UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de Botucatu

ANATOMIA DE RAÍZES DE *Eucalyptus grandis* Hill ex Maiden EM DIFERENTES PROFUNDIDADES DO SOLO SOB UM CONTEXTO DE REDUÇÃO DE CHUVAS

MAËLA PERON GOMIDE

Dissertação apresentada ao Instituto de Biociências, Campus de Botucatu, UNESP, para obtenção do título de Mestre no Programa de Pós-Graduação em Ciências Biológicas (Botânica), AC: Morfologia e Diversidade Vegetal.

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Sumário

GOMIDE, M. P. **ANATOMIA DE RAÍZES DE *Eucalyptus grandis* W. Hill ex Maiden EM DIFERENTES PROFUNDIDADES DO SOLO SOB UM CONTEXTO DE REDUÇÃO DE CHUVAS.** 2016. 47p. DISSERTAÇÃO (MESTRADO) – INSTITUTO DE BIOCIÊNCIAS, UNESP – UNIVERSIDADE ESTADUAL PAULISTA, BOTUCATU.

Resumo – Em espécies perenes de ambientes sazonais, o estabelecimento de um sistema radicular profundo é uma das estratégias de adaptação dessas espécies ao estresse hídrico. Nessas plantas, é bem estabelecido que a maior parte da absorção de água e íons ocorre em raízes finas não-suberizadas e que as porções mais jovens das raízes são as mais ativas nesse processo. Uma vez que modelos de mudança climática preveem uma redução de chuvas de até 20% entre a primeira e a última década do século XXI em grande faixa do globo terrestre, incluindo o Brasil, entender o efeito da redução da precipitação sobre a anatomia das raízes faz-se cada vez mais importante. O objetivo desse trabalho foi estudar a anatomia de raízes de *Eucalyptus grandis* em diferentes profundidades do solo e investigar o efeito da limitação da precipitação nos aspectos estruturais dessas raízes. Um delineamento experimental foi estabelecido em junho de 2010 com um clone de *E. grandis* em uma plantação da Estação Experimental de Ciências Florestais de Itatinga (EECFI), SP, Brasil. Dois tratamentos (dois regimes de água) foram aplicados em blocos separados de 2010 a 2014: tratamento TO (controle) sem a intercepção de chuvas e tratamento TR com interceptação de 37% das chuvas por lâminas de plástico transparente. Em 2014, trincheiras de até 17 metros de profundidade foram abertas no solo, sendo uma para cada tratamento. Cada trincheira foi centralizada entre quatro indivíduos de *E. grandis*. Raízes finas laterais foram coletadas a 0-50cm e 12-16m de profundidade do solo e separadas em quatro categorias de acordo com seu diâmetro: $Ø > 2mm$; $1< Ø \leq 2mm$; $0,5< Ø \leq 1mm$; e $Ø \leq 0,5mm$. As amostras foram processadas segundo técnicas usuais em anatomia vegetal. Análises morfométricas foram realizadas em secções transversais ao microscópio de luz com auxílio do software Olympus Cell B. Os resultados foram submetidos ao teste t (de Student) e U-Mann Whitney; outros à ANOVA dois fatores e ao teste de Tukey. Raízes com diâmetro $Ø > 2mm$ e $1< Ø \leq 2mm$ coletadas em TR, e raízes com diâmetro $0,5 < Ø \leq 1mm$ coletadas em TO nas maiores profundidades do solo apresentaram maior área total ocupada pelo xilema; raízes com diâmetro $Ø > 2mm$, $1< Ø \leq 2mm$ e $0,5 < Ø \leq 1mm$ coletadas em TR nas maiores profundidades também apresentaram maior proporção da área total ocupada pelo xilema pela área total da porção funcional da raiz; nas raízes profundas dessas três categorias coletadas em TO e TR os elementos de vaso eram menos abundantes, porém com maior diâmetro do que as raízes mais superficiais. O aumento

do diâmetro dos elementos de vasos nas raízes profundas remete a um aumento da condutividade hidráulica, porém pode aumentar o risco de embolismo. Além disso, raízes profundas dessas mesmas três categorias coletadas em TR apresentaram numerosos grupos de fibras gelatinosas no floema e tilos nos elementos de vaso. Fibras gelatinosas apresentam potencial para armazenamento de água enquanto que tilos podem representar uma estratégia contra o embolismo em condições de estresse hídrico. Esses dados reforçam a ideia de que a redução de chuva influencia a anatomia das raízes de *E. grandis* nas camadas mais profundas do solo.

Palavras-chave: estresse hídrico, eucalipto, raízes profundas, raízes superficiais, xilema

GOMIDE, M. P. **ANATOMY OF *Eucalyptus grandis* W. Hill ex Maiden ROOTS AT DIFFERENT SOIL DEPTHS IN A RAINFALL REDUCTION CONTEXT.** 2016. 47p. Msc DISSERTATION – BIOSCIENCE INSTITUTE, UNESP – UNIVERSIDADE ESTADUAL PAULISTA, BOTUCATU.

Abstract – In perennial species the establishment of a deep root system is an adaptive strategy to water deficit. In these plants it is well established that most of the absorption of water and ions in perennial woody species occurs in fine non-suberized roots and that younger portions of roots are the most active in this process. Since models of climate changes predict a rainfall reduction in the next years of 20% until the first and the last decade of 21st century in global large scale, including Brazil, to understand the effect of rainfall reduction on the anatomy of these roots becomes even more important. This study aims to study the anatomy of *Eucalyptus grandis* roots at different soil depths investigating the effect of limiting the rainfall in structural aspects of these roots. An experiment was set up in June 2010 with a clone of *Eucalyptus grandis* used in commercial plantations by the Suzano Company in Estação Experimental de Ciências Florestais de Itatinga (EECFI), SP, Brazil. Two treatments (two water systems) were applied in separated blocks from 2010 to 2014: treatment TO (control) without rainfall interception and treatment TR (reduction) with 37% of rainfall interception by transparent plastic sheeting panels. In 2014, trenches of until 17 m of depth were opened in the soil, one for each treatment respectively. Each trench was centered between fours individuals of *E. grandis*. Fine lateral roots with were collected at 0-50cm and 12-16m of depth and separated in four categories according to their diameter: Ø > 2mm; 1< Ø ≤ 2mm; 0.5< Ø ≤ 1mm; e Ø ≤ 0.5mm. Samples were processed according to conventional techniques in plant anatomy. Morphometric analysis was performed in transversal sections at the light microscope Olympus BX 41 using the software Cell B. The results were submitted to the t-student test and U Mann-Whitney test; others to a two way ANOVA and Tukey test. Roots with diameter Ø > 2mm and 1< Ø ≤ 2mm collected in TR, and roots with diameter 0.5 < Ø ≤ 1mm collected in TO in greater depths of the soil presented biggest total area occupied by the xylem; roots with diameter Ø > 2mm, 1< Ø ≤ 2mm and 0.5 < Ø ≤ 1mm collected in TR in greater depths also presented biggest total area of xylem per total area of functional root portion ratio; in deep roots of these three categories collected in TO and TR the vessels were less abundant, however with larger diameter than superficial roots. The increase in diameter of vessels of deep roots refers to an increase in hydraulic conductivity, but can raise the risk of embolism. Furthermore, deep roots of those three categories collected in TR showed

numerous groups of gelatinous fibers in the phloem and tyloses in the vessels. Gelatinous fibers have potential for storing water while tyloses may represent a strategy against embolism in water stress conditions. These data reinforce the idea that the reduction of rainfall influences the anatomy of the roots of *E. grandis* in the deepest layers of the soil.

