JÉFYNE CAMPOS CARRÉRA

CARACTERIZAÇÃO ANATÔMICA E QUÍMICA DO SISTEMA VASCULAR SECUNDÁRIO DO CAULE DO CAFEEIRO (*Coffea arabica* L.) RECEPADO E EM DIFERENTES NÍVEIS DE ADUBAÇÃO

UFLA

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Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-graduação em Botânica Aplicada, área de concentração em Botânica Aplicada, para a obtenção do título de Mestre.

Prof. Dr. Fábio Akira Mori
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ANATOMICAL AND CHEMICAL CHARACTERIZATION OF STEM SECONDARY VASCULAR SYSTEM OF COFFEE TREE (*Coffea arabica* L.) PRUNED AND AT DIFFERENT FERTILIZING LEVELS

Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-graduação em Botânica Aplicada, área de concentração em Botânica Aplicada, para a obtenção do título de Mestre.

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À minha família.
Às mulheres de minha vida: minha mãe, avós, irmãs, tias e primas por me inspirarem e sempre acreditarem no meu potencial.
Dedico

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“Nós devemos ter persistência e, acima de tudo, confiança em nós mesmos. Devemos acreditar que somos talentosos em alguma coisa, e que essa coisa, a qualquer custo, deve ser alcançada”. (Marie Curie)

RESUMO

Coffea arabica L. (Rubiaceae) é uma espécie muito importante para a produção de café no Brasil. Entretanto, a utilização de cultivares altamente produtivas, cujas exigências nutricionais são elevadas, faz com que o cultivo da espécie seja, por vezes, muito dispendioso. Uma saída para a otimização no uso de fertilizantes é conhecer quais os limites suportados por *C. arabica* a nível estrutural (anatômico) que não comprometam sua produtividade. Além disso, buscar alternativas para uso dos resíduos de recepa, poderia aumentar a renda do produtor e garantir um destino sustentável à biomassa subutilizada. O objetivo desse trabalho, deste modo, foi: 1) caracterizar e 2) comparar a anatomia do xilema secundário e a composição química do sistema vascular secundário do caule de *Coffea arabica* cv. Topázio, submetido à fertilização distinta, visando analisar como as possíveis diferenciações encontradas no xilema secundário, além de verificar o aproveitamento de substâncias a partir de resíduos oriundos de cultivo. Para isso, foram coletadas amostras de *C. arabica* cv. Topázio MG 1190, na área experimental do setor de cafeicultura da Universidade Federal de Lavras (UFLA), a partir de um experimento implantado no ano de 2010, que passou por poda do tipo "recepa baixa" no ano de 2015. O experimento recebeu variação em níveis de adubação à 10, 40, 70, 100, 130 e 160%, conforme a recomendação nutricional indicada para *C. arabica*. Amostras de caules ortotrópicos e plagiotrópicos foram caracterizados quanto a anatomia e constituição química (estrutural e não estrutural) do sistema vascular secundário do caule, seguindo metodologia usual, adaptadas à espécie. Foram observadas diferenças anatômicas entre os tratamentos e regiões do caule e na funcionalidade do xilema nos ramos plagiotrópicos. A alteração na anatomia do xilema possivelmente ocorreu para atender a funcionalidade, mostrando a plasticidade do tecido da espécie. Também foram encontradas quantidades diferenciadas de componentes químicos estruturais e não estruturais em ramos ortotrópicos residuais de recepa, entre os diferentes tratamentos. Os resultados revelaram não apenas a plasticidade do caule do cafeeiro quando submetido à fertilização diferenciada mas também apontaram seu potencial no reaproveitamento de compostos químicos quando há a ocorrência de recepa.

Palavras-chave: Cafeicultura. Anatomia do cafeeiro. Recepa.

ABSTRACT

Coffea arabica L. (Rubiaceae) is a very important species for the production of coffee in Brazil. However, the use of highly productive cultivars, whose nutritional requirements are high, makes the cultivation of the species sometimes very expensive. One way to optimize the use of fertilizers is to know which limits are supported by *C. arabica* at a structural (anatomical) level that do not compromise its productivity. In addition, seeking alternatives for prunning residues could increase the income of producers and ensure a sustainable destination for underutilized biomass. The objective of this work, in this way, is to characterize and compare the anatomy and chemical composition of the secondary vascular system of the stem of *Coffea arabica* cv. topázio, submitted to different fertilization levels, aiming to analyze how the possible differentiations found on secondary xylem, besides to verify the use of substances from cultive residues. For this, samples of *C. arabica* cv. Topázio MG 1190 were collected in the experimental area of the coffee sector from Universidade Federal de Lavras (UFLA), from one experiment implemented in the year of 2010, which undergone low-pruning in the year of 2015. The experiment received a fertilizing variation in 10, 40, 70, 100, 130 and 160%, according to the nutritional recommendation indicated for *C. arabica*. Orthotropic and plagiotropic stem samples were characterized as anatomy and chemistry (structural and non-structural) of secondary vascular system of the stem, following the usual methodology, adapted to the species. Anatomical differences were observed between the treatments and stem parts and in xylem functionality in plagiotropic stem. Anatomical differences in xylem probably occurred to answer to functionality, showing the plasticity of the tissue on the specie. Differentiated amounts of structural and non-structural chemical components were also found in pruning residual orthotropic stem, in the different treatments. The results revealed not only the plasticity of the coffee stem when submitted to differentiated fertilization, but also pointed out its potential in the reuse of chemical compounds when prunning is conducted.

Keywords: Coffee cultivation. Coffee tree anatomy. Pruning.

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1 INTRODUÇÃO

O café é uma bebida apreciada em todo o mundo, sendo uma peça cultural na história de muitos países, seu consumo independe de classes sociais. Os cafeeiros compreendem muitas espécies do gênero *Coffea* L. (Rubiaceae), destacando-se no Brasil, *Coffea arabica* L. e *C. canephora* Pierre, conhecidas no mercado como: arábica e conilon/robusta, respectivamente (VIEIRA, 2017).

Coffea arabica L., nesse sentido, responde por mais de 3/4 de toda a área em cultivo (formação e produção) no país, sendo o estado de Minas Gerais o maior produtor nacional (COMPANHIA NACIONAL DE ABASTECIMENTO, 2018).

Na safra de 2018, estima-se que a cultura do cafeeiro tenha movimentado cerca de R$ 18,6 bilhões, sendo um dos responsáveis por manter o crescimento do segmento primário do produto interno bruto (PIB) do agronegócio brasileiro, fato ocorrido principalmente devido à alta do dólar, aumento da demanda doméstica e elevação externa (BARROS et al., 2018).

No que diz respeito à produtividade de café no Brasil, os números são considerados, no geral muito bons, entretanto o país precisa buscar ainda mais estratégias de otimização de produção, visando assegurar a sua competitividade frente a outros países produtores (INTERNATIONAL COFFEE ORGANIZATION, 2018; VIEIRA, 2017). A otimização nesse caso pode ocorrer por meio da diminuição dos custos de produção. De acordo com a Companhia Nacional de Abastecimento (2017), os custos com fertilizantes podem representar até 24,73% do custo operacional das lavouras de café.

Não obstante, utilização de cultivares de *Coffea arabica* altamente produtivos faz com que a demanda por nutrientes aumente, tendo em vista que as principais áreas produtoras de café possuem solos com níveis baixos de nutrientes, sendo a fertilização uma etapa essencial para o sucesso do cultivo (VIEIRA, 2017).

A relevância dos nutrientes está associada à muitos processos ocorrentes nas plantas, seja por sua função bioquímica ou fisiológica, estando portanto, diretamente relacionados com a qualidade e a produtividade apresentada, tendo em vista que eles podem alterar a sua conformação estrutural e influenciar a suscetibilidade das espécies frente às condições adversas (DICKISON, 2000).

A adaptabilidade da plantas às diversas condições ambientais é também dependente das modificações ocorrentes no caule, principalmente porque é ele que conecta os vários órgãos da

planta, transportando substâncias que possibilitam a comunicação celular a longa distância (SANCHEZ; NEHLIN; GREB, 2012).

Embora a estrutura do caule de *Coffea arabica* já tenha sido descrita por Dedecca (1957), estudos que contemplem as possíveis diferenças provocadas pela variação nutricional no sistema vascular secundário necessitam ser desenvolvidos. E para mais, estudos como este auxiliam na compreensão das estratégias delineadas pela espécie, as quais influenciam na sua produtividade, tão importante para a economia do país.

Além disso, à qualidade do café são atribuídas inúmeras substâncias (AHSAN; BASHIR, 2019). Os compostos presentes no cafeeiro são frequentemente associados às folhas (RAMIRO, 2003), flores (MAYER; CARMELLO-GUERREIRO; MAZZAFERA, 2013) e frutos (GOULART, 2007), faltam informações mais aprofundadas sobre sua ocorrência no caule. Tais informações contribuiriam para ampliar a visão de como os compostos estruturais, como celulose, hemicelulose e lignina (metabólitos primários) e metabólitos secundários podem ser influenciados pelas mudanças decorrentes na adubação.

Dessa forma, objetiva-se com esse trabalho comparar a anatomia, com foco na estrura e funcionalidade do xilema secundário, bem como determinar e comparar a composição química do sistema vascular secundário do caule de *Coffea arabica* L. cv. topázio submetido à níveis de adubação distintos e que havia passado por poda do tipo recepa baixa, visando, com isso: a) analisar como as possíveis diferenciações encontradas na anatomia do caule, com ênfase ao xilema secundário, dos indivíduos poderiam influenciar sua produtividade e b) Como os resíduos gerados pela recepa poderiam ser aproveitados na recuperação de produtos químicos ou possível geração de novos produtos.

2 REFERENCIAL TEÓRICO

2.1 Café: contexto histórico e mercado

A história de cultivo do cafeeiro é milenar. Na Etiópia se deu o descobrimento e início do consumo como estimulante por meio de uma polpa doce, macerada, misturada em banha ou como suco fermentado no ano de 575 d.C. (VIEIRA, 2017). Mesmo assim, é contado que os etíopes desconheciam todo o potencial do café, que foi percebido pelos árabes, quando levado à região da "Arábia Feliz" (atual Iêmen) passando a ser cultivado por volta do ano 1000 d.C. (GALETI, 2004).

O consumo coletivo do café, iniciado nas *Kaveh Kanes* (cafeterias), na cidade de Meca (Arábia Saudita), e, mais tarde em países da Europa, fez com que o produto fosse popularizado e sua demanda se elevasse, com consequente interesse, por parte dos países em produzir (GALETI, 2004).

O cafeeiro arábica (*Coffea arabica*) foi então introduzido no Brasil da Guiana Francesa, primeiramente no Pará, no ano de 1727, espalhando-se então para o Maranhão e estados vizinhos, chegando na Bahia por volta de 1770, no Rio de Janeiro em 1774, nos Estados de São Paulo e Minas Gerais em 1825 e no Paraná em 1920 (VIEIRA, 2017).

A partir daí, houve um grande desenvolvimento no cultivo de "café" no país, ocupando posição de destaque na oferta mundial logo na primeira metade do século XX (TOSI; FALEIROS, 2017), e permanecendo durante muitas décadas como sendo o principal produto de exportação (SAKON et al., 2012).

A produção de café no Brasil, hoje se dá pelo do cultivo de duas espécies, *Coffea arabica* e *C. canephora*, a partir de diferentes cultivares, sendo as indicações de cultivo variáveis entre as regiões produtoras (FERRÃO et al., 2005; VIEIRA, 2017).

Ao longo do histórico de produtividade do café, a produção brasileira oscilou, devido a variação do preço no mercado mundial, condições climáticas desfavoráveis e mão-de-obra com custo elevado (VIEIRA, 2017).

Segundo a Companhia Nacional de Abastecimento (2018), a área plantada de cafeeiro arábica no Brasil apresenta pequenas variações decorrentes dos ciclos plurianuais de preço do café, porém tem se mantido estável por dez anos, enquanto que para as áreas em produção, dependentes dos ciclos de bienalidade do café, ora com produtividade baixa, ora com produtividade alta é observada maior variação.

No ano de 2018, devido a safra ser de bienalidade com produtividade alta, a área de produção de "café", aumentou cerca de 1,6%, passando de 1.481.541 para 1.505.201ha, enquanto que a produtividade subiu aproximadamente 27,4%, saindo de 23,1 para 29,5 sacas/ha; semelhantemente, a produção de café foi elevada a uma taxa de quase 29,4%, resultando numa mudança de 34.249,1 para 44.333,4 mil sacas beneficiadas (COMPANHIA NACIONAL DE ABASTECIMENTO, 2018).

Ainda assim, de acordo com Barros et al. (2018), mesmo apresentando queda em relação à 2017, os valores internos do café subiram em maio de 2018, impulsionados pela alta do dólar, aumento da demanda doméstica e elevação externa.

Já no mercado mundial, no ano de 2017, a importação de café esteve na ordem de 126 milhões sacas, com a União Europeia respondendo por quase 63% de todo o volume; as exportações (safra de 2017/18), por outro lado, foram de aproximadamente 113 milhões sacas, com uma queda do valor em cerca de 1,46% em relação à safra anterior, os principais exportadores foram Brasil, Vietnã e Colômbia, representando 25,68, 23,90 e 10,80%, respectivamente do valor total exportado (INTERNATIONAL COFFEE ORGANIZATION, 2018).

