VICENTE LUIZ NAVES

COFFEE TREES UNDER RAINFALL EXCLUSION:
EVIDENCES FOR CANOPY ACCLIMATION TO WATER
SHORTAGE

LAVRAS-MG
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> Tese apresentada à Universidade Federal
> de Lavras, como parte das exigências do
> Programa de Pós-Graduação em
> Agronomia/Fisiologia Vegetal, área de
> concentração em Ecofisiologia Vegetal,
> para a obtenção do título de Doutor.

Prof. Dr. João Paulo Rodrigues Alves Delfino Barbosa
Orientador

Dr. Serge Rambal
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ACCLIMATION TO WATER SHORTAGE
ÁRVORES DE CAFÉ SOB EXCLUSÃO DE CHUVA: EVIDÊNCIAS DA
ACLIMATAÇÃO DO DOSSEL À REDUÇÃO DA DISPONIBILIDADE HÍDRICA

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Tese aprovada em 23 de fevereiro de 2018.
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Prof. Dr. Paulo Eduardo Ribeiro Marchiori   UFLA
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2018

Àquele que está sentado no trono e ao Cordeiro, pois a Ele
seja o louvor, e a honra, e a gloria, e o domínio pelos
séculos dos séculos.

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À minha esposa Lucelene e filha Ana Clara que me
encheram de esperança estando sempre ao meu lado.

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ABSTRACT

The intensification and amplification of dry periods, as predicted by climatic scenarios for the years 2015-2050, indicate that there is a probability that the south of Minas Gerais could become inapt for coffee production, the same way the coffee crop is conducted nowadays. In the field, drought usually occurs as a multidimensional stress, resulting in a decrease in the amount of rainfall, concomitantly accompanied by high temperatures, which normally increases the evapotranspiration leading to soil water scarcity. The role of water in growth, development and yielding of coffee trees has a remarkable importance in different phenological phases of the crop. For instance, water must be freely available during the period of fast fruit expansion to make sure that a large yield of high-quality coffee beans happens. However, due to the unpredictability of the occurrence and magnitude of the dry periods, as well as the lack of knowledge of the plant limits, the coffee crops might be placed in a condition of vulnerability to maintain its vital activities without declining its yielding and productivity. The aim of this study was to verify if a reduction of ¼ of the rainfall would affect the dynamic of leaf area and source-sink relationship in a six-year-old coffee crop subjected to a rain exclusion system. The experiment was composed of one treatment of rain exclusion, one with the system but with no exclusion, and one without the system and no exclusion. Our measurements verified that the reduction of ¼ rainfall through the exclusion system affected the stem, branches and leaf area growth rates, which, in the case of the branch leaf area was highly correlated with the normalized difference vegetation index and the next-year-production data, as well as the microclimatic values such as temperature, vapor pressure deficit, evapotranspiration of the crop, and the reduction of the rainfall load. The drought monitoring models did not find any trend of longer drought duration for the following decades. Furthermore, our study rendered a new tool to validate the use of nondestructive leaf area index (LAI), since leaves are in the interface between plants and the atmosphere, precisely obtaining LAI is important to give an idea of coffee tree canopy acclimation to water shortage.

Keywords: Rain Exclusion. Growth Models. Drought Monitoring Models. NDVI. LAI.

RESUMO

A intensificação e amplificação de períodos secos, conforme previsto por cenários climáticos para os anos 2015-2050, indicam que há probabilidade de que o sul de Minas Gerais possa se tornar inapropriado para a produção de café, da mesma forma que se realiza hoje em dia. O papel da água no crescimento, desenvolvimento e rendimento dos cafeeiros tem uma importância notável em diferentes fases fenológicas da cultura. Por exemplo, a água deve estar livremente disponível durante o período de expansão rápida do fruto para garantir que grãos de café de alta qualidade sejam produzidos. No entanto, devido à imprevisibilidade da ocorrência e magnitude dos períodos secos, bem como a falta de conhecimento dos limites da própria planta, os cafezais podem ser colocados em condições de vulnerabilidade para manter suas atividades vitais sem diminuir seu rendimento. O objetivo principal deste trabalho foi verificar se a redução de ¼ da carga pluviométrica afetaria a dinâmica de crescimento da área foliar, assim como a relação fonte e dreno das árvores de café. Para tanto instalamos um experimento com um sistema de exclusão de chuva em um cafezal de seis anos, composto de um tratamento com exclusão de chuva, um tratmento com cobertura mas sem exclusão e um tratamento sem cobertura e sem exclusão. Os dados obtidos possibilitaram verificar que a redução de ¼ da precipitação através do sistema de exclusão afetou as taxas de crescimento da área foliar, do caule e de ramos. A dinâmica da área foliar foi altamente correlacionada com o índice de vegetação normalizada, dados de produção, assim como com os dados microclimáticos de temperatura, déficit de pressão de vapor, evapotranspiração da cultura e da redução da carga pluviométrica. Os modelos de monitoramentos da seca não encontraram nenhuma tendência de aumento na duração dos périodos de seca para a região do sul de Minas Gerais. Além disso, nosso estudo rendeu um nova ferramenta para validar o uso de metodologias não-destrutivas de obtenção do índice de área foliar (IAF), visto que as folhas estão na interface entre plantas e atmosfera, a obtenção precisa do IAF é importante para dar uma ideia da aclimatação das árvores de café à redução da disponibilidade hídrica.

Palavras-chave: Exclusão de Chuva. Modelos de Crescimento. Modelos de Monitoramento de Seca. NDVI. LAI.

SUMMARY

1 INTRODUCTION

Coffee is a beverage extracted from a tropical crop and it is the most important commodity in international agriculture, generating nearly 90 billion dollars every year and involving around 500 million people into processing the product from cultivation to final consumption. Coffee is the fifth most important agribusiness Brazilian product with over 36 million 60-kg coffee bags exported in the period from January to December 2015, generating revenues of US$ 6.15 billion during that period (MAPA, 2018). *Coffea arabica* L. represents 62% of the coffee consumed worldwide and the south of Minas Gerais is one of the largest producing regions of arabica coffee in Brazil and it is naturally predisposed to a rainfall regime which could compromise soil water availability in certain periods of the year.

The intensification and amplification of dry periods, as predicted by climatic scenarios for the years 2015-2050 and future perspectives (BURKE et al., 2006; FRIEDLINGSTEIN et al., 2006; DAI, 2013; COOK et al., 2014; IPCC, 2014), may indicate a great probability that this region could become inapt for coffee production, the same way it is realized nowadays (ASSAD et al., 2004, NUNES et al., 2010, CAMARGO, 2010). Climatic variations characterized by either dry episodes or irregular distribution of rainfalls have been observed with frequency in the southern region of Minas Gerais since 2010 (MELLO et al., 2016). Water scarcity has already affected various social segments in this region, causing economic damage, with pronounced effect in the 2013/2014 drought, which caused harvest losses of nearly 20% in relation to the predictions for that year (CONAB, 2014). In the field, drought is a multidimensional stress and it is usually a result of rainfall decrease, accompanied by a normal increase in temperature that leads to increase in crop evapotranspiration causing water shortage.

In fact, there is ample evidence of climate variability at a wide range of time scales all over South America, from intraseasonal to long-term oscillations (IPCC, 2014). In many subregions of South America, this variability in climate is normally associated with phenomena called El Niño and La Niña that are opposite phases of what is known as the El Niño-Southern Oscillation (ENSO) that already produce impacts with important socioeconomic and environmental consequences, which could be exacerbated by further global warming and its associated extreme events. For instance, La Niña is associated with drought in southern Brazil (IPCC, 2014). Thus, if La Niña was to increase, the south of Minas Gerais would be exposed to water shortage of up to 20% in some regions.

In recent decades, physiological and growth models for plant responses to drought, and concurrent stresses, such as high temperature and irradiance have been the subject of intense research. However, clearer data addressing several points related to the physiological and

growth responses that the coffee crops will have in the field when subjected to lower water availability in the south of Minas Gerais are practically nonexistent, and those data need to be analyzed to render more trustworthy advice for the coffee growers and plant modelers interested in the behavior of coffee trees in the field. Moreover, there is also a lack of climatic models addressing future perspectives for this region. Therefore, it is necessary to obtain field data so that the management of the coffee crop in conditions of water shortage can be improved, and additionally more acute prediction of the impacts of extreme events in future scenario be modelled. Those responses could be responded by utilizing a system of rainfall exclusion in long-term studies.

In view of what was described above, it is necessary to study the responses that coffee trees will present when facing the reduction of the rainfall available for their growth and yield in the field. Thus, the objective of this study was to develop models to evaluate the impact of rainfall reduction upon the shoot growth rates focusing mainly on the dynamics of leaf area, and the behavior of the source-sink relationship on the growth and yielding of the coffee trees. Besides we focused on developing some monitoring models focusing on drought for the south of Minas Gerais, since drought monitoring and prediction models are practically nonexistent for this region.

Our hypothesis was that the reduction of ¼ rainfall through a system of rainfall exclusion would affect the dynamic of leaf area and source-sink relationship in terms of the vegetative and reproductive effort of the coffee trees, since carbon storage reduction and its effects in the allocation of carbon into different parts of the trees can also be expected in coffee crops facing water shortage. Moreover, we have correlated plant growth rates, branch leaf area (BLA), leaf area index (LAI) and yield with microclimatic data such as temperature and the reduction of rainfall load, as well as with some vegetation data such as crop evapotranspiration (ETc), and the normalized difference vegetation index (NDVI).

2 STATE OF THE ART

2.1 Lessons and outputs from the systems of exclusion

First, the importance of understanding the acclimation of coffee trees in the field, especially when subjected to a condition of water shortage, emerges from the fact that the coffee plants growing in a greenhouse would certainly differ from the trees in the field. Moreover, converting greenhouse data into trustworthy inferences that could be extrapolated into the field crops requires caution. Therefore, the demand of more field studies specially related to water shortages in long-term droughts is fundamental to improve our knowledge towards so important matter.

As already mentioned, the perspective of intensified extreme events, assessed by several model simulations conducted by various authors (BURKE et al., 2006; FRIEDLINGSTEIN et al., 2006; DAI, 2011b; DAI, 2013; IPCC, 2014), which predict a major decline in net ecosystems productivity - reduction of the carbon sinks - as a result of the impacts of climate change in various tropical ecosystems, especially in response to drought within the next few decades, has elicited many studies of the impacts caused by rain reduction on the acclimation of plants in the field (RAMBAL et al., 2003; FISHER et al., 2007; JENTSCH et al., 2007; LIMOUSIN et al., 2008; RODRIGUEZ-CALCERRADA et al., 2011; ERBS et al., 2012; LIMOUSIN et al., 2012). Therefore, such response of carbon storage reduction and its effects in the allocation of carbon into different parts of the plant could also be expected in coffee crops when facing the reduction of rain in the field. However, inferences about the intensity of such decline in the growth and productivity of the coffee trees in the field is still a matter of uncertainty, which could be responded by the system of exclusion in long-term studies.

In the last two and a half decades, the intensification of experiments with reduced rainfall supply in the field were possible by taking advantage of large-scale rain shelter structures, which could exclude the rain from the field plots while allowing other aspects of the environment to remain largely unchanged. Rain shelters are commonly built based on two different concepts. The first uses fixed structures which are permanent systems that could intercept the entire or partial precipitation on the area, thus offering quantitative control of water supply (SVEJCAR et al., 1999; FAY et al., 2000; YAHDJIAN; SALA, 2002; DE BOECK et al., 2011; RODRIGUEZ-CALCERRADA et al., 2011). However, two possible disadvantages of this approach are a continuous reduction in incident radiation, depending on the roof material and/or the extension of such covering, as well as an unavoidable impact on the microclimate, e.g. air temperature and air moisture, depending on the structure even in the wind force. The second fixture frequently chosen is the use of movable shelters, which cover

the experimental area only in the case of precipitation. By doing so, impacts on incident radiation and microclimate can largely be reduced. However, movable rain shelters need more complex technical facilities and elaborate controlling mechanisms for automatic operation, besides being more costly (FOALE et al., 1986; BEIER et al., 2004). Therefore, the most important aspect in a rain exclusion system experiment is to find ways that maximize the scientific response with economic and technological perspectives without causing undesirable artifacts.

The choice of the experimental site to install the rain collectors or shelters is also a very important point to be taken into consideration. The surface runoff water should naturally be excluded from the sheltered plot area. Therefore, the general location should be on a gently sloping land out of the path of natural drainage lines. On a very flat site, grading of the surrounds is advisable to raise the experimental area slightly above the overall soil level, but the top soil of the plot area should be left undisturbed. It is desirable on most soil types to have a barrier to lateral movement of subsurface water around the outside of the sheltered plot and also between replicate areas within the plot. Clay soils with deep cracks, for example, will allow rapid lateral movement of water through the cracks, and sandy soils will allow slower water flow and also root extension across plot boundaries (FOALE et al. 1986). Nonetheless, neither of the previous examples define the soil that was used in our experiment, described ahead in the section Material and Methods.

2.2 Soil water limitations and standardized precipitation-evapotranspiration index

2.2.1 Soil water limitations

Soil moisture is a key variable in the processes related to the water transfer from the soil to the atmosphere through the soil-plant-atmosphere-continuum (SPAC), and its amount could be estimated by the difference of water entering and leaving the soil profile. The simplest water balance methods use a differential function between the precipitation and the potential evapotranspiration. Therefore, the water availability or the amount of water stored in the soil for the plant to uptake is the difference in the quantity of water between the field capacity (FC) and the permanent wilting point (PWP) of that soil. At FC, the soil is wet and contains all the water it can hold against gravity, at PWP, the soil is dry, and the plant can no longer extract additional water. The water available for the plant in terms of time is expected to be greater for clayey and organic soils compared to sandy soils. If we know the water availability and the rate at which this water is being depleted by the crop, then we can determine the necessary quantity of water for the plant. Apart from irrigation scheduling, this information can also be useful in crop growth modeling and prediction of yields (LOPEZ et al., 2017).

The PWP corresponds to the inferior limit of available water. This moisture condition severely restricts the absorption of water by the plants, which will die if there is no replacement of the water in the soil. However, the practical value of PWP determination is reduced, serving only as an overall guide towards the inferior limit of available water content for the plants (ARRUDA et al. 1987). Furthermore, PWP determination is complicated and its value is only a reference, since the wilting symptoms in the plant happen much earlier than the soil water reaches the PWP, nevertheless the plant activity just ceases after this referential point (ARRUDA et al., 1987; JONG VAN LIER, 2000). In general, the PWP may be determined in the laboratory, by the retention curve method. In this method, the moisture value of the wilting point is represented by the balance moisture with tension of 1.5 MPa. To draw the retention curve, soil moisture values are obtained after submitting samples to different tensions in the Richards Extractor (SILVA et al., 2014). Despite its dynamic character, for practical purposes of irrigation, field capacity is usually obtained with tension value of 0.01 MPa in sandy soils and 0.033 MPa in clay soil. On the other hand, the moisture corresponding to the permanent wilting point is obtained with the tension of 1.5 MPa.

One should always have in mind that the dynamics of the water movement in the soil is a function of the water properties (TAIZ; ZEIGER, 2006) as well as a function of the soil characteristics, such as mineralogy, texture, organic matter and physical structure, that by acting together transform the soil into a hydric capacitor that allows the water retention, storage and availability to the plants (JONG VAN LIER, 2000; REICHARDT; TIMM, 2004; REICHERT et al. 2011). The dystrophic Red Latosol found in almost 50% of the savanna region, and intensively used by coffee crops nowadays, is usually a low-density soil with a very high porosity and a structural balance between macro and micropores that allows high drainage as well as high water retention due to its extremely stable granular structure caused by the oxidic mineralogy (FERREIRA; FERNANDES; CURI 1999). However, it is important to highlight that this elevated hydric retention does not imply in high water availability to the plants, since low water retention may occur in the tension range related to intermediate-sized pores (RESENDE; SANS; DURÃES, 1996). Besides, Latosols usually suffer an abrupt transition from very large pores to very small ones, especially Latosols possessing either clayey or very clayey nature and gypseous mineralogy, which furnish water retention curves with steep declines in the water content without large variations in the soil water potential (SILVA et al., 2014). Therefore, it is worth remembering that the Brazilian Savanna Oxisols (Latosols) are fast-drying, due to the bimodal pore distribution described above (CARDUCCI et al., 2013;

2016) as well as because of the slope of the water retention curves, represented by van Genuchten's model (SILVA et al., 2015).

2.2.2 Standardized precipitation-evapotranspiration index - SPEI

Drought is one of the main natural causes of agricultural, economic, and environmental damage, being essentially a climatic phenomenon, it is firstly seen as consequence of an abnormal decrease of precipitation. A severe drought is apparent after a long period without precipitation, but it is difficult to determine their onset, extent, and end. Thus, it is very difficult to objectively quantify the drought characteristics in terms of intensity, magnitude, duration, and spatial extent (VICENTE-SERRANO et al. 2010). The threat of climate change is now recognized as one of the major problems for the planet Earth in the twenty-first century. According to the IPCC (2014) the temperatures on the Earth surface have risen globally, with some main significant regional variations. This warming intensifies the global hydrological cycle (MILLY et al., 2002). The consequence of global warming is not the change in averages but the overall increase of extreme events. Among the extreme meteorological events, droughts are possibly the most slowly developing ones but that often have the longest duration, and at the moment the least predictable among all atmospheric hazards. Studies on how climate change will affect various ecosystems have been conducted as an international effort on many fronts (MISHRA; SINGH, 2010).

However, unlike other extreme conditions such as floods, the analyzes of drought scenarios due to climate change impacts have not been explored fully. Amongst recent studies on understanding drought impacts, Szep et al. (2005) have found that local soil moisture conditions in East Hungary became drier in last century, parallel to changes in other parts of Europe. Andreadis and Lettenmaier (2006) have examined agricultural and hydrological droughts in the USA, and have observed that droughts have, for the most part, become shorter, less frequent, and cover a smaller portion of the country over the last century except southwest and some parts of the west, due to specific trends in drought characteristics, that are mostly opposite to those for the rest of the USA, especially in the case of drought duration and severity, which have increased. In another study, Mishra et al. (2009) highlighted the changes in drought severity-area-frequency due to climate change scenarios and compared with historical droughts for Kansabati River basin in India.

It is now accepted that droughts in future scenarios will pose a threat to several regions and climate sensitive economic sectors, specifically agriculture, and have therefore the need for the assessment of potential impacts of climate change on crop production at various scales. This will help develop measures and forms of measurements of drought predictability to reduce

agricultural vulnerability and thereby secure livelihoods of those who depend on agriculture. Among some drought measurements frequently used in the past, there was the accumulated precipitation deficit, or the accumulated departure from normal. Some examples of early criteria include the following: 1) fifteen consecutive days with no rain, 2) twenty-one days or more with precipitation less than one third of normal, 3) annual precipitation that is less than 75% of normal, 4) monthly precipitation that is less than 60% of normal, and 5) any amount of rainfall less than 85% of normal. Most of these definitions or indices were valid only for their specific application in their specific region. Indices developed for one region may not be applicable in other regions or contexts, because the meteorological conditions that result in drought are highly variable around the world and specific for each sector. Indices developed to measure the intensity of meteorological drought, for instance, were inadequate for agricultural, hydrological, or other applications (HEIM, 2002).

For this reason, much effort has been devoted to developing techniques for drought analysis and monitoring. Among these, objective indices are the most widely used, but subjectivity in the definition of drought has made it very difficult to establish a unique and universal drought index (HEIM, 2002). Furthermore, in the field, drought is a multidimensional stress and besides being a result of rainfall decrease, it is also accompanied by a normal increase in temperature, which increases evapotranspiration causing the water shortage (VICENTE-SERRANO et al. 2013). Therefore, some practical issues become important in an analysis of agricultural drought. These include: time scale, probability, precipitation deficit and the evapotranspiration index to be used. Thus, frequency, duration and intensity of drought all become functions that depend on the implicitly or explicitly established time scales.

Standardized Precipitation-Evapotranspiration Index (SPEI), whose data can be found at a specialized site dedicated to drought measurements (VICENTE-SERRANO et al. 2013; VICENTE-SERRANO et al., 2010), is a drought indicator or index (D) that is calculated by means of a climate water balance at monthly time step, i.e. D (equation described in Material and Methods) is the difference between precipitation and potential evapotranspiration. Therefore, different SPEIs can be obtained for different time windows representing the cumulative water balance over the previous months (VICENTE-SERRANO et al. 2013).

It is commonly accepted that drought is a multi-scalar phenomenon since the period where the soil water limitations impact various components of the ecosystem differ noticeably depending on several factors ranging from type, age and stage of plants or crops, type of soil, crop management to period and duration of drought, just to cite a few factors. Therefore, drought is a phenomenon that may occur simultaneously across various time scales (e.g., a

specific short period of dryness embedded within longer-term droughts). Different SPEIs are obtained for different time-scales representing the cumulative water balance over the previous months, providing windows of the aggregation and specific determined month. For example, to obtain the 6-month SPEI, first a time series is constructed by the sum of D values from five months before to the current month. To obtain SPEI series comparable in space and time, it is necessary to transform the D series using equal probability to a normal distribution with a mean of zero and standard deviation of one so the values of the SPEI are really in the standard deviations and the deficits of the seasonal effects. A log-logistic probability distribution function is fitted to the data series of D, as it adapts very well to all time scales. Monthly climate data for Lavras area are provided by the Climate Research Unit (CRU) of the University of East Anglia - United Kingdom and the Instituto Nacional de Meteorologia - Brazil (INMET).

SPEI uses both precipitation and ET_0 to quantify drought severity. Thus, the concepts of actual evaporation (ET_a) and reference evaporation (ET_0) should be clarified since there is a discussion on what are the factors that drive drought severity and which variables are relevant for quantifying drought severity. ET_a is the water lost under real conditions (i.e. considering the water available in the soils, the vegetation or crop type and state, physiological mechanisms, climate, etc). ET_0, on the other hand, represents the evapotranspiration rate of a reference surface (a well-watered hypothetical grass reference crop with specific characteristics).

Several authors suggested that considering ET_a is better than ET_0 when a drought index is defined since ET_a and not ET_0 would determine the surface water balance and the drought conditions (DAI, 2011a; JOETZJER et al. 2013). The proponents of this idea (i.e. the use of $P - ET_a$) explain that, compared to ET_0, ET_a would always be a better estimation of the amount of water really transferred to the atmosphere. Thus, ET_a would allow for a better estimation of the soil water balance than ET_0 (BEGUERIA et al. 2014). Whether the SPEI goal would be simulating the true water balance of the soils, as other indices such as the Palmer drought severity index do, then using ET_a instead of ET_0 would be a better option for the SPEI. But that is really not the case: the idea behind the SPEI is to compare the highest possible evapotranspiration (what the authors call the evaporative demand by the atmosphere) with the current water availability. Thus, precipitation (accumulated over a period) in the SPEI stands for the water availability, while ET_0 stands for the atmospheric water demand. ET_a would be a poor estimator of this demand, since it depends in turn on the current water availability. On the other hand, the very definition of ET_0 indicates that it refers to the maximum amount of water that would be transferred to the atmosphere by the soils and vegetation if there were no water

supply deficit. Using ET_0 as an estimator of the true evaporative demand seems, thus, a more convenient choice for SPEI.

Furthermore, SPEI is also a very interesting product when it comes to analyzing drought on a regional basis because the database furnished by the CRU of the University of East Anglia is related to a large pixel, which, in turn, covers a large piece of ground terrain. Besides one may analyze drought over long-time series (more than a century) and above all, NPP in the Southern Hemisphere appeared to be more sensitive to SPEI indicated by high correlation values than in boreal regions where significant negative relations were observed (CHEN et al. 2013). Due to temporal, spatial, sensorial and probabilistic parametrization of this product, SPEI has been largely used to monitor, accompany and predict present and future drought events on a regional and global scale.

2.3 Drought, water availability and the mechanisms controlling growth and primary production of coffee trees

Climatological drought refers only to a period in which rainfall fails to keep up with potential evapotranspiration (ET_0). However, particularly in the tropics, drought episodes are remarkably aggravated by both high sun radiation and high temperature, so drought should be considered as a multidimensional stress (DAMATTA et al., 2003, VICENTE-SERRANO et al. 2013). Many authors consider drought as an environmental factor that produces water deficit in plants, which is initiated when low water potential Ψ_w develops and cell turgor begins to fall below its maximum value. In relation to many other stressful events (*e.g.*, frosts), the stress caused by drought usually does not suddenly occur but develops slowly and increases in intensity the longer it lasts. Therefore, the time factor plays a crucial role in plant survival and/or maintenance of productivity under drought conditions, especially with adult perennials, which the stress might take a longer time to creep into the tree.