Key words: drought stress, eucalyptus, deep roots, superficial roots, xylem

Introdução geral

Os modelos de mudança climática preveem um aumento da temperatura e secas mais frequentes (SHEFFIELD; WOOD, 2008; IPCC, 2013) e uma redução de chuvas de até 20% entre a primeira e a última década do século XXI em grande faixa do globo terrestre, incluindo o Brasil (IPCC, 2013). No entanto, as previsões podem diferentes para cada país, região ou bioma, segundo dados do PAINEL BRASILEIRO DE MUDANÇAS CLIMÁTICAS (2015). Modelos prevem que a maioria do bioma Cerrado sofrerá um aumento de temperatura em torno de 4ºC até o final do século (MARENGO et al., 2009). Projeções severas indicam um aumento que pode chegar a até 6ºC na região de transição com a Amazônia enquanto que as projeções menos severas apontam para um aumento de 2ºC na parte leste do bioma. Para a precipitação, as projeções mais severas indicam uma diminuição de 20 a 50% em relação aos valores atuais na parte central e sul do Cerrado. Porém, as projeções menos severas indicam uma redução de 30% nas partes central e sul do bioma. Marengo et al. (2010) prevê mudanças na distribuição das chuvas ao longo do ano no Cerrado. Na região norte-nordeste, ocorrerá um aumento de 20 a 30 dias na duração da estação seca. Por outro lado, para a região centro-sul do Cerrado um aumento no volume de chuva na forma de tempestade é esperado (PAINEL BRASILEIRO DE MUDANÇAS CLIMÁTICAS, 2015).

É um fato conhecido que episódios frequentes e severos de seca são responsáveis por mudanças importantes na estrutura e função das florestas úmidas tropicais como, por exemplo, o aumento na mortalidade de árvores adultas e da capacidade inflamável da floresta (NEPSTAD et al., 2004; VAN NIEUWSTADT; SHEIL, 2005). Quando, durante uma seca severa, um esgotamento da umidade do solo sucede, isso pode promover o fechamento dos estômatos nas plantas, diminuição da área foliar (NEPSTAD et al., 1994) e redução na produção de madeira (NEPSTAD et al., 2004). Num cenário no qual as secas episódicas serão mais frequentes e intensas (COX et al. 2004; MEIR; COX; GRACE, 2006), torna-se importante saber a resposta e a adaptabilidade das espécies perenes às condições de seca para melhor entender como os ecossistemas de florestas tropicais irão reagir frente a essa situação.

Sabe-se, que no caso de espécies perenes, o estabelecimento de um sistema radicular profundo, é uma estratégia de adaptação ao estresse hídrico (DAVID et al., 2013). De acordo com Schenk e Jackson (2005), considera-se um sistema radicular profundo, aquele que possui o sistema no qual 5% ou mais das raízes estão localizadas abaixo de 2 metros de profundidade. O sistema radicular das espécies perenes é capaz de alcançar maiores

profundidades no solo podendo, inclusive, transpor camadas rochosas (MAEGHT; REWALD; PIERRET, 2013). Sabemos que as profundidades de enraizamento estão relacionadas com as características do clima e do solo e que os sistemas radiculares profundos ocorrem provavelmente mais em ecossistemas sazonais limitados pela água de clima quente temperado a tropical (SCHENK; JACKSON, 2002a, b). Os ecossistemas sazonais são limitados pela água, pois a precipitação anual é inferior ao potencial anual de evapotranspiração e costumam enfrentar períodos extensos sem precipitação.

Durante os períodos de seca, o sistema radicular de espécies perenes é capaz de disponibilizar a água das camadas mais profundas do solo e garantir o suprimento hídrico a essas plantas (NEPSTAD et al., 1994; KLEIDON; HEIMANN, 2000; CHRISTINA et al., 2011). Trabalhos demonstram a ocorrência da redistribuição hidráulica no solo, onde a água das camadas mais úmidas é transferida pelos sistemas radiculares profundos até as camadas mais secas do solo (CALDWELL; RICHARDS, 1989) e que esse fenômeno ocorre em espécies perenes durante os períodos de seca (BURGESS et al., 2000). Essa redistribuição e disponibilização de água pelos sistemas radiculares profundos podem influenciar sistemas climáticos, contribuindo significativamente para o ciclo hidrológico em biomas tropicais (OLIVEIRA et al., 2005). Pesquisas realizadas na Amazônia comprovaram que a destruição de sistemas radiculares profundos, provocada pelo desmatamento apresenta impacto direto nas condições climáticas (KLEIDON; HEIMANN, 2000). Nesse contexto, para o estabelecimento de modelos de circulação da água nos ecossistemas é evidente a importância da caracterização morfológica e anatômica dos sistemas radiculares profundos.

Pesquisas sugerem que as camadas mais profundas de solo podem ser fontes de nutrientes significantes (MCCULLEY et al., 2004; DA SILVA et al. 2011). Trabalhos também sugerem que esses sistemas de raízes profundas têm a capacidade de expandir o volume de solo accessível para a captação dos nutrientes (MCMURTRIE et al., 2012; LACLAU et al., 2013). Outro aspecto, que se sabe há décadas, é que as raízes exercem ações de intemperismo físico-químico em seu ambiente (BORMANN et al., 1998). Com sua pressão de crescimento sobre o solo elas podem originar expansões físicas em solos rochosos e a pressão pode apressar a dissolução química dos minerais (RICHTER; MARKEWITZ, 1995; RICHTER et al., 2007). Sabe-se ainda que as raízes profundas têm um papel ativo na distribuição de dióxido de carbono e na acidez do solo, contribuindo para o ciclo de carbono (MAEGHT; REWALD; PIERRET, 2013). Além disso, deve se ressaltar que essas raízes são recursos para a fauna, comunidades de fungos e bactérias que vivem no solo (MAEGHT; REWALD; PIERRET, 2013).

Apesar de sua grande importância ecológica e fisiológica, os estudos sobre raízes profundas de espécies perenes ainda são escassos (SCHENK; JACKSON, 2002a, b, 2005). A maior parte das pesquisas com sistemas radiculares enfoca o papel de raízes superficiais na nutrição mineral das espécies arbóreas perenes, sendo a contribuição relativa das raízes profundas pouco documentada (SCHENK; JACKSON, 2002a, b). Esse fato pode ser justificado pelas dificuldades metodológicas e custos excessivos envolvidos nos processos de coleta e amostragem (MAEGHT; REWALD; PIERRET, 2013). São poucos os estudos envolvendo sistemas radiculares em profundidades de solo superiores a cinco metros, sendo a maioria dos trabalhos focada nos aspectos funcionais dessas raízes (SCHENK; JACKSON, 2002 a, b, 2005; CHRISTINA et al., 2011; LACLAU et al., 2013).

