing for improved yield potential, it ultimately resulted in higher $g_{\text{leaf-res}}$ (Figs 7 and 8; Table 6) due to increased stomatal leakiness (Duursma et al., 2019). Conversely, the more conservative water transport and carbon acquisition strategies of other genotypes resulted in lower yield but had as the main benefit the lower $g_{\text{leaf-res}}$, which, according to our simulations, represents a great advantage under drought conditions (Figs 6, 7, and 8; Table 6). Thus, while a clear trade-off between yield potential and resistance to embolism is apparently not present within *C. canephora*, the leaves of the most productive genotypes are likely to be more vulnerable to DIM due to their increased leaf water leaks (Figs 6 and 8; Table 6). In this regard, it is important to note that, although $g_{\text{leaf-res}}$ on a leaf area basis varied significantly, but minimally (1.42-fold; Table 5), across genotypes, such differences increased substantially when integrated into their canopy leaf area (2.52- fold; Table 5), thus further evidencing the higher water leaks of the most productive genotypes and strengthening the link between $g_{\text{leaf-res}}$ with yield potential and foliar DIM in *C. canephora* (Fig. 6). Importantly, the relative contribution of those leaf water leaks in driving the canopy dieback on *C. canephora* can be further increased with the rise in atmospheric temperatures, also predicted for all Amazonian coffee-producing regions (IPCC, 2021), since $g_{\text{leaf-res}}$ have been shown to increase drastically under high temperatures, especially for non-desert species (Bueno et al., 2019; Billon et al., 2020).

In conclusion, our results evidence the challenges of sustaining coffee production in the Amazon region in a climate change scenario that predicts significant alterations in rainfall patterns that can lead to more frequent and intense drought events, which can also limit the water availability for irrigation. In fact, the leaves of all the studied genotypes had a high vulnerability to DIM. It is important to note that this high vulnerability to DIM of leaves from the studied genotypes will not necessarily be translated into the dieback of the entire plants since coffee species are likely to use the hydraulic segmentation strategy (e.g. stems are more resistant to embolism than leaves) (Rodriguez-dominguez et al., 2018), as suggested by the intense leaf flush after the resumption of rainfall. However, given the large impact of canopy loss on coffee production, and considering the relatively small plasticity to drought of leaf traits on *C. canephora* (Silva et al., 2013), our results suggest that the cultivation of the studied genotypes in the Amazon region is seriously threatened. Given the above, the selection of genotypes with improved drought tolerance is pivotal to anticipate the deleterious impact of water restriction in the region. The existence of highly productive genotypes able to sustain high water transport efficiency with relatively lower vulnerability to embolism could open new venues to the generation of genotypes with higher production stability under drought conditions. The reduction in leaf water leaks should be one of the major targets to increase leaf survival of such genotypes, but the tight coordination between carbon assimilation with $g_{\text{leaf-res}}$, and the uncertainties regarding how this trait will respond to the predicted rise in atmospheric temperature in the Amazon region, imposes a great challenge to this task. Although needing further investigation, especially on coffee species, a possible alternative could be the introgression with other genotypes with improved cuticle composition that reduces residual transpiration since the stomatal water leaks represent just a fraction of $g_{\text{leaf-res}}$.

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Tables

Table 1. 1 Abbreviations of the measured traits and their respective units

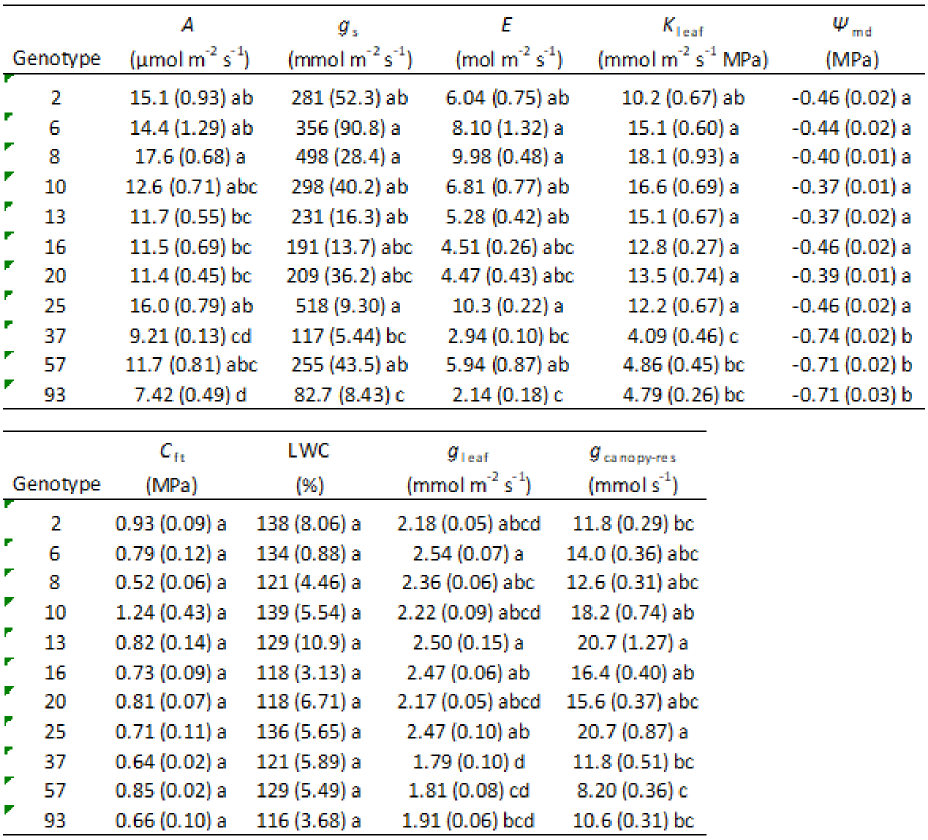
Abbreviation	Trait	units
SD	Stomatal density	stomata cm^{-2}
SS	Stomata size	μm^2
f_{gc}	Fraction of epidermis allocated to stomata	%
g_{wmax}	Theoretical maximum stomatal conductance	mol m^{-2} s^{-1}
V_{d}	Vein density	mm mm^{-2}
C_{ft}	Leaf bulk capacitance	mol m-2 MPa-1
LWC	Leaf water content	%
Ψ_{md}	Midday water potential	MPa
$\mathrm{K}_{\mathrm{leaf}}$	Leaf hydraulic conductance	mmol m^{-2} s^{-1} MPa
E	Transpiration rate	mol m^{-2} s^{-1}
$g_{\mathrm{leaf\text{-}res}}$	Leaf minimum conductance	mmol m^{-2} s^{-1}
$g_{\mathrm{canopy\text{-}res}}$	Canopy minimum conductance	mmol s^{-1}
Ψ_{tlp}	Turgor loss point	MPa
P_{50}	Ψleaf inducing 50% loss of leaf hydraulic conductance	MPa
P_{88}	Ψleaf inducing 88% loss of leaf hydraulic conductance	MPa
HSM_{50}	Hydraulic safety margin	MPa
HSM_{88}	Hydraulic safety margin	MPa
THF	Time to hydraulic failure	Days
A	Net carbon assimilation	µmol m^{-2} s^{-1}
g_{s}	Stomatal conductance	mmol m^{-2} s^{-1}

Table 1. 2: Leaf morphoanatomical traits for 11 genotypes of *C. canephora* cultivated in Western Amazon. Values followed by the same letter do not differ significantly among genotypes at the $P < 0.05$ level using the Tukey test.

Genotype	*SD* (stomata mm^{-2})	*SS* (µm^{2})	f_{gc} (%)	g_{wmax} (mol m^{-2} s^{-1})	V_d (mm mm^{-2})
2	407 (2.96) abc	247 (3.81) bc	19.7 (0.16) ab	2.82 (0.02) bc	11.8 (0.30) abc
6	427 (5.69) ab	299 (3.08) abc	29.6 (0.60) a	3.26 (0.02) ab	9.77 (0.14) c
8	431 (19.4) ab	284 (1.45) abc	19.5 (0.10) ab	3.21 (0.01) ab	10.9 (0.33) abc
10	394 (6.95) abc	245 (3.59) bc	17.5 0.37) b	2.75 (0.04) bc	10.5 (0.66) bc
13	426 (7.79) ab	259 (8.97) abc	19.6 (0.43) ab	3.01 (0.05) abc	9.57 (0.36) c
16	415 (8.46) abc	312 (1.42) ab	21.5 (0.05) ab	3.23 (0.01) ab	8.93 (0.33) c
20	426 (5.67) ab	306 (2.12) ab	21.8 (0.37) ab	3.28 (0.01) ab	12.1 (0.47) abc
25	446 (3.47) a	335 (2.71) a	30.4 (0.45) a	3.59 (0.02) a	9.87 (0.26) c
37	367 (13.3) c	235 (4.50) c	15.2 (0.12) b	2.48 (0.02) c	14.0 (0.56) ab
57	379 (14.3) bc	229 (8.43) bc	17.2 (0.50) b	2.62 (0.02) bc	13.4 (0.18) ab
93	387 (3.01) abc	241 (2.47) bc	16.7 (0.39) b	2.64 (0.01) bc	14.5 (0.43) a

SD - stomatal density, *SS* – stomata size, f_{gc} – fraction of epidermis allocated to stomata, g_{wmax} – theoretical maximum stomatal conductance, V_d – vein density.

Table 1. 3: Leaf traits associated with gas exchange and water relations for 11 genotypes of *C. canephora* cultivated in Western Amazon. Values followed by the same letter do not differ significantly among genotypes at the $P < 0.05$ level using the Tukey test.



Genotype	A (µmol m^{-2} s^{-1})	g_s (mmol m^{-2} s^{-1})	E (mol m^{-2} s^{-1})	K_{leaf} (mmol m^{-2} s^{-1} MPa)	Ψ_{md} (MPa)
2	15.1 (0.93) ab	281 (52.3) ab	6.04 (0.75) ab	10.2 (0.67) ab	-0.46 (0.02) a
6	14.4 (1.29) ab	356 (90.8) a	8.10 (1.32) a	15.1 (0.60) a	-0.44 (0.02) a
8	17.6 (0.68) a	498 (28.4) a	9.98 (0.48) a	18.1 (0.93) a	-0.40 (0.01) a
10	12.6 (0.71) abc	298 (40.2) ab	6.81 (0.77) ab	16.6 (0.69) a	-0.37 (0.01) a
13	11.7 (0.55) bc	231 (16.3) ab	5.28 (0.42) ab	15.1 (0.67) a	-0.37 (0.02) a
16	11.5 (0.69) bc	191 (13.7) abc	4.51 (0.26) abc	12.8 (0.27) a	-0.46 (0.02) a
20	11.4 (0.45) bc	209 (36.2) abc	4.47 (0.43) abc	13.5 (0.74) a	-0.39 (0.01) a
25	16.0 (0.79) ab	518 (9.30) a	10.3 (0.22) a	12.2 (0.67) a	-0.46 (0.02) a
37	9.21 (0.13) cd	117 (5.44) bc	2.94 (0.10) bc	4.09 (0.46) c	-0.74 (0.02) b
57	11.7 (0.81) abc	255 (43.5) ab	5.94 (0.87) ab	4.86 (0.45) bc	-0.71 (0.02) b
93	7.42 (0.49) d	82.7 (8.43) c	2.14 (0.18) c	4.79 (0.26) bc	-0.71 (0.03) b

Genotype	C_{ft} (MPa)	LWC (%)	g_{leaf} (mmol m^{-2} s^{-1})	$g_{canopy\text{-}res}$ (mmol s^{-1})
2	0.93 (0.09) a	138 (8.06) a	2.18 (0.05) abcd	11.8 (0.29) bc
6	0.79 (0.12) a	134 (0.88) a	2.54 (0.07) a	14.0 (0.36) abc
8	0.52 (0.06) a	121 (4.46) a	2.36 (0.06) abc	12.6 (0.31) abc
10	1.24 (0.43) a	139 (5.54) a	2.22 (0.09) abcd	18.2 (0.74) ab
13	0.82 (0.14) a	129 (10.9) a	2.50 (0.15) a	20.7 (1.27) a
16	0.73 (0.09) a	118 (3.13) a	2.47 (0.06) ab	16.4 (0.40) ab
20	0.81 (0.07) a	118 (6.71) a	2.17 (0.05) abcd	15.6 (0.37) abc
25	0.71 (0.11) a	136 (5.65) a	2.47 (0.10) ab	20.7 (0.87) a
37	0.64 (0.02) a	121 (5.89) a	1.79 (0.10) d	11.8 (0.51) bc
57	0.85 (0.02) a	129 (5.49) a	1.81 (0.08) cd	8.20 (0.36) c
93	0.66 (0.10) a	116 (3.68) a	1.91 (0.06) bcd	10.6 (0.31) bc

A – net carbon assimilation, g_s – stomatal conductance, E – transpiration rate, K_{leaf} – leaf hydraulic conductance, Ψ_{md} – midday water potential, C_{ft} – leaf bulk capacitance, LWC – leaf water content, $g_{leaf\text{-}res}$ – leaf minimum conductance, $g_{canopy\text{-}res}$ – canopy minimum conductance.

.

Table 1. 4: Leaf traits associated with drought tolerance for 11 genotypes of *C. canephora* cultivated in Western Amazon.