Mesmo estando entre os líderes de exportação e possuir um bom histórico de produtividade do café, o Brasil necessita continuar investindo em estratégias que visem otimizar a produção (principalmente no que diz respeito a diminuição dos custos) com vistas a se manter competitivo no mercado mundial.

2.2 O gênero *Coffea* L.

Coffea L. pertence à família Rubiaceae, reúne cerca de 103 espécies (DAVIS et al., 2006) em dois subgêneros *Coffea* subgen *Coffea* e *Coffea* subgen. *Baracoffea* (J.-F. Leroy) J.-F. Leroy e tem como principais centros de origem a África e Madagascar (AGUIAR, 2005; DAVIS et al., 2006; DAVIS; RAKOTANASOLO, 2008).

No entanto, levando-se em consideração a relação morfológica e filogenética que *Coffea* possui com *Psilanthus* Hook.f. (Rubiaceae), o número de espécies, assim como a caracterização morfológica, são ampliados e a distribuição é redesenhada, incluindo a Ásia tropical e a região da Australásia (DAVIS et al., 2011).

A morfologia da flor, nesse caso, é de grande relevância na distinção entre os dois gêneros, já que *Coffea* apresenta flores com anteras e estigmas proeminentes e estilete alongado,

enquanto *Psilanthus* apresenta anteras e estigmas inclusos, que não passam o tubo formado pela corola, além de estilete curto (GUERREIRO-FILHO et al., 2007).

As espécies de *Coffea* tem como habitat natural as florestas úmidas, ainda que algumas espécies ocorram em áreas de floresta estacional decidual (MAURIN et al., 2007). As espécies mais importantes de *Coffea*, no âmbito econômico, são *C. arabica*, *C. canephora* e *C. liberica* W. Bull ex Hiern, por serem as espécies empregadas no cultivo do "café", o que é possibilitado devido as altas taxas de produção e de certo modo, facilidades na colheita que elas oferecem (MAURIN et al., 2007; VIEIRA, 2017).

2.3 *Coffea arabica* L.

A espécie *Coffea arabica* L. foi primeiramente classificada como *Jasminum arabicum* Jussieu, e, somente no ano de 1737, foi incluída por Linnaeus no gênero *Coffea* (SOUZA et al., 2015); dados filogenéticos suportam a hibridização de *C. arabica* a partir de *C. canephora* e *C. eugenioides* (MAURIN et al., 2007), estando ela, atualmente incluída na seção *Eucoffea*, subseção *Erythrocoffea* (GUERREIRO FILHO et al., 2007).

Originada na região da Etiópia, *Coffea arabica* em sua formação mais primitiva, ocorre na altitude de 1200 a 2000m, onde a precipitação anual está por volta de 1200 e 2000 mm e a temperatura média, 16,5 e 22,5º C (VIEIRA, 2017).

A distribuição do cafeeiro arábica está relacionada com sua história de cultivo e por isso ele pode ser encontrado em diferentes países, com ocorrência confirmada em pelo menos 26, pertencentes aos continentes africano, americano e asiático (TROPICOS, 2018).

Coffea arabica é uma espécie perene, tetraploide (2n = 4x) (VIEIRA, 2017), caracterizada por seu porte arbustivo e lenhoso (FERREIRA; SANTOS; CHAVES FILHO, 2014), com copa, geralmente em formato cilíndrico, que pode apresentar ramificações laterais (ALVES, 2007).

Segundo Alves (2007), a parte aérea de *Coffea arabica* originalmente é composta por um ramo ortotrópico principal e ramos plagiotrópicos primários e secundários, formados a partir de gemas cabeça-de-série e seriadas, que estão localizadas nas axilas foliares.

As folhas mais desenvolvidas são de formato elíptico, com bordas onduladas e lâminas brilhantes de tonalidade verde escura na face adaxial e clara na abaxial, apresentam domácias glabras e tem comprimento variável (ALVES, 2007; GAMA et al., 2017; VIEIRA 2017). A

estrutura anatômica também é variável e pode sofrer modificações como resposta à diversos fatores como a disponibilidade hídrica, a luminosidade e a adubação (GRISI et al., 2008).

As axilas florais do cafeeiro produzem gemas apenas uma vez e suas inflorescência do tipo glomérulo axilar portam flores brancas com cinco pétalas e sépalas, dois pares de bractéolas, pedicelo curto (ALVES, 2007; VIEIRA 2017), disco nectarífero e coléteres que secretam diversos compostos (MAYER; CARMELLO-GUERREIRO; MAZZAFERA, 2013).

Os frutos, a depender do estágio de maturação recebem o nome de chumbinho, verde-cana ou cereja e se caracterizam por serem drupas elipsoides de coloração variada, comumente, amarelas ou vermelhas, que possuem exocarpo delgado, mesocarpo carnoso e endocarpo fibroso; as sementes, por sua vez, são oblongas, plano-convexas (ALVES, 2007; VIEIRA 2017) e possuem endosperma esverdeado contendo carboidratos, proteínas, óleos, ácido clorogênico, entre outros compostos (GOULART et al., 2007), e espermoderma, película prateada, aderida ao endosperma (ALVES, 2007).

2.4 Anatomia do sistema vascular secundário do caule do cafeeiro

O sistema vascular é constituído por dois tecidos complexos denominados xilema e floema, que possibilitam a distribuição de seivas por todo o vegetal; o desenvolvimento desse sistema permitiu que as plantas pudessem atingir maior porte e ocupar diversos ambientes (CASTRO; PEREIRA; PAIVA, 2009).

Em se tratando de sistema vascular secundário no caule, o câmbio vascular, um meristema lateral, é responsável pela produção de novas células que integrarão o xilema e o floema secundários, a partir de divisões periclinais; formando, nesse sentido, xilema para o interior e floema para a periferia, em relação à zona cambial (ESAU, 1974).

De acordo com Metcalf e Chalk (1957), o gênero *Coffea* apresenta o sistema vascular variando quanto à disposição entre as espécies, além disso, no floema é possível encontrar agrupamento de fibras e de células secretoras; células taniníferas ainda são relatadas, assim como cristais que podem ser achados como aglomerados, ráfides ou apenas areia cristalífera (METCALF; CHALK, 1957).

No que diz respeito ao sistema vascular secundário do caule do cafeeiro, Dedecca (1957) estudando *Coffea arabica*, observou que a zona cambial era estratificada e os raios libero-lenhosos podiam ser uni ou bisseriados com 8 a 10 células; o xilema secundário, no que lhe concerne, apresentou elementos de vaso com placas de perfuração simples, paredes laterais com

pontoações alternadas, com comprimento variando de 700 a 900 µm e diâmetro de 35 a 40 µm. O floema secundário e a zona cambial nesse estudo foram pouco descritos.

2.5 Relação entre nutrição mineral e anatomia do sistema vascular

Os elementos minerais essenciais são responsáveis pelo controle de vários processos que ocorrem nas plantas, e desta forma, condições de deficiência ou excesso desses nutrientes podem provocar transformações visíveis na química e anatomia delas, como resultado da modificação metabólica causada (DICKISON, 2000; TAIZ et al., 2017).

De acordo com Dickison (2000), a porcentagem de tecido vascular, córtex e medula está diretamente relacionada aos níveis de cálcio, que também tem influência sobre a diferenciação de células parenquimáticas; o autor ainda destaca o papel do boro na manutenção da capacidade meristemática do câmbio vascular, chamando atenção para necroses que podem ser estendidas ao xilema.

Ademais, o papel do nitrogênio tem sido demonstrado por meio de nitrato de amônio, onde plantas submetidas a esse composto, apresentaram aumento na proporção de xilema e consequente condutividade, possivelmente relacionados com a regulação da expressão de genes no xilema secundário (HACKE et al., 2010).

Ainda sobre a regulação e fluxo de nutrientes minerais no xilema, Metzner et al. (2010), em estudo de *Phaseolus vulgaris* L. (Fabaceae), observaram que o parênquima os vasos estabelecem trocas rápidas e estáveis de magnésio, potássio e cálcio, principalmente por via apoplástica, ao contrário da medula, floema e câmbio, onde as trocas foram mais lentas.

Experimentos tem mostrado que o suprimento inadequado de nitrogênio levou a uma redução do xilema e incremento de floema, enquanto que uma relação direta entre o suprimento de fósforo e o incremento de formação de xilema secundário, bem como o tamanho de elementos de vaso pode ser observada (FROMM; LAUTNER, 2016).

A fertilização com potássio, cálcio e magnésio pode aumentar as divisões periclinais no câmbio vascular; tendo sido observado, inclusive, que a adubação com potássio tem levado mais ao desenvolvimento de elementos de vaso em comparação com as fibras jovens (FROMM; LAUTNER, 2016). Tais elementos de vaso também apresentam tamanho significativamente aumentado devido a relação desse nutriente com a expansão celular (FROMM; LAUTNER, 2016).

O cálcio é requerido não somente para compor a parede celular, mas para garantir a integridade da membrana celular e atuar como um “mensageiro” para a ocorrência de divisão

celular em resposta à sinais ambientais, desta forma, plantas deficientes em cálcio têm demonstrado redução em incremento de xilema secundário, comprimento de elementos de vaso e fibras, no tamanho da zona cambial e carregamento do floema (FROMM; LAUTNER, 2016).

Cuzzuol et al. (2013), em estudo de *Paubrasilia echinata* (Lam.) Gagnon, H.C. Lima & G.P. Lewis (anteriormente conhecida como *Caesalpinia echinata* Lam.) (Fabaceae) (LEWIS, 2015), observaram que a variação da nutrição de nitrogênio, fósforo e potássio levou a alterações na frequência e diâmetro de elementos de vaso, comprimento e espessura de parede de fibras e acumulação diferenciada de amido nos parênquimas (axial e radial). A variação no suprimento de macronutrientes também tem evidenciado modificações na composição da parede celular (FROMM; LAUTNER, 2016; CUZZUOL et al., 2013).

2.6 Efeito dos nutrientes em *Coffea arabica* L.

Os nutrientes citados como indispensáveis ao bom desenvolvimento das principais cultivares de *Coffea arabica*, são nitrogênio, fósforo, potássio, cálcio, magnésio, enxofre, boro, cobre, ferro, manganês e zinco (VIEIRA, 2017). A ordem de importância, assim como a quantidade exigida de cada elemento é modificada de acordo com a idade e as necessidades de vegetação e produção (VIEIRA, 2017).

Garcia Júnior et al. (2003) afirmam que a deficiência nutricional está entre as principais causas da redução da qualidade e produtividade do cafeeiro. A desordem nutricional pode então levar a uma série de distúrbios e desenvolvimento das principais doenças relatadas para o cafeeiro, haja vista que aumenta sua suscetibilidade (MESQUITA et al., 2016).

O balanço nutricional adequado, por meio da interação entre diversos nutrientes tem sido relatado como peça-chave para uma boa resposta das plantas de café à doenças. Por exemplo, o desequilíbrio entre nitrogênio e potássio, podem favorecer o desenvolvimento da *Cercospora coffeicola* Berk & Cook (Mycosphaerellaceae), fungo causador da cercosporiose (MESQUITA et al., 2016), cujo progresso de lesão é também influenciado por meio da interação entre potássio e cálcio (GARCIA JÚNIOR et al., 2003).

Doses elevadas de nitrogênio e potássio, conforme Pérez et al (2017), podem aumentar a incidência de mancha areolada, causada pela bactéria *Pseudomonas syringae* pv. *garcae* Young, Dye & Wilkie (Pseudomonadaceae). Ainda, a mancha areolada no cafeeiro, pode ter a severidade reduzida com a combinação de cálcio e potássio em diferentes concentrações (CARVALHO, 2016).

Diferentes doses de potássio aplicadas em *Coffea arabica*, influenciam teores de outros nutrientes (N, K, Ca, S e B) e reduzem tanto a incidência quanto a severidade da mancha de Phoma, provocada pela *Phoma tarda* (R. B. Stewart) H. Verm (Didymellaceae), evidenciando a importância da nutrição mineral balanceada para as plantas de café (LIMA et al., 2010).

2.7 Composição química de plantas de *Coffea arabica*

Os nutrientes minerais influenciam na qualidade do café por sua função no metabolismo do cafeeiro e pelo acúmulo de substâncias químicas que compõe o aroma e o sabor da bebida (MARTINEZ et al., 2014).

No que tange a composição química das plantas de cafeeiro, esta pode ser dividida em estrutural e não estrutural, sendo a primeira correspondente à fração de celulose, hemicelulose e lignina, enquanto que a segunda engloba os extrativos e as cinzas.

Os valores para holocellulose (celulose e hemicelulose, juntas) em cultivares de *Coffea arabica*, tendem a estar entre 50,63 e 61,48% a depender do sistema de cultivo; de forma semelhante, a espécie também investe em um elevado quantitativo de lignina, que atinge até 32,35% do montante (LEITE et al., 2014; LEITE et al, 2015).

As cinzas, que correspondem à porção inorgânica, estão em diferentes cultivares de *Coffea arabica* em torno de 1,3% e os extrativos variam de 6,71 à 17,24 %(LEITE et al, 2015). Os extrativos constituem um amplo grupo de substâncias (ROY; PAKDEL; BROUILLARD, 1990), sendo produtos do metabolismo primário e secundário, são também conhecidos como metabólitos.