Existem alguns trabalhos com anatomia de raízes profundas com 20m de profundidade de algumas espécies (MCELRONE et al., 2004; Johnson et al., 2014; Rhizopoulou; Kapolas, 2015). McElrone et al. (2004), estudando *Juniperus ashei* J.Buchholz, *Bumelia lanuginosa* (Michx.) Pers., *Quercus virginiana* var. *fusiformis* (Small) Sarg. e *Quercus sinuata* Walter, compararam a estrutura do xilema e a vulnerabilidade a cavitação de raízes superficiais e raízes profundas. Os autores mostraram que os elementos de vaso do xilema nas raízes profundas apresentavam maior diâmetro, sugerindo que a estrutura das raízes profundas minimiza a resistência ao fluxo hidráulico e maximiza a absorção de água. De forma semelhante, Johnson et al. (2014) compararam aspectos anatômicos de raízes de *Sideroxylon lanuginosum* Michx. e *Quercus virginiana* var. *fusiformis* (Small) Sarg. coletadas a 20 m de profundidade com raízes localizadas em camadas mais superficiais do solo (< 10 cm de profundidade) e encontraram que as células condutoras do xilema de raízes mais profundas possuem diâmetro maior, propondo sua maior condutância e vulnerabilidade ao embolismo. Além disso, Rhizopoulou e Kapolas (2015) estudaram *Capparis spinosa* L. durante a época de seca e identificaram raízes dessa espécie a uma profundidade de 20m através de sua anatomia, com elementos de vaso do xilema com diâmetro maior que nas raízes superficiais. Deduzindo que um sistema radicular profundo nessa espécie estava diretamente associado ao período de seca enfrentado por ela.

É bem estabelecido que a maior parte da absorção de água e íons nas espécies lenhosas perenes ocorre em raízes finas não suberizadas (KRAMER; BOYER, 1995; SCHENK; JACKSON, 2002a, GAMBETTA et al., 2013). Sabe-se também que as porções mais jovens das raízes (porção apical *sensu lato* incluindo zona de pêlos radiculares) são as mais ativas nesse processo (TAIZ; ZEIGER, 2006; GAMBETTA et al., 2013).

O fluxo de água nas raízes pode seguir múltiplos caminhos, através de rotas apoplásticas, simplásticas, e/ou transcelulares. Em raízes não suberizadas, o caminho apoplástico foi identificado como sendo predominante. No entanto, a condutividade hidráulica do caminho apoplástico pode ser alterada devido a mudanças químicas nas paredes celulares, tais como a deposição de suberina em células da exoderme e da endoderme (GAMBETTA et al., 2013). Nesse contexto, sabe-se que sob condições de estresse hídrico ocorre maior suberificação em camadas de células das porções apicais da raiz, alterando sua permeabilidade a água (LESHEM, 1970; VANDELEUR et al., 2009). Raízes suberizadas possuem baixa atividade fisiológica devida à grande proporção de células mortas (PREGITZER et al., 2002) e possuem um papel limitado na absorção de água e íons (WELLS; EISSENSTAT, 2003; GUO et al., 2008b; GU et al., 2014), mas são importantes para o transporte, ancoragem e estoque de nutrientes (PREGITZER, 2002).

Raízes finas mostram estruturas e/ou funções extremamente variáveis entre si (PREGITZER et al., 2002). Estudos apontam que o potencial de absorção de água e nutrientes das raízes finas depende da sua arquitetura (EISSENSTAT et al., 2000; PREGITZER et al., 2002; GUO et al., 2008a). Esse fato é particularmente observado em condições de estresse hídrico sazonal em que as plantas geralmente adotam duas estratégias: (i) uma estratégia de "tolerância" ao estresse hídrico, produzindo raízes mais finas que podem sobreviver à seca periódica do solo e/ou (ii) uma estratégia de "prevenção", causando a morte das raízes finas na superfície do solo e promovendo o crescimento rápido de outras raízes em áreas mais úmidas (mais profundas) (HUANG; DUNCAN; CARROW, 1997). Assim, com a redução de chuvas e consequente redução da disponibilidade de água nas camadas superficiais do solo, a proporção de raízes finas profundas pode aumentar, favorecendo o fornecimento de recursos para as árvores.

Por outro lado, muitos estudos envolvendo anatomia de raízes de plantas em condições de estresse hídrico já existem identificando diferentes estratégias anatômicas a essa condição. Algumas modificações anatômicas às secas seriam: diminuição do diâmetro dos vasos de metaxilema da raiz reduzindo a área cortical transversal (VASELATTI et al., 2001); presença de elementos de vasos mais estreitos em espécies perenes para reduzir a tendência ao embolismo (TYREE; ZIMMERMANN, 2002; CARLQUIST, 2012; VON ARX; ARCHER; HUGHES, 2012); maior quantidade de vasos de menor calibre e redução do cilindro vascular (DURANTE; MASEDA; FERNÁNDEZ, 2011); menor número de camadas de células corticais que reduz os custos metabólicos da exploração do solo, permitindo a exploração mais profunda do solo, maior aquisição de água, e melhor crescimento em condições de

estresse hídrico (CHIMUNGU; BROWN; LYNCH, 2014). Vale destacar que Kadam et al. (2015) mostrou que o diâmetro do estelo e a plasticidade de desenvolvimento do xilema respondem melhor ao estresse por déficit hídrico em trigo que no milho, devido ao fato de um possuir mais plasticidade enquanto o outro é tolerante à seca. Isso mostra que algumas plantas possuem mais plasticidade que outras, podendo responder de forma diferente às condições de seca.

A família Myrtaceae possui uma distribuição predominantemente pantropical, embora se concentre na região neotropical e na Austrália. Myrtaceae inclui 130 gêneros e cerca de 4000 espécies, sendo que 22 gêneros e 1000 espécies são brasileiros, o que a torna uma das maiores famílias da flora nativa (SOUZA; LORENZI, 2012). Dessa família podemos destacar o gênero *Eucalyptus* spp., que possui muitas espécies de rápido crescimento, sendo importante fonte de biomassa para produção de papel, madeira para construção de cercas e postes, painéis de fibras, carvão vegetal e lenha industrial (TURNBULL, 1999; SOUZA; LORENZI, 2012). As plantações de eucalipto estão em expansão nas regiões tropicais ocupando cerca de 20 milhões de hectares (BOOTH, 2013). A espécie mais produtiva do gênero *Eucalyptus* ocorrente em regiões subtropicais quentes e úmidas é *Eucalyptus grandis* (GONÇALVES et al., 2013).

Vários mecanismos foram descritos para esse gênero que permitem que as árvores suportem a seca (WHITEHEAD; BEADLE, 2004). Dentre eles está o acesso à água das camadas profundas do solo através de uma extensão de suas raízes até o lençol freático (COHEN et al., 1997). No caso do *E. grandis* ocorre um aumento do sistema radicular profundo para ampliar a captação de água em períodos onde a quantidade de água vinda das camadas superficiais do solo diminui (DYE, 1996). Estudos envolvendo espécies de *Eucalyptus* demonstram que, em condições de clima árido, suas raízes são tão profundas que alcançam até 60 m profundidade no solo (STONE; KALISZ, 1991).