A limited water supply is the main environmental abiotic factor affecting coffee yield and growth. Under field conditions, the performance of coffee trees in terms of growth, biomass accumulation, and yield depends upon its ability to acclimate to stresses and environmental changes, improving mechanisms of specific tolerance that involves a complex network of molecular and biochemical processes (WANG et al., 2003). Abiotic stresses, such as high temperatures and low water availability, pose serious limitations to agriculture, reducing in more than a half the potential production of main crops (BRAY et al., 2000). In fact, studies upon the effect of drought in carbon assimilation in coffee plants demonstrate that water deficit often leads to a reduction in photosynthesis, increase in stomatal closure, leaf shed and twig burn (die back). Besides the severity in the attacks of bugs and diseases with the increase in

drought duration and intensity (KUMAR; TIESZEN, 1976; DAMATTA et al., 2003; SILVA et al., 2004; LIN, 2007).

Even though great progress has been achieved in understanding the drought effects upon the decrease of photosynthesis, there is no unified concept of the events that cause a reduction in the efficiency of photosynthesis (TEZARA et al., 1999; CORNIC, 2000; LAWLOR; CORNIC, 2002; CHAVES; OLIVEIRA, 2004). Some reports have suggested the importance of stomatal closure in restricting CO_2 supply to the cell metabolism, especially under mild water stress (CORNIC, 2000), while other studies suggest that photosynthesis could be more straightforwardly limited by non-stomatal factors, specially through a direct effect of the drought upon the synthesis of ATP, therefore leading to a restricted ATP supply in the cells (TEZARA et al., 1999; LAWLOR; CORNIC, 2002). Besides, some studies also show that shoot growth rates can be restricted before there is any evidence of differential stomatal closure between irrigated and non-irrigated trees (WORMER, 1965).

For many years it has been accepted that as the soil dries, water uptake is reduced and leaf water status declines. There is no doubt that soil drying changes leaf water relationships and that these changes influence the biochemical and physiological processes involved in the growth and development of the shoot. They are common responses. However, there are those shoot responses to soil drying, which occur in the absence of any detectable change in leaf water status. Because such regulation almost certainly also occurs in tandem with the hydraulic effects of the soil drying, it is crucial to comprehend its mechanistic basis. Therefore, according to Davies and Zhang (1991) variables such as leaf conductance and growth rates may be more useful indicators of plant stress than the more commonly used variables of leaf water relations.

Several simultaneous processes control and drive the growth of plants, so it is not simple to understand and model the plant growth; photosynthesis is only one of the several components driving the plant growth. Besides the photosynthetic process, the form in which the biomass is invested into the different plant organs and tissues, the feedbacks among organs, tissues and processes, and the plant capacity of acquiring additional resources, as well as the metabolic costs and expenditures (specific tissue respiratory rates) are factors that are also involved in the control of plant growth (KORNER, 2006). The strategies of plant investment in relation to process regulated by shoot sinks are important aspects for the analysis and modelling of morphological plasticity, especially in stress situations and heterogenous environments (LAMBERS; POOTER, 1992), where a constant supply of determined resources is not temporarily present to the plant.

Most of the growth models use the photosynthetic process as the main drive of plant growth, however this paradigm of vegetal growth based on the control of the source (photosynthesis) fails in presenting an argument based on effects (KORNER, 2006; FOURCAUD et al., 2008). The plant primary production depends on several other factors besides the assimilated carbon. Therefore, the plant growth rate is not simply determined by the photosynthetic rate of assimilated CO_2 per unit of leaf area, but also depends on the allocation of resources to the leaf area (quantity of photosynthetically active tissues built) and the leaf longevity (for how long the leaf tissues will be able to photosynthesize). Besides, the carbon is used inside the plant to both growth and respiration, and the portion used by respiration varies greatly among species, being generally augmented with the reduction of the plant growth rate (LAMBERS et al., 2008).

The plant growth is a consequence of meristem functioning and ultimately its expansion depends on turgor and cell wall extensibility. During growth, plant cells secret proteins called expansins, which unlock the framework of cell-wall polysaccharides, permitting turgor-driven cell enlargement. Inhibition of cell expansion under drought stress involves both the reduction in turgor and the loss of cell wall extensibility. Loss of cell wall extensibility also involves changes in polysaccharide content and structure in the cell wall (BLUM, 2010). Besides, the meristems are very active regions during favorable moments of the year, consuming very large quantities of sugars and therefore being a potent sink in the coffee trees. Thus, the positioning and temporal activity of meristems determine the plant morphology and the crop growth rate (KORNER, 1991) resulting in its form and architecture. In models developed a decade ago, Luquet et al. (2006) concluded that plant growth and development depend on the supply of assimilates (furnished by photosynthesis) as well as the demand based on the plant organogenesis. This way, against the hypothesis used for the development of many structural-functional models, where the growth is controlled by the source activity, Korner (2003) furnishes evidence that the growth of trees, independent of climatic zone is controlled by sink activity. By making use of non-structural carbohydrates (NSC) concentration in plant tissues and the hypothesis that low NSC indicate low source activity and high sink activity and high NSC concentration demonstrate that photosynthesis furnishes enough assimilates or even beyond the needs for plant growth, Korner (2003) demonstrated that the NSC pools in trees are generally high during unfavorable periods of plant tissue formation. Even though the influence of genes associated to photosynthesis, respiration and plant development are of great importance, in natural conditions, the sink activity will more often drive growth than the source

one, since the sink activity is frequently controlled by finite soil resources such as water and nutrients as well as by developmental restrictions (FOURCAUD et al., 2008**).**
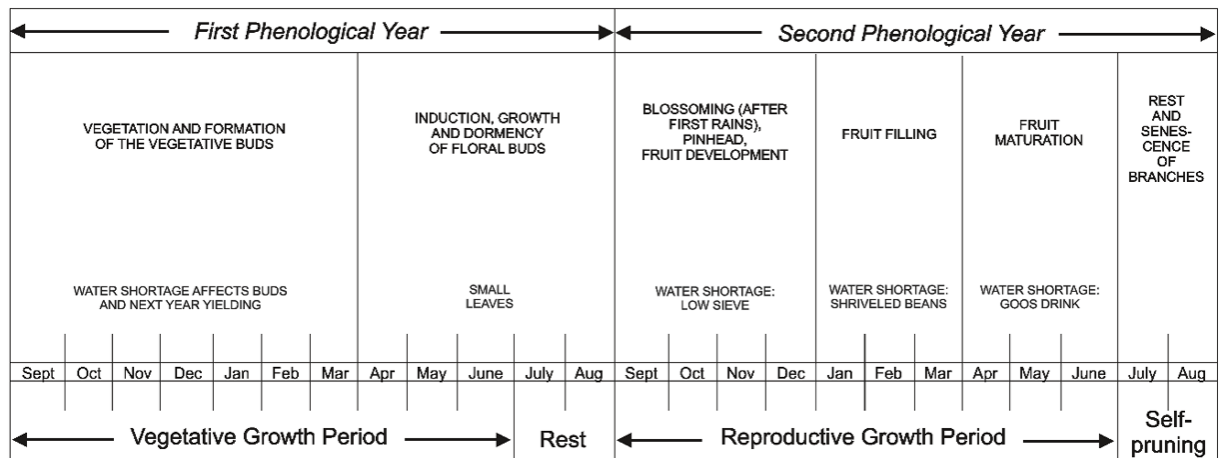
2.4 Water availability and the coffee phenological phases

Most of the crops blossom and fructify in the same phenological year. The Arabic coffee is a special plant that takes two years to complete its phenological cycle (TABLE 2.1). In the first year the vegetative branches are formed with axillary buds in the nodes, during long-day months. During this first year of vegetative growth water shortage may affect next-year production by reducing its vegetative growth. In January it is summer in the south hemisphere, and as the days start to become shorter again, the vegetative buds are induced by photoperiodism into reproductive buds (CAMARGO; CAMARGO, 2001).

By the month of April (middle fall), right after March equinox, as shorter days with less than thirteen-hour-light begin to have an effect, the axillary buds are intensively induced into floral buds that begin to develop. These floral buds proceed to mature and, when ripe, become dormant waiting for anthesis, then there is a significant increase in water potential in the dormant buds. The hydric shock, caused by the rain or irrigation, is the main factor triggering blossoming. A noticeable increase in the air moisture (RH), even if the coffee plants do not get direct rain on the shoot, may induce blossoming. This may also happen with buds kept wet for several days with a cotton wool ball.

The second phenological year begins with the blossoming, followed by the development of pinheads that precedes the expansion of coffee beans until their full size. Water deficits during this phenological moment may reduce significantly the crop yield, producing more low-sieved coffee beans. After the process of filling and ripening of beans, there is the senescence and death of terminal plagiotropic branches, known as self-pruning, a process highly affected by water shortage. In the spring of the second year, new branches will sprout, restarting the production cycle which will lag into the following year.

Table 2.1. Scheme of the phenological phases of Arabic coffee highlighting its two-year cycle in normal tropical conditions of cultivation.

First Phenological Year											Second Phenological Year											
VEGETATION AND FORMATION OF THE VEGETATIVE BUDS							INDUCTION, GROWTH AND DORMANCY OF FLORAL BUDS				BLOSSOMING (AFTER FIRST RAINS), PINHEAD, FRUIT DEVELOPMENT				FRUIT FILLING			FRUIT MATURATION			REST AND SENES-CENCE OF BRANCHES
WATER SHORTAGE AFFECTS BUDS AND NEXT YEAR YIELDING							SMALL LEAVES				WATER SHORTAGE: LOW SIEVE				WATER SHORTAGE: SHRIVELED BEANS			WATER SHORTAGE: GOOS DRINK			
Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June	July	Aug
Vegetative Growth Period										Rest		Reproductive Growth Period										Self-pruning	

Source: CAMARGO & CAMARGO (2001)

2.5 Leaf area dynamics and canopy acclimation to drought

The leaf area is an important variable to acclimating the plant to the drought. The size of the leaf area controls the fluxes of water and carbon in the plant canopy, its adjustment is an efficient means of facing the unpredictability of water shortage in a dry environment. The leaf area adjustment and acclimation to water availability have been demonstrated in many types of plants varying from shrubs to trees (SPECHT 1972; GRIER; RUNNING 1977; GHOLZ 1982; JOFFRE; RAMBAL 1993; MARTINEZ-VILALTA et al., 2009; LIMOUSIN et al., 2012).

Beyond the adjustments in leaf area, the morphological responses at the leaf level generally lead to changes in optical properties that culminate in modifications in the pattern of the canopy reflectance, which in turn, may be studied by remote sensing technology used to monitor plant functioning (SIMS & GAMON, 2002; BLACKBURN, 2007; YANG et al., 2007). Therefore, the use of the NDVI may allow accessing and evaluating the crop yielding (PONTES et al., 2005, BERNARDES et al., 2012). Since NDVI is considered a good estimator of biomass, it is quite used to evaluate the crop productivity and possess a good correlation with the LAI temporal dynamics (SAKAMOTO et al., 2012; SANCHEZ et al., 2012).

Yet the mechanisms that control the long-term adjustment in relation to water stress such as changes in the leaf area have received less attention than the physiological controls of short duration, such as stomatal closure, these mechanisms are the ones that are associated to the phenotypical plasticity of the plants. And, being expressed at the level of modular subunits (meristems, leaves and branches) contribute to the formation of the plant canopy, which is an integrative product of modular plasticity in several of these levels (DE KROON et al., 2005).

The knowledge of growth dynamics of the leaf area combined with the longevity of the leaf in the canopy, as well as the growth responses of branches and twigs, principally in plants that keep their leaves for more than one season may contribute to elucidating the relationship between carbon fixation and its fate in coffee trees under low water availability.

There are different methodologies aimed at quantifying leaf area, which may or may not be destructive (ILKAEE et al., 2011). In the first case, cutting the leaf is necessary, and measuring is performed using traditional methods such as copying on graph paper, photographing, or using a planimeter (FALLOVO et al., 2008). However, the greatest limitation of such methodology is the impossibility of taking successive measurements through time on the same leaf. Additionally, the resulting defoliation may alter other experimental measurements (FALLOVO et al., 2008). However, methodologies that allow measurement of leaf area accurately and nondestructively are very important, as they are less economically costly and enable measurements on the same leaf over time, making it possible to describe accurate patterns and rates of growth, besides ensuring the assertiveness in predicting the crop productivity and yield (POCOCK; EVANS; MEMMOTT, 2010; WATSON, 1952; WHITE et al., 2010, BARBOSA et al. 2012). Some authors developed and adjusted equations to estimate leaf area from individual leaves of coffee, based on the measurement of the length and width of various leaves of the coffee tree (UNIGARRO-MUNOZ, 2015). Therefore, by making use of those foliar values we could access higher scales such as branch leaf area and canopy leaf area with a few adjustments and additional methods.

According to West et al. (1997) most of the variations related to the size of an individual, in this case the leaf volume in the canopy, can be characterized by power laws following the formula $Y = Y_0 \cdot M^b$, where Y is the variable of interest, Y_0 is a constant of normalization, M is the body mass of the organ and b is the potency exponent. Similarly, Negash et al. (2013) verified that the power equation $Y = b_1 \cdot d_{40}^2$ presented a high statistical significance ($R^2 = 0,90$) to estimate the total and partial biomass of the coffee trees in their study. The reliability of the estimation declined in the order of stem > branches > twigs with leaves. (NEGASH et al., 2013). Thus the measurements of LA in addition to other fundamental size variables such as stem diameter are of great importance to agronomic, forest and agroforest engineers who are in search of solving practical and economic problems, to ecologists and ecophysiologists who are interested in climate and environment, and as well as to theoretical evolutionists and ecosystem modelers who want to build up mathematical representations with practical and theoretical applications (LE ROUX et al, 2001; FOURCAUD, 2008). Thus, the

measurements of stem diameter and/or its growth rates seem to be an interesting variable to estimate the biomass of coffee tree canopies or the processes related to its growth.

2.6 Sink-source relationship in branches of coffee trees

The central influence of sink activity over the photosynthesis and in the partitioning of carbon is documented in several woody species, such as citrus (IGLESIAS et al., 2002; RIVAS et al., 2007), mango (URBAN et al., 2004), coffee (FRANCK et al., 2006) and peach (LI et al., 2007; DUAN et al., 2008). Under conditions of sink demand decline, the accumulation of the end-product in source-leaves is frequently understood to be the factor that restricts net photosynthetic rate (A) (PAUL & PELLNY, 2003). There is an assumption that the growth of too big starch grains could either deform the chloroplasts or hinder CO_2 from arriving to the carboxylation sites (NAFZIGER & KOLLER, 1976), while an accumulation of soluble sugars could repress the gene expression related to photosynthesis (SHEEN, 1990). However, the sink effect on A is still a cause of discussion because the decline in the sink demand is not ubiquitously found to cause a reduction on A, despite the accumulation of end-products (LUNN & HATCH, 1995).

In coffee plants, it is well stablished the interdependency between source and sink organs (leaves and branches) and the trade-offs between the sink organs (fruit and branches) (ALVES, 2008; DAMATTA et al., 2008). Some studies show that the leaf area required to fulfill the energetic demands of a coffee grain is of 20 cm^2. Therefore, it is easy to conclude that to support large productions, the coffee plants must possess a high LAI which will allow the leaves to fix enough quantities of carbon to satisfy the grain filling (ALVES et al., 2011).

Regarding the fruitful branches, the great majority of the researches with coffee plants have been conducted by the manipulation of source-sink relationships through the controlled fruit removal or phenological studies to determine changes in shoot growth. In those cases, it was shown that the growth of branches is smaller in plants with full fruit load compared to those with reduced fruit load (AMARAL; RENA; AMARAL, 2006). According to Alves et al. (2011), coffee plants with no fruit load manifested a larger root growth and root density than those plants with full fruit load only in deeper layers of the soil. These results contrast with some results published by other authors taking into consideration the coffee plant source-sink relationship. There are reports that show coffee fruit as carbohydrate-preferential sinks, limiting the growth of other organs, especially the roots (BRAGANÇA et al., 2007). In this case, the amplitude of the explanations refers to the accumulation of high proportions of the total assimilates in the fruit, limiting the mobilization of these sugars to the roots with negative effects on their growth.

Therefore, even though the relationships between fruit and leaves, and between fruit and branches have already been well established for the coffee tree, it is still necessary to study such relationships for understanding the carbon trade-offs among the different sinks and the influence of sink strength in the growth of certain parts of the coffee plant such as leaves, branches and even the fruit under conditions of low water availability, because the comprehension of the physiological process that control the dynamic carbon allocation, as well as the trade-offs in coffee plants subjected to low water availability may produce more precise growth models for the coffee trees when facing extreme events, which, so far, is scarce.

3 MATERIAL AND METHODS

3.1 Experimental area

This study was carried out in a coffee crop (variety Mundo Novo - IAC 379-19) that belongs to the Department of Agriculture - Coffee Division, situated on the campus of the University Federal of Lavras (UFLA), Lavras county (21º13'40''S and 44º57'50''W GRW), southern region of Minas Gerais, Brazil (FIGURE 3.1). The experimental area has a westbound slight declivity of 12% with a leveled planting and the soil is a deep dystrophic Red Latosol with granulometric characteristics described in Table 2 in this section. The crop management also makes use of mulching covering the soil and minimum tilling, which creates protective conditions against erosion and superficial rainfall runoffs. The coffee trees were 6 years old and nearly 2.2-m tall and 2.0-m crown planted in a 3.8m x 0.8m plantation density.

Figure 3.1 - Aerial view of the experimental area highlighting part of the coffee crop object of study (inset box). Site: 21°13'41"S 44°57'43"W. Source: Google Maps 2017.

3.2 System of exclusion, climatic and soil variables and experimental design

In April of 2015, it was installed in the experimental area a system of rain exclusion. Each individual system of rainfall collectors was composed of six eucalyptus posts of 3.2 m high buried up to 0.60 m deep in the soil with three posts arranged on each side of the coffee line and 2.5 m away from each other along the line. Translucid polypropylene tiles turned into gutters were fixed on top of woody beams of 5x7 cm with three metallic supports so that the gutters could be correctly positioned on top of the three posts making up a total of $4.8m^2$ of rain exclusion area. Therefore, this system was designed to perform a nearly exclusion of ¼ of the rain in the plots. This value was based on the IPCC report that predicts reductions of up to 20 % of rainfall for the southeastern area of Brazil (IPCC, 2014) and on the relative soil water content described in the Material and Methods, since lower values could cause problems for the intended experimentation. Each system had its own rainwater reservoir of 200L so that no rain collected by the gutters would spill to the ground. The experimental design completely randomized was composed of three treatments with three plots, each containing seven plants, resulting in the following treatments: a) System with rainfall exclusion (SWE) – gutter system that collect nearly ¼ of the rainfall; b) System with no rainfall exclusion (SWNE) – perforated gutter system that allows the water of the rain to go through the gutters; and c) Control (NNE) – no gutter system at all.

The plots were fully instrumented so that the environmental conditions of area could be monitored as follows: 1) a pluviometer type Ville de Paris was installed on a post positioned at two meters above the soil in the middle of an open area with no obstacle on its way; 2) three thermo-hygrometers model HT500 were installed in three plots of each treatment at 1.5 m above the soil for measuring temperature and relative humidity, collecting data every 30 minutes throughout the experimental period; and 3) nine puncture tensiometers were installed at 0.30 m deep in each of the nine experimental plots and the measurements were taken every two days during the experimental period to access the water potential of the soil (FIGURE 3.2).

The *VPD* was calculated by making use of equations 3.1, 3.2 and 3.3:

$$e_s = 0.61078^{((17.269 \times T \div (237.3 + T))} \tag{3.1}$$

$$e_a = \frac{RH \times e_s}{100} \tag{3.2}$$

$$VPD = e_s - e_a \tag{3.3}$$

where e_s is the pressure of saturation, e_a is the actual pressure of the atmosphere, T is the temperature in degrees Celsius, RH is the relative humidity and the *VPD* is the vapor pressure deficit of the atmosphere.

Figure 3.2 - Photographs from the Exclusion System (A) and the tools used for monitoring the environmental conditions in the experimental area: Pluviometer type Ville de Paris in an open area (B), 50-cm puncture tensiometer (C) and the thermo-hygrometer HT500 (D).

3.3 Drought monitoring models

3.3.1 Relative soil water content, frequency of drought duration, long-time drought duration, unpredictability index and drought return period

We used a soil water budget model in which we reduced the rain input R by 20, 30 and 40%. We further compared these results with those obtained with the current rainfall as a control. The budget of water of the soil layer has been simulated at a daily time step by using a bucket-type model with a limited storage capacity and mathematically represented by a

difference equation in which the daily change in soil water storage, ΔS, equals rain input, R, minus outputs, that is deep drainage, D, minus actual evaporation, AET (equation 3.4):

$$\Delta S = R - AET - D \tag{3.4}$$

Stored water in the soil, S, is up bounded by the so-called field capacity, FC. When the soil water storage exceeds the field capacity threshold extra water flows down as deep drainage. Actual evaporation AET extracts water from the soil compartment. AET is a fraction of potential evaporation PET both related to the amount of stored water in the soil and to PET itself. PET was calculated by using the Priestley-Taylor's equation. Daily values have been calibrated for our regional applications with the standard PET parameter of 1.26 that corresponds to the original equation. The net all-wave radiation term has been linearly related to global incoming short-wave radiation. For reducing PET to AET, Linacre (1973) proposed a simplified algorithm. AET rate equals whichever is the less of (1) the PET rate, or (2) $AET_{max}\left(\frac{S}{FC}\right)^2$. Finally, the model may be summarized by two coupled equations 3.5 and 3.6:

$$AET = min\left[AET_{max}(\frac{S}{FC})^2 , \ PET\right] \tag{3.5}$$

$$S(t+1) = min[S(t) + R - AET, FC] \tag{3.6}$$

AET_{max} is usually fixed arbitrarily to 4 mm per day. Retention properties of the clay soil that covers plot area have been derived from Saxton and Rawl (2006). Percent clay fraction ranges from 44 to 64 and sand fraction ranges from 30 to 32 (TABLE 3.1). Assuming the soil displays a minimum of coarse (rock and stone) fraction, field capacity may be estimated from 0.42 to 0.49 cm^3 H_2O cm^{-3} soil corresponding to water contents reached at water potential of -0.03 MPa. The wilting points at -1.50 MPa were 0.30 and 0.35 cm^3 H_2O cm^{-3} soil, respectively (TABLE 3.2). As first guess, we retained an active rooted layer of 50 cm with a field capacity of 240 mm, corresponding to a rooting pattern observed on coffee varieties with shallow root system.

Table 3.1 – Granulometric characteristics of the dystrophic Red Latosol where the coffee trees were planted.

Depth (cm)	Particles		
	Clay (%)	Silt (%)	Sand (%)
0-20	44	24	32
60-80	64	6	30

Table 3.2 – Soil physical properties used to run the relative soil water content (RSWC) curve.

Layer n°	Depth (cm)	Coarse fraction (%)	Field capacity [1]	WSFC (mm)	β [3]	Wilting point [2]	AWC (mm)	Percent root
1	0-30	32	0.420	149.7	11.2	0.298	36.3	0.60
2	30-60	30	0.490	179.7	13.4	0.352	34.8	0.40

[1] At -0.033 MPa of soil water potential

[2] At -1.5 MPa of soil water potential

[3] The exponent of the retention curve

The relative frequency of drought duration at RSWC<0.7 and RSWC<0.4 was obtained by calculating the ratio of similar drought events corresponding to each drought category in relation to the total number of drought events recorded in the same time-scale and category for different rain exclusions, i.e. 0, 20, 30 and 40% of exclusion.

The long-term series of drought duration were calculated by plotting each drought duration along its respective time of occurrence and calculating the linear trend along the time series from 1980 to 2014.

The unpredictability index was calculated by taking the daily values of the coefficient of variation for the RSWC in control and 30% of rain exclusion plotted over the time series from 1980 to 2014.

The relative frequencies of events for the years 2015 and 2016 in the control and 25% rain reduction were obtained using equation 3.7:

$$f(r) = \frac{n(r)}{\Sigma n} \qquad (3.7)$$

where n(r) is the number of events with a specific rainfall record divided by the number of years, whereas the return period is just the inverse of that ratio, i.e. the number of years divided by the number of specific rainfall events recorded.

3.3.2 SPEI with the log-logistic distribution

SPEI is a drought indicator or index (*D*) that is calculated by means of a climate water balance at monthly time step, i.e. the difference between precipitation (*P*) and potential evapotranspiration (*ET₀*) displayed by equation 3.8:

$$D = P - ET_0 \tag{3.8}$$

Different SPEIs are obtained for different time-scales representing the cumulative water balance over the previous k months given by the equation 3.9:

$$D_n^k = \sum_{i=0}^{k-1}\left(P_{n-i} - ET_{0\ n-i}\right) \tag{3.9}$$

Where *k* in month is the time windows of the aggregation and *n* is the considered month. The *D* values are undefined for *k* > *n*.