Os metabólitos, que podem ser gerados pelo cafeeiro (ou pelo metabolismo de qualquer planta) estão diretamente relacionados com seu crescimento e desenvolvimento, ou podem ser ativados pelo seu sistema de defesa como forma de garantir a capacidade de sobrevivência e propagação, possuindo, nesse sentido, grande relevância funcional, haja vista que permitem resposta à diversos estímulos ambientais (TAIZ et al., 2017).

Entre os metabólitos do cafeeiro, a cafeína figura entre os alcalóides mais conhecidos, presente em altas concentrações em *Coffea arabica*, sendo atribuídas a esse composto, propriedades alelopáticas, inseticidas e fungicidas (EVERT; EICHHORN, 2014).

Estima-se que o teor de compostos fenólicos nas folhas de *Coffea arabica* esteja em torno de 5%, considerado característico da espécie, representa um valor elevado em comparação com outras espécies (RAMIRO, 2003).

Os lipídios nos grãos de café (*Coffea arabica*) são encontrados em abundância nas células parenquimáticas endospérmicas, podendo assumir formato globular, distribuem-se

homogeneamente pelas células ou concentram em locais próximos a parede celular, cuja integridade garante a manutenção desses compostos tão importantes para a qualidade da bebida (GOULART et al., 2007).

Amiloplastídeos, fenóis e um exsudato contendo polissacarídeos, substâncias pécticas, alcaloides, proteínas e substâncias lipofílicas podem ser observados em células de coléteres em flores de *Coffea arabica* (MAYER; CARMELLO-GUERREIRO; MAZZAFERA, 2013).

Grãos de amido, ao que parece, também podem ser armazenados em células parenquimáticas da medula e radiais do xilema em caules ortotrópicos e plagiotrópicos (FERREIRA, SANTOS, CHAVES-FILHO, 2014). Dedecca (1957) encontrou cristais em formato prismático nas células de parênquima radial, determinando como uma característica de espécies pertencentes à seção *Eucoffea*.

No que se refere à terpenoides, alguns componentes encontrados em análise química das flores de *Coffea arabica* também foram localizados na bebida de café preparada, dos quais podem ser citados os monoterpenos voláteis: limoneno, linalol, α-terpienol e geraniol, mostrando que esses compostos responsáveis pelo aroma conseguem ser conservados mesmo após o processo de torrefação (AKIYAMA, 2008; EMURA et al., 1997; TERRA et al., 2013).

A identificação e a localização desses compostos no sistema vascular secundário do caule auxiliam na compreensão de fenômenos relativos à fisiologia do cafeeiro, principalmente aqueles voltados ao sistema de defesa e reserva para uso posterior na conversão de energia.

3 CONSIDERAÇÕES GERAIS

O cafeeiro (*Coffea arabica*) tem grande importância cultural e econômica para o Brasil e garantir sua produtividade significa assegurar a manutenção de *status* de maior produtor no cenário mundial. Embora seja muito estudado do ponto de vista agronômico e que hajam trabalhos com enfoque à botânica da espécie, faltam informações sobre as características anatômicas de cultivares produtivas, principalmente relacionadas à anatomia do xilema do caule, quando submetido à diferentes fatores ambientais, como a modificação na adubação, o que poderia ser de grande valia para compreender os níveis de adaptação do cafeeiro.

Também é pouco explorada a composição química do caule do cafeeiro, que possui uma vasta habilidade de síntese de compostos, comprovadamente presentes em outras partes da planta, como folhas, flores e frutos. A detecção de compostos químicos de interesse, poderá ressaltar o potencial para aproveitamento sustentável por inúmeras indústrias, quando da aplicação de técnicas culturais e indispensáveis, como a poda.

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ARTIGO 1 – MODELO PLANT AND SOIL JOURNAL

XYLEM STRUCTURE AND FUNCTIONALITY OF COFFEE TREE STEM UNDER DIFFERENT FERTILIZATIONS (VERSÃO PRELIMINAR)

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Abstract

Aim: The structure of stem is modified under different environmental conditions, in this sense, the objetive of this work was understand how secondary xylem anatomical characteristic vary along nutrients supply, besides to evaluted the impact of this on functional features, helping in the comprehesion of productivy phenomena of coffee crop.
Methods: Using ortotropic and plagiotropic stem samples, which were submitted to different levels of macronutrients fertilization, anatomical analysis were conducted, following usual methodology. Ten different characteristics for wood anatomy had been processed, encompassing axial and radial systems, also functionality features like vulnerability and mesomorphy index, as well as, theorical hydraulic conductivity were estimated, for each treatment and part stem
Results: The results showed variable behavior for anatomical characteristics along the treatments and stem parts, sometimes to mantain its functional feature (ortotropic stem), and other times to improve efficiency or safety in transport (plagiotropic stem).
Conclusions: Knowing xylem structure and functionality along fertilization, is possible to see coffee tree stem plasticity and limitations, which can be of great help in planning coffee cultive, avoiding loss with surplus fertilization.

Keywords: arabic coffee, plant nutrition, coffee productivity, stem anatomy.

Abbreviations

DV Diameter of vessels
FL Fiber lenght
FW Fiber width
Kh Theorical hydraulic conductivity
LW Lumen width
MI Mesomorphy index
RF Ray frequency
RL Ray lenght
RW Ray width
VF Vessel frequency
VI Vulnerability Index
VL Vessel length
WT Wall thickness

1. INTRODUCTION

Coffee productivity in Brazil is dictated, in part, by its biennial phenomenon that causes fluctuations in total production (Companhia Nacional de Abastecimento 2018). Biennial phenomenon in arabic coffee (*Coffea arabica* L., Rubiaceae), the most expressive in production (International Coffee Organization 2020), is a particular feature that consists in the period of time required for the plant to complete its phenological cycle, representing a competition of a physiological nature between vegetative and reproductive functions, observed in six phases (Camargo and Camargo 2001).

Biogical knowledge of coffee tree, this way, is essential for explain the coffee productivity. From a botanical point of view, *Coffea arabica* plant shoot is composed by an ortotropic main stem and primary and secondary plagiotropic stems, formed from axillary leaf buds (Alves 2007). The last completed work which described coffee stem anatomy was carried out in the 1950s (Dedecca 1957), since that works foccusing the anatomy of vegetative parts in coffee tree productive cultivars were made (Ferreira, Santos and Chaves Filho 2014), but do not detail all xylem characteristics.

Xylem is a system vascular tissue responsible for conduct water with nutrients, storage substances and give strength for organs (Ohtani et al. 2017). Being fundamental for growth, xylem provides substrates to form new cells. Present in stem, is continuous and also interconnect different part of plants, like roots and leaves (Kim et al. 2014), making it important to analyze the modify factors that could impact in plant development.

In plants used for its importance in cultive, like coffee tree (*Coffea arabica*), understand how its xylem structure and function is crucial in the establishment of factors related with high productivity (Gama et al. 2017), principally due to coffee tree cultivars demand a lot of nutrients and the productive regions have soils with nutritional deficiences (Vieira 2017).

Besides that, bearing in mind that many external factors could alter plant anatomy (Sett 2017), and consequently xylem structure, study if and how fertilization could change the vascular system anatomy, with focus in xylem, configure an important step in the delimitation of coffee tree structural limitations and helps to planning better which fertilization is most recommended, avoiding wastage in coffee cultive.

Fertilization can achieve until 24,73% in operational costs to maintain coffee cultivation in Brazil (Companhia Nacional de Abastecimento 2017). Being Brazil the major export country of coffee in world, followed by Vietnam and Colombia (Internacional Coffee Organization 2020), planning with emphasis to fertilization needs is important to reduce costs of production and favour brazilian economy, maintaining the competitivity of the country in global scenario and warrant the world coffee demand.

Although there are many information about modifications caused for abiotic factors in plant anatomy, lack data of coffee stem xylem plasticity when submitted to different environmental conditions, principally in varied fertilizing. Therefore, the aim of this study was analyze xylem anatomy of coffee tree stem when it was in different fertilizing levels, the impact of it in functional parameters of secondary xylem and presenting the characteristics of stem, which would had higher performance in productivity.

MATERIAL AND METHODS

1.1 Site characterization and sampling

The material of stem used were collected from *Coffea arabica* L. (cv. Topázio MG1190) tree, which composed an experiment located at Federal University of Lavras (Minas Gerais state, Brazil), situated on 21°14'06" S and 45°00'00" W, at 910 m elevation. Having the mean weather conditions: 1460 mm of rainfall and 20.4° C of temperature (Dantas, Carvalho, Ferreira 2007).

In addition, experiment comprised a randomized-completeblocks design were different fertilizing parameters for three macronutrients: nitrogen, phosphorus and potassium (N-P-K), being six treatments: T1, T2, T3, T4, T5 and T6, corresponding to 10, 40, 70, 100, 130 and 160 of percentual fertilizing (Figure 1), respectively, from the estabilished for Guimarães et al (1999) like basis fertilizing for coffee cultive, to know N (3 to 5 g of urea per plant), P (20 g of P_2O_5 per plant) and K (20 g K_2O per plant). Macronutrients were applied in fertigation system.

Fertigation system in the experiments was composed by a control unit (pumping system, sand filter and grille, fertilizers injector, pressure gauges and connections), main line of PVC tube (PN80), derivation lines of PVC tube (PN40), lateral lines with flexible tubes of polyethylene PN 40, drippers and regulators. Drippers (nominal flow: 3.8 $L.hour^{-1}$) were spaced every 30 cm in line. Fertigation control was conducted according climatologic data from meterologic station registered in specialized insitution.

The experiment was conducted in the years of 2010 to 2018, and at 2015 year, low-pruning technique was made, generating a new orthotropic stem. The material analyzed was this new orthotropic stem (with three years) and samples of vegetative and reproductive parts of the most productive plagiotropic stem (Figure 1). After collected, coffee stem samples were fixed with $FAA_{70\%}$ for 48 hours and maintened in alcohol in 70% concentration (Johansen 1940).

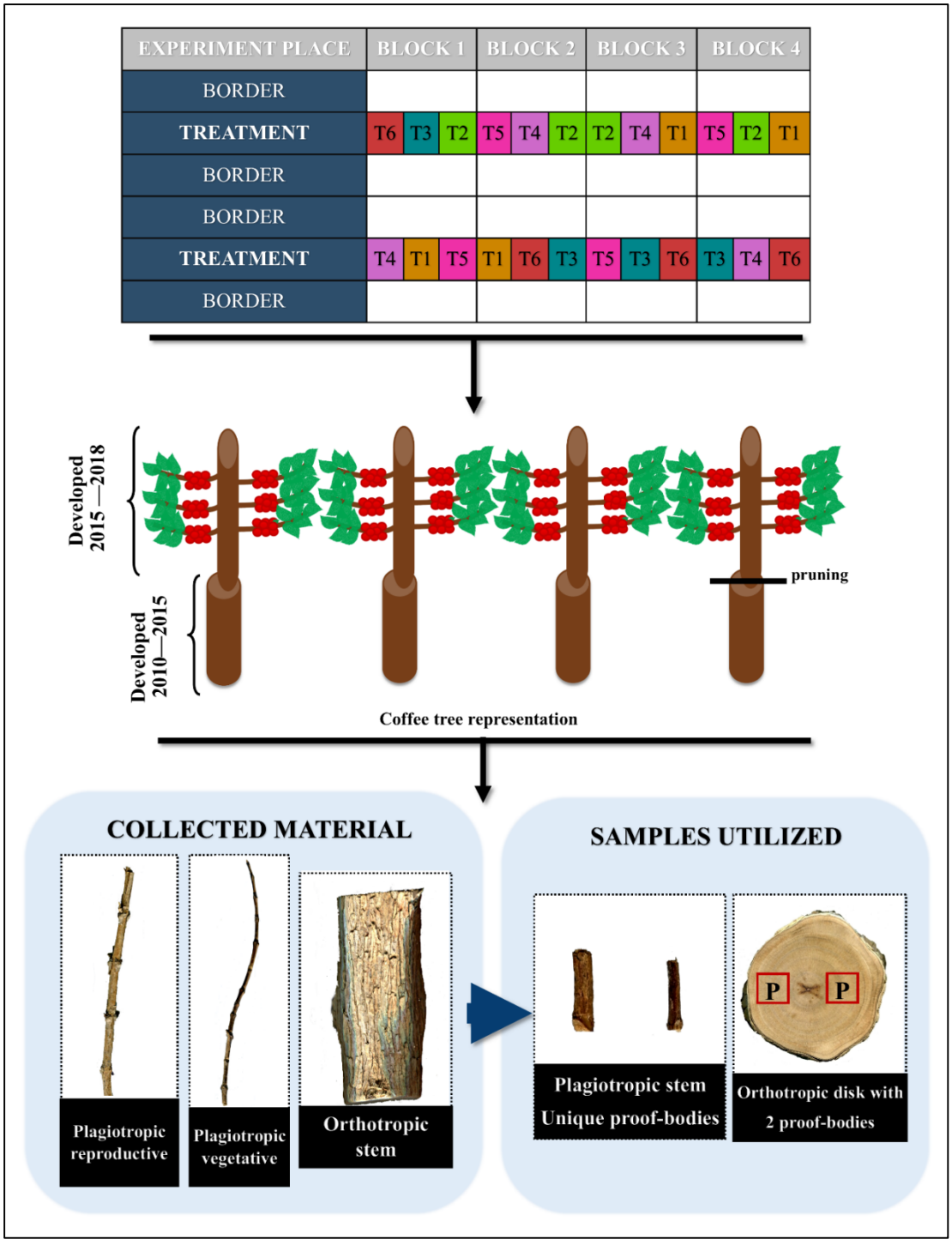

Figure 1- Experimental design and sampling used at analysis

1.2 Prepare of samples for anatomical tests

From ortotropic stem samples, were retired two disks with 5 cm height, while of plagiotropic stem were unfolded samples with aproximatedly 3 cm from the start of reproductive and vegetative regions. Proof-bodies were made in two points of median region of ortotropic samples and how the plagiotropic one were small, it was adopted like a unique proof-body (Figure 1).