Genotype	Ψ_{TLP} (MPa)	P_{50} (MPa)	P_{88} (MPa)
2	-1.72 (0.07) ab	-1.06 [-0.99; -1.13] a	-1.42 [-1.25; -1.54] a
6	-1.56 (0.04) a	-1.57 [-1.45; -1.66] c	-2.10 [-2.00; -2.16] c
8	-1.72 (0.06) ab	-1.33 [-1.24; -1.40] b	-1.62 [-1.52; -1.74] ab
10	-1.59 (0.05) ab	-1.13 [-1.08; -1.19] a	-1.72 [-1.62; -1.78] b
13	-1.72 (0.03) ab	-1.28 [-1.09; -1.40] abc	-2.03 [-1.81; -2.30] bc
16	-1.82 (0.04) ab	-1.13 [-1.07; -1.16] a	-1.46 [-1.35; -1.56] a
20	-1.79 (0.04) ab	-1.80 [-1.70; -1.90] cd	-2.10 [-2.03; -2.15] c
25	-1.70 (0.02) ab	-1.94 [-1.83; -2.02] d	-2.32 [-2.10; -2.43] d
37	-1.85 (0.01) b	-1.39 [-1.29; -1.46] b	-1.76 [-1.65; -1.87] b
57	-1.61 (0.03) ab	-1.44 [-1.35; -1.49] bc	-1.78 [-1.67; -1.88] b
93	-1.82 (0.01) ab	-1.34 [-1.33; -1.51] ab	-1.99 [-1.68; -2.13] bc

For the Ψ_{TLP}, values followed by the same letter do not differ significantly among genotypes at the $P < 0.05$ level using the Tukey test; whereas for P_{50} and P_{88}, significant differences across genotypes were considered when their respective confidence intervals (numbers in brackets) did not overlap). Traits abbreviation as in Table 1. Ψ_{tlp} - turgor loss point, P50 – Ψ leaf inducing 50% loss of leaf hydraulic conductance, P88 – Ψ leaf inducing 88% loss of leaf hydraulic conductance.

Table 1. 5: Variation in leaf morphoanatomical and physiological traits associated with carbon assimilation, water relations, drought tolerance, and yield among 11 genotypes of *C. canephora* cultivated in Western Amazon.

Trait	Min	Max	Mean	Ratio
SD	368	446	410	1.21
SS	229	335	273	1.46
f_{gc}	15.2	30.4	20.8	2.00
g_{wmax}	2.48	3.59	2.99	1.44
V_d	8.93	14.6	11.4	1.63
C_{ft}	0.52	1.24	0.79	2.41
LWC	117	139	128	1.19
Ψ_{md}	-0.74	-0.37	-0.50	0.50
K_{leaf}	4.09	18.1	11.6	4.43
E	2.14	10.4	6.05	4.84
$g_{leaf\text{-}res}$	1.79	2.54	2.22	1.42
$g_{canopy\text{-}res}$	8.22	20.7	14.6	2.52
Ψ_{tlp}	-1.85	-1.56	-1.72	0.84
P_{50}	1.06	1.94	1.40	1.83
P_{88}	1.42	2.32	1.85	1.63
HSM_{50}	-0.70	0.24	-0.32	-0.35
HSM_{88}	-0.36	0.62	0.13	-1.73
THF	28.5	74.5	44.3	2.61
A	7.42	17.6	12.6	2.37
g_s	0.08	0.52	0.28	6.26
Yield	0.67	2.50	1.41	3.71

The table shows the overall minimum (Min), maximum (Max), mean (SE), and the ratio of the maximum: minimum traits values across genotypes. *SD* - stomatal density, *SS* – stomata size, f_{gc} – fraction of epidermis allocated to stomata, g_{wmax} – theoretical maximum stomatal conductance, V_d – vein density, C_{ft} – leaf bulk capacitance, LWC – leaf water content, Ψ_{md} – midday water potential, K_{leaf} – leaf hydraulic conductance, *E* – transpiration rate; $g_{leaf\text{-}res}$ – leaf minimum conductance, $g_{canopy\text{-}res}$ – canopy minimum conductance, Ψ_{tlp} - turgor loss point, P50 – Ψ leaf inducing 50% loss of leaf hydraulic conductance, P88 – Ψ leaf inducing 88% loss of leaf hydraulic conductance, HSM_{50} – hydraulic safety margin at P50, HSM_{88} – hydraulic safety margin at P88, THF – time to hydraulic failure, *A* – net carbon assimilation, g_s – stomatal conductance.

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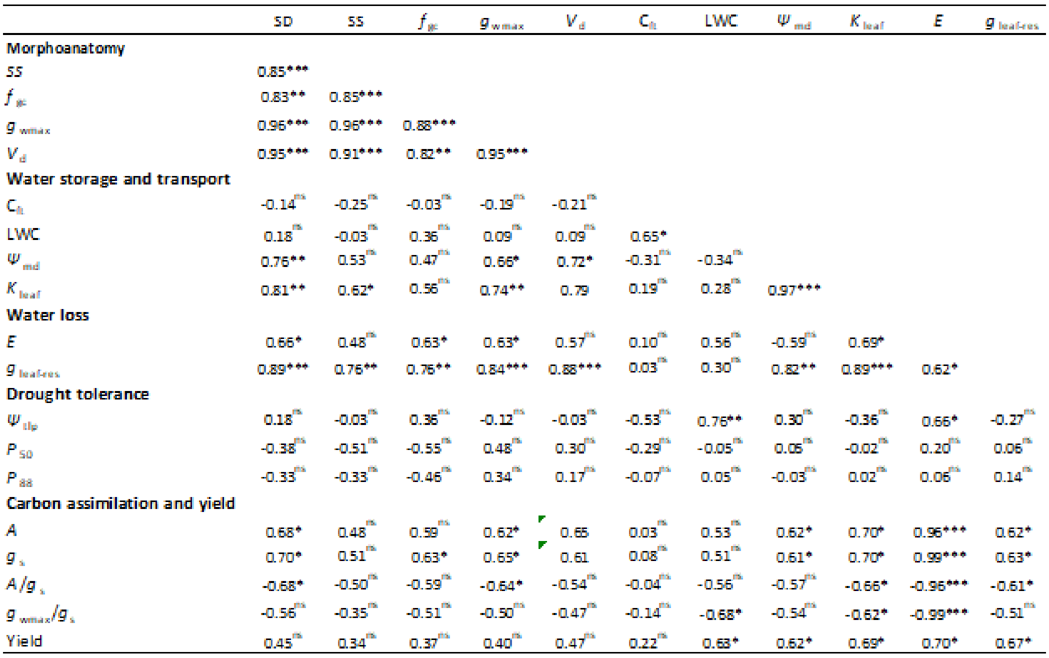
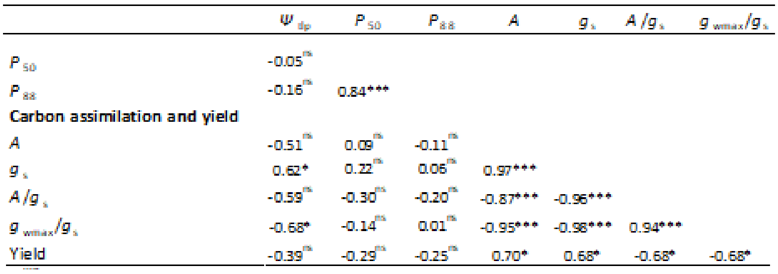
Table 1. 6: Pearson correlation between leaf morphoanatomical and physiological traits associated with carbon assimilation, water relations, drought tolerance, and yield among 11 genotypes of *C. canephora* cultivated in Western Amazon.



	SD	SS	f_{gc}	g_{wmax}	V_d	C_{ft}	LWC	Ψ_{md}	K_{leaf}	E	$g_{leaf\text{-}res}$
Morphoanatomy											
SS	0.85***										
f_{gc}	0.83**	0.85***									
g_{wmax}	0.96***	0.96***	0.88***								
V_d	0.95***	0.91***	0.82**	0.95***							
Water storage and transport											
C_{ft}	-0.14^{ns}	-0.25^{ns}	-0.03^{ns}	-0.19^{ns}	-0.21^{ns}						
LWC	0.18^{ns}	-0.03^{ns}	0.36^{ns}	0.09^{ns}	0.09^{ns}	0.65*					
Ψ_{md}	0.76**	0.53^{ns}	0.47^{ns}	0.66*	0.72*	-0.31^{ns}	-0.34^{ns}				
K_{leaf}	0.81**	0.62*	0.56^{ns}	0.74**	0.79	0.19^{ns}	0.28^{ns}	0.97***			
Water loss											
E	0.66*	0.48^{ns}	0.63*	0.63*	0.57^{ns}	0.10^{ns}	0.56^{ns}	-0.59^{ns}	0.69*		
$g_{leaf\text{-}res}$	0.89***	0.76**	0.76**	0.84***	0.88***	0.03^{ns}	0.30^{ns}	0.82**	0.89***	0.62*	
Drought tolerance											
Ψ_{tlp}	0.18^{ns}	-0.03^{ns}	0.36^{ns}	-0.12^{ns}	-0.03^{ns}	-0.53^{ns}	0.76**	0.30^{ns}	-0.36^{ns}	0.66*	-0.27^{ns}
P_{50}	-0.38^{ns}	-0.51^{ns}	-0.55^{ns}	0.48^{ns}	0.30^{ns}	-0.29^{ns}	-0.05^{ns}	0.06^{ns}	-0.02^{ns}	0.20^{ns}	0.06^{ns}
P_{88}	-0.33^{ns}	-0.33^{ns}	-0.46^{ns}	0.34^{ns}	0.17^{ns}	-0.07^{ns}	0.05^{ns}	-0.03^{ns}	0.02^{ns}	0.06^{ns}	0.14^{ns}
Carbon assimilation and yield											
A	0.68*	0.48^{ns}	0.59^{ns}	0.62*	0.65	0.03^{ns}	0.53^{ns}	0.62*	0.70*	0.96***	0.62*
g_s	0.70*	0.51^{ns}	0.63*	0.65*	0.61	0.08^{ns}	0.51^{ns}	0.61*	0.70*	0.99***	0.63*
A/g_s	-0.68*	-0.50^{ns}	-0.59^{ns}	-0.64*	-0.54^{ns}	-0.04^{ns}	-0.56^{ns}	-0.57^{ns}	-0.66*	-0.96***	-0.61*
g_{wmax}/g_s	-0.56^{ns}	-0.35^{ns}	-0.51^{ns}	-0.50^{ns}	-0.47^{ns}	-0.14^{ns}	-0.68*	-0.54^{ns}	-0.62*	-0.99***	-0.51^{ns}
Yield	0.45^{ns}	0.34^{ns}	0.37^{ns}	0.40^{ns}	0.47^{ns}	0.22^{ns}	0.63*	0.62*	0.69*	0.70*	0.67*



	Ψ_{tlp}	P_{50}	P_{88}	A	g_s	A/g_s	g_{wmax}/g_s
P_{50}	-0.05^{ns}						
P_{88}	-0.16^{ns}	0.84***					
Carbon assimilation and yield							
A	-0.51^{ns}	0.09^{ns}	-0.11^{ns}				
g_s	0.62*	0.22^{ns}	0.06^{ns}	0.97***			
A/g_s	-0.59^{ns}	-0.30^{ns}	-0.20^{ns}	-0.87***	-0.96***		
g_{wmax}/g_s	-0.68*	-0.14^{ns}	0.01^{ns}	-0.95***	-0.98***	0.94***	
Yield	-0.39^{ns}	-0.29^{ns}	-0.25^{ns}	0.70*	0.68*	-0.68*	-0.68*

The table shows the correlation coefficients and their significance: ns, nonsignificant; ***, $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.05$. SD - stomatal density, SS – stomata size, f_{gc} – fraction of epidermis allocated to stomata, g_{wmax} – theoretical maximum stomatal conductance, V_d – vein density, C_{ft} – leaf bulk capacitance, LWC – leaf water content, Ψ_{md} – midday water potential, K_{leaf} – leaf hydraulic conductance, E – transpiration rate; $g_{leaf\text{-}res}$ – leaf minimum conductance, $g_{canopy\text{-}res}$ – canopy minimum conductance, Ψ_{tlp} - turgor loss point, P50 – Ψ leaf inducing 50% loss of leaf hydraulic conductance, P88 – Ψ leaf inducing 88% loss of leaf hydraulic conductance, HSM_{50} – hydraulic safety margin at P50, HSM_{88} – hydraulic safety margin at P88, THF – time to hydraulic failure, A – net carbon assimilation, g_s – stomatal conductance.

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Figures

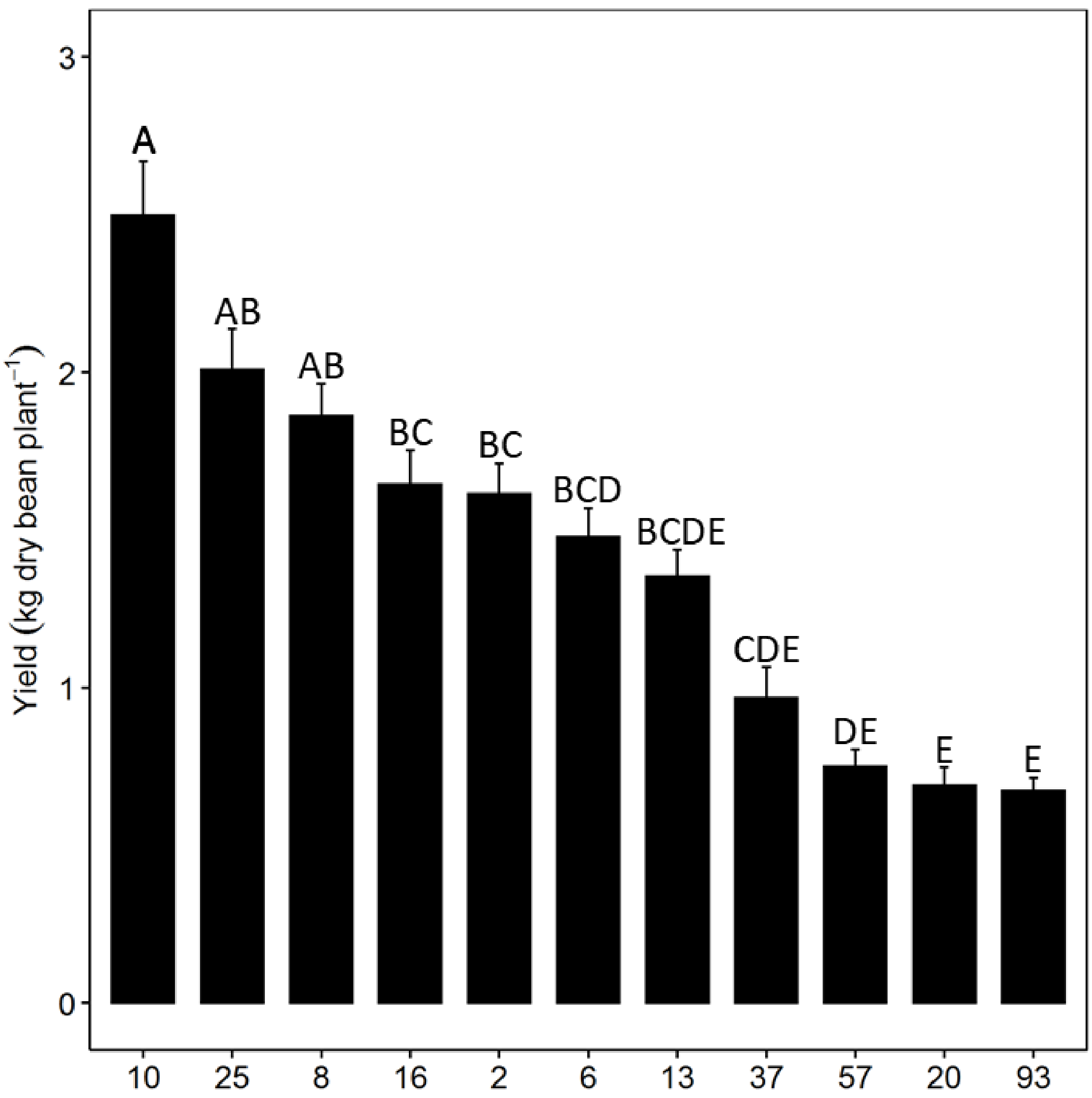

Figure 1. 1: Differences in coffee yield across 11 genotypes of *C. canephora* cultivated in the Western Amazon. The graph shows the mean values for each genotype (n = 4). Different letters denote statistically significant differences through the Tukey test (P < 0.05)