A log-logistic distribution was used to obtain the SPEI series in standardized z units (mean=0, SD=1) (VICENTE-SERRANO et al., 2010). The probability distribution function of a variable *D* according to a log-logistic distribution is given by equation 3.10:

$$F(D) = \left[\left(1 + \frac{\alpha}{D-\gamma}\right)^{\beta}\right]^{-1} \tag{3.10}$$

Where *α*, *β*, and *γ* indicate scale, shape and origin parameters, respectively. This function was chosen as the best distribution function to fit *D* series (Vicente-Serrano et al., 2010). Finally, SPEI data were calculated by standardizing *F*(*D*). More details are provided in Vicente-Serrano et al. (2010).

The maps for the drought evolution regarding SPEI-6 January over the previous three years before the installation of the rain exclusion system and in the years following along the experiment were also obtained using the SPEI time series straight from its site at http://spei.csic.es/index.html.

3.4 Analyses of the coffee crop variables

3.4.1 Leaf water potential and crop evapotranspiration in the field

Leaf water potential (Ψ_{leaf}, MPa) of the coffee trees was determined using a Scholander type pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, California, USA). For that purpose, five fully expanded leaves were excised from plagiotropic branches situated in the middle of both sides of the line in four coffee trees per plot before dawn monthly.

The calculation of ET_c used in the empirical models was performed by applying equation 3.11:

$$ET_c = K_c * ET_a \tag{3.11}$$

where K_c is the crop coefficient, which presents a linear correlation with LAI (PEREIRA et al., 2011) and ET_a is the actual evapotranspiration (ET_a) expressed also on a unit leaf area basis of

the reference surface ($ET_{LA} = ET_a \ LAI^{-1}$) derived from the Penman-Monteith equation (MARIN et al., 2005; ALLEN et al., 1998; PEREIRA et al., 2011). The K_c depends on the size and age of the crop (SILVA et al., 2009; PEREIRA et al., 2011) and K_c values of 1.1 are normally found for six years old coffee crops (SILVA et al., 2009). In this work, we used $K_c =$ 1.0 to calculate ET_c, which was similar to the average value found by Marin et al., (2004) in their work.

3.4.2 Normalized difference vegetation index (NDVI), leaf area index (LAI), branch leaf area) BLA, stem diameter, source-sink relationship and total production

The NDVI was measured with the aid of a portable sensor (GreenSeeker®, Trimble Navigation Ltd., Sunnyvale, CA, USA), top and lateral measurements of the canopy were taken by placing the sensor from nearly 0.8 to 1.0 m away from the trees, every fifteen days throughout the experimental period. We also correlated NDVI with plant indices such as LAI and BLA.

The LAI of coffee tree was obtained through a methodology proposed by Barbosa et al. (2012), which consists of measuring the main orthotropic stem height and the length of plagiotropic branches in at least five different positions in the tree canopy every fifteen day. The values were plugged into formulas obtained by the authors as a form of estimating the leaf area of the coffee trees in four trees per plot (n = 36).

The BLA was accessed by photographic data of one branch in the superior middle part (BLAs) and one in the inferior middle part (BLAi) on the east side of the plantation from three trees per plot, summing up 54 samples (n = 54), through which the mean branch leaf area (BLAm) was calculated. Photographs of the branches were taken monthly, from November 2015 to October 2016, by placing the branches upon a millimeter screen so that the growth of leaf area on the branches could be obtained. Then the photographs were analyzed using the program Image Tool Version 2.01 Alpha 4, which allowed us to register the BLA of the coffee trees (TABLE 3.3).

The stem diameter at 1.30 m high of four trees per plot (n = 36 plants) were measured with the help of a digital caliper on the trees, previously marked with a waterproof ink, in the months of March and July of 2016 to estimate the rate of growth during that reduced growth season.

The relationship between source and sink capacity was also evaluated through accompanying the branches that have suffered flower removal and those which were intact, making up 72 sampled branches. The measurements of leaf number, length and diameter of branches were collected every two weeks.

Table 3.3 – Partial sample of photographs taken from superior and inferior branches of coffee trees. This example is only a small representation of inferior branch leaves approximately 10% of the total taken.
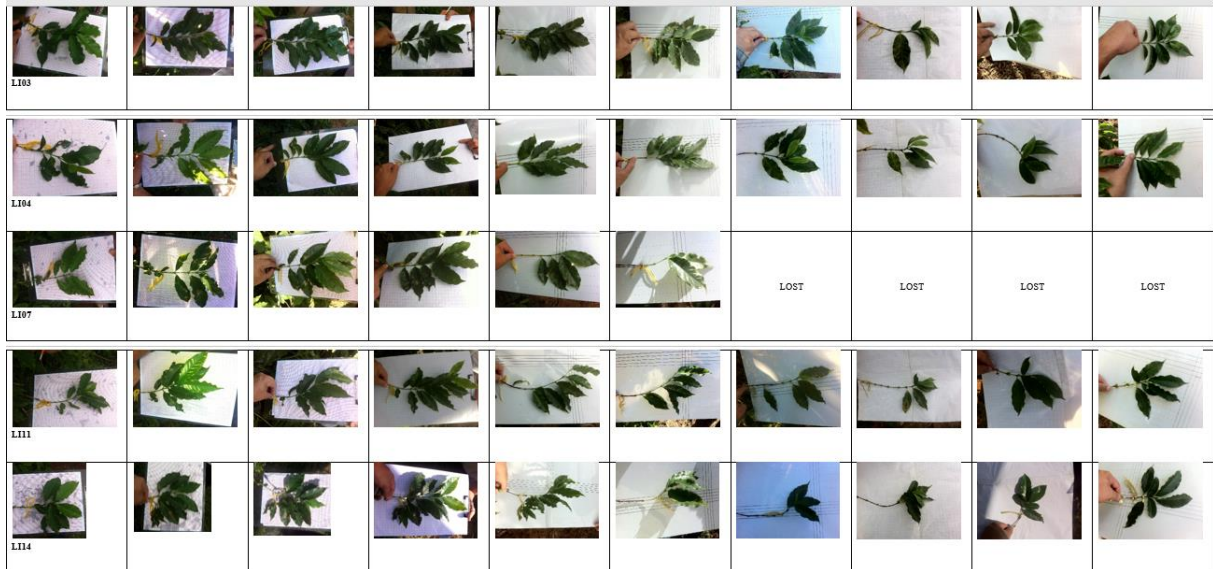
The productivity of the coffee trees was obtained at both harvest seasons in June 2016 and June 2017. The plants were traditionally harvested by manual breaking the coffee beans onto a cloth on the ground, being blown from the leaves using a sieve and bagged separately for each plot and then dried on the ground of a covered terrace until constant mass. For the 2016 harvest the entire plot with 5 plants were harvested furnishing a low number of samples (n = 9). However, during the 2017 harvest four plants by plot were harvested making up a larger number of samples (n = 36).

We also calculated the biennially index by making use of equation 3.12:

$$B = \frac{[Y_1 - Y_2]}{Y_1 + Y_2} \tag{3.12}$$

where Y_1 and Y_2 are the coffee yield in two consecutive years and the index is the coefficient between the difference of yields in modulus divided by their sum.

3.4.3 Relative water content, specific leaf area, chlorophyll and macronutrient content

The relative water content was obtained by equation 3.13:

$$RWC = \frac{FM - DM}{SM - DM} \times 100 \tag{3.13}$$

Where *FM* is the fresh mass of the leaf, *DM* is the dry mass of the leaf and *SM* is the mass of the leaf at full saturation. The *RWC* express the water content in per cent at a given time as related to the water content at full turgor. The values were obtained fortnightly by collecting

completely expanded leaves from the middle-third part of three coffee trees per plot making up 27 samples from December 2015 to July 2016.

The specific leaf area, which is defined as the ratio of leaf area to dry mass, was obtained fortnightly by collecting disks of known area of completely expanded leaves from the middle-third part of three coffee trees per plot making up 27 samples from December 2015 to July 2016.

The indirect quantification of chloroplastic pigments (chlorophyll) was realized with the aid of a portable chlorophyll meter AtLeaf+, every fifteen days in completely expanded leaves and the values were converted into mg.m^{-2}.

The macronutrient content present in the leaves of the coffee trees were determined following the methodology suggested by Malavolta (1997) in the Chemistry Department of UFLA. The sampling was collected in the months of October 2015 (spring), December 2015 (summer), March 2016 (fall) and July 2016 (winter); by collecting four mature leaves from each cardinal point (north, south, east and west) of the middle-third part of three coffee trees per plot that composed a single sample for each plot making up a total of 36 samples for the whole period.

3.5 Further statistical analyses

3.5.1 ANOVA

All variables were subjected to the ANOVA and the statistical significant ones were subsequently tested by Tukey's at $P < 0.05$. If the data were neither normal nor of the same size, the non-parametric Kruskal-Wallis's test would be performed. For big data, such as relative leaf water content, normalized difference vegetation index and chlorophyll the cleaning of outliers to normalize data was performed when necessary.

3.5.2 Regression analysis

Regression analysis generates an equation to describe the statistical relationship between one predictor variable (independent variable) and the response variable (dependent variable). In our case, all the regression analyses developed linear regression curves that fit better the following statistic model displayed by equation 3.14:

$$Y_i = \beta_0 + \beta_1 x_i + \varepsilon_i \qquad to \ i = 1, ..., n \qquad (3.14)$$

Where Y_i and x_i are the values observed for each variable, β_0 and β_1 are, respectively, the vertical intercept and slope of the regression line, and ε_i is the random error term.

3.5.3 Pearson's correlation

The Pearson's correlation measures the degree of correlation between two variables (and the sense of this correlation whether positive or negative). Therefore, this coefficient

varies between -1 and 1 and it is larger the closer it comes to any of the extreme values, being calculated applying equation 3.15:

$$r = \frac{\sum_{i=1}^{n}(x_i-\bar{x})(y_i-\bar{y})}{\sqrt{[\sum_{i=1}^{n}(x_i-\bar{x})^2][\sum_{i=1}^{n}(y_i-\bar{y})^2]}} \qquad (3.15)$$

Where, x_i and y_i are the values observed for each variable and $\bar{x}$ and $\bar{y}$ are the means of the same variables.

4 RESULTS

4.1 Soil drought limitations in today climate: outputs from a soil water simulation and evolution of SPEI

January SPEI-6 maps for the region of Lavras-MG displayed a gradual evolution of the drought (FIGURE 3), during the previous years before the installation of the rainfall exclusion system in the field from January 2012, which presented the lowest drought intensity with an index around $> 2.33,$ culminating to the highest drought intensity with a drought index around < -2.33 in January 2015; SPEI-6 for January 2016 showed a reasonable recovery from the drought intensity with a SPEI value around 0.84 (FIGURE 4.1).

Figure 4.1 - Maps for the evolution of SPEI-6 January from 2012 to 2016. The colored bar indicates the drought indices varying from <-2.33 to >2.33 according to the standard precipitation-evapotranspiration index. The icon displays the location of the experimental site.
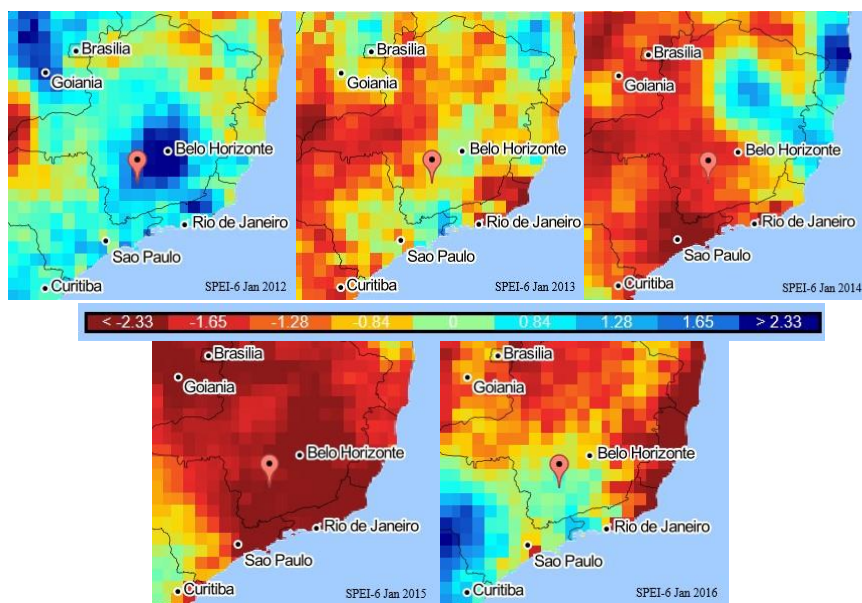
A model simulation for relative soil water content (RSWC) (FIGURE 4.2) was developed according to previously described method and based on a dystrophic Red Latosol, whose physical characteristics for water retention are displayed in Tables 2 and 3. From the picture we can identify a continuous lowering pattern of RSWC towards the winter months of august, reaching its trough in September and then bouncing back to higher figures by the end of spring and beginning of summer months. Therefore, towards the end of August and throughout the whole month of September the intensification of the water removal through

percolation and evapotranspiration brings the RSWC values to lower 0.40 (severe drought) with a possibility of lower figures in certain drier years.

Figure 4.2 - Simulated soil drought pattern from relative soil water content from 1980 to 2013. The blue line is for the mean values of RSWC. The grey area represents the standard deviation. Hatched areas are for respectively, the mild drought with RSWC<0.7 and for the severe drought with RSWC<0.4.
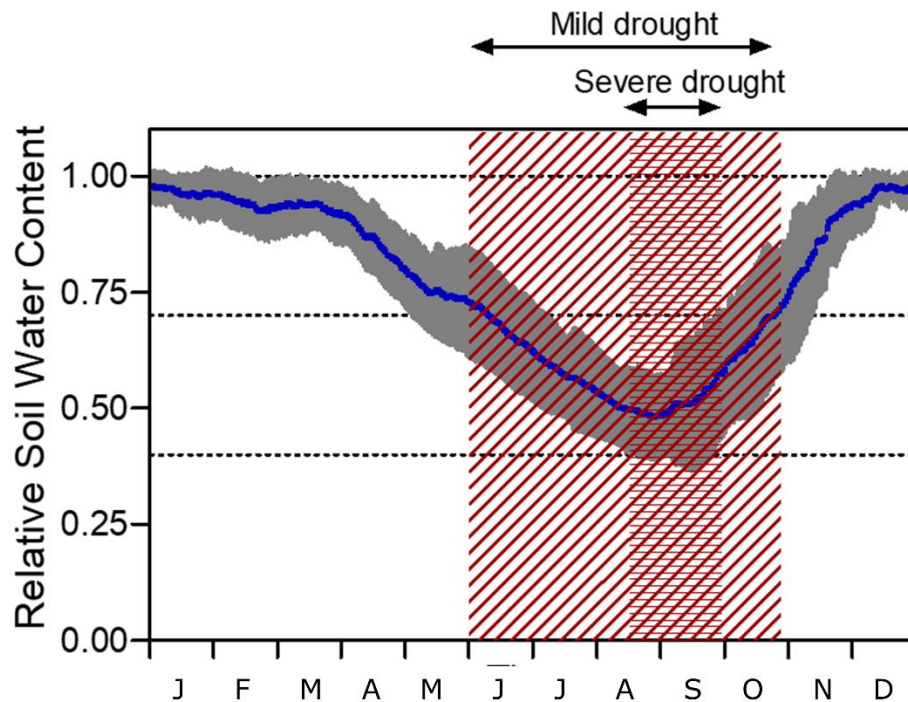
Theoretical modelling for relative frequency of drought duration presented a positive displacement of drought duration curves towards longer days with a mild water shortage (RSWC<0.7) as expected, nonetheless without a significant change in the relative frequency of those events (FIGURE 4.3A). The results also show that for a given time-scale mild droughts occur most frequently and extreme droughts occur least frequently in the area. On the other side, the relative frequency of severe drought (RSWC<0.4) suffered a significant reduction with a reduction of available water (FIGURE 4.3B). According to the model the increase in drought duration would have mean values varying from the normal 130 days towards 175 days for RSWC<0.7, and from the normal average of ten days of severe drought towards a figure of

over 22 days at RSWC<0.4 with a reduction of nearly 36% in the frequency of that event under a rain exclusion of 30% (FIGURE 4.3).

Figure 4.3 Relative frequency of drought duration at RSWC<0.7 (A) and RSWC<0.4 (B). Blue line is for the control plot. Green, orange and red lines for 20, 30 and 40% of rain exclusion.
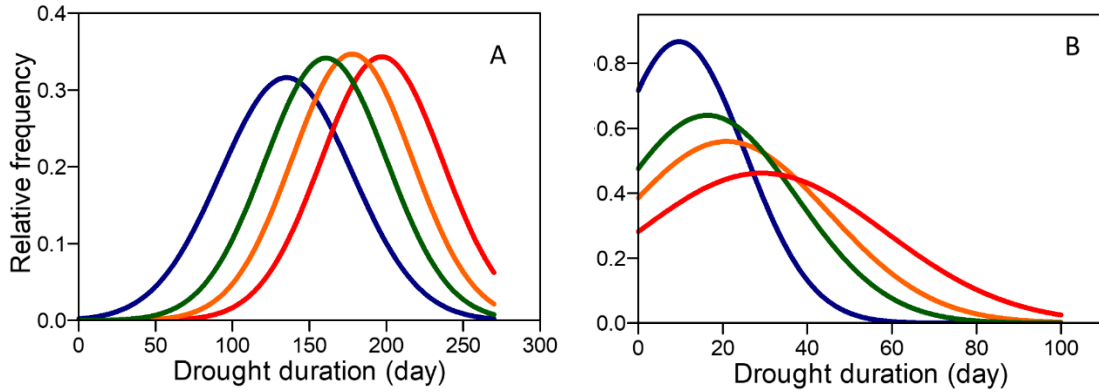
The long-term series of drought duration from 1980 to 2014 (FIGURE 4.4) did not display a clear tendency of longer drought durations periods either for mild drought at RSWC<0.7 (orange line) or severe drought at RSWC<0.4 (purple line) from 1980 to 2014 and the linear trend (orange strait line) displayed in the graph for mild stress was not significant, although the line showed a certain elevation towards the present moment (FIGURE 4.4).

Figure 4.4 - Long-time series of drought durations for mild drought at RSWC<0.7 (orange line) and severe drought at RSWC<0.4 (purple line) from 1980 to 2014. The linear trend (orange straight line) in mild drought duration is not statistically significant.
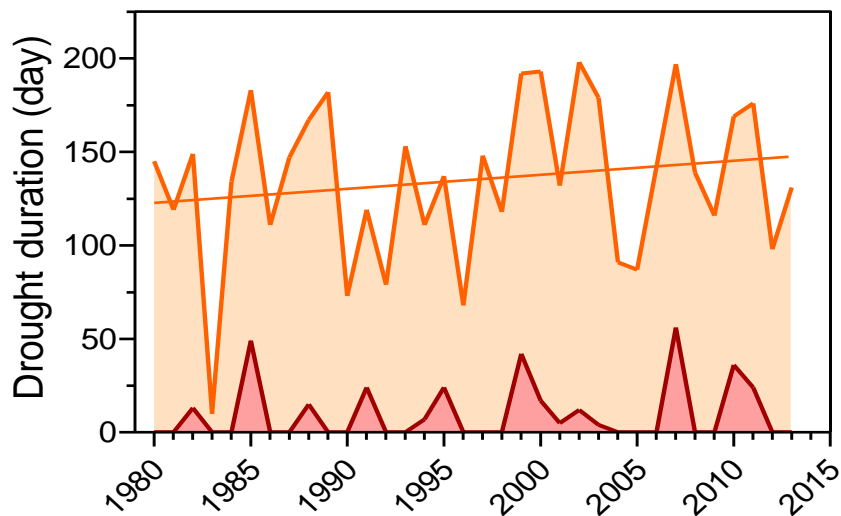
Regarding the unpredictability index, we observe that the months of November, December and January have the most unpredictable soil water content comparing the control (blue line) to 30% rain exclusion (red line), represented by the detachment of both curves in those months (FIGURE 4.5). It indicates that with a reduction of 30% in water available in the soil, the end of the drought period becomes less predictable, and the reduction may disturb the system for a longer time. Besides, we can also observe a certain elevation of the unpredictability index in the critical months of March and April that is a very important transitional period from the active to the reduced growth for coffee trees.

Figure 4.5 - Time course of the unpredictability index from daily values of the coefficient of variation for RSWC calculated over the years 1980-2014. Blue line control and red line presents 30% rain reduction.
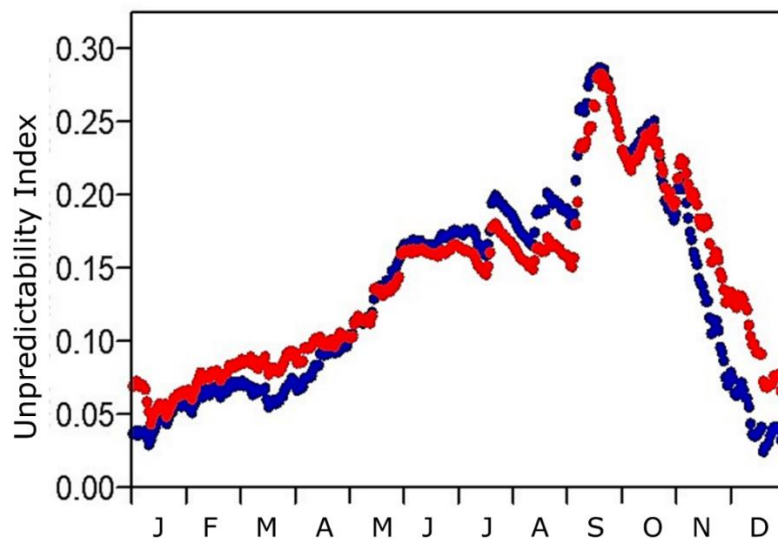
Furthermore, our theoretical simulations presented a higher return period with the rain exclusion experiment SWE (TABLE 4.1) compared to control, regarding individual years 10.2 years for SWE in 2015 compared to 4 years in the control, 13.9 years for SWE in 2016 compared to 4.1 for the control and an astounding figure of 140.3 years for the return period for both years in a row in SWE in comparison to 16.7 years for the control. Furthermore, the model established such occurrence with a very low probability frequency of 0.007, reinforcing the high unpredictability of such events in the future, and therefore indicating a low probability of increase in this kind of event in the future.

Table 4.1. Changes in frequency and return period (in years) resulting from the rain exclusion experiment (SWE) for 2015, 2016 and for both years in a row.

Years Analyzed	Control		SWE (25% reduction)	
	Frequency	Return period	Frequency	Return period
2015	0.248	4.0	0.099	10.2
2016	0.242	4.1	0.072	13.9
2015+2016	0.060	16.7	0.007	140.3

The time series for September SPEI-6 (FIGURE 4.6) displayed a significant correlation with mild drought duration (RSWC<0.7) over the years 1980-2014 and no significance with severe drought duration (RSWC<0.4). Furthermore, other series such as SPEI-3, SPEI-9 and SPEI-12 did not show any significant correlation with drought duration over the years 1980-2014.

Figure 4.6. Comparison of September SPEI-6 and mild drought duration over 1980-2013. The blue line represents September SPEI-6 and the red line indicates drought duration. Pearson r = -0.67 and P <0.0001.
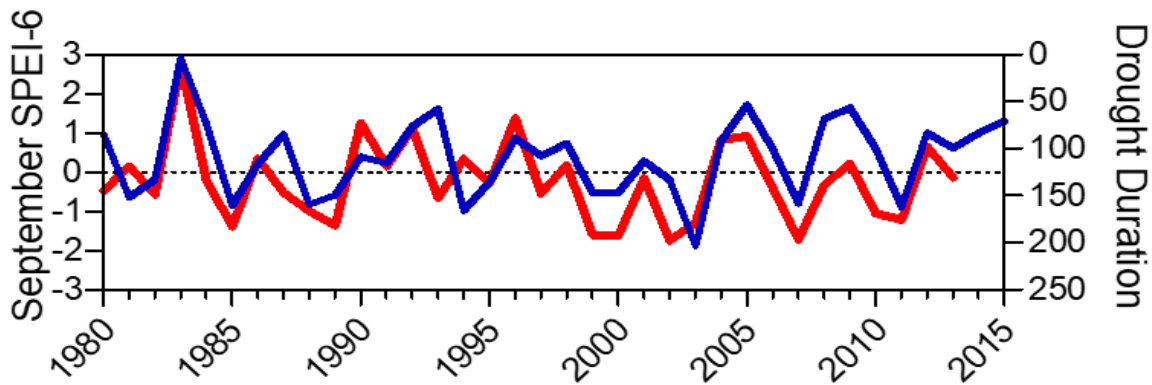
Regarding the empirical data from the field collections, the exclusion system removed on average ¼ of the rainfall in each SWE plot (FIGURE 4.7A), which made up a total of nearly 281 mm of rainfall being excluded from each of the three 20.4 m^2 plots throughout the experimental period from June 2015 up to May 2016. We verified a total of 1,171 mm of rainfall without the exclusion system and 890 mm in the plots with the exclusion system. In relation to the climatic normal average, where the average yearly rainfall from 1991 to 2004 has a value of 1,460 mm, it represented a departure of nearly 19.8% for the plots with no exclusion and 39% for the plots with the exclusion system. Furthermore, the rainfall profile for

the years 2013 and 2014 shows the reduction in precipitation of previous years to the installation of our exclusion system (FIGURE 4.7B).