Histological cuts were unfolded from proof-bodies in transverse and tangential sections, with 10 μm thickness, following to staining in alcahol safranin and usual increasing reagent sequence for slide assemble with Entellan®, an adapted method of the proposed by Siegloch and Marchiori (2015). To visualize xylem cells separeted, macerated technique also was conducted using longitudinal fragments of stem samples (Franklin 1945). After cell dissociation technique, temporary slides were assembled with glycerin and safranin stain.

1.3 Quantitative anatomy and functionality analysis

Histological sections and dissociated material were photographed using optical light microscope (Olympus BX41) with attached camera (Pixel link, PL A662). The images were processed with ImageJ software (1.45 version), where were extracted the follow anatomical characteristics: diameter of vessels, number of ray cells, vessel, fiber and ray lenght, ray and fiber width, fiber cell wall and lumen thickness and frequency per mm^2 for vessels and rays. Were utilized thirty mensureaments, per block and treatment. All anatomical xylem classification were made according IAWA (1989) and its adaptations by Coradin and Muñiz (1992).

The funcionality analysis led in consideration theorical hydraulic condutivity (Kh) by Hagen-Poiseuille, Vulnerability Index (VI) and Mesomorphy Index (MI) (Box 1).

Box 1 – Formulas of functional characteristics

FUNCTIONAL CHARACTERISTIC	***FORMULAS***	***REFERENCES***
Theorical hydraulic condutivity (Kh)	$Kh = (\pi \rho w / 128\eta) \times VD \times Dh^4$	Rungwattana and Hietz 2017
Vulnerability Index (VI)	$VI = \frac{D}{VD_2}$	Carlquist 1977, Scholz 2013
Mesomorphy Index (MI)	$MI = VI \times L_{ve}$	Carlquist 1977, Scholz 2013

Description: *Kh unit*: kg.m.MPa−1. s−1; η (viscosity of water at 20°C): 1.002 × 10−3 Pa; ρw (density of water) 998.2 kg/m3 at 20°C, *VD*: vessel density (m²); *Dh*= hydraulically weighted vessel diameter (m); *D*= vessel diameter (µm); VD_2= vessel density (per mm^2). L_{ve}= vessel element lenght.

2.4 Data analysis

The statistical analysis consisted in comparisons of characteristics values obtained during anatomical analysis (general characteristics and functionality) for all treatments, using *dbc* (randomized-blocks design) of *ExpDes.pt* package from the statistical software R (3.6.0 version) (R Core Team 2019) ANOVA test, followed by Shapiro-Wilk normality test and Tukey's LSD (Lowest Significant Difference) test, with 95% confidence interval were used.

2. RESULTS

2.1 Anatomical characteristics under different fertilizations

All anatomical characteristics presented a variable behavior along the treatments for ortotropic stem (Table 1). Diameter of vessels (DV) were high in T4 and T6 (adequate and upper nutrientes supply), but was low in T1 (the lowest fertilizing level). In turn, the major value for vessel lenght (VL) was seen in T1, differing statistically of other treatment. Ortotropic stem submitted to T1 also invested in number of vessels per mm² (VF), since was the highest one in this characteristic (Table 1).

Concerning to fibers, T1 presented longer fibers, which were thinner and lower lumen, exhibiting T2, wider fiber and lumen. Fiber wall thickness (WT) were high in T2 and T3, decreasing in T4 and T5, getting thick again in T6 (Table 1). Ray lenght (RL) and width (RW) means were lowest in T6, while T3 and T1 highlighted for present high values in these characteristics, respectively. Rays were much more frequents in T5, being lesser in T1 (Table 1).

In reproductive part of plagiotropic stem, diameter of vessels (DV) were high in T3, T4 and T6, and lowest in T1 and T2.Vessels were longest in T4, T3 and T1. Follow treatments: T2, T4 and T5 presented more vessels per mm² and T6 the lowest frequency (Table 2). In all fiber characteristics T6 was upper. In the same way, T5 only was not less in lumen width (LW), which were achieved for T1 (Table 2). Referring to rays, ray lenght were high in T3 and lowest in T5 and T6, while ray widht best values were in T1 and T5, being T5 which had less frequent rays. T4 showed thinner rays and T1 and T2 reached highest value to ray frequency (Table 2).

Differently of reproductive part, plagiotropic vegetative presented the highest value for diameter and length of vessels in T6, and lowest values for these characteristics could be seen in T2. Vessel frequency (VF) was high in almost all treatments, excepting T3 and T6.

In fibers T6 also had highest means for fiber lenght (FL) and lumen width (LW), having the highest values for fiber width (FW) and wall thickness (WT), T3. T1 had thinner fiber and lumen. Ray lenght and width were high in T3, presenting T6 and T4 lowest means for these features, respectively. T5 showed best frequency of rays and T4 the lowest value.

Establishing relations between ortotropic and plagiotropic stem, despite fertilization, is possible to see how diameter and frequency of vessels change along the plant shoot (Figure 2). Porosity duplicate from ortotropic to plagiotropic reproductive, and quadruple when ortotropic and plagiotropic in vegetative portion are compared (Table 2 to 4).

Radial system is also sensibly modificated along the stem. While ortotropic stem invested in longer and wider rays, plagiotropic stem, in reproductive and vegetative parts, have shorter and thinner, but much more frequent rays (Table 2 to 4).

2.2 Functionality analysis

Since the stem collected were at the same place and received water supply, the analysis of functionality was conducted considering fertilization like the modify factor. No functional characteristics analysed were modified for fertilization in ortrotropic stem (Table 4), although they were depending on DV, VL and VF, which were statistically different between the treatments (Table 1).

A distinct behavior were observed for plagiotropic stem. Plagiotropic reproductive in T1 highlighted from of others statistically, reaching highest values for VI and MI, but at the same time, were less efficient in conduction, getting the lowest Kh (Table 4). On the

other hand, in plagiotropic vegetative T3 achieved higher averages for VI and MI, and T2 the lowest ones. Once again for have highest means in VI and MI, T3 was not so able in conduction. T4, in this case, was the most functionally prepared to conduct, attested with high Kh (Table 4).

Table 1 – Anatomical characteristics of coffee tree ortotropic stem submitted to different fertilizations. DV: Diameter of vessels; VL: Vessel length; FL: Fiber lenght; FW: Fiber width; LW: Lumen width; WT: Wall thickness; RL: Ray lenght; RW: Ray width; VF: Vessel frequency; RF: Ray frequency. EVC (%): Experimental variation coefficient. SE: Standard error.

TREATMENT	ANATOMICAL CHARACTERISTICS									
	DV	VL	FL	FW	LW	WT	RL	RW	VF	RF
	µm								N/mm²	
T1	36,95* **c**	630,06 **a**	1250,97 **a**	21,18 **c**	8,63 **c**	6,27 **abc**	792,8 **a**	46,84 **a**	77,34 **a**	20,41 **c**
T2	38,58 **b**	570,05 **b**	1182,85 **c**	23,47 **a**	10,49 **a**	6,49 **a**	676,85 **b**	41,58 **bc**	56,25 **b**	22,22 **c**
T3	37,87 **bc**	577,24 **b**	1203,96 **bc**	23,45 **a**	10,37 **a**	6,54 **a**	822,06 **a**	40,37 **c**	61,20 **b**	26,22 **ab**
T4	42,97 **a**	585,02 **b**	930,79 **d**	21,36 **bc**	9,31 **b**	6,03 **bc**	643,08 **b**	41,53 **bc**	66,41 **ab**	25,15 **b**
T5	39,23 **b**	569,42 **b**	1177,80 **c**	21,79 **bc**	9,88 **ab**	5,95 **c**	569,09 **c**	44,14 **ab**	67,97 **ab**	27,57 **a**
T6	42,24 **a**	565, 25 **b**	1221,98 **ab**	22,21 **b**	9,5 **b**	6,36 **ab**	491,59 **d**	34,13 **b**	59,64 **b**	21,97 **c**
EVC (%)	15.97	20.77	15.26	16.91	28.27	22.4	38.06	32.62	37.86	20.95
SE	0.24	4.51	6.6	0.14	0.1	0.05	9.44	0.5	1.77	0.38

* Means followed by the same letter, in the column, do not differ from each other at 5% probability by Tukey's test.

Table 2 – Anatomical characteristics of coffee tree plagiotropic (reproductive) stem submitted to different fertilizations. DV: Diameter of vessels; VL: Vessel length; FL: Fiber lenght; FW: Fiber width; LW: Lumen width; WT: Wall thickness; RL: Ray lenght; RW: Ray width; VF: Vessel frequency; RF: Ray frequency. EVC (%): Experimental variation coefficient. SE: Standard error.

TREATMENT	ANATOMICAL CHARACTERISTICS									
	DV	VL	FL	FW	LW	WT	RL	RW	VF	RF
	µm								N/mm²	
T1	27,07* **c**	702,30 **a**	1011,56 **c**	17,42 **d**	6,51 **c**	5,46 **cd**	521,31 **bc**	34,79 **a**	159,64 **ab**	70,57 **a**
T2	26,30 **c**	630,79 **b**	1040,43 **b**	19,07 **bc**	7,08 **bc**	6,0 **b**	618,42 **b**	29,53 **bc**	170,31 **a**	71,35 **a**
T3	31,91 **a**	685,35 **a**	1062,05 **b**	19,27 **b**	6,91 **bc**	6,18 **ab**	627,35 **a**	34,31 **ab**	159,64 **ab**	59,37 **b**
T4	30,98 **a**	714,58 **a**	1038 **bc**	18,26 **cd**	7,11 **b**	5,58 **c**	557,29 **b**	28,17 **c**	165,89 **a**	60,68 **b**
T5	29,89 **b**	639,85 **b**	970,13 **d**	17,41 **d**	7,04 **bc**	5,19 **d**	503,82 **c**	34,82 **a**	178,65 **a**	61,98 **b**
T6	31,65 **a**	646,84 **b**	1221,98 **a**	22,21 **a**	9,5 **a**	6,36 **a**	491,59 **c**	34,13 **ab**	139,84 **b**	65,37 **ab**
EVC (%)	16.41	20.18	13	19.73	34.36	23.91	37.54	64.3	21.82	26.06
SE	0.18	5.04	5.12	0.14	0.09	0.05	7.74	0.78	3.27	0.98

*Means followed by the same letter, in the column, do not differ from each other at 5% probability by Tukey's test.

Table 3 – Anatomical characteristics of coffee tree plagiotropic (vegetative) stem submitted to different fertilizations. DV: Diameter of vessels; VL: Vessel length; FL: Fiber lenght; FW: Fiber width; LW: Lumen width; WT: Wall thickness; RL: Ray lenght; RW: Ray width; VF: Vessel frequency; RF: Ray frequency. EVC (%): Experimental variation coefficient. SE: Standard error.

TREATMENT	ANATOMICAL CHARACTERISTICS									
	DV	VL	FL	FW	LW	WT	RL	RW	VF	RF
	μm								N/mm²	
T1	26,76* **bc**	583,46 **bc**	851,64 **b**	16,02 **d**	6,26 **c**	4,88 **c**	438,77 **b**	26,60 **bcd**	296,61 **a**	73,44 **b**
T2	24,55 **d**	546,55 **d**	855,01 **b**	17,98 **ab**	7,40 **b**	5,29 **ab**	428,71 **b**	25,98 **cd**	300,52 **a**	67,19 **bc**
T3	27,88 **a**	592,64 **b**	875,15 **ab**	18,63 **a**	7,66 **ab**	5,48 **a**	577,45 **a**	33,02 **a**	219,01 **b**	66,41 **bc**
T4	27,54 **ab**	597,13 **ab**	821,54 **c**	16,94 **c**	7,40 **b**	4,77 **c**	430,62 **b**	24,64 **d**	311,46 **a**	62,76 **c**
T5	25,92 **c**	565,40 **cd**	810,36 **c**	17,43 **bc**	7,22 **b**	5,11 **bc**	357,92 **c**	27,38 **bc**	294,53 **a**	87,5 **a**
T6	27,95 **a**	622,91 **a**	898,22 **a**	18,27 **ab**	8,2 **a**	5,03 **bc**	345,14 **c**	28,95 **b**	240,36 **b**	66,93 **bc**
EVC (%)	15.94	18.84	13.89	20.19	32.36	28.27	36.71	36.81	27.88	24.51
SE	0.16	4.11	4.41	0.13	0.09	0.05	5.88	0.38	5.21	1.25

*Means followed by the same letter, in the column, do not differ from each other at 5% probability by Tukey's test.