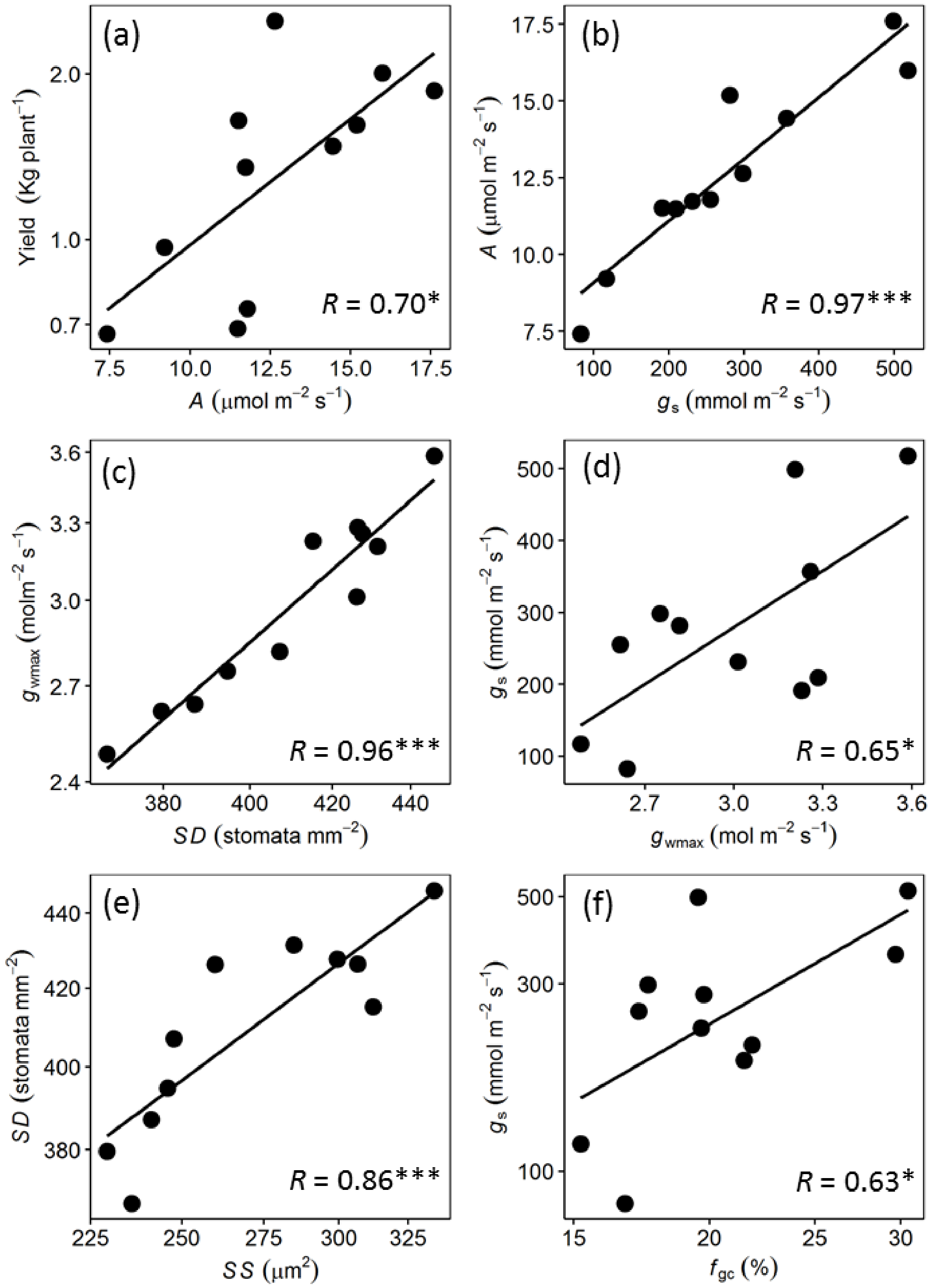

Figure 1. 2: Relationships between (a) coffee yield and the light-saturated CO_2 assimilation rate (A), (b) A and stomatal conductance (g_s), (c) theoretical maximum stomatal conductance (g_{wmax}) and stomatal density (SD), (d) g_s and g_{wmax}, (e) SD and stomata size (SS), and (f) g_s and the fraction of epidermis allocated to stomata (f_{gc}) across 11 *C. canephora* genotypes cultivated in the Western Amazon. Significance of Pearson correlation coefficients: ns, nonsignificant; ***, $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.05$. Each circle represents the mean values for each genotype (n = 4).

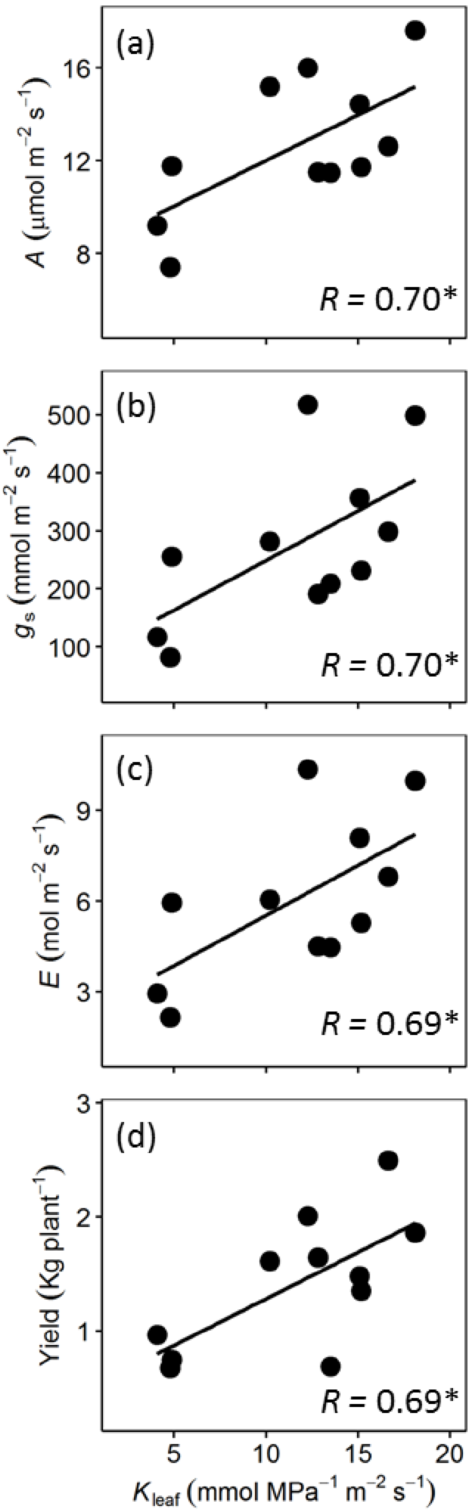

Figure 1. 3. Relationships between (a) light-saturated CO_2 assimilation rate (*A*), (b) stomatal conductance (g_s), (c) transpiration rate (*E*), and (d) coffee yield with the leaf hydraulic conductance (K_{leaf}) across 11 *C. canephora* genotypes cultivated in the Western Amazon. Significance of Pearson correlation coefficients: ns, nonsignificant; ***, $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.05$. Each circle represents the mean values for each genotype (n = 4).

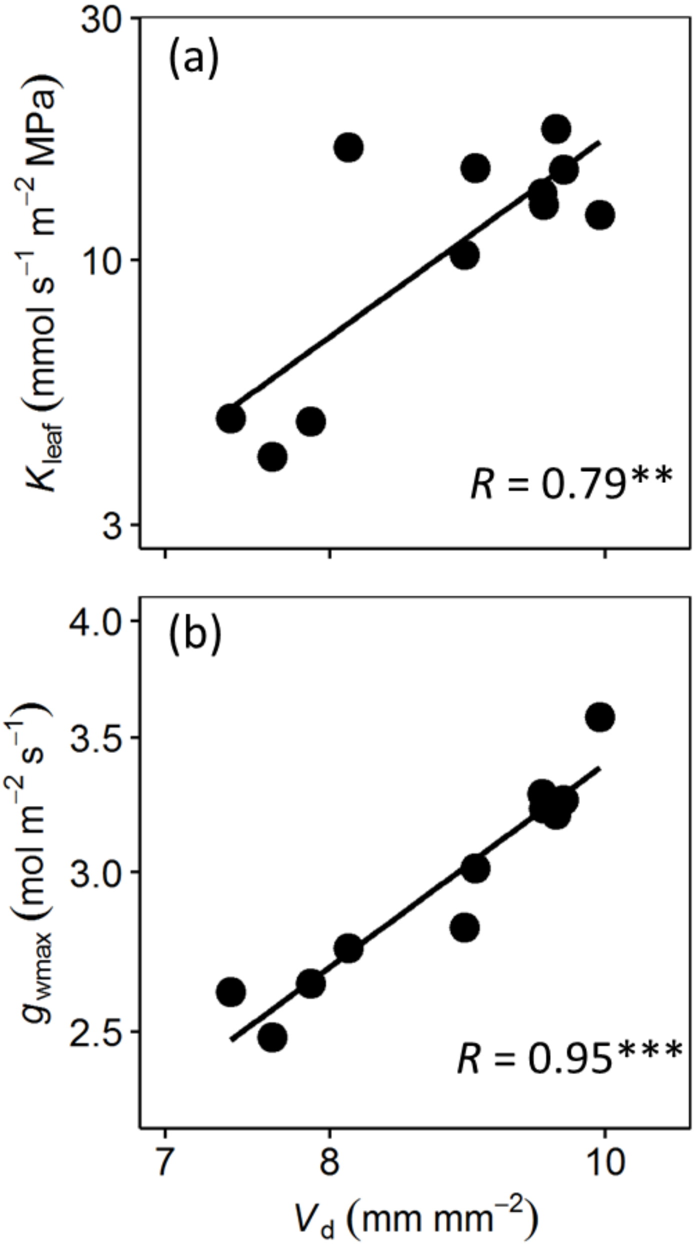

Figure 1. 4. Relationships between (a) leaf hydraulic conductance (K_{leaf}) and (b) theoretical maximum stomatal conductance (g_{wmax}) with vein density (Vd) across 11 *C. canephora* genotypes cultivated in the Western Amazon. Significance of Pearson correlation coefficients: ns, nonsignificant; ***, $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.05$. Each circle represents the mean values for each genotype (n = 4).

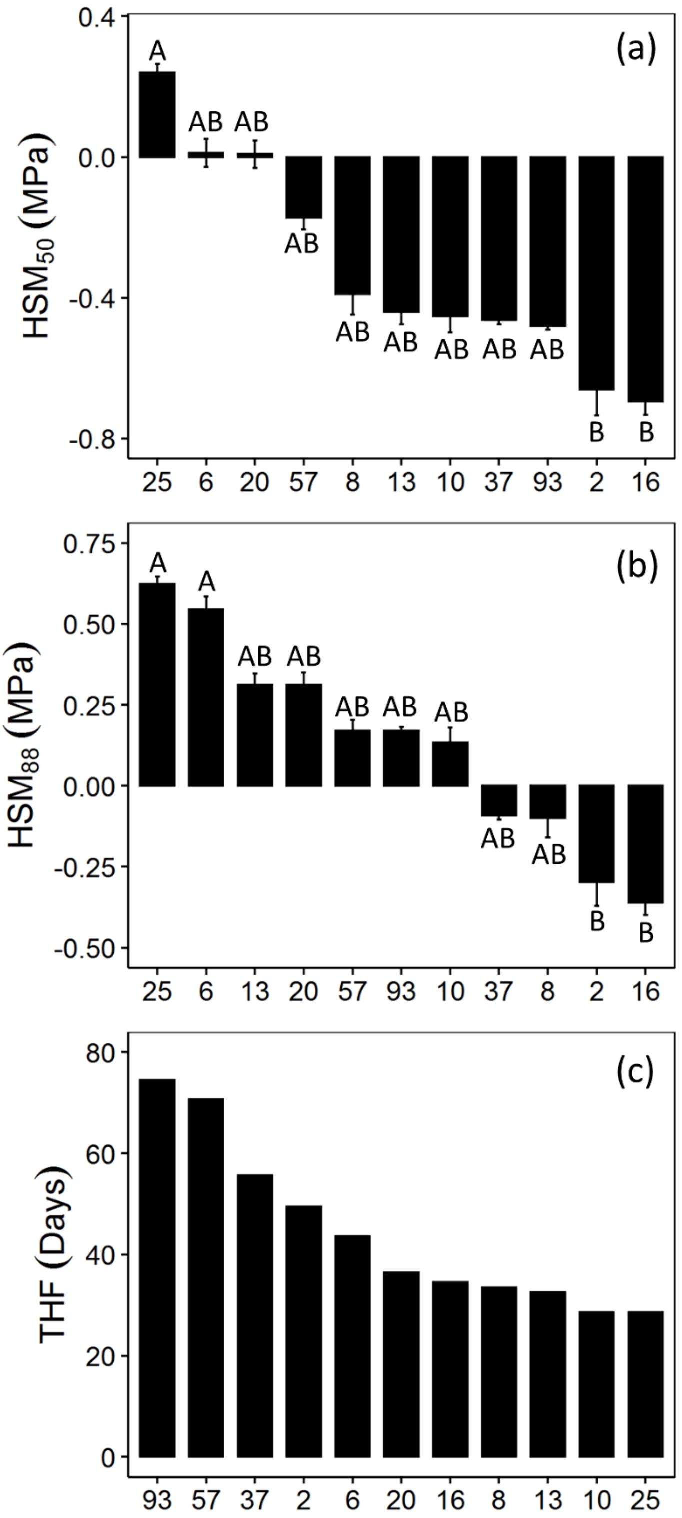

Figure 1. 5. Variation in the leaf hydraulic safety margins (HSM) across 11 *C. canephora* genotypes cultivated in the Western Amazon (a, b). The HSM$_{50}$ ($\Psi_{tlp} - P_{50}$) is shown in (a), and the HSM88 ($\Psi_{tlp} - P_{58}$) is shown in (b). The black bars represent the mean values for each genotype (n = 4). Different letters denote statistically significant differences through the Tukey test ($P < 0.05$). The graph also shows the variation in the simulated time to hydraulic failure (THF) across the studied genotypes (c).