We observed that the ET_c went up at the beginning of the raining period (November 2015) and kept high up to the end of the month of April 2016 when the rainfall had already practically ceased, causing an intense removal of water from the soil at that period (FIGURE 4.7A). Higher values of VPD in the period of March and April 2016 also contributed for the elevated rate of evapotranspiration in the field then. From November 2015 to August 2016, the values obtained for accumulated ET_c (782 mm) were very close to the precipitation in SWE treatment (809 mm), with the precipitation being just 3.45 % above the ET_c for the same period; whereas in the NNE and SWNE treatments (1,066 mm) the precipitation stayed on average 36.3% above the ET_c. If ET_0 had been considered in our calculation, the value accumulated from November 2015 to August 2016 would have been (884 mm), that would have been nearly 13% higher than ET_a, therefore it would theoretically have increased the stress level of the system.

Figure 4.7 - Crop evapotranspiration and monthly rainfall from the plots with exclusion system (SWE) and without the exclusion system (NNE and SWNE) during the experimental period (A); long-series of station values of precipitation and crop evapotranspiration from January 2013 to January 2017 (B).
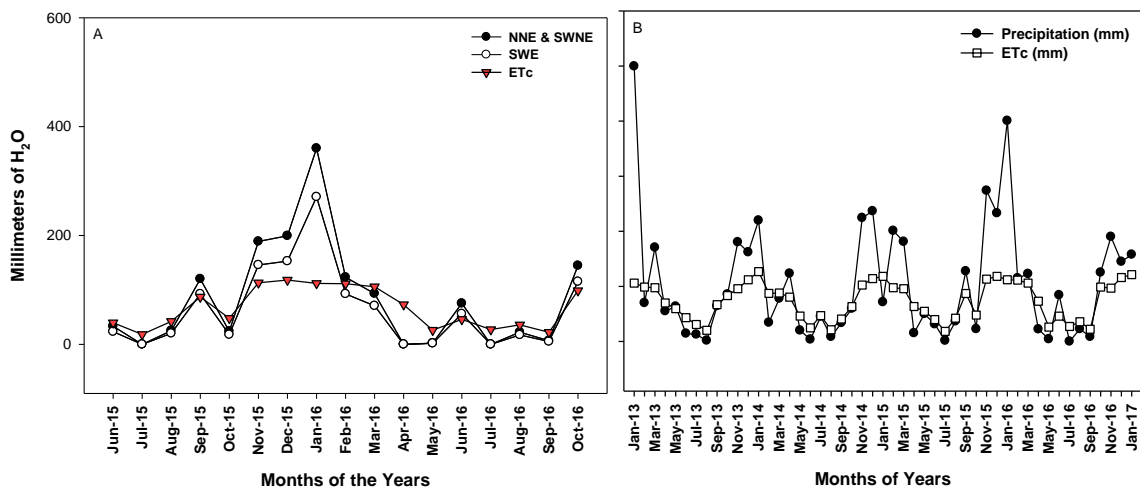
The soil water potential (Ψ_{soil}) at 30 cm of depth, in the months of reduced rainfall (August and October 2015; April and May 2016; and from July to October 2016) were lower than from November 2015 to March 2016 where the values of Ψ_{soil} were usually kept high (FIGURE 4.8). However, it did not display any differences between the treatments (NNE, SWNE and SWE) and its values were apparently high during the whole experimental period

in relation to what one could expect from a stressed environment. Therefore, the Ψ_{soil} seems to not have characterized an intense water stress at that soil depth for the coffee trees.

Figure 4.8 - Monthly values of soil water potential throughout the experimental period from August 2015 to October 2016. The bars represent the mean standard error at P<0.05.
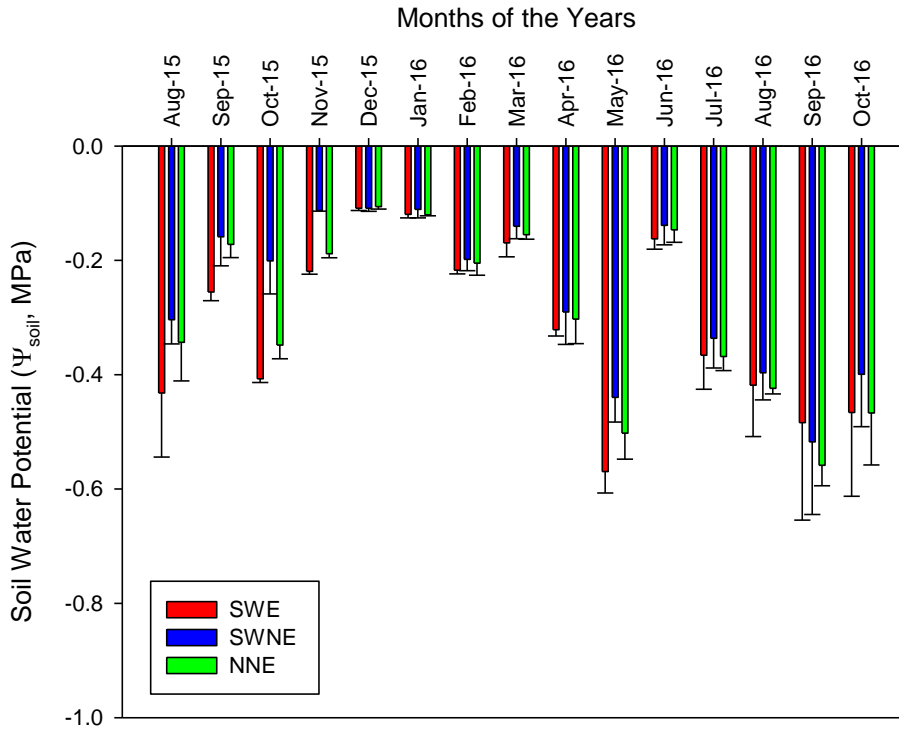

4.2 Temperature, relative humidity and vapor pressure deficit

Regarding the temperature, the measurements collected throughout the experimental period did not show any differences between the plots with and without the exclusion system. The maximum and mean temperatures of both plots with and without the exclusion system varied more intensely at the end of spring and beginning of the summer (months of October, November and December 2015) due to the common change of sunnier days (high radiation) into cloudier ones (lower radiation) and vice-verse during those months (FIGURE 4.9A). Whereas the minimum temperatures did not show any peaks and troughs during the whole experimental period. In general, the mean temperature increased in the month of September 2015 (spring) and started to decrease again in the month of May 2016 toward its lowest figure in July (FIGURE 4.9A). In general, we observed a very good adhesion of both plot and station temperatures with a few nonsignificant detachments from each other in the months of

November 2015 and February 2016 for the maximum and November 2015 for the mean temperatures.

Figure 4.9 - Monthly maximum, mean and minimum air temperature (A), relative humidity (B) and vapor pressure deficit (C) in the coffee field throughout the experimental period from June 2015 to October 2016 for Lavras-MG.
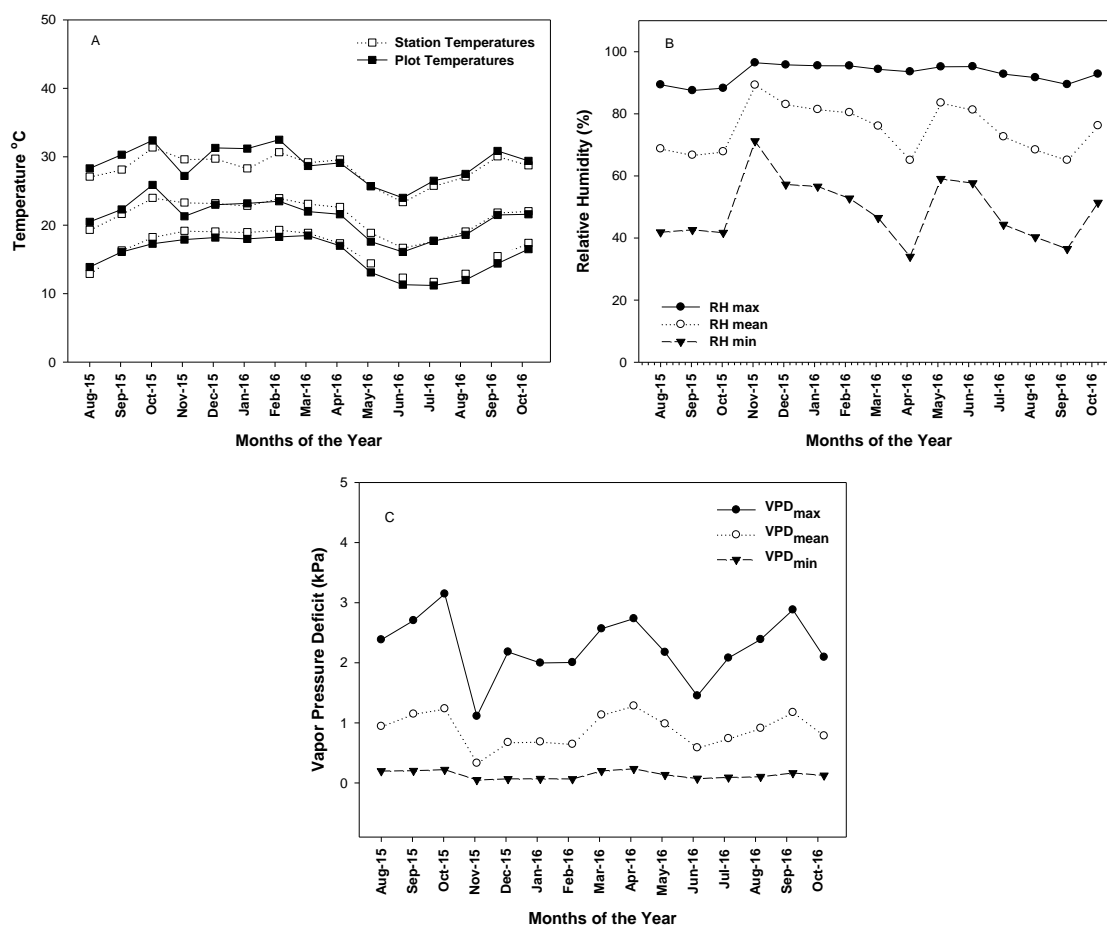
The maximum relative humidity of the air fluctuated less than the minimum RH which, by the way, was similar in behavior to the mean RH, presenting larger amplitude of values during the experimental period and showing lower values in the winter months of July, August and September with a pronounced drop in the month of April 2016 (FIGURE 4.9B). The VPD showed three peaks: October 2015, April 2016 and September 2016, and two troughs: November 2015 and June 2016 during the experimental period (FIGURE 4.9C). The peaks where mainly influence by low RH and high temperatures, especially in the month of October 2015 that displayed the highest VPD peak for the studied period. The month of October presented the highest peak because the temperature was visibly high for the peaks and contrariwise for the troughs (FIGURE 4.9C).

4.3 Leaf water potential (Ψ_{leaf}) and relative water content (RWC)

The predawn leaf water potential (Ψ_{leaf}) did not reveal any statistical differences between the treatments throughout the whole experimental period (FIGURE 4.10). There was only a significant reduction in the months of July, August and September of 2015 in relation to the other months, without any differences between the treatments though. Yet the predawn Ψ_{leaf} did not reveal any statistical differences between the treatments, we can observe from the data that, in the most critical months for water availability in the soil, that is July 2015, August 2015, September 2015 and September 2016 the Ψ_{leaf} for the treatment SWE had a tendency of being a little higher than the NNE treatment, which could indicate some form of adjustment in SWE leaves.

Figure 4.10 - Leaf water potential (Ψ_{leaf}) of six-year-old coffee trees, collected before dawn from June 2015 to October 2016. The bars represent the mean standard error at P<0.05.
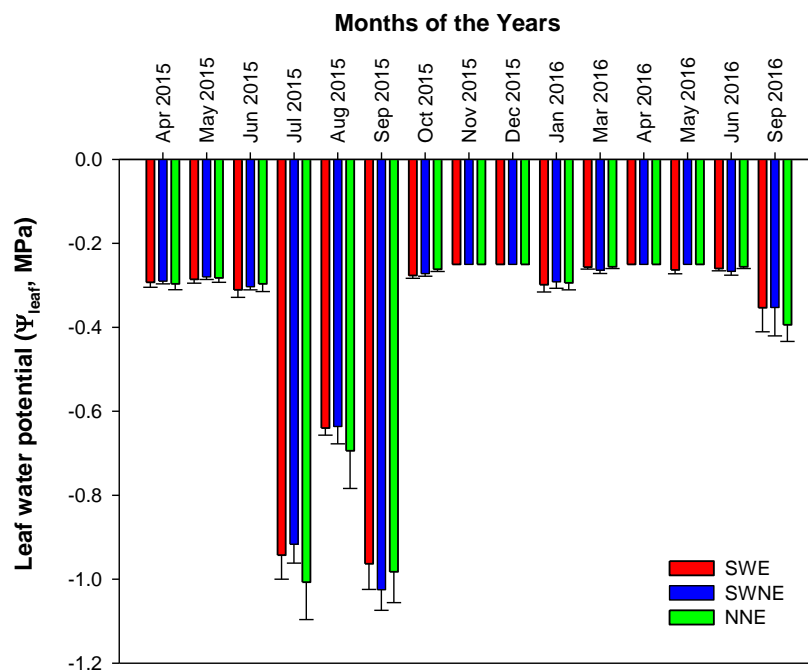
We observed some significant adjustments of the RWC in relation to the natural reduction of water availability that happened during the period of analysis from the active growth period towards the winter months for all coffee trees. In general, the values of RWC did not differ significantly between the treatments (FIGURE 4.11). Nonetheless, as a general trend, there was a linear decrease in the RWC specially in SWE and NNE, which showed

values of R=0.766 and 0.735 respectively, and the SWNE treatment with R=0.456 also showed the same tendency of reduction although it was less conspicuous, which can be identified by a lower value of b=0.0323 in its equation when compared to the other treatments b=0.0608 for SWE and 0.0623 for NNE (FIGURE 4.12). The data for SWNE also presented two deviant peaks in the months of January and July 2016 and a trough at the beginning of April 2016 in relation to the other two treatments (FIGURE 4.11), which are difficult to explain with our data. However, the trend of overall RWC reduction was evident throughout the experimental period, with the first samples in December 2015 displaying on average higher RWC values around 81% and declining to an average of 72% in the last measurements in July 2016 (t-test P=<0.001). However, reaching values below 65% in SWE at the end of May 2016, before the timely rains of June 2016.

Figure 4.11 - Fortnight values of relative water content (RWC) in leaves of six-year-old coffee trees throughout the period from December 2015 to July 2016. The bars represent the mean standard error at P<0.05.
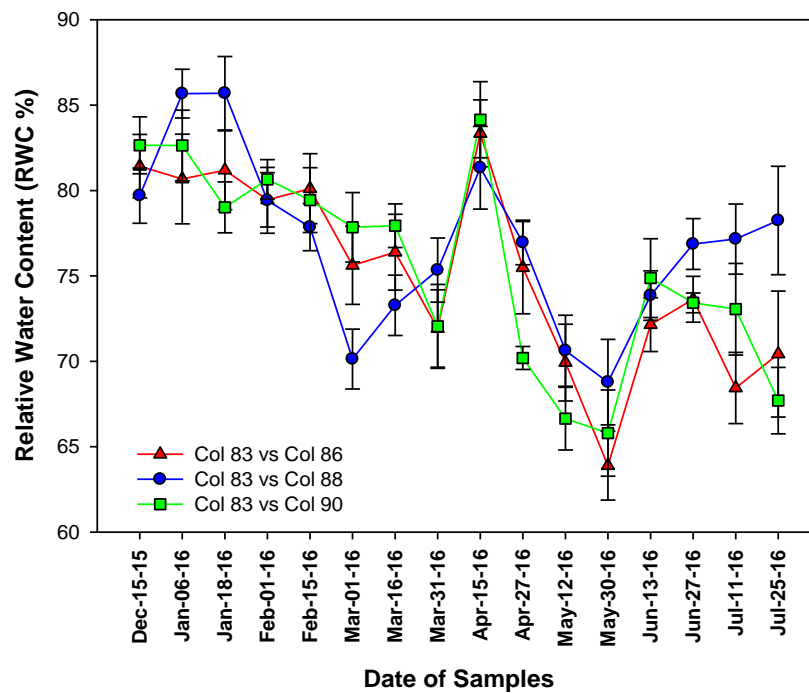
Figure 4.12 - Regression curves for RWC of coffee trees in three experimental conditions: (A) - with exclusion system, (B) - with cover but without exclusion and (C) – with no exclusion from December 2015 to July 2016 Fortnightly values of relative water content (RWC) throughout the period from December 2015 to July 2016.
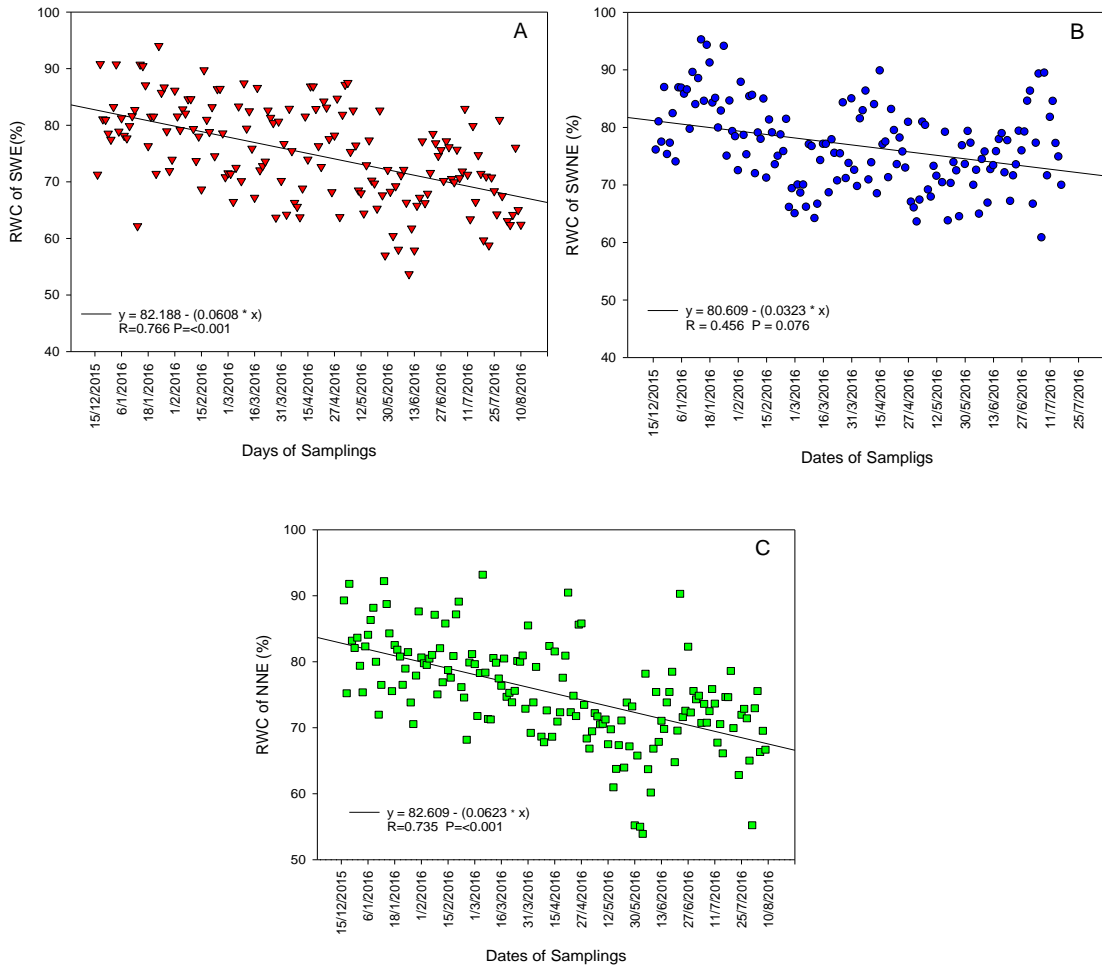
As an overall trend, the SLA presented an increase in its values from December 2015 to March 2016, and afterwards there was a sensitive decline of the SLA in all three treatments (FIGURE 4.13). Considering the means for the entire experimental period (FIGURE 4.14), the SLA of the coffee plants was higher for the SWE treatment than NNE, with SLA for SWNE amply varying between the other two treatments. We could also observe that the period of February and March 2016 were the months were SLA present the highest values for SWE in relation to the other two treatments (FIGURE 4.13).

Figure 4.13 - Temporal series of specific leaf area (SLA) of six-year-old coffee trees from December 2015 through July 2016. The bars represent the mean standard error at P<0.05. The asterisk indicates significant difference by Tukey at P<0.05.
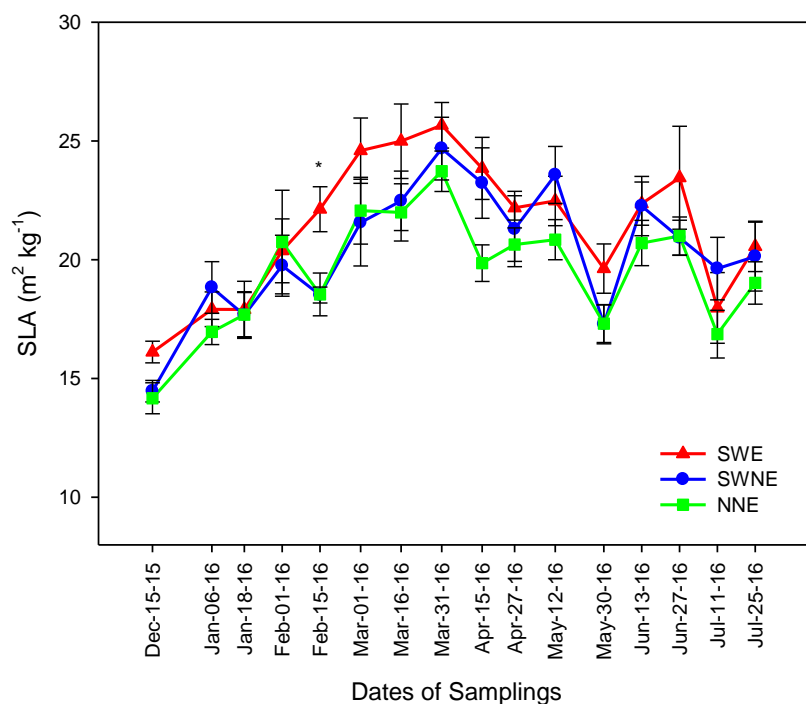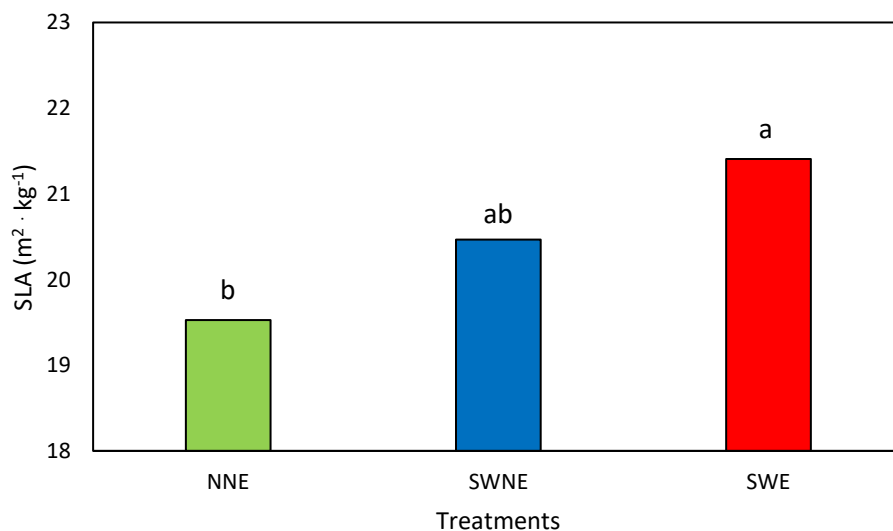

Figure 4.14 - Mean specific leaf area (SLA) from the middle third of six-year-old coffee trees for the experimental period from December 2015 to July 2016.