Table 4 – Functional characteristics of coffee tree stems (ortotropic and plagiotropic) submitted to different fertilizations. VI: Vulnerability Index; MI: Mesomorphy index; Kh: Theorical hydraulic conductivity.

TREATMENT	FUNCTIONAL CHARACTERISTICS								
	Ortotropic stem			Plagiotropic reproductive stem			Plagiotropic vegetative stem		
	VI	MI	Kh (kg.m MPa^{-1} s^{-1})	VI	MI	Kh (kg.m MPa^{-1} s^{-1})	VI	MI	Kh (kg.m MPa^{-1} s^{-1})
T1	0,5* **n.s**	310,88 **n.s**	7,01 $x10^{-8}$ **n.s**	0,9 **a**	564,76 **a**	3,89 $x10^{-8}$ **b**	0,09 **bc**	55,37 **bc**	1,92 $x10^{-7}$ **ab**
T2	0,73 **n.s**	410,23 **n.s**	5,29 $x10^{-8}$ **n.s**	0,16 **b**	102,65 **b**	1,08 $x10^{-7}$ **a**	0,08 **c**	46,82 **c**	1,77 $x10^{-7}$ **abc**
T3	0,65 **n.s**	381,66 **n.s**	5,63 $x10^{-8}$ **n.s**	0,21 **b**	145,22 **b**	1,24 $x10^{-7}$ **a**	0,13 **a**	76,19 **a**	1,48 $x10^{-7}$ **c**
T4	0,65 **n.s**	380,06 **n.s**	6,97 $x10^{-8}$ **n.s**	0,19 **b**	137,36 **b**	1,25 $x10^{-7}$ **a**	0,09 **bc**	54,16 **c**	2,08 $x10^{-7}$ **a**
T5	0,63 **n.s**	357,89 **n.s**	6,47 $x10^{-8}$ **n.s**	0,17 **b**	111,09 **b**	1,29 $x10^{-7}$ **a**	0,09 **bc**	50,52 **c**	1,86 $x10^{-7}$**ab**
T6	0,72 **n.s**	404,91 **n.s**	1,86 $x10^{-2}$ **n.s**	0,23 **b**	146,67 **b**	1,08 $x10^{-7}$ **a**	0,12 **ab**	73,03 **ab**	1,64 $x10^{-7}$ **bc**

*Means followed by the same letter, in the column, do not differ from each other at 5% probability by Tukey's test. **n.s**: not significant.

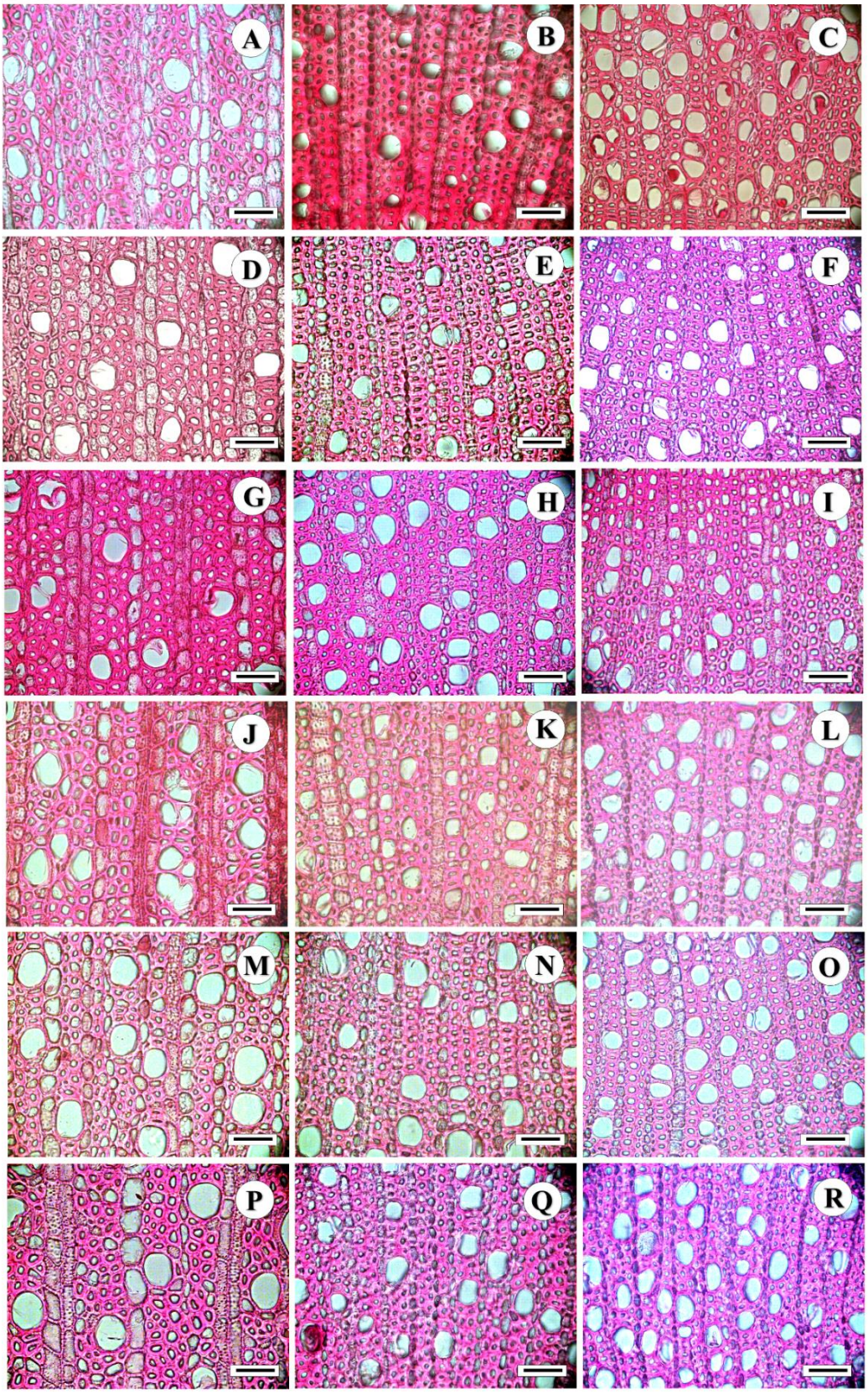

Figure 2 – Secondary xylem aspect of coffee tree ortotropic and plagiotropic stem submitted to different fertilizations (cross section). A, D, G, J, M, P: Ortotropic stem; B, E, H, K, N, Q: Plagiotropic stem, reproductive part; C, F, I, L, O, R: Plagiotropic stem, vegetative part. A to C: **T1**; D to F: **T2**; G to I: **T3**; J to L: **T4**; M to O: **T5** and P to R: **T6**. Scale bar: 50µm.

3. DISCUSSION

Diameter of vessels (DV) were expected to be high with fertilization increase, how was seen in ortotropic and plagiotropics stem, since fertilization based in elevated amounts of macronutrients, like nitrogen, tends to increase the diameter of vessels (Hacke et al. 2010). It is worth to point that, even if T5 were amongst upper macronutrients supply, its DV was one of the lowest. Calling attention for nutrients interactions inside plants which are complex. Moreover, concentration of one nutrient can inhibit or reduce of the other (Fageria 2001), becoming it hard to predict an ascendent behavior, as long as, fertilization is added.

Another factor with variations was frequency of vessels. Vessels were much more frequents in ortotropic and plagiotropic stem when diameter was low. This is a known strategy for try to mantain the flux of water and nutrients, adapting the xylem structure according the demand (Queiroz-Voltan et al. 2014) and mantaing also the xylem functionality, as could be seen when functional characteristics did not differ between the treatments, in ortotropic stem (Table 4).

Similar adaptation behavior could be observed in *C. arabica* cv. Topázio MG 1190 leaves, when plants were submitted to various levels of N-P-K fertilization; leaves adjusted its anatomy to attend gas exchange and coffee productivity (Gama et al. 2017).

Alter vessel frequency also is an approach to get the transport more safe (Carlquist 2001; Dickison 2000; Ribeiro and Barros 2006), but it, at the same time, can let to less efficient transport, as were could be comproved when, in plagiotropic stem, the treatments which had the highest values for VI and MI, had lowest Kh either, being viewed na inverse relations (Table 4).

The more complete work leading in consideration some xylem characteristics of stem was described by Dedecca (1957), his description pointed *C. arabica* cv. typica cramer having diameter of vessels from 35 to 40 µm and vessel length between 700 and 900 µm, comparing with *C. arabica* cv. Topázio MG 1190, in exceptions of T1 and T4 of plagiotropic reproductive, it showed decreased in vessel length.

The difference of N-P-K supply, besides modify frequency and diameter of vessels from *Paubrasilia echinata* (Lam.) Gagnon, H.C. Lima & G.P. Lewis (known

before as *Caesalpinia echinata* Lam.) (Fabaceae) (Lewis 2015), varied its fiber lenght and wall thickness too (Cuzzuol et al. 2013). For *Coffea arabica* cultivar analysed, fiber characteristics behavior were different in treatments, in ortotropic and plagiotropic stem. Decreased wall thickness in T4 and T5 of ortotropic stem indicate probable fast growing of tissue, while in plagiotropic stem the highlight of T6 in almost all fiber characteristics could be explained by surplus fertilization, which caused celular growing.

In literature, there is no informations about how xylem radial system is affected under different fertilizations. Ray lenght, width and frequency presented variation along treatments and stem parts, this could be another attempt of structural adjustment to fit all celular types in same space or invest in storage places if plant required great amounts of reserve substances. In the last case, it would be particularly interesting to *Coffea* productive cultivars with high energetic demand for the growth of tissues.

Concerning to functional features, vulnerability and mesomorphy index, VI and MI, stablished by Carlquist (1977), indicate the risks in transport, caused for cavitation and embolism, when the structure is modified, based principally in vessel characteristics. The classification is made this way: When VI is below 1,0, xylem is adapted for safe tranport, being commonly found in species present in water-restriction environment, in contrast, VI above 3,0 is characteristic of mesic environment (Scholz et al. 2013). In turn, MI below 30 indicate true xeromorphy condition and when it is next to 200 or upper this value, xylem is mesomorphic (Scholz et al., 2013).

Xylem in ortotropic and plagiotropic stem is consider, in general, mesomorphic and well-adapted for safe transport, independent of fertilization (Table 4), with some differences on its index degree. While fertilization could not alter VI and MI of ortotropic stem as a whole, in plagiotropic reproductive T1 highlight from the other been the most safe for conduct, on the other hand, analysing VI and MI for plagiotropic vegetative, T3 has more safety (Table 4). Comparing with results found by Deddeca (1957), Topázio cultivar decreased the vessel lenght in almost treatments, it occurs probably to strenghten transport efficiency, since the demand for productivity requires more water and nutrients.

Acording to Resende (2019), the productivity of *Coffea arabica* plant, submitted to the same conditions of fertilizing analyzed in this work, was greater on

129.5% level and lower at 31.9%, changing from 23.8 bags/hectare to 92.6 bags/hectare, representing, this way, a increase in the order of 74.2 per cent. Assuming that secondary xylem structural conditions are ideal next to 130% level (or T5), and with results found here, had stayed evident that joint frequency of vessels and rays tends to favor the productivity, for warrant a secure transport, but also have a well developed system of storage (Table 1 to 3).

4. CONCLUSION

Secondary xylem of coffee tree stem (ortotropic and plagiotropic), submitted to different fertilizations were analysed. The results showed a variable behavior for anatomical characteristics in both stem. In ortotropic stem, higher values, with exception of ray frequency in the treatment which were used recommended fertilization plus 30 percent, had been in treatment bellow recommended and complete dose (100 percent).

Plagiotropic stem, in turn, excepting ray frequency in plagiotropic reproductive, all other anatomical features higher values were found from less 30 percent to plus 60 percent since the recommended and complete dose. Fertilizing altered anatomical characteristics of secondary xylem, possibly to answer the functionality of coffee tree stem.

Moreover, modified structure, only influenced plagiotropic stem functional features. Xylem structural and functional informations reported here, besides being valuable in the botanic point of view, since coffee tree wood anatomy works are scarce, help to understand plant development and probable fluctuations in coffee productivity.

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ARTIGO 2 - MODELO INDUSTRIAL CROPS AND PRODUCTS JOURNAL

FERTILIZING *vs.* COFFEE TREE CHEMICAL COMPOSITION: RELATIONS AND NEW OPPORTUNITIES FOR CULTIVE WASTE DESTINATION (VERSÃO PRELIMINAR)

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Abstract

Coffee tree chemical composition is diverse. A lot of compounds can be found in various parts of the plant and the recovery of these substances from defective beans and coffee ground already happens. Despite that, little is known about coffee tree stem chemical composition when its submitted to different fertilization. Aiming establish probable chemical relations and point new forms for cultive waste reuse, samples of coffee tree stem treated with variation in macronutrients, and which undergone low-pruning technique were collected, being the portion of vascular system evaluated under the optics of structural and non-structural chemistry (histochemistry and chromatography techniques). Statistical differences between the treatments for all structural and non-structural components could be seen. Moreover, several compounds could be localized punctually and throughout the rays in vascular system. Also, the phenolics: chlorogenic acid, vanillin, resveratrol and the alkaloids: caffeine and trigonelline, could be detected in different amounts in the treatments. The results showed coffee tree stem residue potential for biorefinary, pharmacological and cosmetic industries, given a sustainable and profitable destination to a underused biomass.