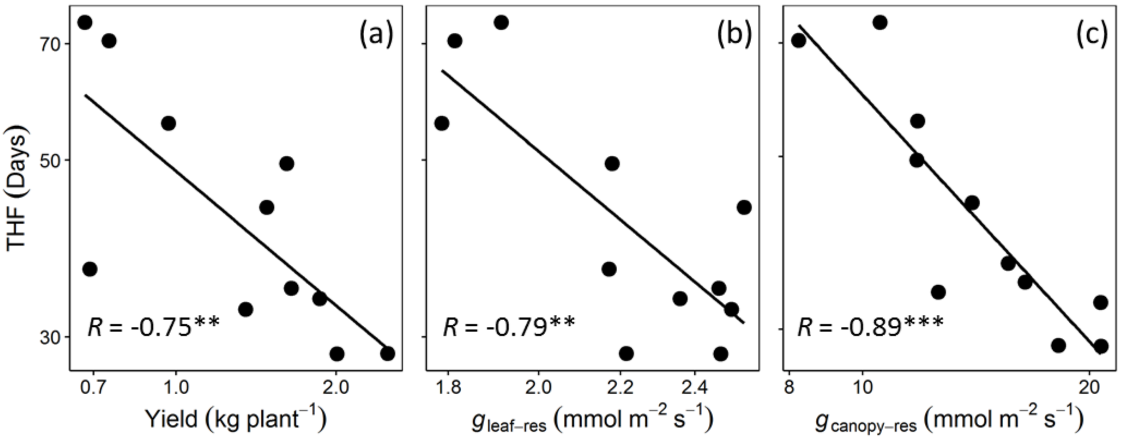

Figure 1. 6. Relationships between the time to hydraulic failure of leaves (THF) and (a) coffee yield, (b) leaf minimum conductance ($g_{\text{leaf-res}}$), and (c) canopy residual conductance ($g_{\text{canopy-res}}$) across 11 *C. canephora* genotypes cultivated in the Western Amazon. Significance of Pearson correlation coefficients: ns, nonsignificant; ***, $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.05$. Each circle represents the mean values for each genotype (n = 4).

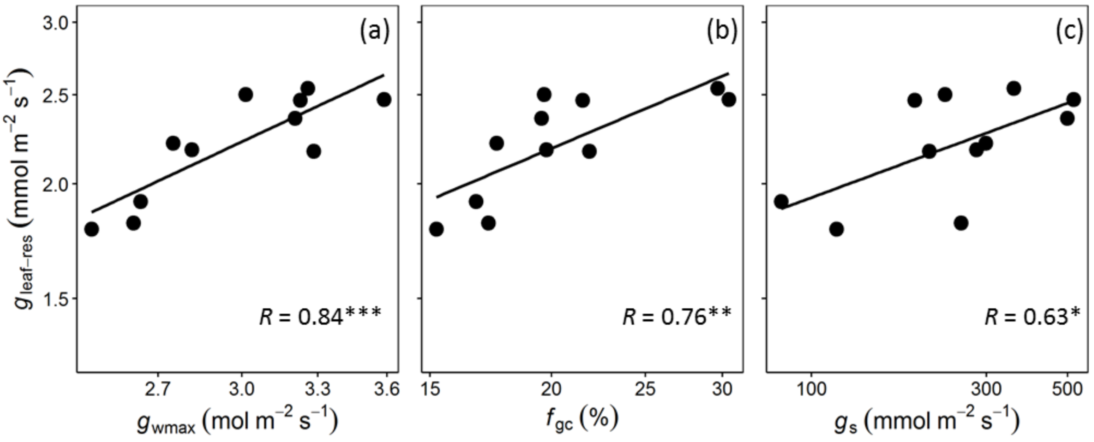

Figure 1. 7. Relationships between the leaf minimum conductance ($g_{leaf\text{-}res}$) and (a) the theoretical maximum stomatal conductance (g_{wmax}), (b) the fraction of the epidermis allocated to stomata (f_{gc}), and (c) the stomatal conductance (g_s) across 11 *C. canephora* genotypes cultivated in the Western Amazon. Significance of Pearson correlation coefficients: *ns*, nonsignificant; ***, P ≤ 0.001; **, P ≤ 0.01; *, P ≤ 0.05. Each circle represents the mean values for each genotype (n = 4).

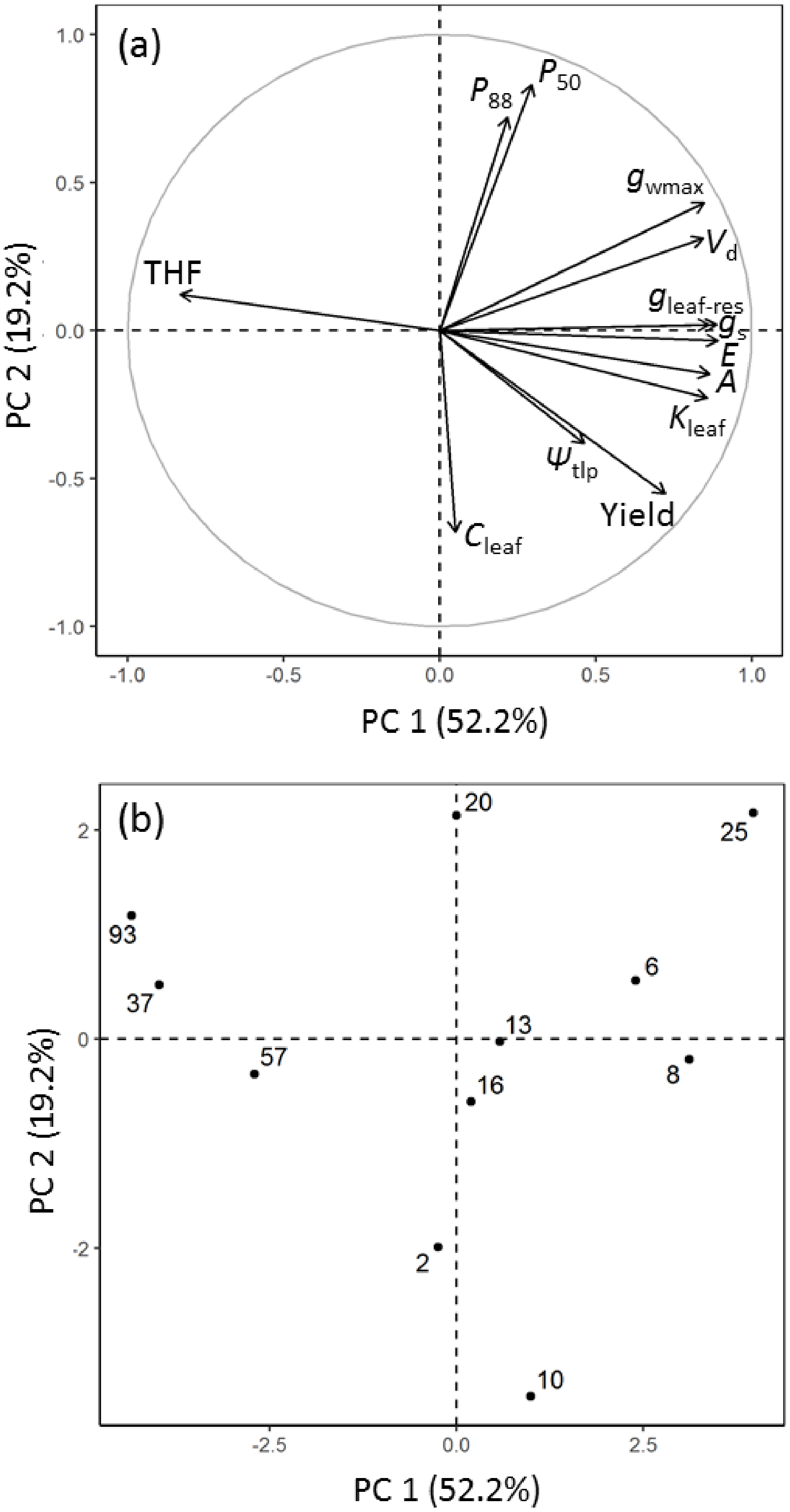

Figure 1. 8. Principal component analysis (PCA) with the mean values of the physiological and morphoanatomical leaf traits of 11 genotypes of *C. canephora* cultivated in the Western Amazon. The figures show the variation in traits scores (a), and genotypes scores (b) along the first two PCA axes with the percentages of explained variation given. g_{wmax} – theoretical maximum stomatal conductance, V_d – vein density, C_{leaf} – leaf bulk capacitance, K_{leaf} – leaf hydraulic conductance, E – transpiration rate;

$g_{\text{leaf-res}}$ – leaf minimum conductance, Ψ_{tlp} - turgor loss point, HSM_{50} – hydraulic safety margin at P50, HSM_{88} – hydraulic safety margin at P88 (P50 and P88– Ψ leaf inducing 50% and 88% loss of leaf hydraulic conductance, respectively), THF – time to hydraulic failure, *A* – net carbon assimilation, g_s – stomatal conductance. Genotypes: 10 - 010, 25, 8 – 08, 16 – BRS1216, 2 – AS2, 6 – 06, 13 – BRS3213, 37 – BRS3137, 57 – BRS2357, 20 – BRS3220, 93 – BRS3193.

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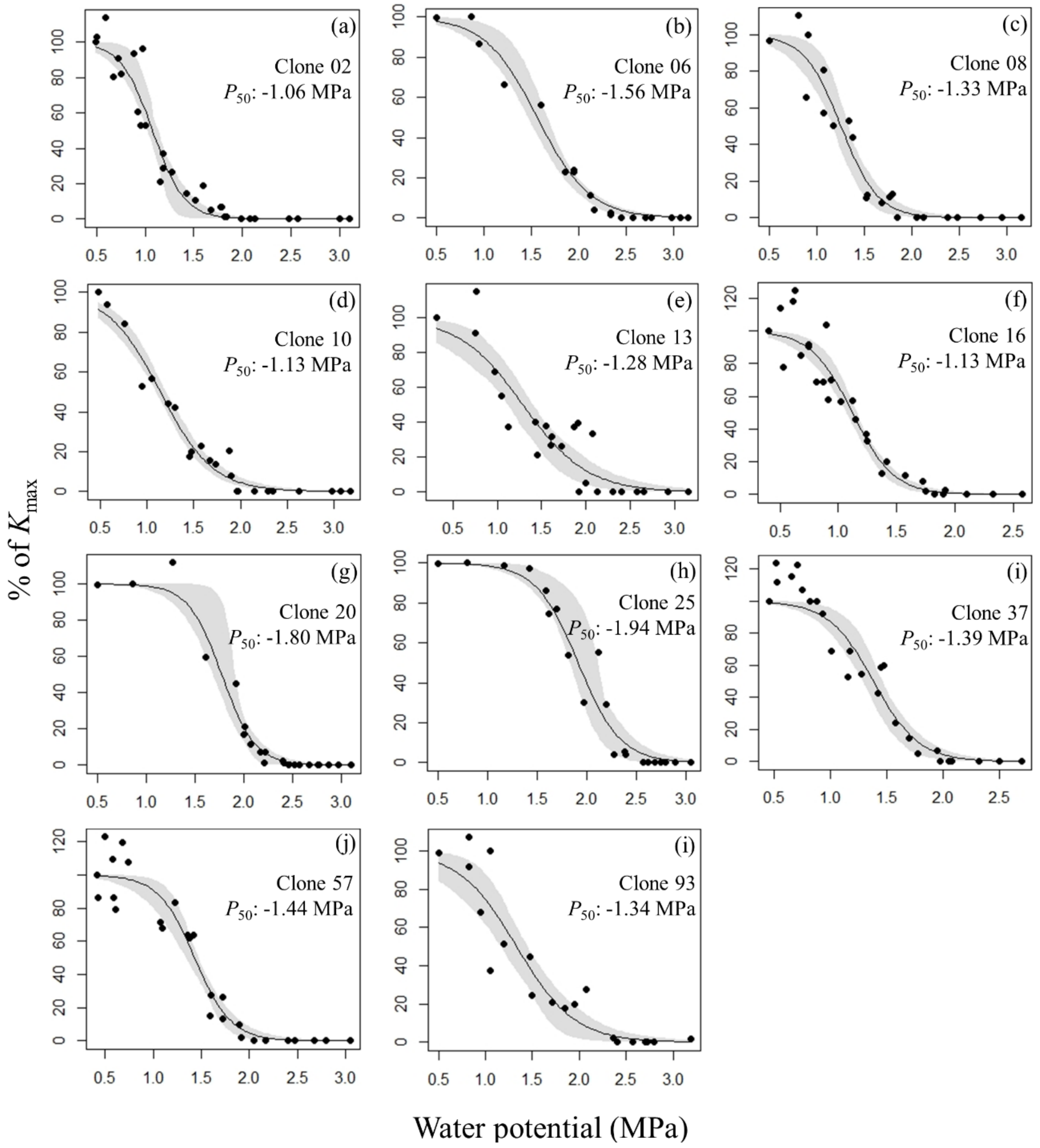

Figure 1. 9. Vulnerability curves of leaves from 11 genotypes cultivated in the Western Amazon. The graphs show the reductions in leaf hydraulic conductance from max (K_{max}) as a function of the decline in leaf water potential (Ψ_{leaf}) with dehydration.