4.4 Leaf area adjustment, shoot growth and yield of coffee trees

The BLAm for the treatments did not show any statistical differences between them during the months of the raining season from October 2015 to February 2016, after which there was a continuous difference between the treatments with a more pronounced reduction of the BLAm in the SWE treatment after March 2016 persisting up to the last data sampling in October 2016 with a stronger tendency of reduction for that treatment (FIGURE 4.15A). As it could be observed from FIGURES 17B and 17C, both BLAs and BLAi had the same behavior with a more pronounced reduction of leaf area in SWE after the month of March 2016. During the setting of the samplings, BLAi was established with a certain nonuniformity of samples. Even though BLAi was statistically alike within the treatments at the beginning of the analysis, such nonuniformity rendered somehow higher figures for BLAi in the SWNE treatment throughout the experiment period. Nonetheless, it did not compromise the analysis.

Figure 4.15 - Monthly series of mean branch leaf area (BLAm) (A), that is the mean values of superior branch leaf area (BLAs) (B) and inferior branch leaf area (BLAi) (C) of six-year-old coffee trees from November 2015 through October 2016. The bars indicate the mean standard error. The asterisk indicates significant differences by Tukey at P<0.05.
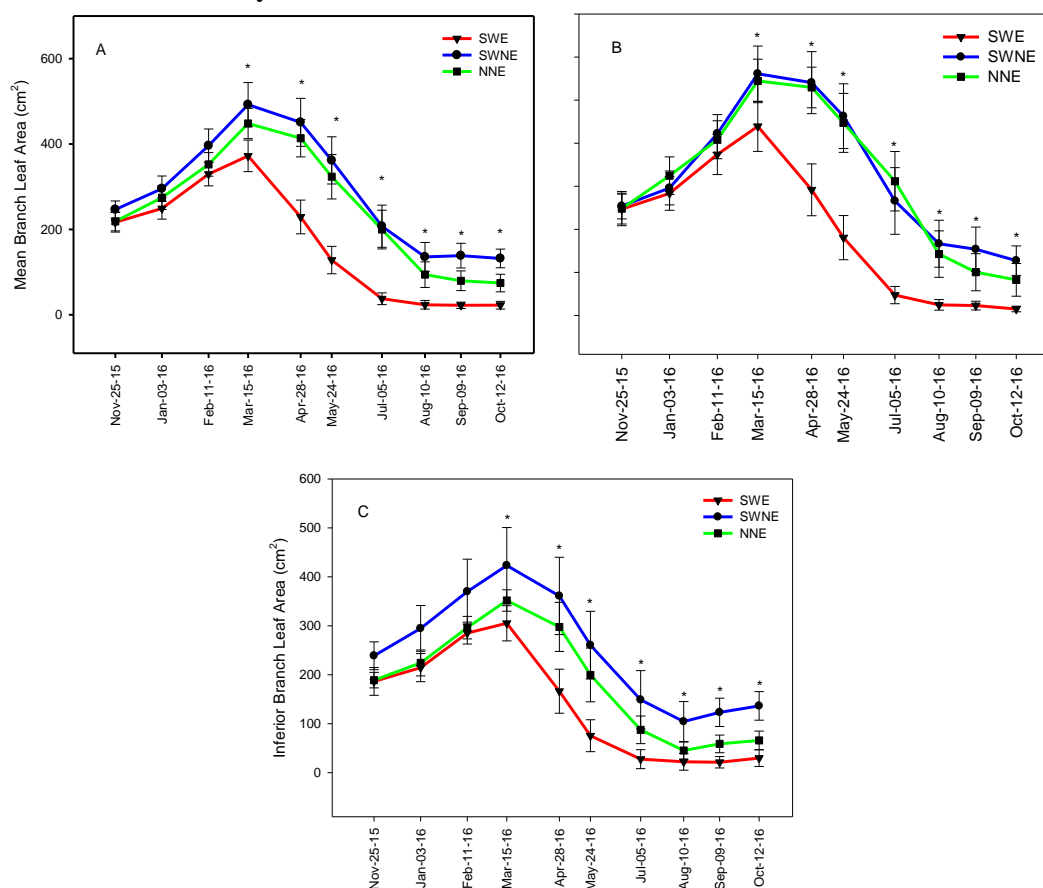
The growth rates of BLAm showed, in the month of March 2016, a more intensive reduction in the treatment SWE than in the other two treatments (FIGURE 4.16), demonstrating a sensitive influence of the rain exclusion on the growth rates of BLA. That reduction trend in the rates continued in April and May 2016. Nonetheless, after May 2016 the SWE trees recovered their growth rates faster than the SWNE and NNE ones, leveling off the growth rates with the other two treatments by September 2016 (FIGURE 4.16).

Figure 4.16 - Growth rates of mean branch leaf area (BLAm) of six-year-old coffee trees taken from the superior and inferior middle thirds of the trees from January 2015 through October 2016. The bars indicate the standard error of the means. The asterisks indicate a significant difference between SWE and the other two treatments SWNE and NNE by Tukey's test at P<0,05.
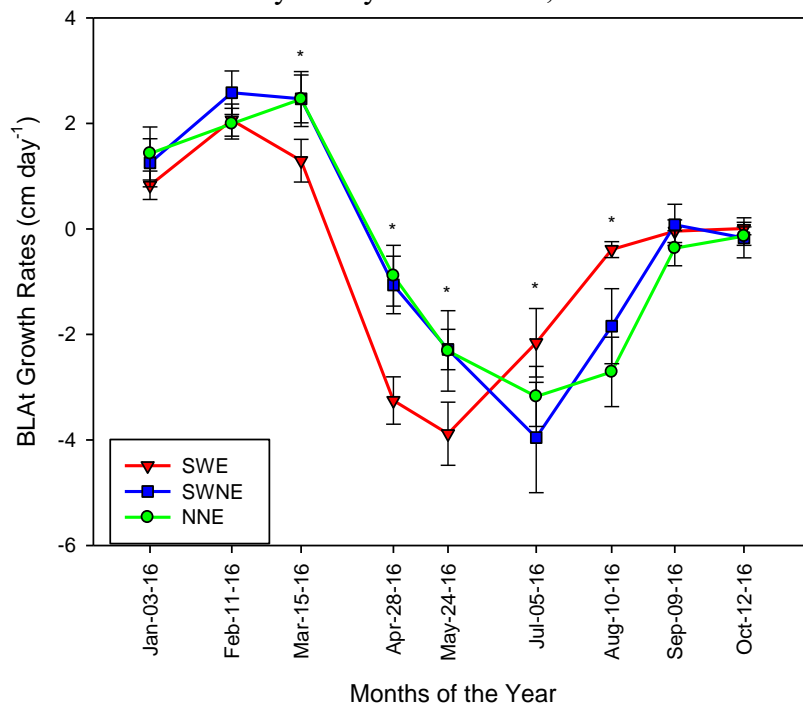
On the other hand, LAI in our study presented higher values for the NNE treatment in relation to the other two treatments SWNE and SWE, which were statistically similar from April 2016 to August 2016 (FIGURE 4.17). Whereas, in the previous months, there were no significant differences between the treatments. However, this result does not seem to correlate with other important measurements obtained by our analyses. In fact, we stablished some

correlations of LAI with BLA, precipitation, temperature and NDVI (TABLE 4.2) to possibly validate the use of the method proposed by Barbosa et al. (2012) to estimate coffee tree LAI in this study, however a low correlation and inconsistency of values of LAI in comparison to NDVI and BLA was a setback.

Figure 4.17 - Monthly measurements of leaf area index (LAI) taken from the six-year-old coffee trees using Barbosa et al.'s method from March 2015 through September 2016. The bars represent the mean standard errors at P<0.05.
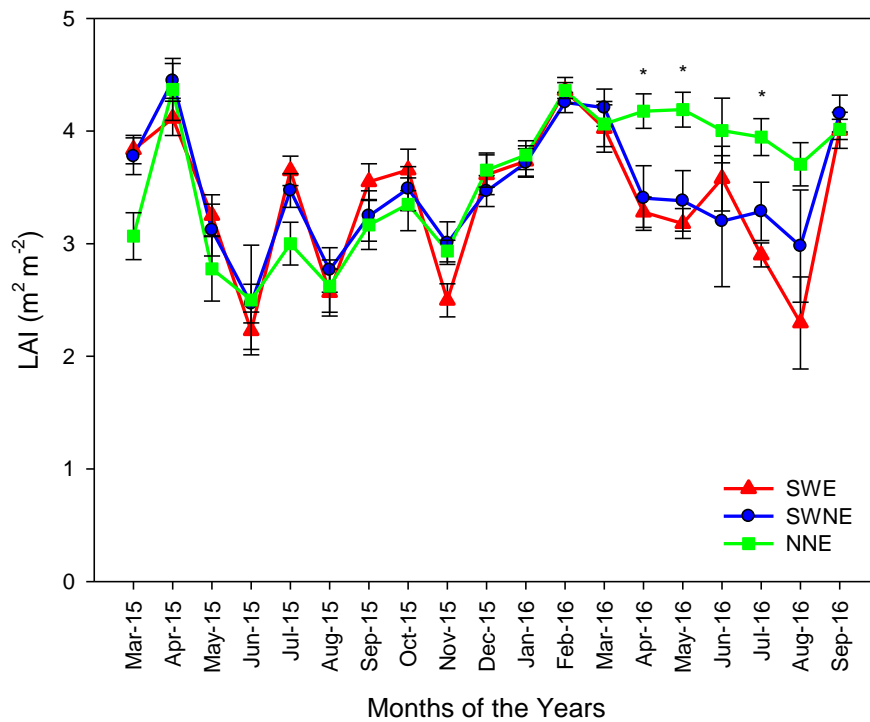
Even though NDVI is a very precise method to make inferences on the state of the vegetation, only perceived significant differences between the rain exclusion treatment (SWE) and the controls (SWNE and NNE) in the month of June 2016 (FIGURE 4.18) compared to BLA, which, already in March, could realize significant differences between the treatments. Nonetheless, the same pattern exhibited by the curves between NDVI and BLA were accused by high level of correlation between them (TABLE 4.2) and one can also visually observe their similar patterns from their graphs (FIGURES 4.15 and 4.18).

Figure 4.18 - Fortnight mean values of normalized difference vegetation index (NDVI) taken from top of six-year-old coffee trees from November 2015 through October 2016 using the GreenSeaker® portable sensor. The bars indicate the standard error of the means. The asterisk indicates a significant difference between SWE and the other two treatments SWNE and NNE by Dunn's test at P<0,05.
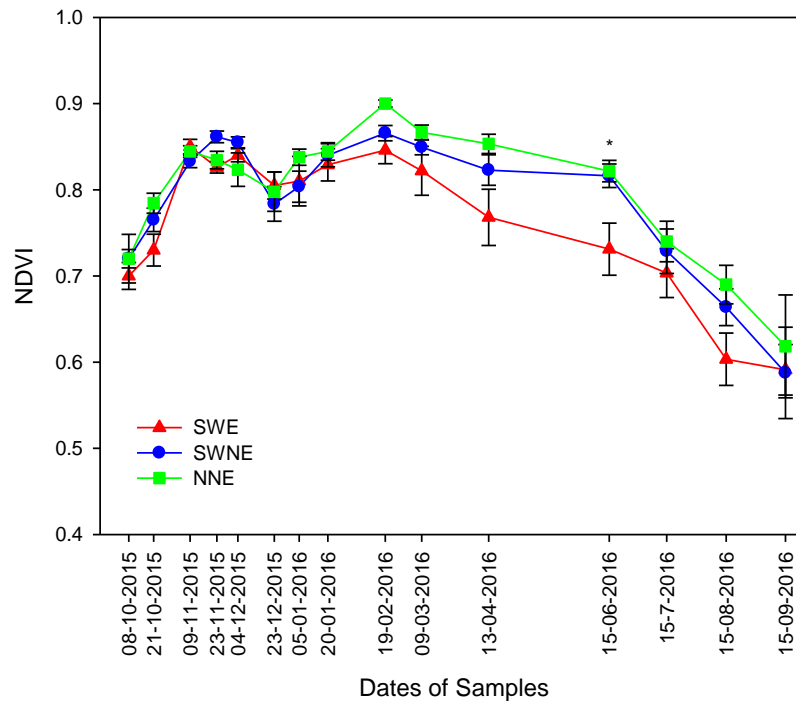
In terms of the correlation stablished: LAI displayed some reasonable correlations with NDVI of the coffee trees in SWE (0.679*) and SWNE (0.614*) treatments, but it did not show any significant correlation with NNE (0.358ns) (TABLE 4.2). On the other hand, the BLAm and BLAs displayed very high correlations with the NDVI in all three treatments (SWE, SWNE and NNE). Besides, LAI also failed in rendering good correlations with the precipitation, temperature and ET_c in most of the cases (TABLE 4.2).

Table 4.2 - Correlations between leaf area index (LAI), mean branch leaf area (BLAm), superior branch leaf area (BLAs), normalized difference vegetation index (NDVI), mean temperature (T_m), rainfall and coffee crop evapotranspiration (ETc) from November 2015 to September 2016 under three rain exclusion treatments. ** $P < 0.001$, * $P < 0.05$ and ns = nonsignificant.

CORRELATIONS	TREATMENTS		
	SWE	SWNE	NNE
LAI x BLAm	0.636*	0.876**	0.293ns
LAI x NDVI	0.679*	0.614*	0.358ns
LAI x T_m	0.488ns	0.606*	-0.086ns
LAI x ET_c	0.725**	0.773**	0.334ns
LAI x rainfall	0.307ns	0.242ns	-0.422ns
BLAm x NDVI	0.851**	0.773**	0.848**
BLAs x NDVI	0.878**	0.793**	0.881**
BLAm x T_m	0.929**	0.864**	0.859**
BLAs x T_m	0.939**	0.775**	0.772**
BLAm x ET_c	0.779**	0.892**	0.908**
BLAs x ET_c	0.812**	0.869**	0.898**
BLAm x rainfall	0.749*	0.427ns	0.430ns
BLAs x rainfall	0.704*	0.287ns	0.304ns
NDVI x T_m	0.791**	0.626*	0.622*
NDVI x ET_c	0.819**	0.766**	0.804**
NDVI x rainfall	0.590*	0.342ns	0.399ns

The number of branch nodes did not show any differences along the growth season from September 2015 to March 2016. However, in April 2016 during the second week of the fall, the SWE treatment displayed a reduction in relation to the other two treatments (FIGURE 4.19). On the other hand, in terms of absolute growth, the total branch length did not show any differences between treatments throughout the entire experimental period (FIGURE 4.20). Nevertheless, the branch length growth rate was also significantly smaller in the SWE treatment compared to the NNE treatment (FIGURE 4.22). Regarding the branch diameter, it did not present any differences between the treatments from September 2015 to March 2016, then in April 2016 it presented a significant reduction in the SWE treatment in relation to the NNE (FIGURE 4.21) with the SWNE treatment between them. In the case of the branch

diameter growth rate, the SWE treatment as well as the SWNE were statistically smaller than the NNE treatment (FIGURE 4.23).

Figure 4.19 - Branch node number from the middle third of six-year-old coffee trees from September 2015 to April 2016. Same letters in each month of year represent a nonsignificant difference at P<0.05.
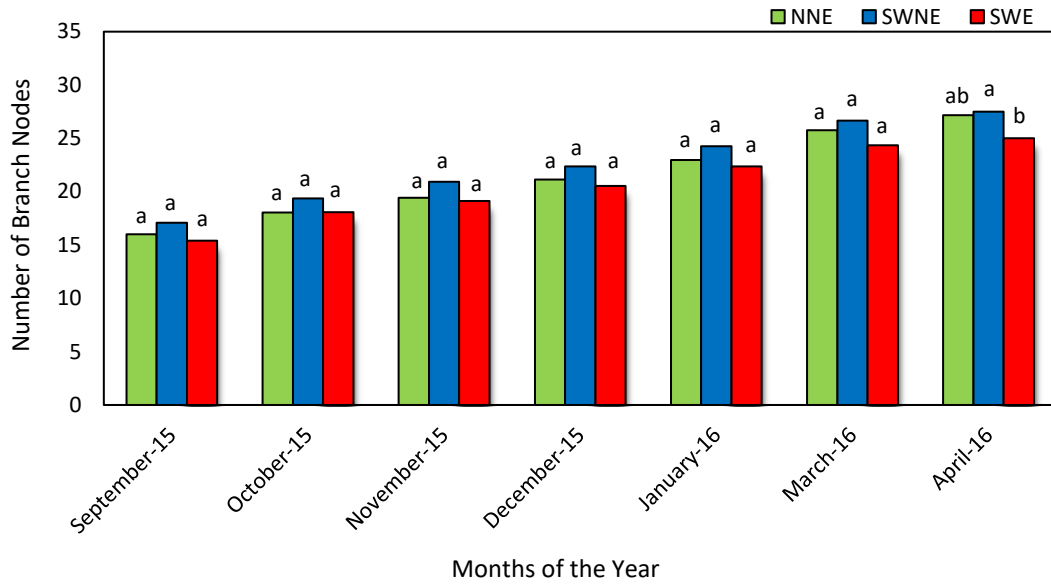
Figure 4.20 - Total branch length from the middle third of six-year-old coffee trees from September 2015 to April 2016. Same letters in each month of year represent a nonsignificant difference at P<0.05.
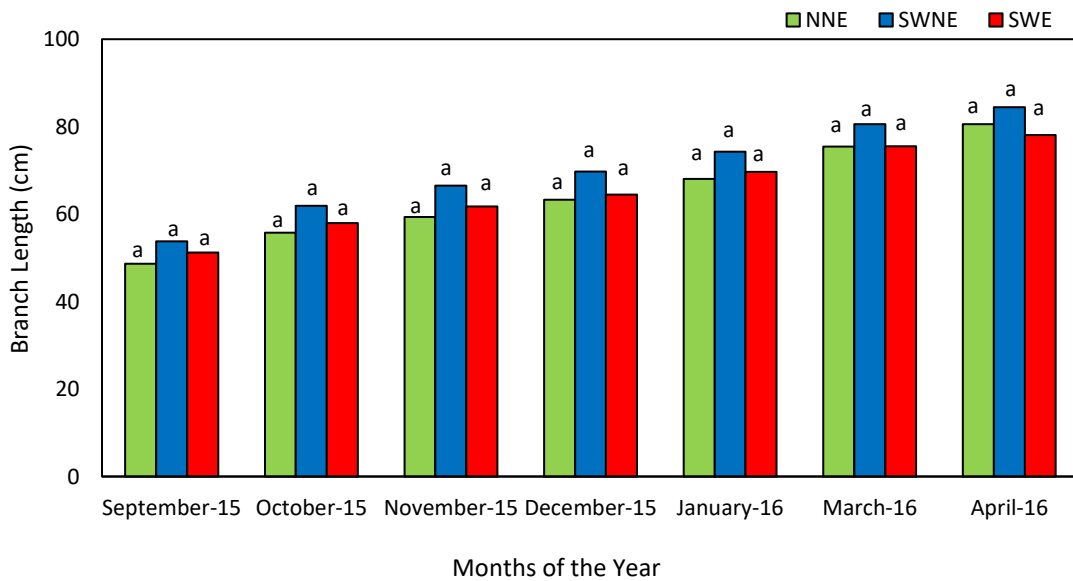
Figure 4.21 - Branch diameter from the middle third of six-year-old coffee trees from September 2015 to April 2016. Same letters in each month of year represent a nonsignificant difference at P<0.05.
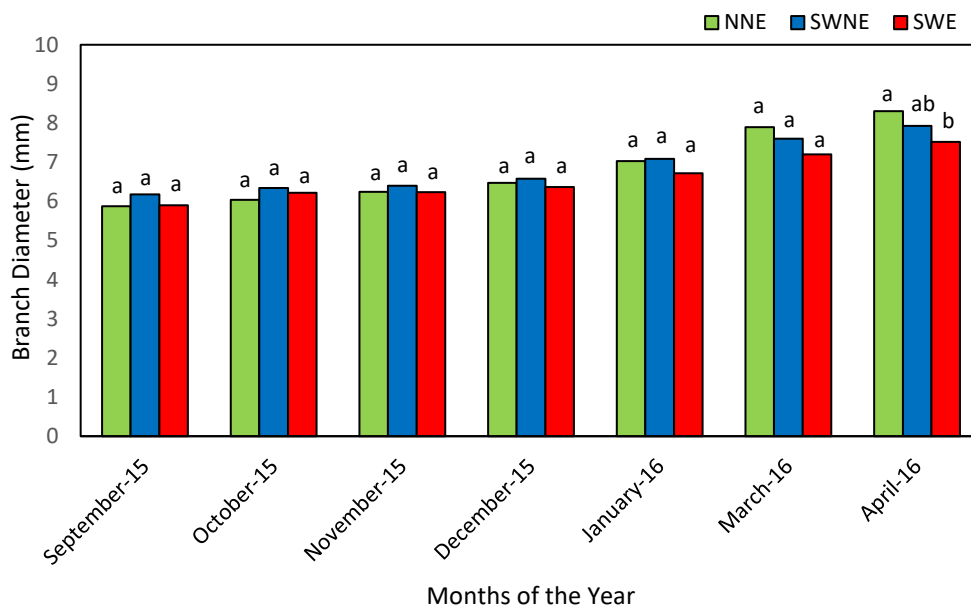
Figure 4.22 - Growth rate of branch length from the middle third of six-year-old coffee trees from September 2015 to April 2016. Different letters represent a significant difference at P<0.05.
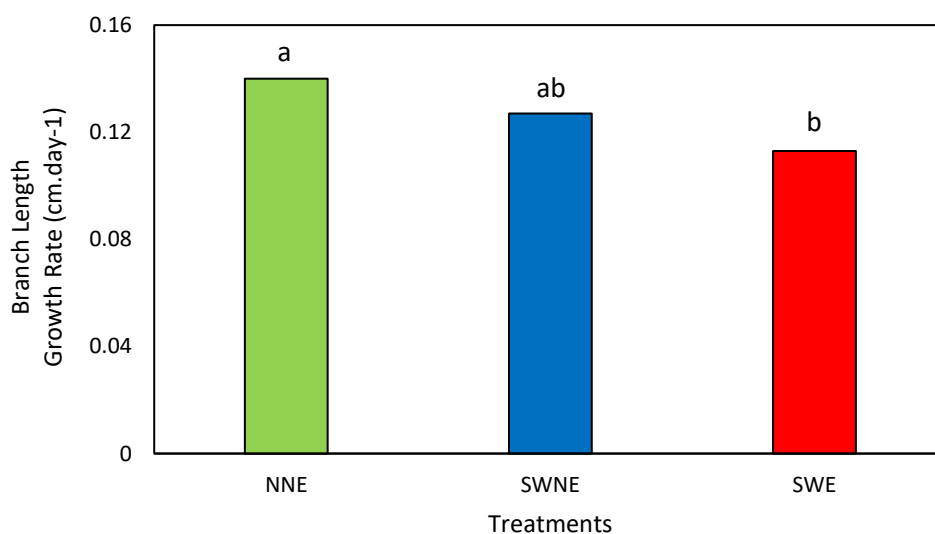
Figure 4.23 -   Growth rate of branch diameter from the middle third of six-year-old coffee trees from September 2015 to April 2016. Different letters represent a significant difference at $P<0.05$.
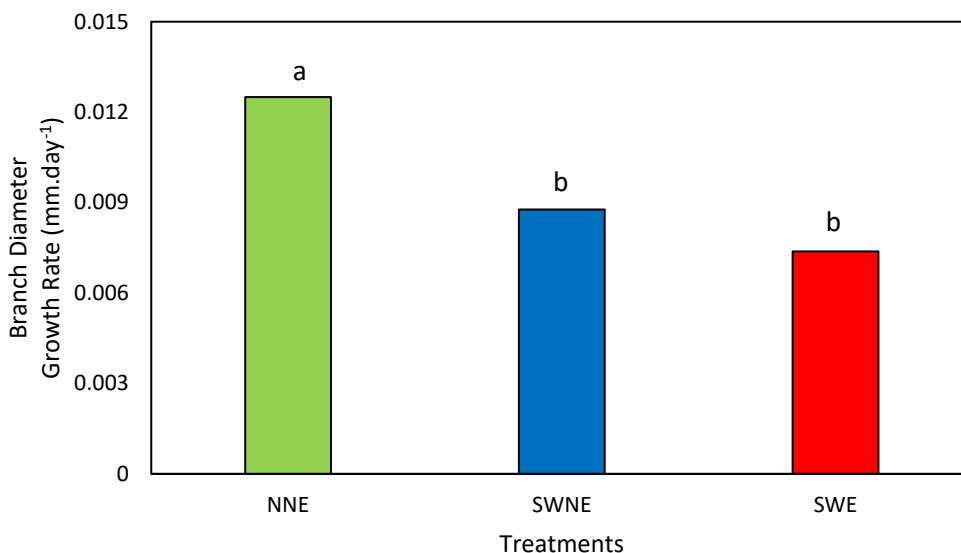
The number of leaves on the twigs in April 2016 did not present any statistical difference, although it showed on average a reduction of nearly two leaves for the SWE branches in relation to the other two treatments the SWNE and the NNE (FIGURE 4.24). There was a reduction in the number of tertiary branches on the plagiotropic branches in the SWE treatment compared to the other two treatments (SWNE and NNE), rendering on average a three-fold decrease in tertiary branches in the SWE treatment in comparison to NNE (FIGURE 4.25).