Existem muitos estudos envolvendo *E. grandis*, tais como: o sequenciamento de seu genoma (MYBURG et al., 2014); trabalhos relacionados a diversos aspectos de sua fisiologia (NOUVELLON et al., 2012; CAMPOE et al., 2012); e trabalhos que abordam mais a parte aérea da planta (CLEARWATER; MEINZER, 2001). Embora existam diversos estudos envolvendo *E. grandis* ainda existem poucos trabalhos sobre as raízes dessa espécie. Dentre os trabalhos com raízes dessa espécie podemos destacar aqueles com as raízes finas em grandes profundidades, que abordam tanto a biomassa (LACLAU et al., 2013) quanto a captação de nutrientes dessas raízes (DA SILVA et al., 2011).

Os trabalhos anatômicos com *E. grandis* são mais escassos e estão relacionados a anatomia da madeira (SEARSON et al., 2004; THOMAS; MONTAGU; CONROY, 2007) e das folhas (BATTIE-LACLAU et al., 2013; BATTIE-LACLAU et al., 2014). E no que se refere aos aspectos morfoanatômicos de raízes profundas dessa espécie, não foram encontrados dados disponíveis na literatura.

Este trabalho teve como objetivo estudar a anatomia de raízes finas de *Eucalyptus grandis* ao longo da profundidade do solo e investigar o efeito da limitação da precipitação nos aspectos anatômicos dessas raízes. Além disso, essa pesquisa foi realizada em consonância com outros estudos que vem sendo desenvolvidos no Brasil pela equipe da *Unidade de Pesquisa em Ecologia Funcional, Biogeoquímica de Solos e Agro-ecossistemas*" do CIRAD – Agricultural Research for Development (Instituição francesa de pesquisa) no intuito de entender os aspectos funcionais das raízes profundas e uso da água em espécies arbóreas em regiões tropicais.

Conforme estabelecido pelo Programa de Pós-Graduação em Ciências Biológicas (Botânica) do IBB, UNESP, os resultados obtidos durante a execução deste Projeto de Mestrado foram reunidos em um artigo científico para publicação, que é apresentado de acordo com as normas do periódico Annals of Botany (A1 – Comitê de Biodiversidade da Capes).

Anatomy of deep and superficial roots of *Eucalyptus grandis* trees under rainfall reduction

Anatomy of deep and superficial roots of *Eucalyptus grandis* trees under rainfall reduction

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Background and Aims The establishment of a deep root system in perennial species is an adaptive strategy against the water stress. During drought periods, these roots, mainly the non-suberized fine roots, are able to withdraw water from deep soil layers and ensure water supply to these plants. Considering that climate models predict a rainfall reduction in the next years, to know the effect of water scarcity on the structure of superficial and deep roots is an important point. Our aim was to analyze the anatomy of fine roots of *Eucalyptus grandis* along a soil depth gradient and to investigate the effects of the rainfall reduction in their anatomical features.

Methods An experiment was set up in June 2010 using adult clones of *E. grandis*. A lot of plants was submitted to 37% of rainfall interception (TR treatment) during 5 years using plastic blades. Lateral fine roots were collected from 0-50cm and 12-16m of depth in the soil from the control treatment (TO) and from TR. These roots were separated according to their diameter: $Ø > 2mm$; $1< Ø \leq 2mm$; $0.5< Ø \leq 1mm$; and $Ø \leq 0.5mm$ and processed according to standard techniques in plant anatomy. The morphometric results were submitted to the t-student test and U Mann-Whitney test; others to a two way ANOVA and Tukey test.

Key Results Roots with diameter $Ø > 2mm$ and $1< Ø \leq 2mm$ collected in TR, and roots with diameter $0.5 < Ø \leq 1mm$ collected in TO in greater depths of the soil presented biggest total area occupied by the xylem; roots with diameter $Ø > 2mm$, $1< Ø \leq 2mm$ and $0.5 < Ø \leq 1mm$ collected in TR in greater depths also presented biggest total area of xylem per total area of functional root portion ratio; in deep roots of these three categories the vessels were less abundant, however with larger diameter than superficial roots. The increase in diameter of vessels of deep roots refers to an increase in hydraulic conductivity, but can raise the risk of embolism. Deep roots, with diameter > 0.5mm from TR exhibited several groups of gelatinous fibers in the phloem and tyloses in the vessels. Gelatinous fibers have potential for storing water while tyloses may represent a strategy against embolism in water stress conditions.

Conclusions The rainfall reduction has a greater effect on the anatomical traits of *E. grandis* roots in deeper layers of the soil than shallow soil horizons and usual rainfall conditions.

Key words: deep root, drought, eucalyptus, superficial root, vessels, xylem

INTRODUCTION

The establishment of a deep root system in perennial species is an adaptive strategy to water stress (David *et al.*, 2013). During periods of drought, the root system of perennial species is able to provide water from the deeper layers of soil and ensure the water supply to these plants (Kleidon and Heimann, 2000; Christina *et al.*, 2011). Under this condition, the root system of these species is able to reach greater depths in the soil and even transpose rock layers (Maeght *et al.*, 2013). Studies with *Eucalyptus* species show that in arid conditions their roots are so deep getting to reach up to 60 m depth in the soil (Stone and Kalisz, 1991). In this sense, Burgess et al., (2000) demonstrated the occurrence of hydraulic redistribution in the soil of a tree species, where the water is transferred by deep root systems from the wet deeper layers to the drier shallower layers.

Redistribution and provision of water by deep root systems can influence weather systems and contribute significantly to the hydrological cycle in tropical biomes (Oliveira *et al.*, 2005). Surveys conducted in Amazonia have shown that the destruction of deep root systems caused by deforestation has direct impact on climate conditions (Kleidon and Heimann, 2000). In addition, the models of climate changes predict rising temperature and more frequent droughts, and a reduction in rainfall of up to 20% between the first and the last decade of 21st century in a large range of the earth, including Brazil (IPCC, 2013). In this context, it is crucial to know the response of ecosystems and their adaptability to this situation.

Researchers suggest that the deepest layers of soil may be more significant sources of nutrients than believed (McCulley *et al.*, 2004; da Silva *et al.*, 2011) and that these deep root systems have the ability to expand the volume of soil accessible to the uptake of water and nutrients (McMurtrie *et al.*, 2012; Laclau *et al.*, 2013). Another aspect that has been known for decades is that the roots exercise physico-chemical weathering on their environment

(Richter and Markewitz, 1995; Richter *et al.*, 2007). It is also known that the deep roots play an active role in the carbon dioxide distribution and soil acidity, contributing to the carbon cycle. It should also be noted that these roots are resources for fauna, fungi communities and bacteria that live in soil at different soil depths (Maeght *et al.*, 2013).