CHAPTER 2

(AGRONOMY – INSS-2073-4395)

SEASONAL VARIATION IN PHYSIOLOGICAL PARAMETERS AND YIELD OF *COFFEA CANEPHORA* IN THE BRAZILIAN AMAZON

Abstract: Climate variation throughout the year affects photosynthesis and other physiological processes correlated with plant development and yield. This study objective to evaluate the changes in the physiological attributes of *Coffea canephora* genotypes over the year in the Brazilian Amazon and assess their relationship with crop yield. The experiment was carried out in three cultivation systems with three genotypes. The evaluations were carried out in four periods: the peak of the dry season (S1); the beginning of the rainy season (S2); the peak of the rainy season (S3); and the beginning of the dry season (S4). A dataset of gas exchange, pigment indices, chlorophyll fluorescence, branch growth, and coffee yield were obtained. The group of gas exchange variables was the main contributor to treatment discrimination and was most affected by seasons. As expected, the values of g_s, E, and A were lower in S1, while the values of $VPD_{Leaf\text{-}ar}$, T_{Leaf}, and IWUE were higher. Our results demonstrate that climatic seasonality affects the photosynthesis of Amazonian Robustas coffee, even under irrigated conditions, particularly in response to increased VPD. The physiological variables analyzed at the leaf level, even in different periods, did not explain the differences in the yield of *C. canephora*.

Keywords: Amazonian Robustas, irrigation, fertigation, photosynthesis, anthocyanin.

2.1. INTRODUCTION

The annual climatic seasonality of a region is mainly characterized by variations in the incidence of solar radiation, temperature, rainfall, wind, air relative humidity [1,2]. These variations can affect morphological, physiological, and biochemical processes, including *C*-assimilation and partitioning, respiration, nutrient uptake, translocation, and whole metabolism, increasing or reducing crop productive potential of the *Coffea canephora* Pierre ex Floehner [3–6]. Approximately, 15% of the Brazilian production of *C. canephora* is from the southwestern Brazilian Amazon, mainly from the state of Rondônia. This region is characterized by two well-defined seasons throughout the year: a rainy season (October to May) and a dry season (June to September). Both seasons have a high average temperature, small thermal amplitude, and high light availability [7,8].

In the rainy season, the high availability of water in the soil, high relative humidity, and low vapor pressure deficit (VPD) favor the opening of the stomata of coffee trees [9–11]. The increased stomatal opening leads to an increase in stomatal conductance (g_s) and, consequently, enhances the diffusion of CO_2 into the leaves [12]. The increase in the internal concentration of CO_2 (c_i) increases the net photosynthetic rate (A). Under these conditions, the risks of irreversible damage to the photosynthetic apparatus (“photoinhibition”) are reduced due to the high demand for energy (ATP and NADPH), even at high temperatures and/or light intensity [13]. On the other hand, during the dry season, water restriction can lead to partial or total closure of stomata throughout the day, decreasing g_s and c_i and thus compromising A [14]. However, in the biochemical phase of photosynthesis, the decrease in CO_2 fixation reduces the energy demand and triggers a cascade effect. This can result in photoinhibition and impairment of the photosynthetic apparatus due to the formation of reactive oxygen species (ROS) [13]. High light intensity and temperature can aggravate this situation [3,15].

Climate anomalies and increasingly frequent and intense droughts in the Amazon [16] have made irrigation crucial for coffee cultivation in the region. Complementary water supply reduces the risk of damage from intense and/or prolonged water deficits, especially when they occur during the reproductive growth phase [3,4,17]. Greater vigor, growth, and coffee yield are expected in irrigated cultivation systems than in dryland farming systems [18,19]. In Rondônia state, there is a predominance of localized irrigation as the management technique in coffee plantations. This irrigation method results in water and energy savings, compared to other methods, and allows for fertigation (aqueous fertilization) [20].

Coffee cultivation in irrigation and/or fertigation systems enables high yield in the Amazon region, with an average yield above 6 Mg/ha/year of clean (green) coffee [21,22]. However, the additional water supply to trees through irrigation may not be enough to avoid the negative effects of drought on coffee photosynthesis [12]. In this case, VPD is a key variable in inducing stomatal opening and closing. High VPD conditions lead to a considerable increase in tree transpiration rates [23,24]. In some situations, it can even overcome the capacity of the tree hydraulic system, which is inherent to the genotype, to absorb and/or transport water from the soil to the leaves, even with water available in the soil [12,25].

The main coffee genotypes grown in Rondônia are the result of the natural hybridization of genotypes belonging to the Group SG1 or “Conilon” [26,27] from the state of Espírito Santo on the eastern Brazilian coast, with other genotypes of an undefined genetic group phenotypically described as “robusta” [21,28], which were introduced from the Germplasm Bank of the Agronomy Institute of Campinas (IAC), Campinas, Brazil. These hybrid materials are locally known as Robustas Amazônicos or Robustas da Amazônia (Amazonian Robustas) [21] and stand out for their high vigor, tolerance to diseases [18], high yield [18,19], and superior drinking quality [28]. However, these genotypes are generally more sensitive to drought than “Conilon” [21]. In recent years, several genotypes of Amazonian Robustas have spread to other producing regions of *C. canephora* in Brazil, mainly in the state of Espírito Santo.

Despite the success of coffee production in Rondônia, little information is available on the ecophysiology of coffee trees grown in the Brazilian Amazon. Therefore, the objective of this study was to evaluate the seasonal variation in physiological variables related to photosynthesis in genotypes of Amazonian Robustas under fertigation, irrigation, and dryland farming, cultivated under field conditions in the Amazon, and to correlate these variables with yield.

2.2. MATERIALS AND METHODS

Experimental area and coffee cultivation

The research was carried out at the experimental farm of the Federal University of Rondônia Foundation (*Fundação Universidade Federal de Rondônia - UNIR*), located in the municipality of Rolim de Moura, Rondônia state, Brazil (11º34’5” S; 61°41’12” W; altitude 275m). The region climate is of type Aw (tropical with dry winter), according to the Köppen classification [8]. The experimental area has a flat topography and dystrophic red-yellow latosol (ferralsols) soil [29]. Precipitation and temperature data of the region were obtained from an automatic station for the study period (Figure 1) from the Meteorological Institute (*Instituto Nacional de Meteorologia - INMET*) of Brazil [30]. In the evaluation days, the average values of maximum, average and minimum temperature, average and minimum relative humidity were obtained (Table 1). From these data, the average and maximum air vapor pressure deficit (VPD) were calculated, using the difference between the vapor saturation pressure (es) and the partial vapor pressure (ea) [31].

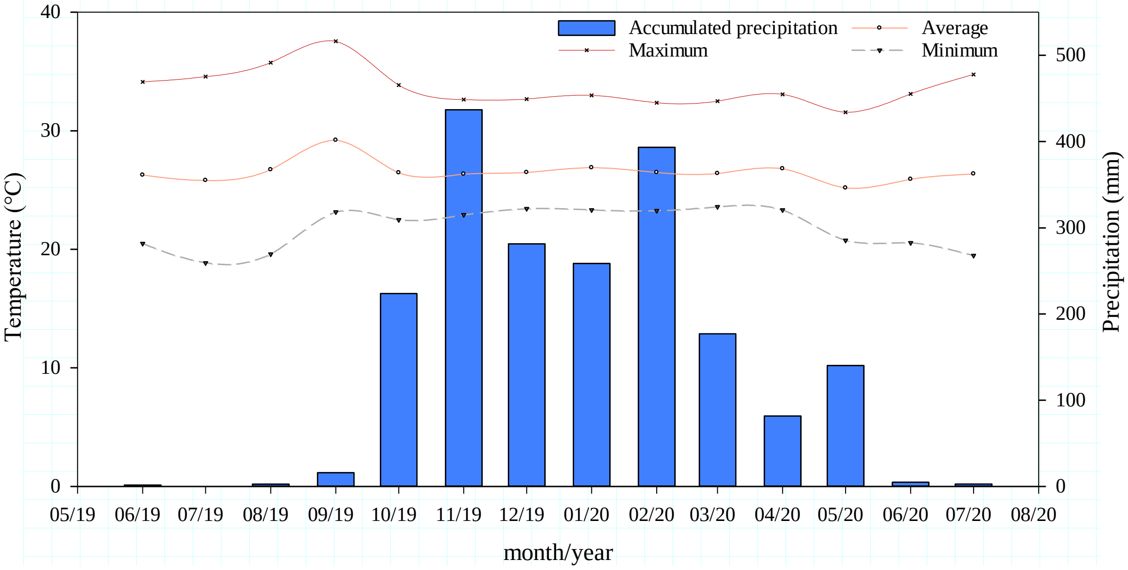

Figure 2. 1: Accumulated precipitation data (blue) and minimum (grey), average (orange) and maximum (red) means temperature of the region during the experimental period.

Table 2. 1. Climatic data of the region during the assessments. Average of daily mean values.

Season	**Rel. Air Humidity (%)**		**Temperature (°C)**			**VPD (kPa)**	
	Average	**Minimum**	**Maximum**	**Average**	**Minimum**	**Average**	**Maximum**
S1	56,5	30,0	36,4	28,7	22,5	1,38	3,31
S2	78,7	50,3	32,7	26,3	22,7	0,59	1,93
S3	83,9	60,7	30,9	25,8	23,1	0,44	1,39
S4	69,0	38,0	33,1	25,8	20,2	0,84	2,46

S1 – peak of the dry season, with evaluations carried out between August 23 and 25, 2019; S2 – beginning of the rainy season, with evaluations carried out between November 21 and 24, 2019; S3 – peak of the rainy season, with evaluations carried out between February 28 and March 1, 2020; S4 – beginning of the dry season, with evaluations carried out between June 26 and 28, 2020. VPD – vapors pressure deficit.

The coffee plantation was established in November 2013, with seedlings of Amazonian Robustas (*Coffea canephora* Pierre ex Floehner) of clonal origin spaced at 3.0 m between rows and 1.5 m between trees. Part of the area was cultivated with supplementary irrigation (fertigation and irrigation systems) and part without irrigation (dryland farming). The average yield of the three previous harvests was 7.99 Mg/ha under fertigation (2018/2019), 7.86 Mg/ha under irrigation (2017/2018), and 6.40 Mg/ha under dryland farming (2016/2017) of green coffee, with no difference between cultivated genotypes. Preliminary analysis showed statistical differences only for the dryland farming system between the 2016/2017 crop and crops of the other two years (unpublished data).

Fertilization of the coffee plantation was carried out according to [32], aiming for a yield of 6.0 to 7.8 Mg/ha of green coffee. In the irrigation and dryland farming systems, the distribution of fertilizers was conventional (manual) in the projection of the coffee canopy area. In the fertigation system, fertilization was carried out using a localized irrigation system after the solubilization of fertilizers. Cultivation treatments were carried out throughout the experimental area according to [33] and the technical recommendations for the Rondônia region [34]. In the fertigation and irrigation cultivation systems, irrigation was carried out with drip systems in June, July, August, and September of 2019. However, irrigation was suspended between July 15 and August 10, 2019. This procedure is common in production units in the region and aims to increase uniformity in flowering and, consequently, in fruit ripening [35].

Experimental design

The experimental area was divided into three separate cropping systems [irrigation (I), fertigation (F), and dryland farming (D)], but in adjacent areas. In each cultivation system the experiment was arranged in split-plot a randomized block design (RBD) with four replicates. Three genotypes [clones “25” (G1), “08” (G2), and “03” (G3)] were evaluated in four seasons (S1, S2, S3, and S4). The three genotypes are highly productive and the most cultivated in Rondônia [22,28,36]. The evaluation periods were selected to assess climatic seasonality effects on crops. The four seasons studied were as follows:

1. S1 – peak of the dry season, with evaluations carried out between August 23 and 25, 2019. During this period, trees were in pre-anthesis (Biologische Bundesanstalt, Bundessortenamt and Chemical Industry scale - BBCH - 58,59 [37]) in the dryland farming system and at the beginning of anthesis (BBCH 61-65) in the irrigation and fertigation systems. In this season there is a reduced rate of vegetative growth.
2. S2 – beginning of the rainy season, with evaluations carried out between November 21 and 24, 2019. During this period, the accelerated growth of the fruit occurs (BBCH 73-75) and the resumption of the vegetative growth rates..
3. S3 – peak of the rainy season, with evaluations carried out between February 28 and March 1, 2020. During this period, the final stage of bean filling occurred (BBCH 77-79). At this season there is high rates of vegetative growth.
4. S4 – beginning of the dry season, with evaluations carried out between June 26 and 28, 2020. In this period, trees had been harvested 20 days before and, atypically, had only partially flowered (BBCH 58-65) (2020/2021 harvest). At this season vegetative growth is stopped (dryland farming system) or reduced (irrigation and fertigation systems).

Assessment of physiological variables associated with photosynthesis

Evaluations of pigment and chlorophyll a (*Chl a*) and b (*Chl b*) indices, *Chl a* fluorescence, and gas exchange were performed out in the morning on fully expanded leaves of the third or fourth nodes from the apex of the plagiotropic branch, chosen from the upper third of the tree [38]. The analyzes were carried out in two plants i.e. two leaves of each experimental unit, whose mean was used for statistical analyses. The specific procedures for the assessment of each variable group are described below.