Keywords: Biomass reuse, arabic coffee, metabolites.

*Abbreviation*s: AP, Axial parenchyma; Cb, Vascular Cambium; ESC, esclereids layer; Ph2, Secondary Phloem; RP1, Xylem ray parenchyma; RP2, Phloem ray parenchyma; V, vessel.

1. INTRODUCTION

Coffee has a great economic and cultural importance. Considered like one of the most common beverage in the world, its moderate comsumption brings benefits, on account of presence combination of complex chemical compounds (AHSAN; BASHIR, 2019). Is estimated that coffee world comsumption have increased by 2.1%

at 2018/19 harvest, with production ensured by two species of *Coffea*, *Coffea arabica L. and Coffea canephora* Pierre ex A. Froehner, known by arabic and conilon coffee, respectively (International Coffee Organization, 2020).

The most expressive in production is arabic coffee (International Coffee Organization, 2020), which owns many cultivars that requires several fertilizing rates to mantain its high productivity (VIEIRA, 2017). Responding to crop handling for yield increase, arabic coffee tree invests on plant shoot, leading to crop closure, also generally loses down-plagiotropic and productive stem, including plant exhaustion and, for this, coffee tree pruning is a fundamental method to be applied over time (THOMAZIELLO, 2013).

Coffee pruning is a common technique used to renovate arabic coffee crops in mountain regions and the best type for its employment is dependent on *Coffea arabica* genotypes, due to the particular genetical characteristics, reflected over growth rates and canopy architecture patterns (RODRIGUES et al, 2017). One of prunning types is low-pruning, which is a drastic method, consisting in cut the ortotropic stem about 20 – 40 cm height, eliminating the plant shoot (THOMAZIELLO, 2013). This technique when applied in *C. arabica* (cv. Topázio MG1190), allowed good recovery of irrigated and non-irrigated plants (REZENDE et al., 2006).

After pruning, a big amount of coffee tree biomass is lost and a sustainable destination needs to be provided. Provenient wood of waste coffee tree already was evaluated to energy purposes (LEITE et al., 2014). Besides that, the potential use of metabolites of spent coffee grounds and green coffee defective beans for new pharmacological product syntehis (MARTO et al., 2016) arouses attention to direct the research on biomass coffee tree, focusing on finding interesting compounds for the industries.

A lot of metabolites groups are synthetized for coffee tree, being many of them responsable in quality of coffee beverage. Metabolites in coffee tree include, alkaloids, phenolic compounds, terpenoids, lipids and starch, and can occur in different plant parts (GOULART et al., 2007; MAYER; CARMELLO-GUERREIRO; MAZZAFERA, 2013; PATAY; BENCSIK; PAPP, 2016).

For plants, metabolites importance lies in the fact of to be able in acess material stocked and defense, when required, warranting its survival in adverse environments, having a ecological importance (YANG et al., 2018). Nutrition figures

out among abiotic factors that affects metabolites production (GOBBO-NETO; LOPEZ, 2007), as well as, relations of structural components content, mediated for changes in some macronutrients concentration (TULLUS et al., 2010).

Stem structural components are cellulose, hemicellulose and lignin. They compose plant cell wall alongside pectins, proteins and water, according to cell wall development (MEENTS; WATANABE; SAMUELS, 2018; VANHOLME et al, 2019). With these components, cell wall is able to establish plant-microbe interactions, protecing cell against potential pathogens (KEEGSTRA, 2010).

In a general way, these plant metabolites and structural components have been extracted and used to create or improve innovate products of pharmacological, food and energy industries, primarly (MORAES-LOVISON et al., 2017; PAVELA;2016; PEREIRA et al., 2017; SECA; PINTO, 2018).

Concerning to extraction of metabolites, it can be realized from different plant parts, according its occurence, using distinct suitable methods. Structural components, in turn, are most commonly obtained from stem in industrial processes or recovered from it, how is made in pulp & paper industry.

We highlight that, albeit studies about chemical composition in coffee tree already been conducted, a chemical study which analyze the potential utilization of waste coffee tree in different fertilizing rates and that undergone low-pruning, needed to be developed.

Therefore, the aim of this study was investigate chimically the vascular system in orthotropic coffee tree stem that undergoes to different fertilizing levels and low-pruning, determinating its structural and non-structural components and verify its potential for sustainable chemical exploitation for several industries.

2. MATERIAL AND METHODS

2.1 Site characterization and sampling

The material of stem used were collected from *Coffea arabica* L. (cv. Topázio MG1190) tree, which composed an experiment located at Federal University of Lavras (Minas Gerais state, Brazil), situated on 21°14'06" S and 45°00'00" W, at 910 m elevation. Having the mean weather conditions: 1460 mm of rainfall and 20.4° C of temperature (Dantas, Carvalho, Ferreira 2007).

In addition, experiment comprised a randomized-completeblocks design were different fertilizing parameters for three macronutrients: nitrogen, phosphorus and potassium (N-P-K), being six treatments: T1, T2, T3, T4, T5 and T6, corresponding to 10, 40, 70, 100, 130 and 160 of percentual fertilizing (Figure 1), respectively, from the estabilished for Guimarães et al (1999) like basis fertilizing for coffee cultive, to know N (3 to 5 g of urea per plant), P (20 g of P_2O_5 per plant) and K (20 g K_2O per plant). Macronutrients were applied in fertigation system.

Fertigation system in the experiments was composed by a control unit (pumping system, sand filter and grille, fertilizers injector, pressure gauges and connections), main line of PVC tube (PN80), derivation lines of PVC tube (PN40), lateral lines with flexible tubes of polyethylene PN 40, drippers and regulators. Drippers (nominal flow: 3.8 $L.hour^{-1}$) were spaced every 30 cm in line. Fertigation control was conducted according climatologic data from meterologic station registered in specialized insitution.

The experiment was conducted in the years of 2010 to 2018, and at 2015 year, low-pruning technique was made, generating a new ortotropic stem. How the objective, was characterize, but also evaluate the potential reuse of cultive waste (losses of stem after low-pruning technique), the material used were provenient from primary ortotropic stem, developed between five years (2010 to 2015). In analysis were used 24 plants (Figure 1).

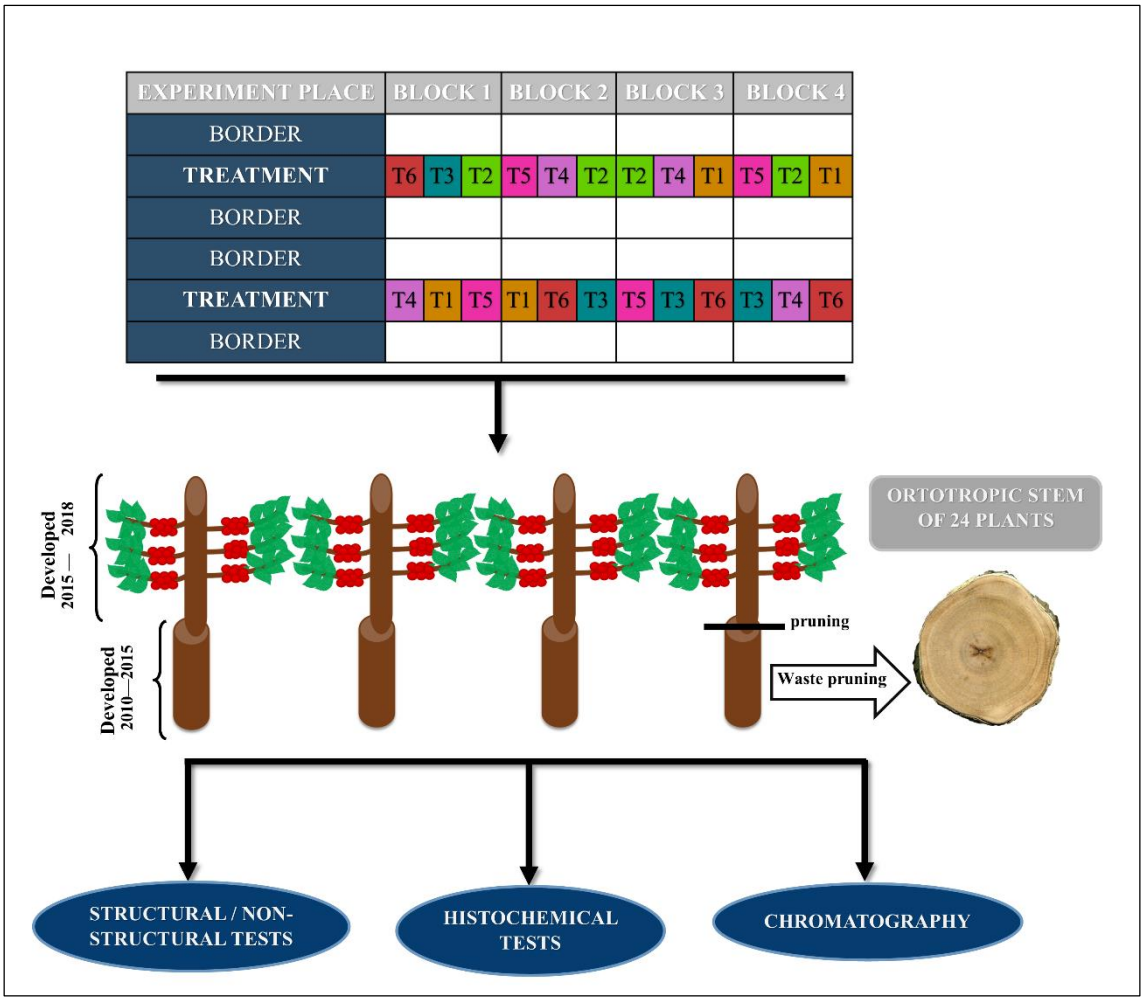

Figure 1- Experimental design and sampling used at analysis.

2.2 Chemical composition analysis

2.2.1 Structural and non-structural components determination

From stem samples collected were removed all external bark (corresponding to outside tissues to secondary phloem), the material, this way, was ground and classificated and the fraction of 60 mesh was used in general extractives and structural componentes chemical analysis. The follow proceedings were developed in three replicates for each sample. Total extractives content were determinated gravimetrically after sucessive Soxhlet extractions with toluol and ethanol for 7h, on each and water for 3h, in agreement with NBR 14660 standard (ABNT, 2004). Using extractives-free material, the acid insoluble (Klason) and acid soluble lignin were determinated acordding a modificated Klason lignin method (mini-sample method) did by Gomide and Demuner (1986). Ash content were determinated using 3 g of material, following after TAPPI T211 om-93 standard. Considering that chemical

composition of the material is equal to a hundred percent, holocellulose value was obtained by subtraction of total extractives, lignin and ash content.

2.2.2 Histochemical Tests

For specify and localize the great extractives groups at anatomical tissues, system vascular samples used in histochemical tests were dried at room temperature. For the analysis, transverse sections were obtained using sliding microtome (LEICA JUNG SM2000), without previous treatment to prepare the material, aiming to warrant the present compounds preservation. After, an adaptation were made to realize histochemical tests directly at optical light microscope (Olympus BX41) with attached camera (Pixel link, PL A662).

To have no doubt of reaction localization, control sections were photographed first and just then the reagent contact with sections were realized, dragging substances with filter paper, during a determined period, according the proposal methodologies. To attest flavonoid presence in tissue, fluorescence microscope was used following usual methodology. The evaluated metabolites and the used reagents are described in (Box 1). Were tested samples of all treatments (T1 to T6), totalizing twenty-four replicates (four replicates per treatment) for each analysis.

BOX 1 - Metabolites evaluated and reagents used on histochemical tests

METABOLITE GROUP	*REAGENTS*	*REFERENCES*
Lipids		
Total	Sudan III Sudan IV	Pearse (1972)
Terpenoids		
Resin-oils	Nadi Reagent	David, Carde (1964)
Phenolic compounds	Ferric Chloride	
General	III	Johansen (1940)
Tannins	Vanillin-	Mace, Howell (1974)
Flavonoids	hydrochloric acid	Charrierè-Ladreix (1976)
	Fluorochromes	
Polysaccharides		
Starch	Lugol	Johansen (1940)
Alkaloids		
General	Dragendorff Wagner	Svendsen, Verpoorte (1983)

2.2.3 Chromatographic analysis

For chromatographic analysis, 2,5 g of composed samples, by the treatments, with 60 mesh and adjusted with moisture were used. The material was transferred to flasks containing 20 mL of methanol in 70% concentration, which remain in ultrassonic bath for 1 h. After this period, filtration was conducted utilizing filter crucible n° 2 in vacum system and syringe filter with 0,45 µm pore.

To determinate the amount and phenolic compounds profile we used high performance liquid chromatography (HPLC) technique. The patterns employed were: gallic, caffeic, chlorogenic, rosmarinic, trans-Cinnamic acids, catechin, vanilllin, ferulic, p-coumaric, o-coumaric, m-coumaric acids and resveratrol, for phenolic compounds, and caffeine and trigonelline to alkaloids. Samples were quantified by external standardization method and the analitical curves were set using 1000 mg L^{-1} diluted of stock solution for each pattern solubilised in methanol. Analitical curves were obtained from linear regression with R^2=0,99 determination coefficient.

Analysis were conducted with high performance chromatograph Shimadzu®, equipped with four LC-20AT high-pressure pumps, SPD-M20A photodiodearray detector, DGU-20A5 degasser, CBM-20A interface, CTO-20AC oven and automatic injector with an auto-sampler SIL-20A model.