Despite its great ecological and physiological importance, studies of deep roots of perennial species are rather scarce. Most research with root systems focuses on the role and activity of superficial roots and the relative contribution of the deep roots is poorly documented (Schenk and Jackson, 2002 *a, b*). This fact can be explained by methodological difficulties and excessive costs of collecting and sampling procedures (Maeght *et al.*, 2013). There are few studies involving root systems in soil depths greater than five meters, being the majority of the work being focused on the functional aspects of these roots (Schenk and Jackson, 2002 *a*, *b*, 2005; Christina *et al.*, 2011; Laclau *et al.*, 2013).With regard to the morphological and anatomical features of deep roots, works are even scarcer and restricted to few species: *Juniperus ashei* J.Buchholz, *Bumelia lanuginosa* (Michx.) Pers., *Quercus virginiana* var. *fusiformis* (Small) Sarg. and *Quercus sinuata* Walter*; Sideroxylon lanuginosum* Michx. and *Quercus virginiana* var. *fusiformis* (Small) Sarg.*; Capparis spinosa* L. (McElrone *et al.*, 2004; Johnson *et al.*, 2014; Rhizopoulou and Kapolas, 2015*).*

To know the anatomy of roots at different depths of the soil is necessary for understanding the role of roots in the acquisition of water and mineral resources in diverse ecosystems; this fact becomes increasingly important, particularly when a decrease in water availability is predict to the next years. In this paper, we analyzed the anatomy of fine roots of *Eucalyptus grandis* W. Hill ex Maiden in different soil depths and investigated the effect of the rainfall reduction on their anatomical traits.

In the literature there is a surprisingly lack of studies on the anatomy of woody plant roots mostly for vascular tissues. Researches on deep roots anatomy in natural ecosystems are

even scarcer due to the methodological difficulties and excessive costs involved in collecting and sampling techniques (Maeght *et al.,* 2013). Despite recent innovations and technological advances, researchers still prefer to use available soil profiles to study deep roots (McElrone *et al.*, 2004; Johnson *et al.*, 2014; Rhizopoulou and Kapolas, 2015). Differently, in this study we used the deep trenches methodology to collect deep roots of *Eucalyptus grandis* in their natural ecosystems. We compared the root anatomy of *E. grandis* along different soil depths and under two treatments of rainfall availability. This is the first study of anatomy of deep roots of *E. grandis* at 12-16m of depth.

MATERIALS AND METHODS

Plant material and area of occurrence

We used *Eucalyptus grandis* as a model species due to the high growth rates of their fine roots that quickly explore deep soil layers. Root samples were collected from *E. grandis* plantations established in the Experimental Station of Forest Sciences of Itatinga (EECFI) (latitude 23º02'S and longitude 48º38'W), São Paulo State, Brazil. In this region, two seasons can be distinguished: a dry season from June to September and a rainy season during the remaining months characterized by high rainfalls, temperature and global radiation. During the last 15 years the average annual rainfall was 1360 mm and the average annual temperature was 20°C, with a mean monthly temperature ranged from 15°C (between June and September) to 25°C (between October and March) (Battie-Laclau *et al.,* 2014). During the collecting period the average temperature was 24°C and the average rainfall was 8.22mm.

The experiment was located at the top of a hill (slope < 3%) at 850 m of altitude. There, the soils are very deep latosols type (> 15m) sandstone-Cretaceous, Marília formation,

Bauru group, with clay content ranging from 14% in the horizon A1 to 23% in the deeper layers (da Silva *et al.*, 2011). The mineralogy consists predominantly of quartz, kaolinite and oxy-hydroxide with acidic layers (pH between 4.5 and 5.0) (Campoe *et al.*, 2012; Maquère, 2008).

Experimental design

An experiment was set up in June 2010 with an *E. grandis* clone used in commercial plantations by the Suzano Company (São Paulo, Brazil). Two treatments (two water supply regimes) were applied in separated blocks. The treatments were as follows: TO: Control nutrition with K^+ application and without rainfall interception (control treatment); and TR: Control nutrition with K^+ application and with approximately 37% of rainfall interception.

The area of individual plot was 864 m^2, with 144 trees per plot spacing in 2m x 3m (a total of 432 trees per treatment). All the experimental trees were fertilized at planting (3.3 g m-2 P, 200 gm-2 of dolomitic limestone and trace elements) and at three months of age (12 g N m-2) (Laclau *et al.*, 2009). From September 2010, the rains were partially intercepted on the TR plot using transparent plastic sheet panels which enables PAR transmission, mounted on wooden structures with height ranging between 1.6 and 0.5m (Figure 1 A, B). Different highs of wooden sticks helped drain the water flow coming from the rain. Plastic sheets covered 37% of the site in the TR plot and this percentage was determined by area calculation. Openings were left around the stems of the trees and between rows allowing a homogeneous distribution of rainfall not intercepted when possible. The drain stem was not deleted from the portion, given the small contribution of the total input of water in the soil and the disproportionately greater contribution of nutrients entering the soil (Laclau *et al.*, 2010). All organic material that fell into the plastic sheets was collected weekly and spread on the floor under the plastic sheets (Figure 1 B).

Root sampling in the field

In January and March of 2015, two trenches with 17m deep were dug into the ground (Figure 1 C), one for each treatment. Each trench was centered among four trees. The trenches were manually dug into the ground with a hoe. The collected land was placed in a bucket, which was pulled outside of the trench for posterior collection of roots. Concrete rings that fit one another were also placed inside the trench forming a concrete column to ensure their stability and the safety of the staff members (Figure 1 C, D). Fine root samples were collected from soil removed from 0-0.2m; 0.2-0.5m; 12-13m; 13-14m; and 15-16m. Roots were also collected from 0.5-12m but couldn't be used in the experiment due to the short time available for this work.

Root categories

We considered the roots collected at 0-50cm as superficial roots and the roots collected at 12-16m as deep roots. After washed in distilled water, the roots were immediately fixed in FAA 50 solution (formalin - acetic acid - 50% alcohol) (Johansen, 1940). After 48 hours, the roots were transferred to 70% alcohol, dissected and carefully separated in categories according to their diameters, using a stereomicroscope Leica (M205 C). The roots were discriminated in four categories according to their diameter: $Ø > 2mm$; $1 < Ø \leq 2mm$; $0.5 < Ø \leq 1mm$; e $Ø \leq 0.5mm$. This categorization of roots in diameters was an attempt to separate the roots according to their vascular maturation.

Light microscopy

Three subsamples were excised from each root category at each depth from both treatments. For roots with $Ø \leq 0.5mm$ diameter, subsamples were excised from the differentiation zone, at 1mm from the apex. Then, the material was dehydrated in alcoholic series and embedded in resin (Leica Historesin). Cross sections (3-4µm) were obtained in a

semi-automated rotary microtome and stained with 0.05% toluidine blue O in phosphate buffer pH 4.7 (O'Brien *et al.*, 1964). Permanent slides were mounted with synthetic resin (Entellan). The most relevant aspects were documented in Olympus BX41 light microscope coupled to an Olympus digital camera.