Leaf pigment index

Chlorophyll, flavonoid, and anthocyanin indices were determined using an instantaneous, non-destructive chlorophyll and polyphenol meter from DUALEX Scientific^{TM} (Force-A, Orsay, France) [39]. The nitrogen balance index (NBI) was calculated as the ratio between the chlorophyll index and the flavonoid index. For S2, the data for these variables were obtained indirectly through statistical imputation using multivariate regression.

In addition, the chlorophyll index were also determined using a Chlorofilog CFL-1030 FALKER chlorophyll meter (Automação Agrícola, Porto Alegre, Brazil) to evaluated theindices of *Chl a* and *Chl b* separately. The ratio of *Chl a/Chl b* and total chlorophyll (*Chlt* = *Chla* + *Chl b*) were calculated.

Chlorophyll a fluorescence

The transient OJIP fluorescence of *Chl a* was measured using a portable FluorPen FP100 fluorometer (Photon Systems Instruments, Drasov, Czech Republic) following the manufacturer's instructions. The leaves were adapted to the dark for 30 min and then exposed to a saturating pulse of blue light (3000 µmol m^{-2} s^{-1}). Data were obtained from fluorescence intensity at points *O* (F_o=$F_{50µs}$), *J* (F_j=F_{2ms}), I (F_i=F_{60ms}), and P (F_p=F_m) and from the fluorescence intensity at=300µs ($F_{300µs}$). From the fluorescence data, the maximum variable fluorescence (F_v=*Fm-Fo*), the ratios F_m/F_o and F_v/F_o, the proportion of F*v* until F_j (V_j) and until F_i (V_i), and the parameters of the *JIP*-test were calculated according to previous reports [40,41]. Specific flow parameters were obtained by primary quinone reduced (Q_a^-), by reaction center (*RC*) (t = 0), and by the absorbed (*ABS/RC*), trapped (TR_o/RC), dissipated (DI_o/RC), and transported (ET_o/RC) energy. In addition, we calculated the maximum (Phi_{Po}) and average (Phi_{pav}) quantum yields of the primary photochemical reactions of photosystem II (PSII) (t=0), quantum yield of energy dissipation (Phi_{Do}) and electron transport (Phi_{Eo}), and the probability of an exciton moving an electron in the electron transport chain beyond $Q_{a.}^-$ (Psi_o). We also obtained the area under the fluorescence curve (Area), the area under the fluorescence curve between $F_{40µs}$ and F_{1s} (FixArea), normalized area (S_m), smallest possible S_m (S_s), approximate initial slope of the fluorescence transient normalized with F_V (M_o), the turnover number of Q_a (N), ratio of reaction centers and the absorbance (*RC/ABS*) and the performance index on the absorption base (PI_{ABS}).

Gas exchange

Using a portable infrared gas measurement system (IRGA; LI-COR – Li 6800), we obtained the following traits: the net assimilation rate of CO_2 (*A*; µmol CO_2 m^{-2} s^{-1}); transpiration rate (*E*; mmol H_2O $m^{-2}s^{-1}$); ambient (external) CO_2 concentration (*Ca*; µmol/mol); internal CO_2 concentration (*Ci*–µmol/mol); stomatal conductance (g_s – mol $m^{-2}s^{-1}$); VPD between the leaf and the air of the IRGA chamber (VPD_L; kPa); and leaf temperature (T_L–°C). The evaluations were carried out with an ambient concentration of 400 µL L^{-1} CO_2 and an irradiance of 1000 µmol m^{-2} s^{-1}. From the equipment data, we calculated the instant carboxylation rate (*A/Ci*), *Ci/Ca* ratio, instant water use efficiency (WUE=*A/E*), and intrinsic water use efficiency (iWUE=A/g_s).

Relative water content (RWC) in leaves

To determine the RWC, four segments measuring 1 cm² were cut from six fresh leaves per experimental unit and weighed on a precision balance to verify fresh mass (FM). The segments were then immersed in distilled water for 24 h to obtain turgid mass (TM). Subsequently, the plant material was placed in an oven at 70°C for 24 h to obtain the dry mass (DM). RWC was calculated according to Equation and only during S1, when the coffee trees were exposed to different conditions of water supply and deficit, according to the treatments.

$$RWC = \frac{FM - DM}{TM - DM}$$

Equation 1

Vegetative growth and production

One vegetative (orthotropic) and two reproductive (plagiotropic) branches were marked on the trees at the beginning of the production cycle and its length was measured monthly between August 2019 and May 2020. From the data obtained, the average daily growth rate (cm/day) was calculated. In the first week of June 2020, all fruits were harvested from four trees in each experimental unit. We then estimated the average yield (Mg/ha) of dried cherry coffee and green coffee.

Statistical analysis

The data of the physiological variables (gas exchange, pigment index, and fluorescence) were subjected to multivariate analysis of variance (MANOVA) according to the joint analysis model (by cultivation system) of experiments in plots (by clone) split in time (by season), using randomized blocks. The residuals were tested for multivariate normality using the generalized Shapiro–Wilk test[42], and for homogeneity of covariance matrices using the Box’s M test. The Box-Cox [43] procedure was used to avoid variables from escaping both assumptions. The bean yield data were subjected to joint analysis of variance and the Fisher's LSD test to compare the means by clones and systems. From the MANOVA performed for the physiological variables, canonical discriminating variables were built to compare the factor levels using ellipses of 95%

confidence. The scores for the first two canonical variables were represented in a biplot [44]. The residual correlation matrix was graphically represented in a correlation network, as described by [45]. We attempted to identify the correlation patterns of groups of physiological variables with bean yield. Additionally, a correlation analysis was carried out between the mean values of VPD, RH, and ambient temperature (Ta) climatic variables of the assessment periods in each season with the mean values of the gas exchange variables (g_s, c_i, VPD_L, and T_L). First-order autoregressive models were fitted to the time series of the plagiotropic and orthotropic branches to estimate average growth (p.med and o.med, in cm/day) and the time dependency parameter (p.ar1, o.ar1). These estimates were subjected to canonical discriminant analysis, as described above, for the comparison of clones and systems.

The analyses were performed in R (version 3.5.3), using the packages “biotools” [46] “candisc” [47], and “qgraph” [48].

2.3. RESULTS

August and September 2019 were characterized by high temperatures, absence of rain (Figure 1), and low RH. In the dry season, RH reached values equal to or less than 20% during at least 13 days. In September, the maximum VPD was estimated to reach above 3.5 kPa, while the average value was around 1.5 kPa. Starting in October 2019, a small decrease in temperature and an increase in RH was verified with a significant accumulation of rain. High precipitation (437 mm) was recorded in November 2019, resulting in high RH, which exhibited little fluctuation throughout the rainy season until May 2020. There was no rainfall in the region in June 2020, resulting in decreased RH. VPD decreased with the increase in RH in October and remained low with small oscillations until May 2020, when it rose again until the experiment was completed at the end of June (Table 1).

The first two canonical discriminating variables, canonical 1 (Can. 1) and canonical 2 (Can. 2), constructed using a MANOVA for physiological variables, retained 75% of the multivariate differences between systems, clones, and periods, with 64.7% in Can.1(Figure 2). The group of variables related to gas exchange (mainly VPD_L, T_L, IWUE, c_i, g_s, E, and A) highly contributed to the discrimination of treatments. A high coefficient was also observed for the anthocyanin index. The variables related to the chlorophyll index had low relative discrimination power. Chlorophyll fluorescence parameters contributed more to the second canonical discriminating variable (Can.2), together with *A* and *A/Ci* (Figure 2), than other variables.

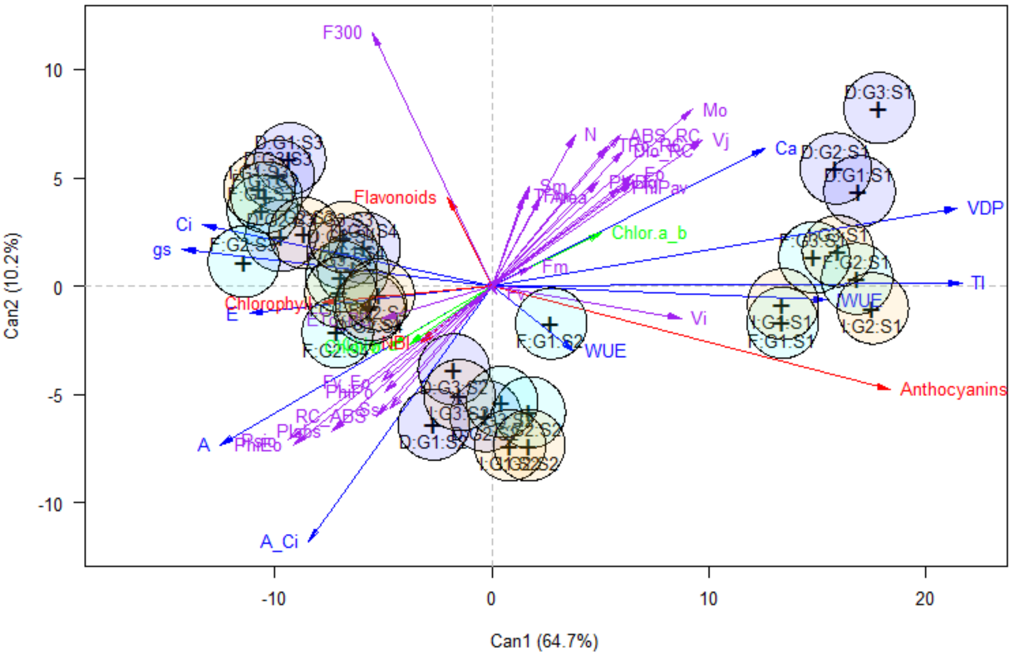

Figure 2. 2. Biplot containing mean scores of the first two canonical (Can1 and Can2) discriminating variables constructed from gas exchange variables, chlorophyll and pigment indices, and chlorophyll a fluorescence trait. The 95% confidence ellipses for the combinations of system, clone, and period are shaded. (Systems: F – fertigation, I – irrigation, D – dryland farming; genotypes: G1 – clone “25,” G2 – clone “08,” G3 – clone “03”; Season: S1 – peak of the dry season; S2 – beginning of the rainy season; S3 – peak of the rainy season; S4 – beginning of the dry season). A - net assimilation of CO_2, E - transpiration; Ca - ambient (external) CO_2 concentration, Ci - internal CO_2 concentration, gs - stomatal conductance, VPD – vapor pressure deficit between the leaf and the air of the IRGA chamber, Tl - leaf temperature, A_Ci - A/Ci, WUE - instant water use efficiency, IWUE - intrinsic water use efficiency. Chlor.a – chlorophyll a index; Chlor.b - Chlorophyll b index, Chlor.a_b – ratio of Chl a/Chl b, NBI - nitrogen balance index, Fo – initial fluorescence ($t=_{50µs}$), Fm – maximum fluorescence, F300 - fluorescence intensity at=300µs, TFm – Fm time, Fv – variable fluorescence, Fv_Fo - F_v/F_o, Vj - the proportion of Fv until F_j (j), Vi - the proportion of Fv until F_i (i), ABS_RC- absorbed, TRo_RC - trapped, DIo_RC– dissipated and ETo_RC - transported energy by primary quinone reduced (Q_a^-) by reaction center in t = 0, PhiPo - maximum and PhiPav – effective quantum yields of the primary photochemical reactions of photosystem II (PSII) (t=0), PhiDo - quantum yield of energy dissipation, PhiEo - quantum yield of electron transport, Psio - probability of an exciton moving an electron in the electron transport chain beyond Q_{a}^{-}. Area - area under the fluorescence curve, Sm - normalized area, Ss - smallest possible S_m, Mo - approximate initial slope of the fluorescence transient normalized with F_V, N - the turnover number of Q_a, PIabs - performance index on the absorption base.

We found a significant effect ($P < 0.05$) of the evaluation period on the physiological variables, in addition to a trend concerning Can. 1 (Figure 2). This effect was superior to that of the system and clone factors (Figure 3). The most contrasting periods were S1 (drought peak) and S3 (rainy peak). In S1, we observed higher values of anthocyanin index, T_L, and VPD_L than those in other periods. In S3 and S4, we detected higher values of g_s, c_i, E, and A than those in other periods. In the latter two seasons, systems and clones caused less dispersion in physiological responses (Figure 2). In S1, we found a significant difference between the dryland farming system ($P < 0.05$) and the fertigation and irrigation systems, considering all clones. Clone “03” differed from clones “25” and “08” when cultivated in the dryland farming system, especially regarding chlorophyll *a* fluorescence and gas exchanges traits (Figure 2).