Separation of material was made using a Shimadzu® -Shim- packGVP- ODS-C18 (4,6 x 250 mm, 5µm) column conected to Shimadzu® -Shim- packGVP- ODS-C18 (4,6 x 5 mm, 5µm) pre-column. Elution solvents utilized for phenolics determination were: acetic acid solution 2% in ultrapure water (Milli-Q Integral Water Purification System) at phase A; methanol, water and acetic acid (70:20:2% v/v) at phase B in gradiente mode (Time - Concentration: 0.01 min – 0%; 5 min – 20%B; 25 min – 40% B; 43 min – 45% B; 50 min – 80% B; 55 min – 0% B; 65 min - Stop). For alkaloids determination were utilized: acetic acid solution 0,5% in ultrapure water (Milli-Q Integral Water Purification System) at phase A; methanol, water and acetic acid (70:20:2% v/v) at phase B. Isocratic mode was conducted with 25% of phase B, during 20 minutes.

Wavelength used was 280 nm (phenolics) and 272 nm (alkaloids), 1,0 mL min-1 flux, oven temperature: 35 ºC (phenolics), 30 ºC (alkaloids); and 20µL injection

volume. To calculate phenolic compound and alkaloids concentration, external standardization method were employed for each pattern analyzed.

2.3 Data Analysis

The statistical analysis consisted in comparisons of the percentual values contents obtained during general extractives, ash and structural component analysis for all treatments, using *dbc* (randomized-blocks design) of *ExpDes.pt* package from the statistical software R (3.6.0 version) (R Core Team 2019), ANOVA test, followed by Shapiro-Wilk normality test and Tukey's LSD (Lowest Significant Difference) test, with 95% confidence interval were used**.**

3 RESULTS AND DISCUSSIONS

3.1 Structural components

Holocellulose fraction (celullose plus hemicellulose content) in stem vascular system represented the greater percentual value comparing to other components. Means had been among 52,91 and 58,23%, treatments T1 to T5 differ statistically only in comparisson to T6, which presented biggest average (Table 1, Figure 2).

Table 1- Chemical properties of coffee tree stem vascular system, per fertilisation treatment. EVC (%): Experimental variation coefficient. SE: Standard error.

Treatment	Holocellulose (%)	Lignin (%)	Ash (%)	Extractives (%)
T1	52,91 **b** (2,34)*	34,96 **a** (1,58)	1,86 **a** (0,25)	10,26 **a** (1,15)
T2	54,19 **b** (4,18)	33,90 **bc** (1,28)	1,80 **a** (0,15)	10,11 **a** (3)
T3	54,00 **b** (2,74)	33,79 **bc** (1,04)	1,64 **b** (0,06)	10,57 **a** (2)
T4	54,86 **b** (2,40)	34,14 **abc** (1,39)	1,58 **b** (0,12)	9,42 **a** (1,58)
T5	53,69 **b** (1,48)	34,77 **ab** (0,89)	1,64 **b** (0,11)	9,91 **a** (1,43)
T6	58,23 **a** (3,64)	33,26 **c** (1,33)	1,57 **b** (0,11)	6,94 **b** (2,51)
EVC (%)	5,08	3,68	8,57	20,12
SE	0,27	0,16	0,02	0,39

*Means followed by standard deviation. Means followed by the same letter, in the column, do not differ from each other at 5% probability by Tukey's test.

The averages found here are common to coffee samples, ever since holocellulose in Mundo Novo and Catuaí (*Coffea arabica* cultivars) tends to be

amongst 50,63 and 61,48% having variable values depending on cultive system (LEITE et al, 2014).

Independent of specie, cellulose and hemicellulose tends to be in great amounts, due to they are the majority compounds found in cell wall, being present in primary wall and the secondary one (MEENTS; WATANABE; SAMUELS, 2018). For this reason, was expected to found a increasing value to holocellulose content as long as fertilizing was incremented, as consequence of tissue growth enabled for macronutrients support.

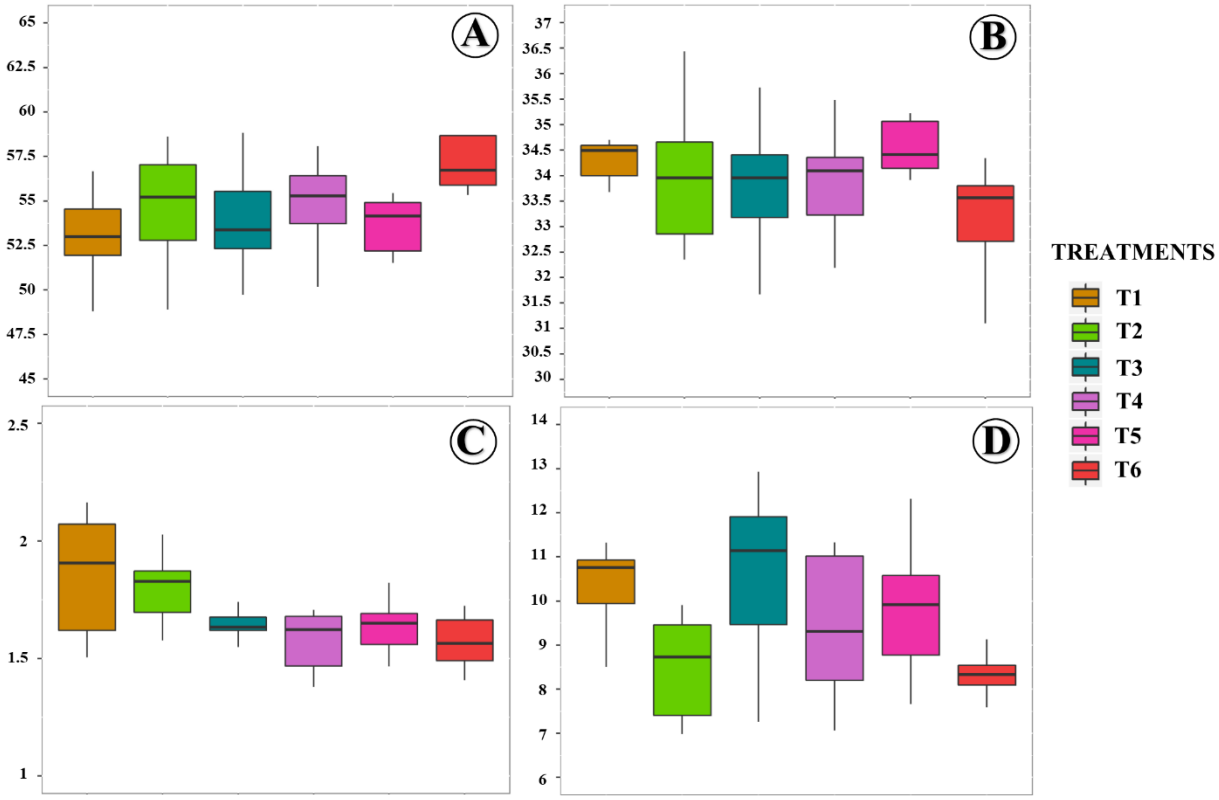

Figure 2 – Chemical composition of coffee tree vascular system. A to D: percentual values. A: Holocellulose; B: Total lignin; C: Ash; D: Extractives.

Concerning to lignin content, a variable behavior was expressed with Tukey's test and a trend to accompany fertilization, like in the other chemical compounds, could not be observed, since lignin content was high in T1, decreased in T2 and T3, to increase again in T4 and T5, finalizing with the lower value in T6 (Table 1, Figure 2).

In a general way, coffee tree presented a high value for lignin content, when compared to trees that achieve high size, like some clones of *Eucalyptus* L'Hér. (Myrtaceae) (NEVES et al., 2011) or even amazon wood trees (MOUTINHO et al, 2016). However, elevated averages for lignin content have been common for other coffee tree cultivars, how seen in Mundo Novo and Catuaí coffee cultivars, which can achieve until 32,35% (LEITE et al, 2015).

The reason why *Coffea* cultivars have been invest in elevated rates of lignin is explained for this compound be considered a inheritable feature that is strongly genetically controled (POKE et al., 2006). Lignin has a great important paper for plants, like a phenolic compound, for example, acts at pathogen attack; confers strenght, principally for secondary vascular system and waterproof vessel elements, reducing contact of water with the hydrophillic cell wall portion (VANHOLME et al., 2019).

Due to lignin has presented a variable value, fertilizing appears not to be a closer relation with it. Leading to query if are not a covered factor behind, that was responsable for increase or decreased lignin content, and which was influenced for fertilizing. Therefore, fertilizing would have a indirect influence at lignin content.

Moreover, despite some nutrients can be envolved with lignin synthesis, an analyze to verify this relation, is difficult to be established, due to assurance of nutricional balance at the moment of lignin polimerization, which could be sensitively modify for the absence or interference in any substrate during the assemble (BARROS et al, 2015).

Nevertheless, some issues remain without an answer: which of lignin functions are preponderant for coffee tree to invest energy (VANHOLME et al, 2013) to produce a high content of lignin even when the resources can be scarce? And what led *Coffea* tree to select high lignin content like a favorable condition for it survival. Maybe develop of deepened studies in biochemistry and epigenetic fields could answer these question.

3.2 Non-structural components

3.2.1 Ash content

Ash content stayed amongst 1,86 and 1,57%, exhibiting larger averages in T1 and T2, that differed of T3 to T6. Second Leite et al. (2015), ash content in wood of

arabic coffee is around 1,34%, which is considered a high value for this characteristic.

Taking in account that ash content was high in T1 and T2, inorganic matter accumulated in stem vascular system of these samples could have contributed for the result, bearing in mind that biomass ash consists in a mixture of inorganic and (a less portion of) organic matter, including minerals, cristalline to semi-cristalline, poorly crystallized mineraloids, grains and aggregated of organic minerals (oxalates) (VASSILEV et al., 2017). During the laboratory analysis, monocristals could be observed in T1 and T2 treatments accumulated in ray parenchyma cells.

3.2.2 Total extractives content

Extractives content had been between 6.94 and 10.57%, with variable values according the treatments. Although, the averages per treatments decreased insofar as fertilisation enhanced, statistical differences could not be observed in T1 to T5 (Table 1). In T6, treatment with higher fertilisation, there was a substantial reduction in total extractives content (Table 1, Figure 2).

Inumerous substances can be included in extractives group (ROY; PAKDEL; BROUILLARD, 1990). These compounds outcomes of primary and secondary metabolism, from different pathways. Besides that, production and accumulation of extractives occur in many conditions, but are mainly associated to stress and defense stimuli (ISAH, 2019).

In *Coffea arabica* wood, depending of cultivar and cultive system analized, percentual of extractives can vary 6,71 to 17,24% (LEITE et al., 2014; LEITE et al., 2015), with potential to present different metabolites (GOULART, 2007; MAYER; CARMELLO-GUERREIRO; MAZZAFERA, 2013)

The supply of some macronutrients can influence the content of metabolites dependent of them. In *Eucalyptus grandis* W. Mill ex Maiden (Myrtaceae) with aproximately 4 years and planted in different places, extractives content was high, where macronutrients, generally were in great amounts (SANSÍGOLO; RAMOS, 2011). The omission of Ca, S and K, macroelements, led to the increase of extractives content more than 40 percent, in a hybrid of *Eucalyptus* (SGARBI; SILVEIRA; BRITO, 2000).

3.2.2.1 Histochemical results

Histochemical tests could clarify which metabolite great groups (considered in extractives content) were present in vascular system tissue. Different compounds

were observed in *Coffea arabica* vascular system stem. The tests were positive for lipids, starch and alkaloids in parenchymatic cells of secondaries xylem and phloem, and in the esclereid layer present in secondary phloem (Box 2, Figure 3).

Box 2 – Results of histochemical reactions per treatment for great metabolic groups

METABOLIC GROUP	TREATMENTS					
	T1	T2	T3	T4	T5	T6
Lipids (total)	+	+	+	+	+	+
Terpenoids	-	-	-	-	-	-
Phenolic compounds (total)	-	-	-	-	-	-
Phenolic compounds (tannins)	-	-	-	-	-	-
Phenolic compounds (flavonoids)	+	+	+	+	+	+
Starch	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+

A difference in the reaction localization was able to be seen. While lipids and starch were all over radial system and pith, alkaloids accumulation appeared only in stretches on radial system or next to pith, as well as could be observed to flavonoids (Figure 3). Although the samples had been tested for other phenolic compounds and resin-oils, the result could not be conclusive to atest its presence in tissue (Box 2).

The abundance of lipids in the ray system can be explained for the fructification. Lipids are commonly found in endospermic cells in coffee seeds, located next to the cell wall; it is supposed that lipids have some influence to beverage quality (GOULART et al, 2007). For plants lipids can be acessed like a source of energy for seed germination (GRAHAM, 2008), and the presence in all ray system indicate the importance of the stem to invest in ray parenchyma, like a big "warehouse", since that the demand to in fruitful cultivars would require great amounts of lipids.

In the same way, starch are accumulated in ray system for its degradation and use in energetic functions (YU et al., 2018), maybe, once again, for fructification or to be acessed when cambium needs energy to form new cells to all the vascular system. Thereby, starch grains are seen frequently in parenchyma xylem cells of *Coffea arabica* stem (FERREIRA; SANTOS; CHAVES-FILHO, 2014).