Anatomical measurements were made in the cross sections using the Olympus Cell B software. In roots under primary growth, we analyzed: root diameter (Gambetta *et al.*, 2013; Gu *et al.*,2014; Kadam *et al.*, 2015); total area of the root; thickness of the cortex (Gu *et al.*, 2014.); number of cortical layers; stele diameter (Gu *et al.*, 2014; Kadam *et al.*, 2015); proportion stele / root; total area of xylem; number of xylem vessels (von Arx *et al.*, 2012; Kadam *et al.*, 2015); and diameter of metaxylem vessels (Vasellati *et al.*, 2001; Kadam *et al.*, 2015). In cross-sections of roots under established secondary growth we measured: diameter of the functional portion of the root; total area of functional portion of the root; area occupied by sclerenchyma; total area of the xylem; number of xylem vessels; and diameter of xylem vessels (von Arx *et al*., 2012; Kadam *et al*., 2015). We called functional portion of the root the part of the root that is in secondary growth and functional, disregarding the obliterated part in primary growth that remains visible but not functional. Comparisons between roots vessels diameter were made between roots with the same total diameter or root portions that had the same maturation of the vascular cambium.

Statistical analysis

Three samples from each root category at each depth from both treatments were used in statistical analysis. Results were analyzed in comparative way between the different depths of the soil in each trench and between the two treatments, using RStudio program and R program (3.2.3 version). Statistical differences were determined with different statistical tests. The obtained data of roots with diameter $Ø \leq 0.5$mm were submitted to U Mann-Whitney test.

In roots with diameter 0.5 < Ø ≤ 1mm, the obtained data were submitted to a two way ANOVA and the means were compared by Tukey test (5% of significance) (Vasellati *et al.*, 2001). In roots with 1 < Ø ≤ 2mm and Ø > 2mm diameter, the obtained data were submitted to a t-Student test when data's were parametric (Johnson *et al.*, 2014) and a U Mann-Whitney test when data's were non parametric.

RESULTS

Distribution of roots along the soil depth

In the TO trench, eucalyptus roots were found up to 14m of depth in the soil. In TR trench, the roots were found until deeper layers of the soil and reached 16m of depth. Roots with 0.5 < Ø ≤ 1mm and Ø ≤ 0.5mm diameter were found in both trenches and in superficial and deep soil. On the other hand, roots with Ø > 2mm and 1< Ø ≤ 2mm diameter were not found in the deep soil in TO trench.

General anatomical features of the roots

The roots with diameter Ø ≤ 0.5mm presented primary growth. Some of them exhibited asymmetric contour (Figure 2A-C) and others had more regular shape (Figure 2D). They exhibited uniseriate epidermis with root hairs, 3-6 layers of cortex with evident endoderm, and stele constituted by parenchymatic pericycle, primary xylem and phloem (Figure 2A-D).

Roots with 0.5 < Ø ≤ 1mm; 1 < Ø ≤ 2mm and Ø > 2mm diameter exhibited secondary growth (Figure 3A-C). They presented periderm with suberized phellem, phellogen and phelloderm, secondary phloem, vascular cambium and secondary xylem (Figure 3A-C). The secondary phloem was comprised by sieve tube members, companion cells and common

parenchyma cells; groups of gelatinous fibres were observed mainly in the outer portion of the phloem (Figure 3A-D). A well-established cambial zone was observed between secondary phloem and xylem (Figure 3B). The secondary xylem was composed by vessels with different sizes, fibres and parenchyma cells (Figure 3A-C). Parenchyma rays pass through the secondary phloem and xylem and their cells accumulate phenolic compounds (Figure 3A-C); these rays were dilated in the outer portion of the secondary phloem (Figure 3A-C).

Anatomical traits of the roots along the soil depth

Under the control treatment (TO), in roots with Ø ≤ 0.5mm diameter (Figure 2 A-D): anatomical characteristics did not differ statistically between superficial and deep roots (Table 1); number of cortical layers was similar in superficial (5 – 6) and deep roots (3 – 4) (Table 1).

In roots with 0.5 < Ø ≤ 1mm diameter (Figure 4A-E), the diameter of the functional portion of the root, the total area of the functional portion of the roots and the total area occupied by the xylem were greater in deep roots (Figure 4C-E) than in superficial roots (Figure 4A-B). Furthermore, the diameter of vessels was wider in deep than in superficial roots (Table 2). Otherwise, the number of vessels was higher for superficial than for deep roots (Table 2). The ratio total area occupied by xylem / total area of the functional portion of the root did not differ statistically between superficial and deep roots (Table 2).

Effects of rainfall reduction on anatomical traits of the roots

In roots with Ø ≤ 0.5mm diameter from superficial soil (Figure 2A, B), morphometric data didn't differ statistically between the two treatments (Table 1). The number of cortical cells layers (Table 1) also didn't differ between TR (4 – 6) and TO (5 – 6). Morphometric data from deep roots with diameter Ø ≤ 0.5mm (Figure 2C, D) did not differ statistically between

the two treatments (Table 1). However, the number of cortical cells layers (Table 1) was higher in roots from TR (3 – 6) than those from TO (3 – 4).

In roots with 0.5 < Ø ≤ 1mm diameter from superficial soil (Figure 4A, B) all the parameters did not differ statistically (Table 2). In roots from deep soils (Figure 4C, D) the ratio total area of xylem / total area of functional root portion were larger in TR than in TO (Table 2). The number of vessels and the other parameters did not differ between the treatments in these roots (Table 2).

In roots with 1 < Ø ≤ 2mm diameter from superficial soil, the vessels were wider in TR than in TO (Table 3) and the number of vessels was higher in TO than in TR (Table 3). The other analyzed parameters did not differ between the two treatments (Table 3).

Concerning the roots with Ø > 2mm diameter from superficial soil, the analyzed structural parameters did not differ between the two treatments (Table 5).

Effect of depth X rainfall reduction in the anatomical traits of the roots

Roots with diameter Ø ≤ 0.5mm from TR (Figure 5A-B) anatomical characteristics did not differ statistically between superficial and deep roots (Table 1). The number of cortical cell layers was the same in superficial (4 – 6) and deep roots (3 – 6). On the other hand, in roots with 0.5 < Ø ≤ 1mm diameter from TR, the ratio total area of xylem / total area of the functional portion of the root were greater in deep roots than in superficial roots (Table 2). Diameter of vessels was also larger in deep than in superficial roots (Table 2). The number of vessels was greater in superficial and smaller in deep roots (Table 2). The diameter of the functional portion of the root, the total area of the functional portion of the root and total area occupied by the xylem did not differ between superficial and deep roots (Table 2).

For roots under TR with diameter 1 < Ø ≤ 2mm (Figure 5 C-D) the total area occupied by the xylem and the ratio total area of xylem / total area of functional portion of the

root were also greater in deep roots than in superficial roots (Table 4). In addition, the diameter of the vessels was also wider in deep than in superficial roots (Table 4). Number of vessels, diameter of the functional portion of the roots and total area of the functional portion of the root also did not differ statistically between superficial and deep roots (Table 4).