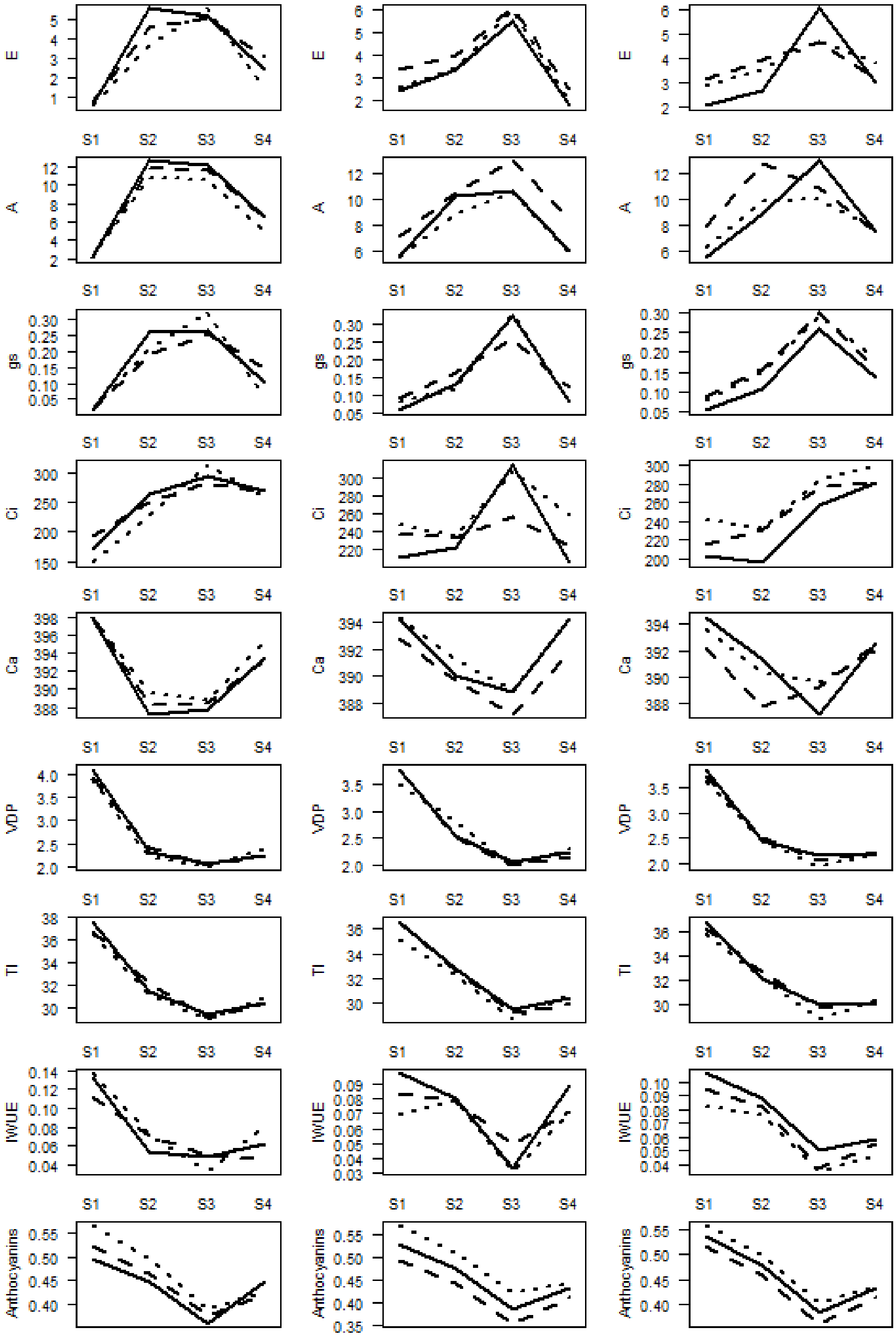

Figure 2. 3. Averages of the physiological variables of coffee with the greatest discriminating power between systems (dryland farming in the left column, irrigation in the center, fertigation in the right), genotypes ("Clone 25," continuous line; "Clone 08," dashed line; "Clone 03," dotted line), and seasons (S1 – peak of the dry season; S2 – beginning of the rainy season; S3 – peak of the rainy season; S4 – beginning of the dry season). E - transpiration rate (mmol H_2O $m^{-2}s^{-1}$), A - net assimilation rate of CO_2 (µmol CO_2 m^{-2} s^{-1}), gs - stomatal conductance (mol $m^{-2}s^{-1}$), Ci - internal CO_2 concentration (µmol/mol), Ca - ambient (external) CO_2 concentration (µmol/mol), VPD – vapor

pressure deficit between the leaf and the air of the IRGA chamber (kPa); Tl - leaf temperature (°C). IWUE - intrinsic water use efficiency (A/g_s).

We observed stronger correlations between variables in the same group of physiological analyses (gas exchange, chlorophyll *a* fluorescence, and pigments indexes) than in those between the groups (Figure 4A). There was a positive correlation between g_s and c_i and between both variables and A (P <0.01). In contrast, *gs* was negatively correlated with VPD_L and T_L (P <0.01). The values of *gs* during the evaluation periods were also negatively correlated with VPD (P <0.1) and positively correlated with RH (P <0.1) (Table 2.2). The variable c_i was negatively correlated with environment temperature (T_e) (P <0.05). For gas exchange variables, the correlations were high within the seasons, whereas the correlations between seasons were low (Figure 4B). We found a correlation between the dried cherry coffee yield and the gas exchange variables in S2, with absolute values around 0.60. In the other periods, these correlations were below 0.15, considering absolute values (Figure 4B).

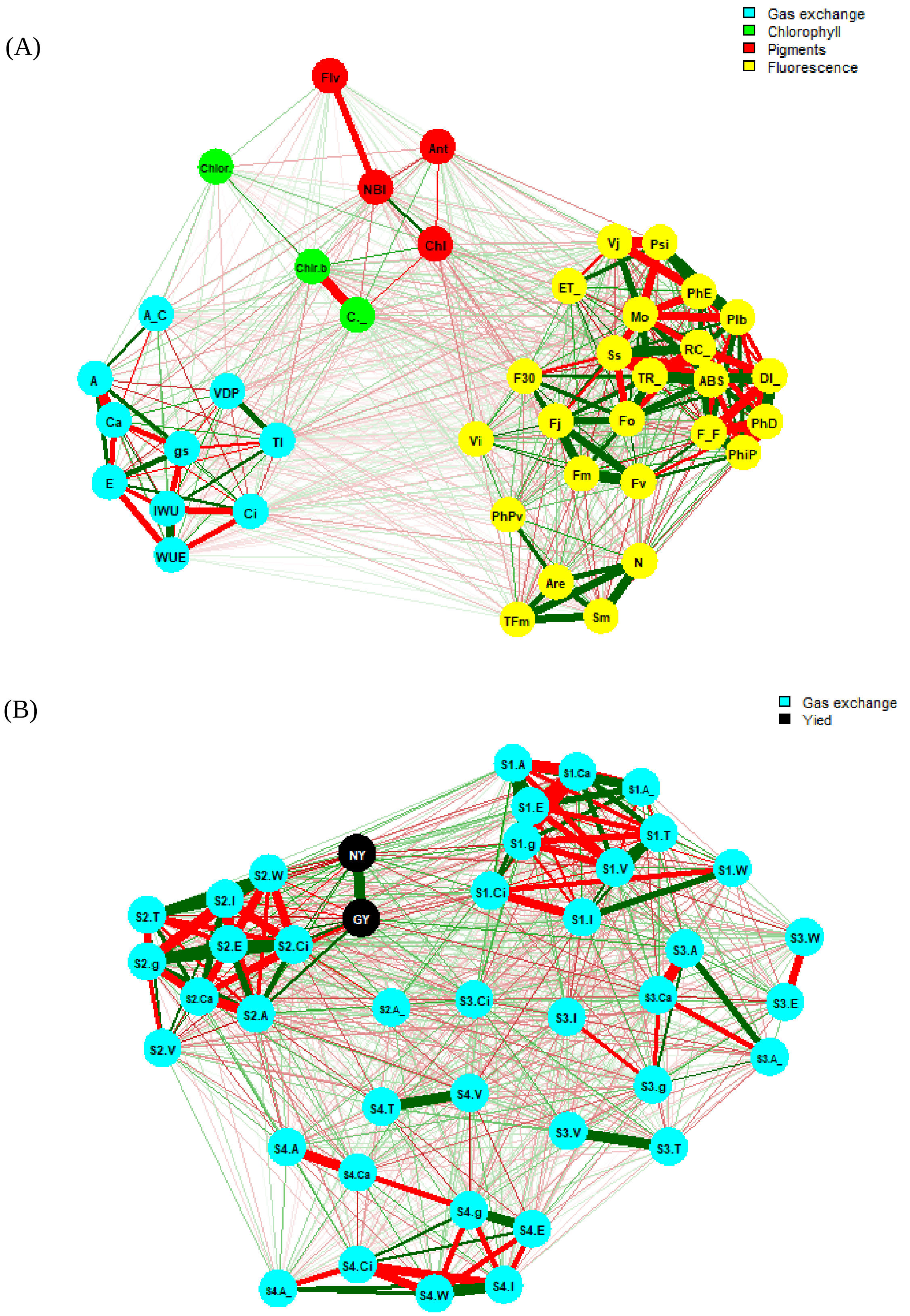

Figure 2. 4. (A) Correlation network for coffee physiological variables. Correlations greater than 0.5, positive (green line) or negative (red line), are highlighted (thicker lines indicate higher correlations). A - net assimilation of CO_2, E - transpiration; Ca - ambient (external) CO_2 concentration, Ci - internal CO_2 concentration, gs - stomatal conductance,

VPD – vapor pressure deficit between the leaf and the air of the IRGA chamber, Tl - leaf temperature, A_C - A/Ci, WUE - instant water use efficiency, IWU - intrinsic water use efficiency. Chlor.a – chlorophyll a index; Chlor.b - Chlorophyll b index, C._ – ratio of Chl a/Chl b, NBI - nitrogen balance index, Chl – chlorophyll idex by Dualex, Flv – flavonoid index, Ant - anthocyanin index, Fo – initial fluorescence (t=$_{50\mu s}$), Fm – maximum fluorescence, F30 - fluorescence intensity at=300µs, Fj – fluorescence in 2ms, TFm – Fm time, Fv – variable fluorescence, F_F - F_v/F_o, Vj - the proportion of Fv until F_j ($_j$), Vi - the proportion of Fv until F_i, ABS - absorbed, TR_ - trapped, DI_ – dissipated and ET_ - transported energy by primary quinone reduced (Q_a^-) by reaction center in t = 0, PhiP - maximum and PhPv – effective quantum yields of the primary photochemical reactions of photosystem II (PSII) (t=0), PhD - quantum yield of energy dissipation, PhE - quantum yield electron transport, Psi - probability of an exciton moving an electron in the electron transport chain beyond $Q_{a.}^-$. Are - area under the fluorescence curve, Sm - normalized area, Ss - smallest possible S_m, Mo - approximate initial slope of the fluorescence transient normalized with F_V, N - the turnover number of Q_a, RC_ - ratio of reaction centers and the absorbance, PIb - performance index on the absorption base. (B) Analysis by season (S1 – peak of the dry season; S2 – beginning of the rainy season; S3 – peak of the rainy season; S4 – beginning of the dry season) was performed concerning the correlation of gas exchange variables and dried cherry coffee (GY) and green coffee (NY) bean yield. A - net assimilation of CO_2, E - transpiration; Ca - ambient (external) CO_2 concentration, Ci - internal CO_2 concentration, g - stomatal conductance, V – vapor pressure deficit between the leaf and the air of the IRGA chamber, T - leaf temperature, A_ - A/Ci, W - instant water use efficiency, I - intrinsic water use efficiency.

Table 2. 2. Correlation of environmental variables (temperature – Te, relative humidity – RH, air vapor pressure deficit – VPD) with gas exchange variables (stomatal conductance - g_s, internal CO_2 concentration – C_i, leaf temperature - T_L, vapor pressure deficit between the leaf and the air of the IRGA chamber – VPD_L).

Environmental variables	Gas exchange variables			
	g_s	*Ci*	T_L	VPD_L
Te	-0.78	-0.98*	0.99*	0.96*
RH	0.94^+	0.92^+	-0.94^+	-0.94^+
VPD	-0.90^+	-0.93^+	0.97*	0.97*

[1] + significant with P <0.1; * significant with P <0.05.

In S1, we found a statistical effect only of the cultivation system when considering the effect of clones and cultivation systems on the RWC of leaves (Figure 5). The RWC was higher in the irrigation and fertigation systems at the three evaluated times than in the dryland farming system. In the predawn, the RWC values in the dryland farming system were, on average, approximately 35% lower than those observed for irrigation systems (Figure 5).

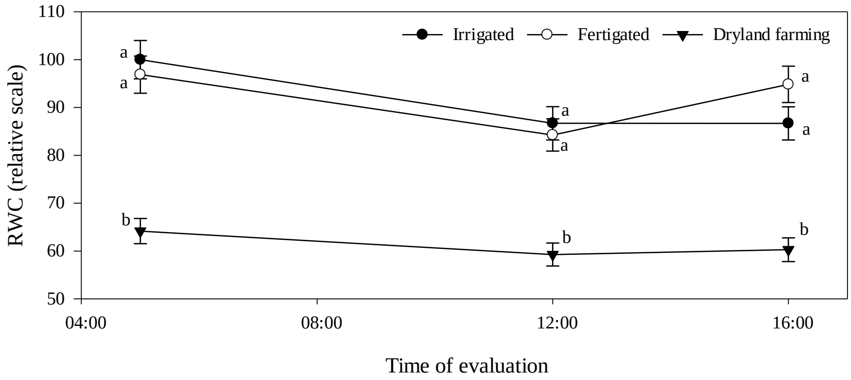

Figure 2. 5: Relative water content (RWC) in the leaves of Amazonian Robustas at the time of the peak dry season (S1) in the dawn (5:00h), at midday (12:00h), and in the late afternoon (16:00h). Cultivation systems (irrigation, fertigation, and dryland farming) with means followed by the same letter do not differ by the Fisher's LSD test (P <0.05). Bars indicate the standard error of the mean.

The first canonical discriminating variable retained 91.7% and 98.1% of the multivariate differences between systems and clones, respectively, when considering the average growth rate of stems and branches (Figure 6). The dryland farming system showed the lowest average growth rate of plagiotropic branches, and clone “25” showed the lowest growth rate of the orthotropic stem. We found a high temporal dependence on the growth rate of stems and branches in the fertigation system (Figure 6).

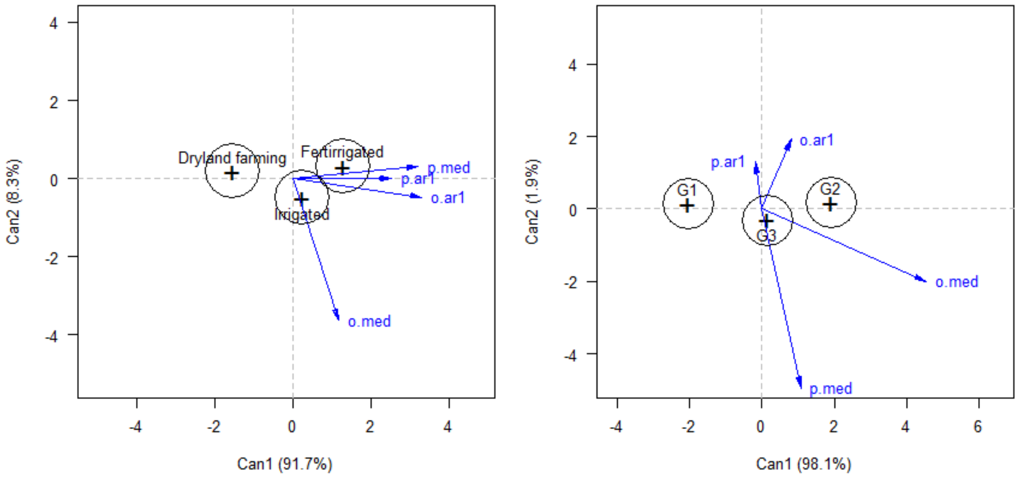

Figure 2. 6: Biplot containing mean scores and 95% confidence ellipses for the first two canonical discriminating variables of systems (left) and clones (right), built from growth variables of plagiotropic and orthotropic branches (p.med and o.med– estimates of the average growth of the plagiotropic and orthotropic groups, respectively; p.ar1 and o.ar1 – parameters of temporal dependence on the growth of plagiotropic and orthotropic branches, respectively).