Both alkaloids and phenolics are "toxic" compounds synthetized for plants like chemical defense when tissue damage occure; phenolic compounds, in addition,

were very important to plants in its transition to the terrestrial environment, due to protect against UV irradiation damage and water loss (KUTCHAN et al., 2015).

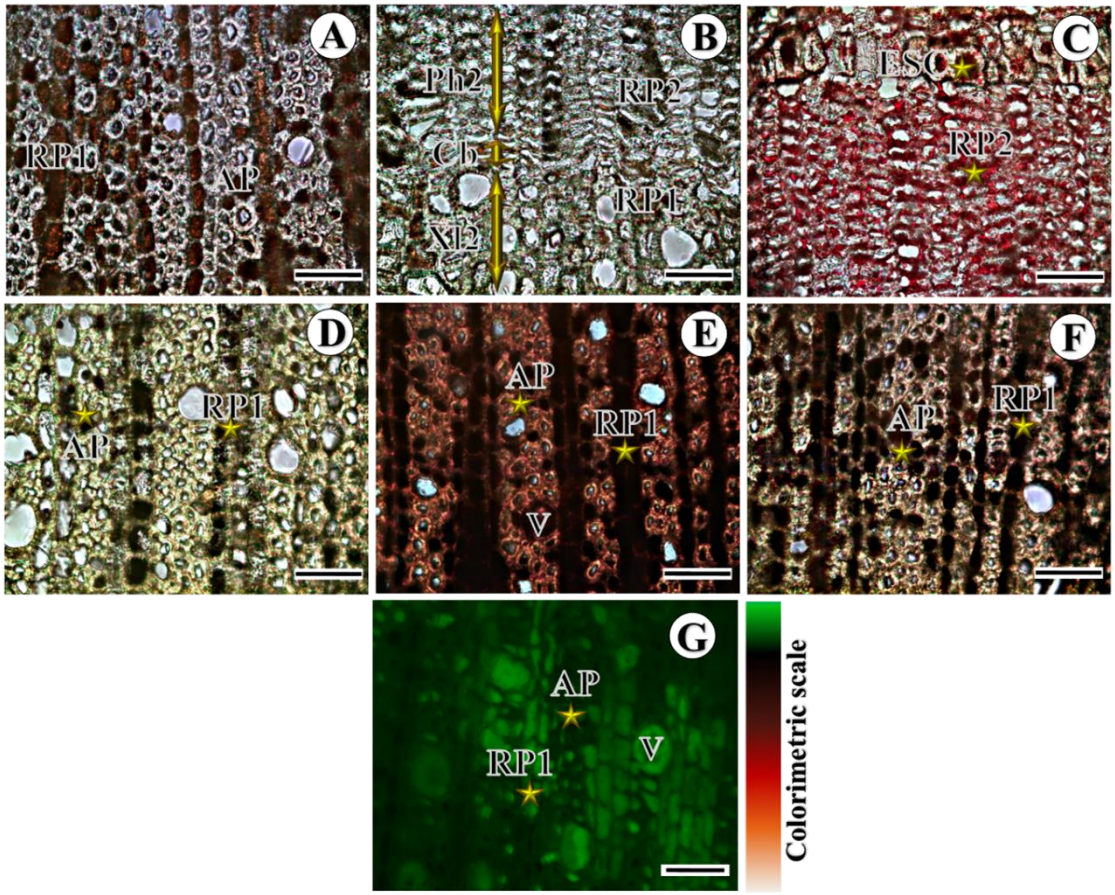

Figure 3 — Positive Histochemical analysis results of *Coffea arabica* L. vascular system, field tested with different fertilizing concentrations. A and B: Control; C: Lipids (Sudan III); D: Starch (Lugol); E and F: Alkaloids (Dragendorff and Wagner's Reagent, respectively); G: Flavonoids (Aluminium chloride). AP: Axial parenchyma; Cb: Vascular Cambium; ESC: esclereids layer; Ph2: Secondary Phloem; RP1: Xylem ray parenchyma; RP2: Phloem ray parenchyma; V: vessel. Scale bar: A to F: 70 µm. Stars: reaction localization.

Like defense substances, they are secondary metabolites products, that can be produced locally in stress conditions, after stimulus triggered by signal transduction, which active regulation pathways of biochemical and physiological processes (ISAH, 2019). This explain why alkaloids and flavonoids could be situated in tissue portions, indicating probable local damages. It is worth pointing out that alkaloids and phenolic compounds are found in other parts of coffee tree, like in flowers (MAYER; CARMELLO-GUERREIRO; MAZZAFERA, 2013).

Terpenoids are secondary metabolites either. They are volatile substances responsable for give to plants and flowers fragrance (YADAV; YADAV; GOYAL, 2014). How terpenoids were not able to seen in vascular system tissue, these compounds can not be produced in ortotropic stem cells or the protocol used in histochemical test was not efective for terpenoids determination, being necessary adaptations in methodology.

3.2.2.2 Chromatography

The HPLC analysis revelead chlorogenic acid, vanillin and resveratrol as phenolic compounds present in stem vascular system of coffee tree. Chlorogenic acid concentration had been between 83,34 and 175,11 mg, with high value in T3 (Table 2). Vanilllin presented the lowest value in T6 and the highest one in T3; whereas was found on resveratrol its high concentration at T3, once again (Table 2).

Table 2 – Phenolic compounds and alkaloids concentration present at stem coffee tree vascular system.

Chemical compound	Treatments (concentration = mg/100g)					
	T1	T2	T3	T4	T5	T6
Chlorogenic acid	152,04	116,25	175,11	112,29	116,36	83,34
Vanillin	1,91	1,61	2,19	1,50	1,71	1,27
Resveratrol	18,83	17,29	21,02	19,96	20,58	18,96
Caffeine	0,15	0,13	0,17	0,13	0,15	0,11
Trigonelline	0,31	0,26	0,27	0,26	0,33	0,21

Chlorogenic acid (CGA) is an esther composed by quinic acid and cafeic acid, being caffeoylquinic acid the prominent CGA in coffee (CAMPOS-VEGA et al, 2015). The majority spent coffee ground analyzed showed relevant quantity of caffeoylquinic acid, with exception of those obtained from mocha coffeemaker (BRAVO et al, 2012). CGA appears to be the most abundant phenolic compound present in coffee tree, since a great amount of this substance also can be found at green coffee beans, when analyzed by HPLC (ESQUIVEL; JIMÉNEZ, 2012).

Averages found for vanillin appeared to be common, since coffee can present until 2,38 mg/g of vanillin, depending on the variety (HECIMOVIC et al., 2011). Vanillin is a polyphenol with a simple molecular structure, produced naturally in orchid “pods” (DIXON, 2011), but its synthesis also can be achieved for the conversion of other phenolic compounds, like ferulic acid, which present a strong

structural similarity with vanillin, being considered the best-explored substrate to obtain it (GALLAGE; MOLLER, 2015).

The another polyphenol detected, resveratrol, is a compound known to be present in grapes and wine (KURSVIETIENE, 2016) and in spent coffee ground at 0,14 mg/g concentration (RAMÓN-GONÇALVES, 2019). Resveratrol have a great importance for plants. It has antioxidant activity and protect against sun damage (KURSVIETIENE, 2016).

The relation between phenolic compounds and fertilization is variable for macronutrients or specie studied. For example, when in great amounts, potassium fertilization caused higher content of phenolic compounds in “eggplant” (*Solanum melongena* L., Solanaceae) (OLIVEIRA et al, 2019), on the other hand, nitrogen higher concentration led to decrease in total phenolic compounds of *Sesamum indicum* L. seeds (Pedaliaceae) (ELHANAFI et al., 2019). Once again, its difficult to predict what will be polyphenols behavior when plants are submitted to different fertilizing, because there are many interactions in nutritional balance order, which could cause fluctuations in phenolic compounds content.

Leading in consideration that nitrogen-based compounds present in coffee tree are alkaloids (caffeine and trigonelline) and distinctives of *Coffea* species (WANDERLEY et al., 2017) was expected to find great amounts in the analyzed samples (Table 2), in comparison to other compounds, what did not happen. This, calls attention to defense estrategies outlined for coffee tree, since phenolics would be the main compounds used for these plants against a pathogen attack, for example.

In agreement with alkaloids synthesis, once they are nitrogen-dependent compounds (KUTCHAN et al., 2015), the deviation of this macronutrient, to produce secondary metabolites to the detriment of growth, at first sight, does not appear propitious. A relation between phenolic compounds and alkaloids production is reported for carbon-nitrogen balance hypothesis.

According to carbon-nitrogen balance hypothesis (CNBH), plants can divert resources to produce secondary metabolites when obtain sufficient material to growth; for this, being luminosity conditions favorable, carbon excess is used to produce phenolic compounds and a opposite environmental condition generate more nitrogen-based compounds, like alkaloids (LERDAU, 2002).

Another point to be highlighted in alkaloids is higher concentration of trigonelline in comparison to caffeine (Table 2). In coffee tree, alkaloids concentration differ according to part of plants, with caffeine being abundant in cotyledons (2% of dry weight) and trigonelline more concentrated in stems (2,5%) and roots (0,34%); furthermore, trigonelline concentration tends to decreased with the age of the tissues (ASHIHARA, 2014).

3.3 Opportunities for coffee waste cultive destination

Coffee residues have been pointed like a feedstock by biorefinery industry, for present high holocellulose fraction, high calorific value in contrast to ash content (RAMBO; SCHMIDT; FERREIRA, 2015). Moreover, coffee pulp waste were tested to produce a cellulose based high value product and the results were satisfactory to adsorb methilene blue from concentrated aqueous solutions, with promises to be used in food packages (ACHABY et al, 2019).

One example of well-succeed value-added product obtained from hemicellulose hydrolysis is xylitol. Xylitol is derived from xylose (released of xylan) fermentation conversion (ARRIZON et al, 2012), it has been indicated like a well accepted low calorie sweetener in confectionaries and healthcare products (DASGUPTA et al, 2017). Representing the majority fraction in coffee tree stem (Table 1), holocellulose could be exploited from pruning residues, give them a profitable destination.

Biorefinery industry also has focusing in lignin recovery, which is incorporated to several products (UPTON; KASKO, 2016). These products seize lignin potencial expressed on high-density and chemical aromatic structure, being fuels, functional materials and value-added chemicals, highlighting on it benzene, toluene and xylene (BTX) and phenols market (STRASSBERGER; TANASE; ROTHENBERG, 2014; WANG et al, 2019). Coffee tree pruning residues, this way, appears like a promising feedstock for lignin reuse by biorefinery, due to around 1/3 of all stem biomass (Table 1) correspond to this compound.

Another substances with pharmacological and cosmetical importance are the metabolite products (phenolic compounds and alkaoides, for example). Phenolic compounds are namely known how antioxidant substances. Results showed in Table 2, point the seize of the residues for extract chlorogenic acid and resveratrol. With

other many important functions, chlorogenic acid (CGA), can be used for the treatment of diabetes, obesity and hypertension, also has antimicrobial effects (NAVEED et al., 2018). In the market, chlorogenic acid can be found supplementing encapsulated products of coffee green beans, with prices ranging from R$ 20 to R$ 130 (GOOGLE SHOPPING, 2020).

Resveratrol, as well as CGA, also has antimicrobial and antidiabetic effects, besides that, when tested promoted cardio and neuroprotection, being either, a strong candidate to be inhibitor in all carcinogenesis stages, in different types of cancer, acting, this way, in treatment and prevention (SALEHI et al, 2018). Although resveratrol be in grapes (*Vitis vinifera* L., Vitaceae) (KURSVIETIENE, 2016), is in the market like capsules of *Polygonium cuspidatum* Siebold & Zucc. (Polygonaceae) extract; a tradicional chinese medicine (WANG; LIU; CHEN, 2013), highlighting the potential of resveratrol to be extracted from different species.

Between the alkaloids analyzed, caffeine is the most commercialized. Caffeine is a psychoactive component (JAIN et al., 2019) used to increase physical and cognitive performances, being sold in market like a biostimulant additive for beverages, used in supplementary food (BEAUCHAMP; AMADUCCI; COOK, 2017). Extraction of caffeine from stem residues would be promissor, since, depending on fertilizing, the amount of this compound can achieve until 1,7 mg x kg^{-1} of concentration (Table 2).

4 CONCLUSION

Coffee stem pruning residues were for the first time analyzed from a chemistry broad vision (structural and non-structural) and showed the ability of coffee tree to produce several compounds. These compounds had different behavior according to fertilizing, holocellulose had a direct relation, while ash and extractives had an indirect one. Lignin presented a variable relation, needing, this way, more studies to justify. Main extractives groups were detected by histochemical tests (lipids, flavonoids, starch and alkaloids) and of the tested compounds by HLPC, only chlorogenic acid, vanillin, resveratrol, caffeine and trigonelline, could be found in coffee stem samples. Being it in variable concentration.

Since these substances are associated with growth, defense and productivity, being responsable also for coffee beverage quality, the results showed here, led to the

start of comprehesion of how these compounds behave when submitted to different fertilizing, and how it impacts in plant development and coffee productivity. In addition, the reuse of stem residue for biorefinary, pharmacologic and cosmetic industries is possible, with promisses to be profitable, including offer a sustainable destination for material till then underutilised.

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