On the other hand for roots under TR with Ø > 2mm diameter (Figure 5 E-F), the total area of the functional portion of the root was greater in deep roots than in superficial roots (Table 6). Similarly in these roots, the total area of xylem and the ratio xylem total area / total area of the functional portion of the root were greater in deep roots than in superficial roots (Table 6). The diameter of the vessels was also higher in deep than in superficial roots (Table 6). Roots of Ø > 2mm diameter showed great variability in statistical characteristics between superficial and deep roots; however the diameter of the functional root portion and the number of vessels did not differ statistically between superficial and deep roots (Table 6).

It is remarkable that roots with diameter $0.5 < Ø \leq 1$mm; $1 < Ø \leq 2$mm and Ø > 2mm from TR showed some notable anatomical characteristics: abundant groups of gelatinous fibres in the phloem (Figure 3 A-D; 4 D-E) and the occurrence of tyloses in several vessels (Figure 5 D, F).

DISCUSSION

In this study, most part of the roots collected presented secondary growth instead of primary growth. Roots in primary growth can be called absorptive roots and exhibit cortical cells that are easily injured under stress (Wells and Eissenstat, 2003), which makes first-order roots the most ephemeral of all orders. Since life expectancy of individual roots in the branching root system varies widely, the presence of a big amount of roots in secondary growth could be a result of the significantly shorter lifespans on average of the roots in primary growth that have the smallest diameter (Baddeley and Watson, 2005).

We separated the roots according to their diameter. So based on our results, most of the analyzed anatomical traits differed significantly among the roots of different diameters. In roots with diameter $0.5 < Ø \leq 1$mm, $1 < Ø \leq 2$mm e $Ø > 2$mm, secondary growth was established and a suberized periderm, secondary phloem, vascular cambium and secondary xylem were observed. Roots under secondary growth are important to the transport, anchorage, and storage of nutrients (Pregitzer, 2002), but have low physiological activity because of the large proportion of dead cells (Pregitzer *et al*., 2002) and play a limited role in absorption (Wells and Eissenstat, 2003; Guo *et al.*, 2008*b*; Gu *et al.*, 2014). However, studies show that the formation of suberized periderm does not completely seal the apoplastic pathway for the secondary growth zone in roots (Steudle, 2000, Gambetta *et al.*, 2013). Furthermore, the well-developed cork layer and secondary xylem can protect roots from environmental stresses and herbivore pressure (Brundrett, 2002), guaranteeing high-order roots long life spans, and consequently, low turnover rates (Wells and Eissenstat, 2003; Guo *et al*., 2008 *a, b*).

Roots in primary growth exhibited epidermis with root hairs, and intact cortex and stele. According to these anatomical characteristics these roots belong to first branching order (Pregitzer *et al.*, 2002; Wells and Eissenstat, 2003; Guo *et al.*, 2008 *b*; Valenzuela-Estrada *et al.*, 2008; Gu *et al.*, 2014) having absorptive functions mainly. In this category, the superficial roots from TO do not differ from the deep roots. In roots with diameter $Ø \leq 0.5$mm the number vessels were more numerous in superficial than in deep roots.

When analyzing the anatomical characteristics according to soil depth from TO, the diameter of vessels and the total area of xylem proved to be determinant in the comparison between deep and superficial roots. For roots in secondary growth, the total area of xylem and diameter of vessels was wider in deep roots than in superficial roots while the number of vessels was higher for superficial than for deep roots. Studies affirm that the number and the

diameter of xylem vessels can be a key factor that influence hydraulic conductance (Tombesi *et al.*, 2010). Such anatomical features observed in deep roots support the idea that *E. grandis* trees can adjust their hydraulic architecture as a strategy in order to improve their hydraulic conductivity in depths from 12-16m. Similar data about the larger vessels diameter were found by some researchers that studied deep roots at 20m of depth (McElrone *et al.*, 2004; Johnson *et al.*, 2014; Rhizopoulou and Kapolas, 2015).

The conductive path reflected by stem length, is the main driver of global variation in mean vessel diameter (Olson *et al.*, 2014). Deep roots of *E. grandis* presented mean vessel diameters (82.4µm) three times larger than superficial. Vessel diameter in stem wood can vary from 5µm in conifer needles to 500 µm in tropical lianas (Hacke and Sperry, 2001). Also, lianas have wider vessels for a given stem diameter than self-supporting plants, like *Trimenia moorei* (Oliv.) Philipson with a stem of 18m long for a mean vessel diameter of 93 µm (Olson *et al.*, 2014). According to this information, the length of the root that reaches great depths could be an important factor related to the *E. grandis* deep roots vessels diameter.

Concerning the influence of rainfall reduction on the anatomical features of superficial roots with $1 < Ø \leq 2$mm, vessels were larger in TR than TO, but fewer in TR and numerous in TO. It is known that the number and diameter of xylem vessels can influence hydraulic conductance (Tombesi *et al.*, 2010). In this case, the larger vessels in superficial roots from TR could indicate an improved hydraulic conductance, while the smaller diameter of vessels in superficial roots from TO could be important to avoid embolism (McElrone *et al.*, 2004). The studies supports the idea that dry environments favor species with narrow conduits that are embolism resistant vessels but conducts very slowly while warm and moist environments favors wider vessels, more vulnerable but conductively efficient (Tyree and Zimmermann, 2002; Carlquist, 2012). In this study, contrarily to the literature, different results were found for superficial roots of *E. grandis* trees in rainfall reduction condition. The presence of larger

vessels in *E. grandis* roots supports the idea that large vessels would be able to conduct larger quantities of water during short periods of rainfall and that the increase in their diameter causes an increase in mass flow (Tyree and Zimmermann, 2002). However more researches are needed to confirm this observation.

In the current study anatomical changes in roots from TR according to soil depth are mainly related to the secondary xylem structure. In roots from deeper soils of TR: the total area of xylem was larger in roots with diameter $Ø > 2mm$, $1 < Ø \leq 2mm$; the total area of xylem / total area of functional root portion ratio was larger in roots with diameter $0.5 < Ø \leq 1mm$; and vessels had larger lumen in roots from these three categories. The presence of vessels with larger lumen in deep roots in secondary growth could be associated with the hydraulic capacity of these roots (Johnson *et al.*, 2014; McElrone *et al.*, 2004) and drought stress. Vessels with widest lumen are more vulnerable to suffer embolism (Hacke and Sperry, 2001; Tyree and Zimmermann, 2002; McElrone *et al.*, 2004) and might also be more susceptible to drought-induced cavitation (Hacke and Sperry, 2001; Tyree and Zimmermann 2002; McElrone *et al.*, 2004; Hacke *et al.*, 2009). In climates with intense but limited drought periods, vessels with larger diameters conduct greater quantities of water during short periods of rainfall because a rise in diameter outcomes an increase in mass flow by the fourth power of the change in diameter (Tyree and Zimmermann, 2002; Tyree and Ewers, 1991; Dettmann *et al.*, 2013).