The coffee yield in dried cherry and green coffee showed similar behavior, with the lowest averages observed in the dryland farming system for all clones (Table 2.3**Erro! Fonte de referência não encontrada.**). Clones “08” and “03' had higher yields in the fertigation system than clone “25.” We found no difference in the fertigation and irrigation systems for clone “25.” The “03” clone had a higher yield than the other two clones in the fertigation and dryland farming systems. In the dryland farming system, its yield was about three and seven times higher than the yields of “25” and “08” clones, respectively. For the variable can.1, we found no difference between cultivation systems concerning clone “03.” The fertigation system showed the lowest values for “08” and “25” clones. In the irrigation and dryland farming systems, clone “08” showed the highest Can.1. Finally, clone “25” had the lowest values in all cultivation systems (Table 2.3).

Table 2. 3. Average values of production systems and coffee genotypes (G1 – ‘clone 25’; G2 – ‘clone 08’ and ‘G3 – clone 03’) for the canonical discriminant 1 (64.7%) and bean yield variables.

	Can.1			Green coffee (Mg/ha)			Dried cherry coffee (Mg/ha)		
	G1	G2	G3	G1	G2	G3	G1	G2	G3
Fertigation	-2.44 bB	0.56 bA	-0.13 aA	1.72aC	2.53aB	4.38 aA	4.08aB	5.37aB	8.70aA
Irrigation	-0.83 aC	1.44 aA	0.63 aB	1.82 aA	1.51bA	2.20bA	3.77aA	3.12bA	4.57 bA
Dryland farming	-1.06 aC	1.36 aA	0.46 aB	0.45bB	0.17 cB	1.29cA	0.94 bB	0.39 cB	2.69 cA
SVC		--			29.75%			26.14%	

[1] Averages followed by the same lowercase letter in the column and uppercase letter in the row do not differ according to Fisher's LSD test at 5% significance.

It is important to highlight that at the end of August and during September, sharp defoliation (abscission) was observed in coffee trees, notably in dryland farming systems, where this phenomenon was visually greater than that commonly observed in the region. In addition, in October, we observed “scalded” and severely damaged leaves in the dryland farming system (field observations).

2.4. DISCUSSION

The long dry season period (especially between August and early October) of approximately 150 days without significant accumulation of rainfall, associated with high temperatures (Figure 1) and high VPD values, imposed unfavorable conditions on the development and production of coffee trees (*C. canephora*) [5,17,49]. The time of the first assessment (S1) showed climatic characteristics and hydric deficit (HD) consistent with those of the drought peak registered for the region in previous years. However, the dry season, which usually ends at the beginning of September, was atypically extended until October. In fact, September 2019 was the driest month in terms of accumulated precipitation and VPD and was the warmest since 2016. On the other hand, in November, the heavy rain resulted in accumulated precipitation above the monthly average recorded in the previous 11 years of approximately 220 mm [30].

[10,50,51]. In the dry season (S1), supplementary irrigation improved the indicators of chlorophyll *a* fluorescence, regardless of genotype. It is important to note that with the persistence of the dry period and high temperatures throughout September, "scalded" leaves of coffee trees under the dryland farming cultivation were observed, which were absent during the evaluations at the end of August (S1). Clone "03" showed the lowest values of fluorescence indicators in the dryland farming system, with effects on fluorescence dynamics (M_o, V_j), quantum efficiency of PSII (Phi_{Po}, Phi_{Eo}, Psi_o, Phi_{Do}), and performance index (PI_{ABS})(Figure 2). These findings suggest that this genotype may be sensitive to photoinhibition [13,50].

The behavior of gas exchange traits reflected a cascade effect, triggered by tree responses to temperature seasonality, RH and VPD (Table 1 and 2). Low values of g_s were observed at the drought peak, a parameter directly related to the stomatal opening. The g_s vary especially in response to changes in environmental conditions, notably, soil moisture, temperature and VPD [14,50–52]. The positive correlations between g_s and E ($P < 0.01$), c_i ($P < 0.01$), and A ($P < 0.01$), and negative correlations with T_L ($P < 0.01$), such as those found in this study (Figure 4 A, B), were expected. Stomatal closure is a crucial and efficient strategy to limit water loss from the leaf to the atmosphere, as it reduces E. However, this process can result in the reduction of c_i and, consequently, of A [38,49,50]. Diffusive factors commonly impose the main photosynthetic limitations on coffee [51,53,54]. Reduced E, in turn, can increase T_L, especially on days of high irradiance and high temperatures [14,15].

In the dryland farming systems, the water deficit worsened the effects of climatic seasonality, resulting in lower values of g_s, c_i, and A than those observed in the systems that received supplementary irrigation (Figure 2 and Figure 3). The RWC values showed a HD in non-irrigated trees since dawn (Figure 5). These results corroborate those of previous studies suggesting that the combination of HD with high temperatures [9,55] and low RH or high VPD reduces A in coffee plants due to diffusive limitations [10,11].

[49,52]. Thus, coffee trees grown in dryland farming systems are more vulnerable to droughts because these events lead to HD associated with high temperatures and/or high VPD in the field [16]. The correlation of g_s and c_i with the climatic variables VPD, RH, and Ta (Table 2.2) implies that these variables are mainly responsible for the seasonal variation observed in A in the irrigation and fertigation cultivation systems. The high VPD can explain the reductions in g_s in coffee trees of the irrigation system in the dry season due to the high temperature and low relative humidity in the region. This sensitivity of genotypes to VPD, associated or not with HD, is worrying and will probably be a critical factor for its cultivation in Rondônia, since climate projections indicate an increase in VPD in Amazon in the decades to come [23].

[10–12][54,56][12]The observed values of A were relatively high in S2 and S3 (Figure 3), considering the pattern normally presented by the species [5,49]. The seasonality of A can explain, at least in part, the seasonality of the growth rate of coffee observed in the region [57]. Fruiting may have contributed to increase A in these seasons [25,58]. However, the increase in A in coffee tree fruiting stage is also associated with the increase in g_s .

The variation observed in the anthocyanin index reveals a less-studied physiological aspect in coffee (*C. arabica* and *C. canephora*). Usually, high anthocyanin accumulation occurs in trees subjected to biotic or abiotic stress conditions [59]. Protection against radiation, especially UV radiation, is considered the main function of anthocyanins [60,61]. They act as a filter, preventing an excessive and harmful amount of energy from reaching the light-harvesting complexes of the photosystems [60,61]. The anthocyanins also are associated with the elimination of ROS resulting from photooxidation [52,59,62] and your synthesis can impede sugar-promoted feedback regulation of photosynthesis in leaves [61]. In this study, the highest anthocyanin content was observed in S1, the peak of the dry season. Interestingly, the increase in the anthocyanin index occurred in parallel with a decrease in the chlorophyll index.

In relation to the physiological variables, few differences were observed in the behavior of the genotypes, in the same system and evaluation period. This may be associated with the common genetic origin of the genotypes. Therefore, similarities are not restricted only to high yields [28,36].

The lower average growth rate of plagiotropic branches in coffee trees cultivated under dryland farming, during the annual cycle evaluated, reduced their productive potential for the next harvest because there were fewer nodes available to form reproductive buds [63–65]. However, a higher average rate of vegetative growth was expected in the dryland farming system because the low fruit yield (Table 2.3) would result in greater availability of photoassimilates to promote vegetative growth [25,64], especially between October and May. As the behavior of the photosynthetic traits was very similar, especially in S2, S3, and S4(Figure 2), it is possible that a large part of *C* was allocated to the root system [64,66]. In addition, the total fixation of *C* by the tree, which is a function of *A* and the total leaf area, may have varied between treatments. The leaf area of a tree depends on the area of each leaf and the number of leaves on the tree. Both parameters were affected by the HD [5,67]. Thus, trees with smaller branches, fewer leaves, and/or smaller leaves have fewer available photoassimilates to sustain growth. The high temporal dependence of branch growth (Figure 6) corroborates this hypothesis. The more pronounced defoliation in coffee trees under dryland farming during the dry season (S1) may also have contributed to a possible reduction in *A* at the plant level [66,67].

In general, the yield obtained in the 2019/2020 harvest was low, considering the history of the experiment described previously (in the Experimental area and coffee cultivation section) and the potential of the genotypes [22,36]. The variable canonical 1 (Can.1), constructed from the MANOVA for physiological variables (view Figure 2), and the bean yield exhibited a different behavior under the treatments (cultivation systems and clones) (Table 3); therefore, other physiological processes not evaluated in this study could have a high influence on coffee yield. Our results suggest that the range of optimal conditions for production, which is variable for each phase of the cycle, in some cases, was narrower than that often recorded for the photosynthetic process, as previously suggested [4]. The magnitude of the effect of climatic variables on each physiological process and yield depends on the cultivation practices, plant genotype, season, light intensity, and duration of stress [3,17]. For example, the final stages of flowering and the beginning of the fruiting (BBCH 59-70[37]) coincided with high temperatures (view Figure 1) between late August and early October. The recorded values in this study were not optimum and were thus harmful to the yield of *C. canephora* when they occurred in the phenological stages in which the trees were found [4]. Thus, the low yield obtained in the 2019/2020 harvest evaluated in this study can be explained largely by climate. Although localized irrigation adopted in irrigation and fertigation systems mitigates the effects of HD, it does not produce significant effects on VPD and Te.

The physiological traits evaluated, including branch growth rate, were similar between the fertigation and irrigation systems. However, fertigation had a positive effect on the yield of clone “08” and, in particular, of “03.” The literature reports the benefits of fertigation on the nutritional status of trees [19,20,68]. This may be sufficient to improve performance in key processes related to yield and even affect coffee tree responses to environmental stresses [49]. However, the yield from clone “25” requires additional analysis to determine the potential benefits of fertigation. Complementary studies are needed to understand the effects of fertigation on the metabolism of coffee trees in Amazonian conditions and determine how the responses can be affected by climate variables, management, and genotypes. From an agronomic perspective, the technical/operational and economic feasibility of the practice must also be considered for recommending it. The high productive potential of clone “03” is already widely known by producers and technicians in the region [22,36]. Our results show that this genotype has mechanisms that allow it to adapt to adverse weather conditions, with or without water deficit, at some critical stages of the production process. Its flowering, which is better distributed over successive blooms, may have reduced the proportion of reproductive structures exposed to the harmful temperatures of September.

Our findings highlight the need for new studies aimed at detailing the specificities of Amazonian Robustas genotypes ecophysiology. We suggest new research to assess the sensitivity and susceptibility to increasing VPD, with or without water restriction. It is also important to assess the hydraulic conductivity of different genotypes and to elucidate the mechanisms that enhance or limit it. In addition, studies should investigate the role of anthocyanins in the metabolism ofcoffee tree. Finally, it is still necessary to investigate the impact of climatic variables (such as temperature and VPD) on the production of Amazonian Robustas genotypes. This will help support the adoption of strategies aimed at the sustainable cropping coffee in the Amazon.

2.5. CONCLUSIONS

For the three studied genotypes of *C. canephora* (clone '25', '08', '03') regardless of the cultivation system there was seasonal variation in physiological variables, especially those related to gas exchange. Lower values were observed in net assimilation rate of CO_2 (A) associated with lower stomatal conductance (g_s) in the dry season (S1). On the other hand, in S1 it was observed higher values in the indices of anthocyanins in the leaves of the coffee trees.

Amazonian Robustas genotypes cultivated in irrigated systems (irrigated and fertirrigated cropping systems) presented greater *A* and better indicators of chlorophyll *a* fluorescence in S1. The ‘clone 03’ presented worse indicators of chlorophyll *a* fluorescence in dryland system in S1.

Coffee yield of three genotypes was lower in the dryland system. However, the ‘clone 03’ had a much higher yield than ‘clone 25’ and ‘clone 08’. The productivity of the coffee plants cannot be explained by the physiological variables analyzed at the leaf level.

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FINAL CONSIDERATIONS

The studies carried out increased the understanding of the physiology and ecophysiology of the Amazonian Robustas with implications for their management and production. They also reveal a great challenge for coffee growing in Rondônia in a possible future with a drier and warmer climate.

The genotypes of Amazonian Robustas (*C. canephora*) evaluated for vulnerability to embolism, showed a narrow or negative hydraulic safety margin (HSM). In turn, the most productive genotypes showed more residual leaf water leakage ($g_{\text{leaf-res}}$), making them more vulnerable to hydraulic failure, as demonstrated by the shorter time to hydraulic failure (THF). The leaves of the eleven genotypes have high vulnerability to drought-induced death (DIM). Although this does not necessarily lead to the plant death, in the case of drought events, it negatively impacts production due to the loss of photosynthetically active area.

The three main genotypes cultivated in the state (clones 03, 08 and 25) show a reduction in A with the increase in VPD due to the drop in g_s. The use of localized irrigation mitigates but does not prevent the negative effect of high VPD on photosynthesis. On the other hand, a certain resilience of the leaf photosystems of these clones was observed. The water deficit associated with high temperatures in pre-anthesis and anthesis (August/September) compromises coffee productivity in Rondônia.

If the current course of search and selection of genotypes persists, considering only the yield aspects, the success of coffee farming in Rondônia state will become increasingly dependent on irrigation. The selection of genotypes with greater drought tolerance is essential to anticipate the deleterious impact of water restriction predicted for the region.