Figure 4.24 - Number of leaves in the twig of branches from the middle third of six-year-old coffee trees in April 2016. Same letters represent a nonsignificant difference at P<0.05.
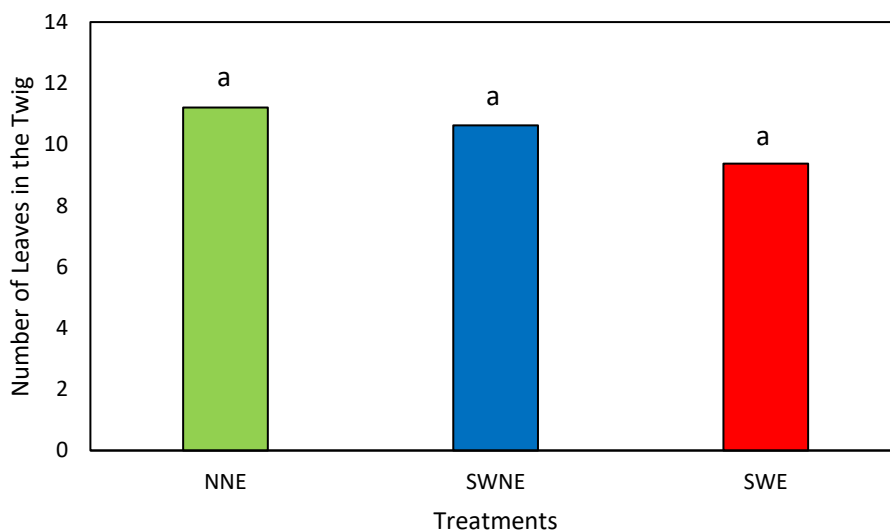
Figure 4.25 - Number of tertiary branches on plagiotropic branches from the middle third of six-year-old coffee trees in April 2016. Different letters represent a significant difference at P<0.05.
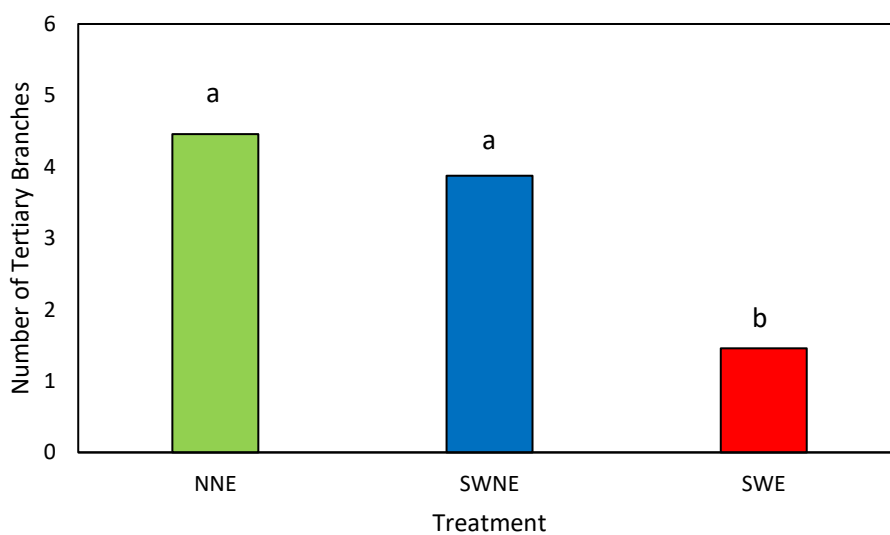
Regarding the diameter of the main orthotropic stems there was no significant differences in absolute values of that variable (FIGURE 4.26). Neither in March 2016 nor in July 2016 there were significant differences between the treatments. However, the treatment with exclusion (SWE) presented lower growth rates than the control (NNE), with the SWNE again fluctuating between both (FIGURE 4.27).

Figure 4.26 - Diameter of main orthotropic stem of six-year-old coffee trees in March 2016 and July 2016. Same letters in each month of year represent a nonsignificant difference between the treatments by Tukey's test at P<0.05.
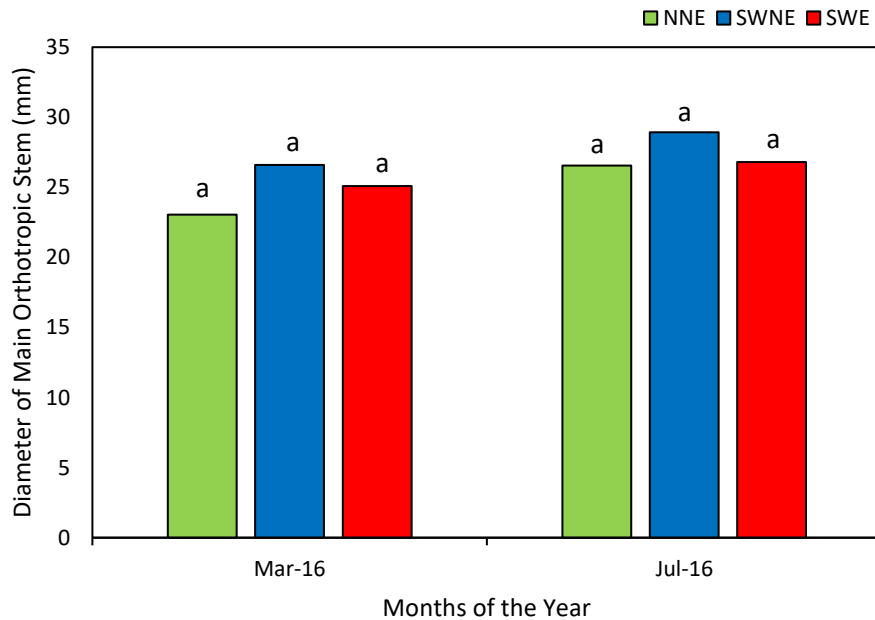
Figure 4.27 - Growth rate of main orthotropic stem of six-year-old coffee trees from March 2016 to July 2016 Same letters in each month of year represent a nonsignificant difference between the treatments by Tukey's test at P<0.05.
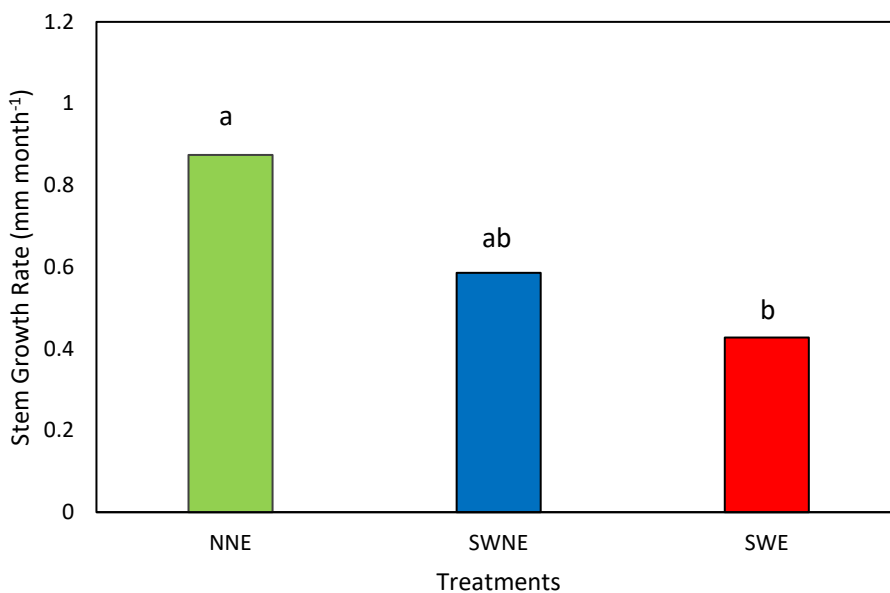

Yield of the coffee trees in 2016 did not show any statistical differences between the treatments (FIGURE 4.28). Nevertheless, the small number of replicates n=3 may have contributed for such insensitivity of the ANOVA to realize differences between the treatments, since the yield in SWE was twice as large than the coffee trees under the NNE treatment. However, in 2017 the coffee trees displayed differences between the treatments and the SWE treatment showed a lower yield than the trees in NNE (FIGURE 4.29).

Figure 4.28 - Yielding of six-year-old coffee trees in kilograms per tree (kg tree^{-1}) in the year 2016. Same letters represent a nonsignificant difference between the treatments by Tukey's test at P<0.05.
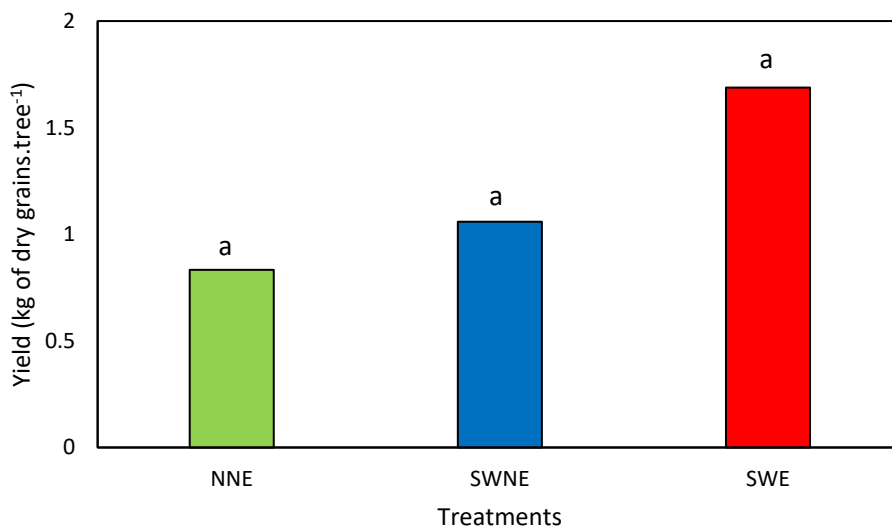
Figure 4.29 - Yielding of seven-year-old coffee trees in kilograms per tree (kg tree^{-1}) in the year 2017. Same letters represent a nonsignificant difference between the treatments by Tukey's test at P<0.05.
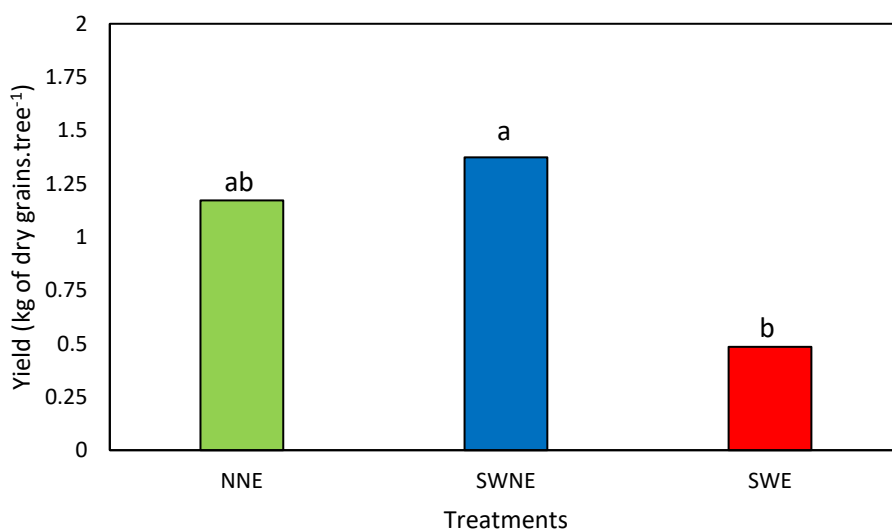
The branches which were subjected to the fruit removal displayed on average more number of nodes by April 2016 than the ones which did not have their fruit removed. (FIGURE 4.30). On the other hand, the total branch length did not show any differences between the branches either with fruit removal or with no fruit removal (FIGURE 4.31). However, the branch length growth rate was larger for the branches with no fruit (FIGURE 4.33). The branch diameter did not render any significant statistical differences between the treatments with or with no fruit (FIGURE 4.32) and the diameter growth rate did not suffer any influences either (FIGURE 4.34). And finally, the number of tertiary branches was statistically larger when the branches were subjected to the fruit removal (FIGURE 4.35), which was a response that has a correspondence with the biennially of the coffee tree.

The statistical analyses did not find any interaction between the treatments of the rain exclusion system and fruit removal. In other words, the variables analyzed demonstrated the same behavior in each of the water regime treatments, whether subjected to the fruit removal or not.

Figure 4.30 - Node number in branches from the middle third of six-year-old coffee trees from September 2015 to April 2016. Same letters in each month of year represent a nonsignificant difference between the treatments by Tukey's test at P<0.05.
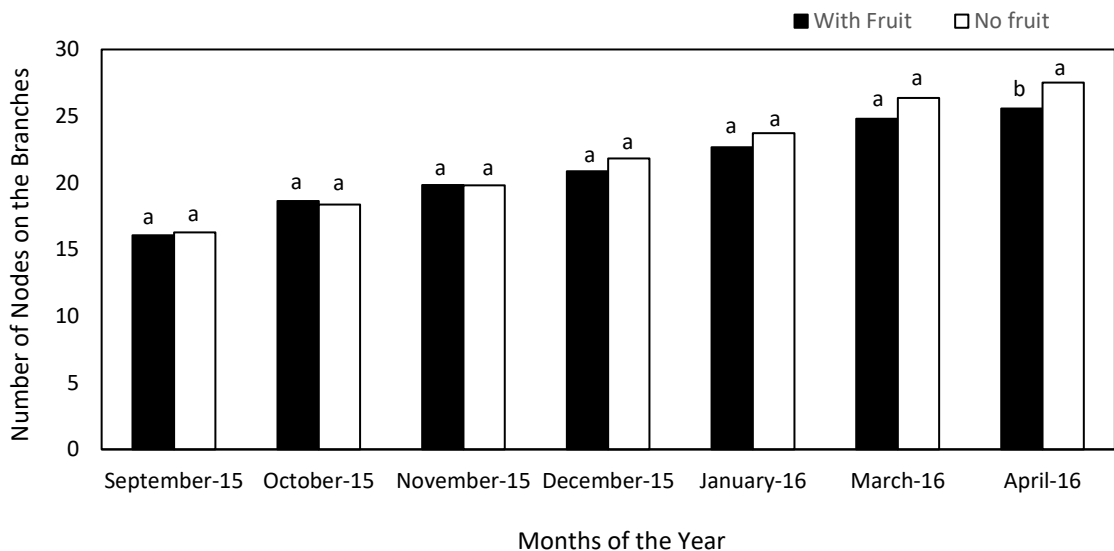
Figure 4.31 - Total branch length from the middle third of coffee trees from September 2015 to April 2016. Same letters in each month of year represent a nonsignificant difference between the treatments by Tukey's test at P<0.05.
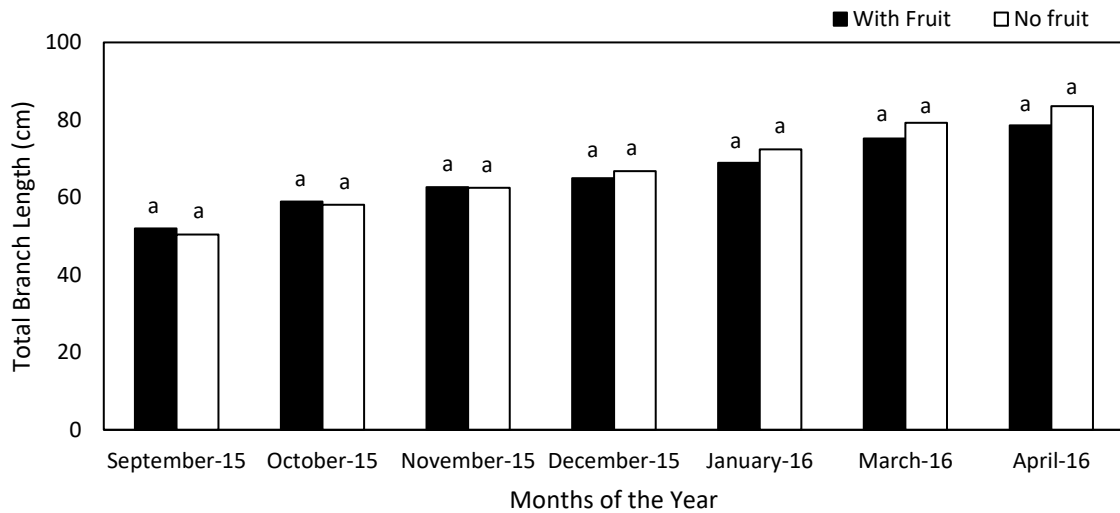
Figure 4.32 - Branch diameter from the middle third of coffee trees from September 2015 to April 2016. Same letters in each month of year represent a nonsignificant difference between the treatments by Tukey's test at P<0.05.
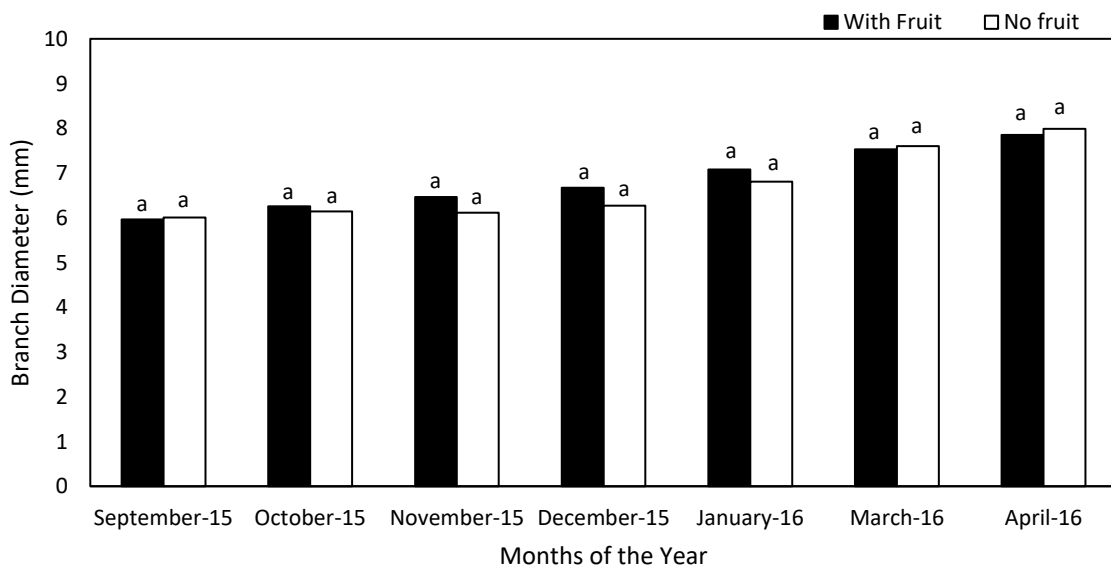
Figure 4.33 - Branch length growth rate from branches of the middle third of coffee trees from September 2015 to April 2016. Same letters represent a nonsignificant difference between the treatments by Tukey's test at P<0.05.
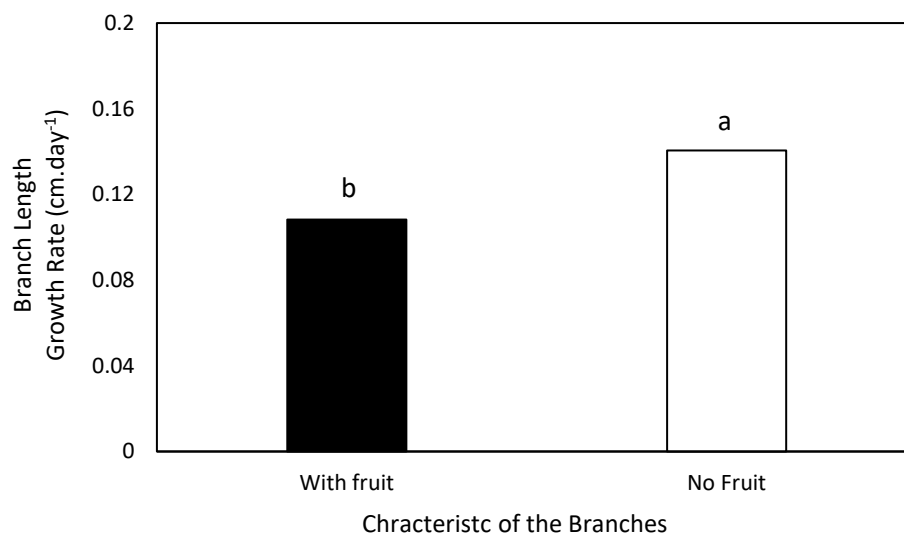
Figure 4.34 – Branch diameter growth rate of the middle third of coffee trees from September 2015 to April 2016. Same letters represent a nonsignificant difference between the treatments by Tukey's test at P<0.05.
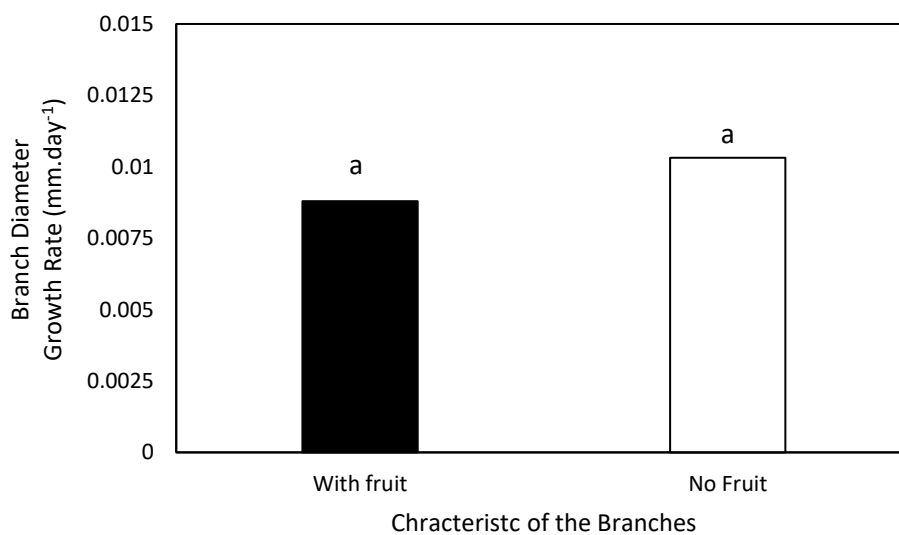
Figure 4.35 - Number of tertiary branches from the middle third of coffee trees in April 2016. Same letters represent a nonsignificant difference between the treatments by Tukey's test at P<0.05.
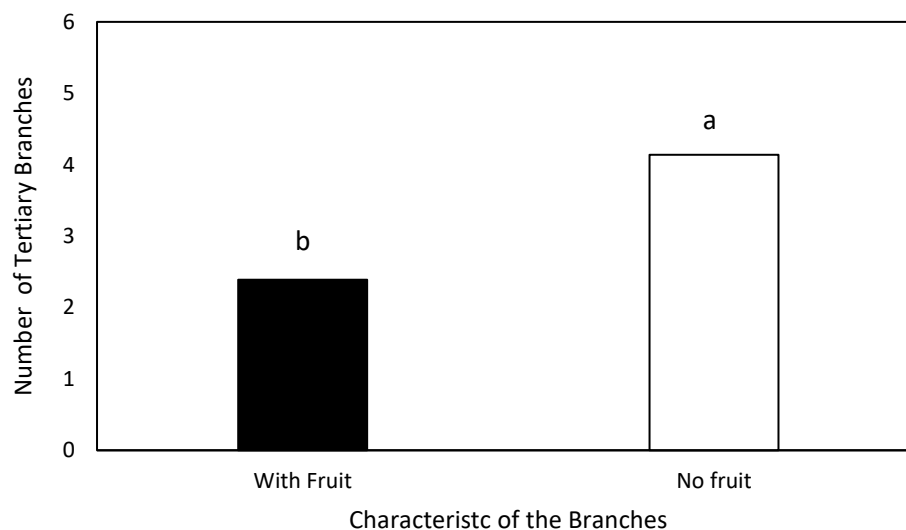

## 4.5 Leaf macronutrient and chlorophyll contents

Regarding the leaf macronutrient analysis, there was not any difference in N content per leaf mass in the treatments, there was only a decline in the last two samplings (March and June 2016) in relation to the first two (October and December 2015). P, K, Mg and S did not show any differences between the treatments either, and Ca was larger in SWE when compared to the NNE and SWNE (TABLE 4.3).

The overall content of chlorophyll did not show any variation between the treatments. However, in some moments during the experiment it was larger in the NNE leaves than in the SWE and SWNE ones, especially in the months of February and March 2016 (FIGURE 4.36).

Table 4.3 - Macronutrient contents in leaves of coffee trees subjected to a ¼ rain reduction through the experimental period from October 2015 to June 2016. Values with the same letters in the columns are not statistically different at P < 0.05 (n=36).

Treatments and Prescriptions *	N	P	K	Ca	Mg	S
	g kg^{-1}					
NNE	27.6 a	1.31 a	19.2 a	7.84 b	3.00 a	2.53 a
SWNE	28.4 a	1.22 a	19.2 a	8.23 b	3.17 a	2.44 a
SWE	26.9 a	1.30 a	19.3 a	9.12 a	3.45 a	2.51 a
*Mills & Jones (1996)	23-30	1.2-2.0	20-25	10-25	2.5-4.0	1.0-2.0
*Malavolta (1997)	27-32	1,5-2,0	19-24	10-14	3,1-3,6	1,5-2,0

Figure 4.36 – Content of chlorophyll of completely expanded leaves from the middle third of coffee trees from October 2015 to July 2016. The asterisks indicate significant differences between NNE and the other two SWE and SWNE by Tukey's test at P<0.05.
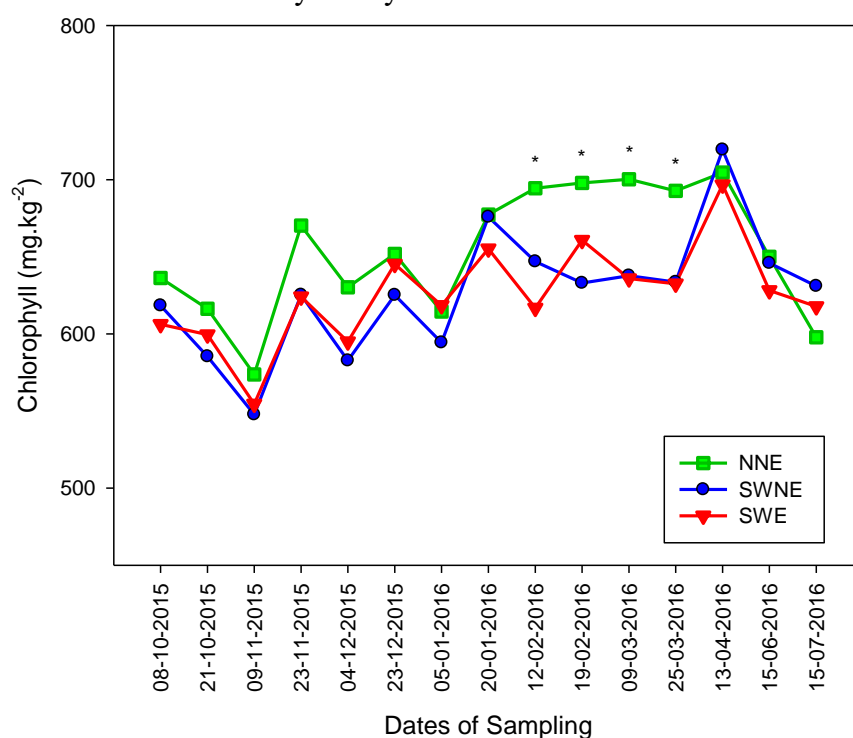
5 DISCUSSION

5.1 Exclusion system and the climatic factors influencing the coffee tree responses

The model we developed to investigate the RSWC for the years 1980-2014 showed the lowest RSWC for the month of September with a RSWC value around 0.40 (FIGURE 4.2), which is alike the value displayed by INMET model (INMET, 2017). Moreover, the models developed by INMET display an increase of RWC from 0.30 (monthly series from 1931 to 1960) to 0.40 (monthly series from 1961 to 1990) found by our calculations and clearly indicating a tendency of elevation in the RSWC during those decades, which somehow corroborates with our calculations that have not found any tendency of increase of drought frequency for future years.

The normal reduction in RSWC in winter months associated with other climatic conditions such as a temperature and RH during specific moments in the south of Minas Gerais favors the crop and permits its superior drinking quality. In fact, coffee trees need a period of a certain water stress to ready the buds for anthesis. However, if this reduction is moderately intensified it would probably affect coffee yielding, since we could expect an increase of certain osmotically active solutes in the plant to deal with the lower water availability for a longer time (VINECKY et al., 2017). In fact, we could even expect a trade-off between yield and quality since it is practically impossible for the coffee trees to keep with high energetic demands of both high yielding (sugars directed to produce biomass) and high quality (sugars directed to a higher concentration of osmotically active solutes). Therefore, the increased days with mild water stress could intensify the production of osmotically active solutes that may have a positive effect in drinking quality.

Our models in association with SPEI drought index have also demonstrated a reduced predictability to distinctly observe the end of a drought duration when the dry spell hits the system during the critical months of November, December or January. That rise in unpredictability was demonstrated by the detachment of the curve and increase in the unpredictability index with the removal of water from the system (FIGURE 4.5). The model indicates that with a reduction of 30% in water available in the soil, the end of the drought period becomes less predictable, and the reduction may disturb the system for a longer time. Although, we have not found significant correlations for SPEI values other than SPEI-6 September (FIGURE 4.6), the maps for SPWI-6 January (FIGURE 4.1) indicate that even during that month in 2015 the system was quite disturbed, which was probably affected by the drastic reduction in rainfall during that period (FIGURE 4.7B).

Therefore, the reduction of available water in the months of November, December or January, which are months of active growth for both vegetative and reproductive organs of the plant, when water should be freely available for them, may affect both shoot and cherry growth. This more severe condition may reflect in next-year yield as well as same-year yield by producing either low-sieved beans (dry periods in November or December) or shriveled and malformed beans (dry period in January). Thus, the climatic patterns that confer the south of Minas Gerais its distinct coffee quality may become threatened by longer droughts or punctual drier periods on critical moments of its phenology.

Nevertheless, the theoretical model showed a rise in the unpredictability index with reductions in water availability in some critical moments as the ones observed by the sequential drought events that happened in the months of February 2014 and January 2015 (FIGURE 4.7B), which were emulated by our system in January 2016 (FIGURE 4.7A). Such occurrences, in association with rather longer dry days for severe drought in the month of September (RWC<0.4) as predicted for the model (FIGURE 4.3B) could cause reductions in the yield as well as significant disturbances in the system. Therefore, predisposing the coffee crops to some systemic instabilities that may lead to unpredictable physiological events such as maybe even an inversion in the alternate bearing (biennially) of the coffee crops. In fact, our empirical data demonstrate that there was a reduction in next-year yielding, with amplification and even an unforeseeable alternate bearing in SWE coffee trees (FIGURES 4.27 and 4.28) with a biennially index of 0.55 in SWE compared to 0.17 in NNE and 0.13 in SWNE.

Although the theoretical models develop in this work gave an idea that such events will be very rare for this region, our exclusion system seems to have worked well so that it allowed the real reproduction of what the theoretical models claimed would have happened if a certain percentage of water were withdrawn from the system. Moreover, the very high return period for such event to happen again also reinforces that the disturbances seen in the coffee trees where caused by the reduction in water availability in the system. However, contradicting some prevision for this region, our theoretical models based on the SPEI drought index could not find any definite pattern or trends of increase in such drought events for future years and otherwise there is even an indication of a wetter climate for the region in the future. In fact, severe drought occurrences are cyclic events and they seem to have a return period of 50-60 years in average (MELLO et al., 2016). Furthermore, some authors have already demonstrated some uncertainties in relation to definite trends of increase in such drought events for future years (BURKE et al., 2008; GORNALL et al., 2010; DAI; ZHAO, 2017).

According to Alègre (1959 apud DAMATTA et al., 2007, p. 486), the optimum annual rainfall range for arabica coffee is between 1,200-1,800 mm. The values obtained with our empirical data: 1,171 mm of rainfall without the exclusion system and 890 mm for the plots with the exclusion system on from June 2015 to May 2016, certainly provided a mildly adverse water condition for the coffee trees growing under the exclusion system, especially during certain moments of the experiment when high values of ET_c and VPD probably caused a more intense water shortage in the superior layers of the soil, such as in March and April 2016. The evapotranspiration of the coffee culture was calculated according to the formula: $ET_c = K_c *$ ET_a. The use of the actual evapotranspiration (ET_a) in this empirical calculation takes into consideration only the water present in the system (precipitation) and would therefore give us an idea of the real removal of water from the soil and its final water balance and not the more abstract idea of a potential energetic balance over the SPAC as the ET_0 would inflict on the system. However, if we consider ET_0 in the calculation we would imply that there was even a higher tension in the system, since its value 884 mm was higher than the precipitation (809 mm) for the same period in the SWE.

Moreover, it is incontestable that the precipitation and temperature possess a strong influence upon the growth of the coffee tree. We found on average high correlation coefficient for the growth of leaf area of branches in relation to the precipitation and air temperature (R^2 = 0.53 and R^2 = 0.88) for the entire experimental period (TABLE 4.2). Demonstrating the important control that those factors have over the leaf area growth. Although, in the field, it can be difficult to distinguish which factor is exerting more influence upon the coffee tree growth at a specific moment. Our study showed that the coffee trees suffered additional growth rate reductions when subjected to the rainfall exclusion system.

In the south of Minas Gerais, the shoot growth of the coffee trees is slow during the dry, cool season (from May to August), and rapid in the rainy, hot season (from October to March), similar response was seen in coffee trees growing in the region of Viçosa (BARROS; MAESTRI, 1974; DAMATTA; AMARAL; RENA, 1999). According to Silva et al. (1997), these growth fluctuations do not seem to be associated to soil moisture since water availability does not alter the overall pattern of growth, although it may affect the rates of shoot growth. However, in our opinion, more precise methods to evaluate shoot growth should be necessary to make such affirmation. Furthermore, this growth periodicity is also apparently independent of reproductive growth, although fruitless trees may outgrow those bearing fruit (SILVA et al., 1997; AMARAL et al., 2001; CASTRO, 2002).

In fact, lowering temperatures would, to a great degree, set off the declining growth rates through the dormant or quiescent phase; by contrast, occasional repression in shoot growth during the intensive growing season seems to be mainly associated to very high temperatures (BARROS et al., 1997). However, we verified a greater decline in the growth rates of SWE plants inside the exclusion system after the month of March 2016, demonstrating that the growth rates were probably affected by the reduced bulk of water availability at the beginning of the quiescent phase, having a synergistic effect of this factor in association with lower temperatures upon the growth of coffee.

The RH has some important roles in the flowering of coffee trees (CARR, 2001) as well as in the control of the stomatal pores (BAUER et al., 2013). The maximum relative humidity of the air fluctuated less than the minimum RH which, by the way, was similar in behavior to mean RH, presenting a larger amplitude of values during the experimental period and showing lower values in the winter months of July, August and September with a pronounced drop in the month of April 2016 (FIGURE 4.9B).

The VPD as well as the relative humidity has a very important role in breaking the flower bud dormancy of coffee trees through a continuous period of dryness, which could last from one to four months. The stress needed to break the bud dormancy can be induced by dry soil and/or a large evaporative demand connected to high temperatures and large VPD of the air (BROWNING; FISHER, 1975). VPD also has a crucial importance in the coffee trees transpiration rates, since it is a totally physical process dependable on the thermodynamics of the environment. The VPD showed three peaks during the experimental period: October 2015, April 2016 and September 2016 (FIGURE 4.9C). The high VPD normally presented in September and October are of vital importance to flower blossoming in that period of the year. Nonetheless, the VPD also has an important role in the transpiration of plants.

Stomatal closure in response to increased VPD is driven by the rapid up-regulation of foliar ABA biosynthesis and ABA levels in angiosperms; however, very little is known about the physiological trigger for this increase in ABA biosynthesis at increased VPD. Explanations range from passive regulation by leaf hydration (water potential) to biochemical regulation. The presence of ABA also inhibits the actions of growth hormones such as gibberellins (WANG, 1998). The ICK1, a cyclin-dependent kinase inhibitor, was induced by ABA, and along with the ICK1 induction there was a decrease in Cdc2-like histone H1 kinase activity. These results suggest a molecular mechanism by which plant cell division might be inhibited by ABA, and the balance between ABA and gibberellins might influence a trade-off between either bud flower induction or vegetative growth.

5.2 Ψ_{leaf}, Ψ_{soil}, RWC, SLA and coffee tree physiological adjustments to water shortage

The predawn Ψ_{leaf} did not reveal any statistical differences between the treatments throughout the whole experimental period (FIGURE 4.10). Many authors use the Ψ_{leaf} as a suitable indicator of water stress in plants. However, Davies and Zhang (1991), believe that variables such as leaf gs and growth rates may be more useful indicators of water stress than the more commonly used variables of leaf water relations. Another important detail is that the values of pre-dawn Ψ_{leaf} were obtained at a time of the day where the leaf usually shows the highest values for that variable. Therefore, causing the Ψ_{leaf} to become insensitive to the water shortage that was developing in the most superficial layers of the soil, besides the trees were subjected to an overall mild water stress that could become more intensified during alternate time of the day such as noon. Anyway, in July and September 2015 the coffee trees displayed values of pre-dawn Ψ_{leaf} around -1.0 MPa, such values usually cause the interruption of primary and secondary growth of the trees (RAMBAL et al., 2003).

Ψ_{soil} at 30 cm of depth did not display any differences between the treatments and its values were apparently high during the active growth season. Therefore, the Ψ_{soil} did not characterize a very intense water stress for the coffee trees during that period. Nonetheless, there was a significant and normal decrease in soil water potential from the wetter months of active growth to the drier ones of the quiescent phase (FIGURE 4.8). One point to consider is that the retention curve for this type of Red Latosol shows that it may reduce its significantly quantities of water without suffering expressive or meaningful changes in Ψ_{soil} (SILVA et al., 2014, 2015).

Normally the coffee root system developed from a transplanted seedling is composed of one or more tap (vertical) roots, plus a superficial layer of lateral roots growing horizontally to the soil surface, and other lateral roots coming from those tap roots but growing downwards at sharp angles to the vertical one. The depth and distribution of those roots may vary due to the chemical and physical properties of the soil, as well as cultural practices such as fertilization, soil covering (mulching) and irrigation (quantity of water available to the roots) (CARR, 2001). The main part of the root system of an arabica coffee tree (60% of the roots) is generally concentrated in the first 0.30 m layer from the soil surface and distributed in a circle of nearly 1.50 m of diameter around the trunk (SAIZ DEL RIO et al., 1961; INFORZATO; REIS, 1974; HUXLEY et al., 1974; ALFONSI et al., 2005). The fact that most of coffee roots are concentrated in the first 30 cm of soil have some important consequences for the entire tree.

On the other hand, the fact that there is a reduction of moisture in the most superficial layers of the soil during long part of the winter months in the south of Minas Gerais is a very important aspect. Firstly, the roots located in the most superficial layers of the soil are the starting point for sending hydraulic signals, which are internal changes in water potential that can propagate from one cell/organ to another, in this case from roots in drying soil to shoot (CHRISTMANN et al., 2007). Secondly, chemical signals from the same dry thin roots located in the most superficial soil cause changes in the transport of plant hormones and other chemical species from one cell or organ to another. For example, soil drying can sensitively increase the synthesis and availability of the plant hormone ABA in the roots (CUTLER; KROCHKO, 1999; THOMPSON et al., 2007; WILKINSON; DAVIES 2002, 2010). This ABA is then transported through the xylem up to the shoot (DAVIES; ZHANG 1991; WILKINSON; DAVIES 2002; DODD, EGEA; DAVIES 2008).

An elevation in VPD, caused by a reduction in RH can also induce both ABA-autonomous signaling in the guard cells (BAUER et al., 2013) as well as a hydraulic change within the shoot (THOMPSON et al., 2007; TARDIEU; DAVIES, 1992, 1993). In the coffee shoot, such signals will control stomatal closing at certain moments of the dry season so that the trees can avoid losing water through transpiration, revealing one of the main strategies for dealing with the reduction of the water bulk in the soil. Alternatively, some of those signals could also be used to trigger leaf area reduction and also leaf shedding (WILKINSON; DAVIES 2010), which would cause the reduction of the evaporative area of the plant by reducing the coffee tree leaf area.

The reduction of the rainfall by the exclusion system coupled to a high evapotranspiration during all the growth season, and particularly in the months of March and April 2016, when high values of VPD intensified soil drying and consequently influenced the bulk of water in the soil, probably affected Ψ_{soil}, especially in the most superficial layers of the soil - up to 30 cm of depth, where a great quantity of the root system is located.

Moreover, coffee leaves always try to maintain a high RWC when subjected to drying conditions and therefore coffee is considered a water-saving species instead of being a plant tolerant to dehydration (BIERHUIZEN et al., 1969; NUNES, 1976; JOSIS; NDAYISHIMIYE; RENARD, 1983; DAMATTA et al., 1993). This characteristic may be attributed to efficient stomatal control upon transpiration (BIERHUIZEN et al., 1969; NUNES, 1976; PINHEIRO, 2004) and low cell wall elasticity or a high bulk modulus (MEINZER, 1990; DAMATTA et al., 1993, 2003; PINHEIRO 2004; PINHEIRO et al., 2005). The coffee leaves are always trying

to adjust its turgor by retaining a high symplastic volume. DaMatta et al., (1993) agree with the fact that to confer drought tolerance to coffee trees under water deficit, the maintenance of a high RWC is more important than an osmotic adjustment by itself, especially because the latter could cause problems for the metabolism of the plant.

DaMatta and Ramalho (2006) affirm that comparative analyses of variables of tissue water relations based on pressure-volume curves suggest that irrespective of water supply coffee leaves show a high RWC at the turgor loss point, usually close to 90 %, a phenomenon largely associated with low cell-wall elasticity (DAMATTA et al., 1993; PINHEIRO et al., 2005). That corresponds to Ψ_w values ranging from –1.5 to –2.2 MPa for irrigated plants, with lower values for non-irrigated genotypes exhibiting some osmotic adjustment, being defined as a net increased solute concentration. Some authors point that the osmotic adjustment is not a general trait observed in coffee under drought conditions (GOLBERG; BIERNY; RENARD, 1984; DAMATTA 2004). Therefore, the mechanism of osmotic adjustment, although it has been generally associated with maintenance of gas exchange in many plant species under drought conditions (TURNER, 1996), could hardly explain the stomatal sensitivity of coffee to drought.

We observed some significant adjustments of the RWC in the face of the stress conditions that happened during the period of analysis for the coffee trees. In general, the values of RWC did not differ significantly between the treatments (FIGURE 4.11). Nonetheless, there was a linear decrease in the RWC specially in SWE and NNE, which showed values of R=0.766 and 0.735 respectively, and the SWNE treatment with R=0.456 also displayed the same tendency of reduction although it was less accentuated, which can be identified by a lower value of b=0.0323 in its equation when compared to the other treatments b=0.0608 for SWE and 0.0623 for NNE (FIGURE 4.12). In general, the leaf RWC values in SWNE demonstrated a different behavior with two deviant peaks from (January and July 2016) and a trough (at the beginning of March 2016) that could hardly be explained. One possible explanation of such strange behavior might come from the fact that the collectors for SWNE treatment were perforated at determinate position and that caused the water to fall in a form of a little cascade that could supposedly have affect the normal characteristics of the leaves being sampled. Thus, for further studies using the system, the researcher would have to develop an adaptation to the holes on the collectors so that it would cause a scatter or sprinkle of the water flow minimizing its cascade effect before the water hits the tree leaves specially under conditions of heavier rains.

However, there was a sharp increase of RWC for all treatments in the middle of May 2016 that may have occurred as a response to stomata closure. Several authors (DAMATTA et al., 2003; SILVA et al., 2004) affirm that stomata naturally reduce their opening toward the winter month and quiescent phase of the trees. This reduction may have allowed the leaves to build up more inner water in their tissues causing the sharp elevation in RWC at that period. During the month of May 2016, there was a strong increase in the VPD (FIGURE 4.9C), which was caused by lower RH and yet high temperatures in the period. Therefore, this high VPD generate by those meteorological conditions did not allow the stomata to open fully, preventing transpiration and causing the water to build up inside the cell, consequently increasing its RWC. Then again, in the month of June 2016 there was a rise in RWC, but this time it was induced by the timely rains that occurred at that period. This elevations of RWC in periods of low RSWC is a strong evidence that the coffee trees always try to keep a high RWC in their leaves, even when subjected to a reduction in the moisture condition of the environment (DAMATTA, 2004). Nevertheless, a trend of overall RWC reduction was evident throughout the experimental period, with the first samples in December 2015 displaying on average higher RWC values around 81% and declining to an average of 72% in the last measurements in July 2016 (t-test P<0.001).

SLA is also a very interesting trait to evaluate the ecophysiological adaptation of the plant to various environmental factors, such as light and water availability. For example, it gives an idea of the photosynthetic efficiency of the leaf and the relative growth rate (RGR) (RGR=LAR x NAR) of a plant is strongly correlated with leaf area ratio (LAR), that is the quantity of leaf area per unit total plant mass, and differences in LAR can be due to variation in SLA, since LAR = SLA x LMR, where SLA is the specific leaf area and LMR is the leaf mass ratio. Therefore, differences in leaf area growth rate depends on the values of SLA (LAMBERS; POOTER, 1992).

Considering the means for the entire experimental period, SLA of the coffee plants was higher for the SWE treatment than NNE, with the SLA for the SWNE fluctuating between the other two treatments (FIGURES 4.13 and 4.14). The mean increase in SLA for SWE seems to go against the results found in several works, which report a decrease in SLA when the plants were subjected to a reduced soil moisture. However, we must be careful in dismissing such data as a deviation from normal finds. Besides, we cannot discard the fact that the coffee trees under the system of exclusion were partially shaded and therefore such condition could have collaborated to the increase in SLA in the SWE individuals. Nonetheless, the SWNE control treatment set up to evaluate the influence of light – i.e. if the collectors would have any effect

over the trees under the exclusion system (SWE treatment) - did present opposite SLA values from the SWE trees during the critical months of February and March 2016 (FIGURE 4.13).

The overall response of SLA for all treatments increased their values from December 2015 to April 2016 and then decreased from the end of April to the end of July 2016 (FIGURE 4.13). This response seems to be adequate to the observations of several authors that report a decrease in SLA values when the coffee trees are subjected to a reduction in the soil moisture. Therefore, the pattern of SLA's increase from December to April and then decrease from April to July, seems to be a quite normal trend for SLA, since during the increase of SLA the leaf area was still in expansion and during its decrease the leaf area was also reducing their growth rates. Some authors describe a positive correlation of SLA and RGR (LAMBERS; POOTER, 1992).

Nonetheless, drought and lower water availability have varying effects on SLA. In a variety of species, most of the times, drought decreases SLA. For example, under drought conditions, leaves are, on average, smaller than leaves on control plants. This is a logical observation, as a decrease in surface area would mean that there would be fewer ways for water to be lost. Species with typically low SLA values are equipped for the conservation of acquired resources, due to their large dry matter content, high content of cell walls and secondary metabolites, and high leaf and root longevity. Which is not the case with the coffee leaf in the field, that has a short permanence in the canopy and has less costly inner foliar structures as well.

In some other species, such as Poplar trees, there was an overall decrease in SLA, but there was an increase in SLA until the leaf had reached its final size. After the final size has been reached, the SLA will then begin decreasing (MARRON et al., 2003). ASPELMEIER; LEUSCHNER (2005) have also found increasing SLA values in plants under water limitation. Birch trees have increased their SLA values as consequence of drought stress, their SLA values significantly increased after two dry seasons. In our experiment, the coffee trees had already gone through some rain shortage in the years of 2013 and 2014 (FIGURE 4.7B), with a register of a sharp reduced water accumulation in the soil caused by the scarcity of rains during that period (MELLO et al., 2016; INMET, 2017). Therefore, the larger SLA observed in the SWE trees, which started to differentiate only after February 15 (FIGURE 4.13), was probably an influence of the water shortage promoted by the exclusion system. Furthermore, the coffee trees under SWNE besides having an overall pattern of lower SLA in relation to SWE also displayed a definite lower SLA during the crucial months of February and March 2016, when many adjustments such as several growth rates, chlorophyll content, and probably other

important 'omics' adjustments were happening in the trees, which were getting ready for the upcoming and less active growth season. Thus, such rise in SLA in SWE trees may also indicate the influence of a reduced soil water availability upon SWE trees.

5.3 Vegetative growth, leaf area, sink-source relationship and coffee yield

Vegetative growth of coffee trees has been associated with several environmental factors, such as temperature, water, photoperiod, irradiance, nutrient supply and other factors intrinsically related to the plant itself such as the reproductive growth (SYLVAIN, 1958). Although it is unquestionable the importance of the control of environmental signaling upon the growth of coffee plants, there is little information about how seasonal differences of those factors affect coffee growth and how it is physiologically controlled (SILVA et al., 2004). For example, according to some authors, coffee shoot growth is not continuous during the active growing season, temporary depression in growth of shoots has been attributed to high temperatures (GINDEL, 1961; BARROS; MAESTRI, 1974; BARROS et al., 1997). In addition, other factors such as nutrient leaching and daytime water stress due to a high evaporative demand are also invoked to account for growth oscillations.

Additionally, according to several authors, there seems to be an asynchronous modulation between rapid vegetative growth and fruit development, suggesting some incompatibility or competition between the two processes that may influence the vegetative growth of coffee trees. In fact, competition between the developing fruit and shoot sinks begin after anthesis, yet the resource requirement for each sink may vary during the growing season. Shoot growth is not uniform and occurs in rhythmic cycles of shoot extension called "growth flushes" and rest of meristem activities (BARTHELEMY; CARAGLIO, 2007). However, climatic factors such as temperature and water availability may modulate the vegetative growth and fruit production in such way that they should not coincide. In most non-equatorial regions and even in coffee's native land, coffee blossoming and fruit development are synchronized to coincide with the rainy period and right after a flush of new leaves so that it can increase the probability that those processes will not rival (CANNELL, 1985). This would allow the development of a satisfactory leaf area to support the succeeding fruit growth. Nonetheless, particularly in full sun crops, coffee trees usually flower abundantly, producing high crop loads, without coinciding with a proportional response in leaf area formation (DAMATTA et al., 2007).

In fact, coffee berries act as main sinks so that dry matter allocation to them may represent more than four times that allocated to branch growth over the annual production cycle (VAAST et al., 2005). Therefore, reduced branch growth and high branch dieback are

commonly observed in such circumstances (DAMATTA et al., 2007). Such responses were observed in our study, SWE trees presented lower BLA; and lower branch length and main stem growth rates (FIGURE 4.15, 4.22 and 4.23); besides a higher number of dieback branches than the other treatments (data not shown), with a relative frequency of 0.61 in SWE treatment in comparison to 0.17 in SWNE and 0.33 in NNE. These responses have been traditionally linked to carbohydrate deficiency (CANNELL, 1985), although in more recent investigations no consistent pattern between shoot growth depression and exhaustion of stored carbohydrate was observed (CARVALHO et al., 1993; CASTRO, 2002; DAMATTA et al., 2008, CHAVES et al., 2012). Furthermore, Chaves et al., (2012) did not find any apparent relation between dieback and differences in photosynthetic rates per unit leaf area, carbon isotope composition, or oxidative stress, as was assessed by the electrolyte leakage from the leaf tissues.

Branch dieback is a phenomenon of intricate nature; it refers to the death of twigs starting from the apex and progressing basipetally. Usually, leaves in those branches become yellow prematurely and fall, leading to extensive defoliation of those trees. The affected branches dry out and are finally shed. The syndrome has been registered in practically all coffee growing countries, resulting in severe loss of both yield and quality of coffee (CLOWES; WILSON, 1977). Although branch dieback was in the past often associated with pathogens like *Colletotrichum*, *Phoma*, and other microorganisms (THOROLD, 1945; CHOKKANNA, 1962 apud DAMATTA et al., 2007), some evidences suggest that the pathological infections are just consequences of tissue disability instead of its primary cause. In fact, no pathogen was found in tissues exhibiting the first symptoms of deterioration and attempts to reproduce dieback symptoms through the inoculation of the pathogens in healthy branches turned out to be unsuccessful (BARROS et al., 1999). However, the occurrence of dieback has been associated with environmental stresses such as soil and atmospheric water deficits, high temperatures, high insolation or the combined effects of these stresses (BARROS et al., 1999; DAMATTA, 2004). As shading can attenuate such adverse environmental effects, a lower incidence of dieback in both shaded and high-density crops is to be expected, which apparently did not happen in SWE trees under the collectors and given an indication of the effect of reduced water availability for those plants.

Some authors mention the importance of sink strength controlling the growth and photosynthesis of many trees, functioning as a special form of drive directing the rates of *A* (DINGKUHN et al., 2006). Even though the sink strength may control the partitioning of carbon to different organs in the trees of Arabica coffee, one must also have in mind that, during the active growth season, the coffee trees have too many demanding sinks acting at the same

time: vegetative and reproductive growth in the shoot (young leaf and beans, respectively), besides the growth of roots that practically happen at the same moment. Moreover, when a plant experiences a moderate reduction in water availability, there are some strategies that the plant might follow to adjust to water shortage, such as investing more carbon in the growth of roots or even more carbon into reproduction (fruit and seeds) as a memory of ecological survival. According to Trewavas (2009) intelligent behavior (adjustment) cannot be divorced from the situation (stress condition) that elicits it. All organisms including plants face highly variable situations in which they must attempt to optimize their survival and at the same time produce the maximal numbers of siblings.

By making use of the notion that plant growth is driven by sink regulation, Dingkuhn et al. (2006) developed a conceptual framework for the analyses and simulation of crop growth. Such framework takes into consideration the feedback effects of the plant's resources status on meristem behavior as well as the mechanisms to cope with momentaneous source-sink imbalances (due to the time elapsing between organ initiation and expansion). These mechanisms include the management of transitory reserves, organ senescence and end-product inhibition of photosynthesis and are generally related to sugar metabolism (FOURCAUD et al., 2008). Therefore, the growth of an organ is the fine-tuned decision of an orchestrated regulation of sinks and their interactions with assimilate sources. Thus, we should always be careful in considering how the intrinsic conditions of a plant such as sink strength of certain organs can control the growth of the coffee trees and how their adjustment to the forces of the environment will eventually drive basal mechanisms such as photosynthesis and respiration (in a broad sense – not only cellular respiration but also in terms of leaf shedding) into changes, specially, when the plants are subjected to a mild water stress.

Coffee trees grow and develop through adjustments of their physiology to changes in their environment. Abiotic changes in the environment occur over the period of years, seasons, and days, but can also happen over minutes and even seconds. In this constant change nature, the coffee trees must adjust their structure and mechanisms rapidly to allow significant growth and reproduction. Moreover, stress effects from numerous treatments [cold, heat, salinity, drought, ultraviolet (UV) light, mineral imbalance, disease, etc., including, surprisingly, ABA] can be remembered and influence not only a later response (GOH; NAM; PARK 2003) but memory of some stresses can also be passed to subsequent generations (DURRANT 1962; MOLINIER et al., 2006). Therefore, responses of coffee trees to repeated cycles of drought would differ from those of single incidences of drought. In fact, coffee trees are usually exposed to repeated cycles of drought that differ in time and intensity. Several mechanisms are involved

in the adjustment to those cycles, and an increased interest in better understanding them, at least in part, due to the discovery of epigenomic changes that trigger drought stress memory in plants. Besides epigenomic changes, there are other aspects that should be considered in the study of plant responses to repeated drought: from changes in "omics" such as transcriptomics, proteomics, and metabolomics, to changes to the classic plant structure; all of which may help the scientists to better understand plant stress memory and its underlying mechanisms.

Structural changes, which, in turn, are the result of changes in "omics" (molecular biology) during previous stress exposure, will severely affect the "omics" and overall physiological response during subsequent stress episodes. Fleta-soriano and Munné-Bosch, (2016) exposed *Silene dioica* plants to reiterated drought in a greenhouse (including two cycles of 6 days of water deficit by withholding water, followed by subsequent periods of six days of recovery), it was found that, despite of the RWC, LMA, maximum efficiency of the photosystem II (F_v/F_m ratio) and the total amount of chlorophylls (Chl a + b) having not differed between double-stressed and single-stressed plants, the Chl a/b ratio was higher in double-stressed plants than in single-stressed plants. It is interesting to note that changes in the Chl a/b ratio were only observed after recovery; this suggests a change in the structure of the photosynthetic apparatus, since it has been reported that there is a reduction in the size of the light harvesting complex of the photosystem II (LHCII) under excess light (CAJANEK et al., 1999; KURASOVA et al., 2000, 2002). This change in the pigment composition of leaves is therefore an indicative of reduction of size of the antenna from LHC in double-stressed plants, which might help plants to reduce reactive oxygen species production and photo-oxidative stress in chloroplasts, if they are challenged by a new stress in the future (FLETA-SORIANO and MUNNÉ-BOSCH, 2016).

Although we have not analyzed Chl a/b ratio in our experiment to analyze such chloroplastic structural adjustment to reiterated drought episodes, we could observe an evident faster resumption of BLA growth rates in SWE plants right after some significant rains in June 2016, which allowed the SWE trees to present higher BLA growth rates than the other two treatments in the following months of July and August of 2016 (FIGURE 4.16), demonstrating a drought memory trait and faster growth rate adjustments of SWE trees in recovering from stress. We must also have in mind that the year 2014 was drier than other years with only 1025 mm of precipitation from September 2014 to August of 2015, nearly 372 mm or 25.5% lower than the climatic normal (1460 mm), which allowed repeated cycle of mild water stress over the SWE coffee trees.

Leaves are the main interface where gas exchanges (inflow of CO^2 and outflow of H_2O) happen in plants and terrestrial plants must deal with the dilemma of either keeping their stomata open to allow the influx of CO_2 and running the risk of foliar desiccation or shutting the stomata to reduce H_2O outflux and concurrently diminishing the CO_2 fixation and consequently reducing the plant production and growth. Therefore, a precise adjustment of the leaf area may also represent at a larger scale an interesting acclimation procedure for coffee plants facing some water shortage. In fact, we could observe a greater reduction in BLAm after the month of February 2016 (FIGURE 4.15A), indicating an adjustment of that trait in SWE trees. Further decreases of BLA in SWE ended up culminating in a higher relative frequency of branch dieback in the treatment. Moreover, it is quite plausible that during the later quiescent period a reduced leaf area would probably diminish the overall impact of water loss, but at the same time a reduced leaf area could also diminish the overall production of *A*, affecting the tree growth rates, since sugars produced at that moment would probably be used to either maintain the basic physiological functions such as the maintenance respiration of tree organs or be used in building up some reserve for the next blossoming and resuming growth for the following active growth season.

LAI is a very important estimator of the production of coffee trees, since it gives an idea of the area involved in the assimilatory processes of the plants. However, a nondestructive and continuous obtainment of such variable throughout an experimental period is not an easy endeavor, besides it is the most difficult trait to deal with when validating growth models for any plant, due to the influence of a huge number of factors upon it. Numerous studies have aimed at scaling up responses of leaf photosynthesis to variations in environmental conditions from the leaf to the canopy, plant, ecosystem and even global level. However, aggregation of fine scale photosynthetic variability (leaf level) to higher spatial (canopy, crop or forest) and temporal scales remains difficult, since systems' functions and responses to environmental conditions are generally modified by scales of time and space. For example, photosynthesis models such as the one by Farquhar et al. (1980) were originally developed to explain CO_2 exchange by leaves to environmental conditions. Such mechanistic process model with timescale of seconds to hours contrasts with other processes that are also important at the individual and ecosystem level with characteristic timescales of days or even longer time periods of weeks and months (EWERT, 2004), such as the growth of a tree leaf area.

Some authors have recently demonstrated that the temporal resolution (hours, days, months or years) of input data has a significant impact on the radiation efficiency use (RUIMY et al., 1994; MEDLYN, 1998; VAN WIJK; BOUTEN, 2002), which gives an idea of the crop

production and is straightforwardly influenced by the leaf area. As the time scale increases, the radiation efficiency use becomes less variable (MEDLYN, 1998), that increase is probably positively correlated with the leaf area. Consequently, in this case other processes in the system (such as respiration and leaf shedding) becomes also very important for explaining the system's response. Therefore, a precise access of LAI data is essential for the calibration of growth model in time and space.

As already mentioned, LAI in our study presented higher values for the NNE treatment in relation to the other two treatments SWNE and SWE from April 2016 to August 2016 (FIGURE 4.17). Nonetheless, these results do not correlate well with other important variables. In fact, we analyzed some correlations of LAI with BLA, precipitation, temperature and NDVI (TABLE 4.2) to possibly validate the use of the method proposed by Barbosa et al. (2012) to estimate the coffee tree LAI in this study. However, our analyses demonstrated some weaknesses in relation to Barbosa et al.'s method and as the proper authors argue the methods used for estimating LAI of coffee trees often show limitations related to the representation of some important aspects of coffee crops, affecting LAI in time and space. Therefore, the correlations developed by our analyses displayed limitations regarding their LAI estimation method. The main problem presented by Barbosa et al.'s method is a lack of precision when measuring the plagiotropic branch, their measurement usually overlooks one crucial variable inside the branch, i.e. leaf demography and by disregarding such crucial parameter the errors build up.

Several authors point to the great importance of the NDVI as a variable to accessing and evaluating crop yielding (PONTES et al., 2005; BERNARDES et al., 2012; SAKAMOTO et al., 2012; SANCHEZ et al., 2012). The correlation displayed by BLAm and BLAs in relation to NDVI, which can be graphically observed by the identical pattern of adjustments of BLAm and BLAs with NDVI in each treatment (FIGURES 4.15A, 4.15B, and 4.18), during the experimental period, reinforces the quality of our methodology to make inferences about the coffee tree LA. We believe that by introducing a few new parameters into our methodology would allow fairer proxies for the canopy volume to be obtained in future studies. Furthermore, these high correlations of BLAm and BLAs show the sensitivity of this variable in relation to the parameters evaluated compared to LAI values obtained from the Barbosa's method. Thus, a few adjustments in terms of data sampling may be necessary so that their methodology can be finely improved.

Coffee trees as most fruit trees experience a biennial mode of fruit production designated "alternate bearing," this is the result of a tree's alternate yield between high fruit

load and low fruit load on a yearly basis. Therefore, the next-year productivity of coffee trees depends straightforwardly on many same-year variables, ranging from exogenous climatic and environmental parameters to endogenous variables such as size and vigor of plagiotropic branches, number of nodes on the branches, frequency of tertiary branches to name a few variables. In their turn, these variables are influenced by the size and number of fruit (sink strength) produced by the trees on the current year.

Alternate bearing intensity may be measured by an index (B), that is the absolute difference in yield in the last two years divided by sum of the last two yields with levels between 0 (no alternate bearing) and 1 (no yield in one of the years) (PEARCE; DOBERSEK-URBANC, 1967). We observed B indices of 0.17 for NNE trees, 0.12 for SWNE and 0.55 for SWE, indicating a strong alternate bearing for the SWE treatment. Among the several variables responsible for next-year yield, our data revealed that the number of branch nodes were significantly reduced in SWE trees by April 2016 (FIGURE 4.19). Another important variable, i.e. number of tertiary branches, which is inversely influenced by same-year fruit load present in the trees (CLOWES; WILSON, 1977), was also smaller in SWE trees (FIGURE 4.25). The reduction in such variables are probably the result of high fruit load (higher sink strength located in the coffee berries), in association with the synergistic effect of the rain exclusion over SWE trees, since the same response was not seen in trees under SWNE and NNE treatments.

Therefore, these results may indicate that the rain exclusion caused this strong alternate bearing in the SWE trees and complementarily inverted its biennially in relation to SWNE and NNE. In fact, besides the high biennially index of 0.55 for SWE trees, those trees were also subjected to an inversion of yield in comparison to SWNE and NNE. In 2016, SWE trees presented a higher yield while the other two treatments presented lower figures compared to the yield in the year of 2017, which was lower for SWE yet higher for SWNE and NNE trees compared to the previous year of 2016. These responses demonstrate a clear inversion in the pattern of biennially or alternate bearing in SWE trees. We theorize that this inversion must have been influenced by the longer mild water stress over the coffee plants. However, the precise mechanism through which this mild water stress could have caused a higher 2016 yield in SWE trees remains obscure.

There are many hypotheses regarding what is involved in determining the strength of a sink. It is quite probable that neither the source (leaf area in our study was reduced after February) that produces sugars to be consumed by the sinks nor the path (phloem) that furnishes a certain sink with an advantageous supply of sugars are the main elements determining which

sink is dominant over another. It seems that the dominant sink itself determines its strength either by competing more successfully for nutrients or by actively sending signals to weaken competing sinks (SMITH; SAMACH, 2013).

Some current models predict that gibberellin acts as the dominance signal, especially controlling the growth of other fruit. Experimental studies suggest that auxin stimulates gibberellin biosynthesis in various shoot tissues as well as seeds. While applications to fruit trees inhibits flowering when applied at the appropriate developmental time point, gibberellin also promotes shoot growth. On the other hand, ABA is a plant hormone that limits cell division, perturbing shoot growth and floral induction. Therefore, ABA may be a possible candidate for the dominance-signaling molecule that mediates meristem growth cessation in response to high fruit load (SMITH; SAMACH, 2013). The hypothesis that ABA is a possible candidate to that chemical signaling controlling source/sink relationship in balance with other hormones, at least in our situation, makes great sense, since ABA is a hormone affecting LA and shoot growth rates negatively (VAN VOLKENBURGH; DAVIES, 1983; SAAB et al., 1990).

5.4 Macronutrients and chlorophyll in coffee leaves

The analysis of leaf mineral content did not reveal any nutritional deficiency for the coffee trees based on the prescriptions given by Mills and Jones (1996) and Malavolta (1997) (TABLE 4.3). Nitrogen was below the lower limit according to Malavolta's prescription in SWE, P was also a little lower for Malavolta's in all treatments as well as Ca^{+2} was a little below both prescriptions in all three treatments. Costa et al. (2006), working with arabica trees with a few levels of fruit thinning, found some consistent variation in leaf mineral content in relation to the thinning, they found increases in calcium (37%) and boron (29%), decreases in potassium (34%) and copper (17%), and unchanged contents for the other macro and micronutrients analyzed with the increase in the fruit load. In our study, only the macronutrients were analyzed and similarly to results found by Costa et al. (2006) there was also an increase in Ca^{+2} in SWE, which was the treatment that displayed the highest fruit bearing although the fruit yielding was not statistically significant according to the ANOVA (P<0.05).

Similarly, DaMatta et al. (2002) did not find any differences in the N content in leaves of *Coffea canephora* as result of water availability for plants, it was only influenced by the content of N in the substrate, being reduced in lower concentration of N. Nonetheless, several authors found that N absorption by crops is automatically reduced under dry conditions, even when the element is present in the soil colonized by roots (HERZOG; GOTZ, 2004; GONZALEZ-DUGO et al., 2005, 2010). Drought decreases the uptake of water and/or nutrient

flux and translocation. Thus, transport, allocation and metabolism of N may serve as adequate indicators for stresses and may help to understand how plants cope with a wide variety of suboptimal environmental conditions (GOTZ; HERZOG, 2000). Gonzalez-Dugo et al., 2010 found that for the same quantity of N applied to the soil, and with lower shoot biomass, especially on the last measurement date, N nutrition status declined under drier treatments. A similar response was found with less fertilizer application, but the differences between irrigation regimes were smaller.

The absorption of N by roots requires the presence of water in the soil, as it is the agent that transports solutes to the soil-root interface (GARWOOD; WILLIAMS, 1967). As N is mainly transported through bulk flow, a lower volume of water in the soil may affect its transport to the soil-root interface, diminishing its absorption by the roots and consequently, diluting this element all over the volume of the tree. The leaves of coffee trees presented a higher SLA in SWE (thinner leaves) (FIGURE 4.13), which is an indicative of a lower N content per leaf area as well as foliar structural changes probably at the level of chloroplasts and the ratio Chl *a/b* (FLETA-SORIANO; MUNNE-BOSCH, 2016) since the SWE leaves displayed a lower chlorophyll content in the same period of February and March 2016. Besides, we must also have in mind that SWE presented reduced BLA after March 2016 compared to NNE and SWNE, indicating again a lower overall content of N in the canopy in SWE trees. *Enterolobium contortisiliquum* a forest species growing in different levels of light without any water stress did not present any differences in N content in terms of leaf mass, even though it had presented lower concentration of N per leaf area in shaded leaves with higher SLA, which, in turn, presented reduced growth compared to the ones growing in full sun (personal information).

In the past, agronomical research about N uptake, and its relation to yield has mainly been considered empirical. The demands of N by crops and the analysis of yield components in relation to N supply were mostly directed toward the overall objective of satisfying crop N requirements (GREENWOOD et al., 1986). A more functional approach linking plant productivity with N supply was developed in plant ecology, however, this approach was mostly conducted toward a quest for adaptive strategies of species under contrasting natural environments (LAMBERS; POOTER, 1992). Research guided in the last two and a half decades has allowed the development of a functional approach for crops based on the ecophysiology of N uptake, N distribution and growth (LEMAIRE, 2001).

In situations where there is plentiful N supply, the variability in the dynamics of N accumulation in crops, under different climatic conditions (for instance, variable available

water content), can be greatly reduced when crop N content (amount of crop N per unit ground area - that in our study resulted in lower growth rates for SWE trees) is related to crop biomass rather than time (GREENWOOD et al., 1986; LEMAIRE; SALETTE, 1984). In several instances, crop N content has been related to crop biomass through the allometric relationship $N_{content} = aW^b$, where W represents crop biomass per unit ground area (LEMAIRE; SALETTE, 1984; GREENWOOD et al., 1990). It consequently renders the following $N\% = a'W^{1-b}$, where N concentration (N%) is the percentage of N in the plant biomass, and where a and a' differ by a factor dependent on the units adopted for $N_{content}$ and W. Critical N concentration (N%$_{critical}$) is defined as the minimum crop N concentration required to achieve maximum crop growth rate (GREENWOOD et al., 1986, 1991), and is analogous to the critical N content (minimum N content necessary to achieve maximum crop growth rate). The introduction of this concept allowed rationalization of the analysis of field data.

N in coffee trees is mainly located in the metabolically active shoot and is thus related more directly to the tree surface than to its volume. At the canopy, the relationship between the tree N content and its surface also results from the close relationship between the shoot N concentration and the incident solar irradiance (HARDWICK, 1987; GASTAL; LEMAIRE, 2002). In general, within the canopy, leaves exposed directly to solar radiation have the highest N concentration, which optimizes N use relative to carbon assimilation (HIROSE; WERGER, 1987; HIKOSAKA et al., 1994). Three-quarters of total reduced N in leaves usually relate to photosynthesis, mostly in the form of RuBP carboxylase and chlorophyll. In many moments, Total N concentration is related to chlorophyll concentration in leaves (TURNBULL et al., 2007). The overall content of chlorophyll did not show any variation between the treatments. However, in a few moments during the months of February and March 2016, it was larger in the NNE compared to SWE trees (FIGURE 4.36). As mentioned above, leaves more directly exposed to the sun generally have higher contents of N and therefore somehow corroborates with our data for a higher chlorophyll content at some specific moments. Therefore, the lower chlorophyll concentration in SWE in the months of February and March (FIGURE 4.36) confirmed by a reduction in NDVI (FIGURE 4.18) at the same period, in complement with a higher SLA (thinner leaves) than SWNE and NNE (FIGURE 4.13) and a smaller BLA (FIGURE 4.15A) happening all of them at the same time (February and March 2016) is an evidence that the N content per ground area of SWE plants were somehow lower than in the control NNE, which, in turn, might also have influenced, concomitantly to the water shortage, the lower growth rates in SWE coffee plants as already demonstrated by our experimental data.

6 FINAL REMARKS

The development of nondestructive empirical models focusing on leaf area is somehow more laborious and precise than the development of theoretical formalistic models, since it is realized by accompanying the whole processes and dynamic of leaf area growth, or whatever organ one wants to measure, more frequently and throughout the entire experimental period. Besides, precision and accuracy should be hallmarks of a good empirical methodology. The use of image processing in remote sense technology has been an important tool to evaluate and validate the real growth of coffee trees in the field. Therefore, in our study, we developed a new method to validate the LAI of coffee trees using BLA images, the precision of that method linked to NDVI allowed us to verify that the coffee trees underwent a mild water stress that caused changes in their leaf area dynamics and this new BLA method could also indicate that the LAI method used in our study needs adjustments so that it may be used in future studies that require accurate precision.

7 CONCLUSION

The simulation models for relative soil water content displayed a natural trend of longer drought time by increasing the percentage of a rain exclusion from the system, which was caused by an increase of unpredictability for the end of the drought time. September SPEI-6 was the drought time series that showed the best correlation with drought duration of a moderate water stress level. Besides, contradicting some previsions for this region, our theoretical modelling did not find any tendencies of increase in the intensification of drought periods for a large area of the south of Minas Gerais.

We empirically also verified that the reduction of ¼ rainfall through the exclusion system affected several shoot growth rates, such as stem, branches and leaf area growth rates, which were highly correlated with the NDVI and in turn related to crop yield, as well as the microclimatic values such as temperature, VPD, ET_c, and the reduction of the rainfall load. Besides, our data also showed that there was a strong alternate bearing ($B = 0.55$), besides an inversion of biennially in SWE trees probably caused by rain exclusion. Therefore, our data indicates that a reduction of ¼ of the rainfall may cause an overall shoot growth reduction and a stronger alternate bearing in coffee plants subjected to a continuous reduction of rainfall load.

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