Deep roots of *E. grandis* with structural features that refers to a greater hydraulic conductivity, such as largest vessels diameter and bigger total area of xylem, suggest the establishment of a deep root system as an adaptation strategy of perennial species to water deficit as per David *et al.* (2013). So in TR, the presence of roots in deeper layers than TO suggest that *E. grandis* trees use a deep rooted system as a source of water to complement

their water necessity, in order to continue to grow during the drought like some other species (Rhizopoulou and Kapolas, 2015).

Roots with diameter Ø > 2mm,1 < Ø ≤ 2mm and 0.5 < Ø ≤ 1mm from deep soils from TR also showed some interesting morphological and anatomical characteristics different from roots from TO. Eucalypt roots showed tortuous aspect, tyloses in the vessels and abundant groups of gelatinous fibres in the bark. First, the soil compaction caused by the depth and the hydraulic reduction can affect the root system architecture, and play direct effect on root morphology (Bengough *et al.*, 2006; Tracy *et al.*, 2012). So, these conditions are probably the cause of morphological changes of roots from deeper layers of the soil in TR. Secondly, our work showed that roots of *E. grandis* have typical gelatinous fibers in the secondary phloem, as found in the tension wood of this species (Washusen *et al.*, 2005). Gelatinous fibers are specialized sclerenchyma cells with an extra non-lignified layer in the innermost portion of the secondary cell wall, composed of mostly cellulose, called G-layer (Esau, 1977). They appear in several flexible organs such as peduncles, contractile roots, tendrils and stems (Mellerowicz and Gorshkova, 2012) and are related to the formation of tension wood in angiosperm (Du and Yamamoto, 2007; Gardiner *et al.*, 2014). Some studies showed that the presence of gelatinous fibres in contractile roots is important to the mechanism of contraction (Zimmermann *et al.*, 1968; Fisher, 2008; Schreiber *et al.*, 2010). However gelatinous fibers may also function as water storage (Paviani, 1978; Chalk, 1989) that occurs because of the large capacity for water absorption of the inner layer, that is cellulosic and hydrophilic (Chalk, 1989; Carlquist, 2001). Since the root system in TR was deeper than TO reaching 16m and that we found a larger amount of gelatinous fibers in roots from TR, we can suggest the importance of these cells in the water storage and as a result of the higher pressure in deeper layers of the soil.

Another remarkable feature of deep roots with secondary growth from TR is the presence of tyloses in great amount in the vessels of the xylem. Tyloses occur when parenchyma cells of the secondary xylem, located proximally to the vessels, make protrusions to the inside of the lumen of the vessels. Such parenchyma cells possess a thicker wall layer composed of celluloses microfibrils in a loose arrangement and abundant pectin. This layer inflates passing through the wall pits and enters the vessel lumen originating tyloses (Evert, 2013). Tyloses are formed as the vessels cease their function since it obstructs fluid penetration and decreases or eliminates the hydraulic conductivity of an occluded vessel (Collins *et al.*, 2009). There are many reasons why the tyloses formation can initiate, mainly cavitation and frost (Cochard and Tyree, 1990) and represent a strategy against embolism in drought condition (Barry *et al*., 2001; Tyree and Zimmermann, 2002; Dettmann *et al.*, 2013); in addition, tyloses serve as pathogen defense mechanism inhibiting the spread of the pathogen to the plant via the xylem (Clérivet *et al.* 2000; Collins *et al.*, 2009). Tyloses production can also affect the water flow causing problems in hydraulic conductivity (McElrone *et al.,* 2010). So, the presence of large vessels in deep roots from TR and the drought stress condition increase the tendency of vessels to suffer embolism. However, deep roots vessels in a drought stress situation could accumulate water for the tree since embolized vessels can still function as water storage organs as they retain some water (Tyree and Zimmermann, 2002).

Recent studies show that the ability of survival and recover to drought periods of the perennial woody plants is intensely related to their resistance to embolism. However, this feature is highly variable among species and is widely determined by differences in the xylem structure (Choat *et al.*, 2012). Though the majority of species use mechanisms of high resistance against the embolism, xylem developmental plasticity and variation in vessel numbers to survive to drought stress conditions, there are many examples of studies that show

plant species with low embolism resistance growing in seasonal drought areas (Choat *et al.*, 2012). This mechanism can be seen in trees of tropical dry forests which use internal water storage, reduce leaf area and access to ground water by deep roots (Choat *el al.*, 2012). In our study, the rainfall reduction appears to have promoted deeper roots in *E. grandis* and played a greater effect on the anatomical traits of roots from deeper layers of the soil leading to structural changes in the xylem as a result of this mechanism.

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Table 1. Morphometric data of roots of *Eucalyptus grandis* with Ø ≤ 0.5mm diameter at superficial (0-50cm) and deep (12-16m) layers of the soil from individuals under the control treatment (TO) and under rainfall reduction (TR) (median).

Morphological traits	Superficial TO	Deep TO	Superficial TR	Deep TR
Diameter of the roots (mm)	0.26	0.20	0.21	0.24
Total area of the roots (cm²)	5.36	2.14	3.68	5.28
Cortical thickness (µm)	82.79	32.57	62.51	37.5
Stele diameter (µm)	87.61	49.63	74.64	69.07
Stele/root ratio (µm)	0.34	0.27	0.31	0.26
Total area occupied by the xylem (cm²)	0.08	0.03	0.07	0.05
Number of vessels	19	8	16	9
Diameter of vessels (µm)	3.49	3.57	2.80	2.14
Total area of xylem / total area of functional root portion ratio (%)	1.75	1.30	1.5	0.97

*Variable with significant difference ($p<0.05$)

Table 2. Morphometric data of roots of *Eucalyptus grandis* with 0.5 < Ø ≤ 1mm diameter at superficial (0-50cm) and deep (12-16m) layers of the soil from individuals under the control treatment (TO) and under rainfall reduction (TR) (mean ± s.d).

Morphological traits	Superficial TO	Deep TO	Superficial TR	Deep TR
Diameter of the functional portion of the roots (mm)	0.45±0.05 B	0.72±0.10 A	0.54±0.06AB	0.55±0.03 AB
Total area of the functional portion of the roots (cm²)	15.76±3.76 B	46.30±15.63 A	23.25±5.14 AB	26.09±3.09 AB
Total area occupied by the xylem (cm²)	4.44±0.28 B	15.60±7.13 A	7.13±1.03 AB	15.54±1.31 AB
Number of vessels	35±9.17 A	14±2.00 B	38.67±2.08 A	10±2.65 B
Diameter of vessels (µm)	23.29±6.53 B	99.36±25.79 A	17.54±2.24 B	117.08±19.87 A
Total area of xylem / total area of functional root portion ratio (%)	29.40±7.72 B	32.87±3.65 B	31.18±4.19 B	59.70±1.97 A

Means followed by the same letter do not differ by Tukey test ($p<0.05$).

Table 3. Morphometric data of roots of *Eucalyptus grandis* with $1 < Ø \leq 2$mm diameter at superficial (0-50cm) layers of the soil from individuals under the control treatment (TO) and under rainfall reduction (TR) (mean ± s.e).

Morphological traits	Superficial TO	Superficial TR
Diameter of the functional portion of the roots (mm)	1.67**	1.66**
Total area of the functional portion of the roots (cm²)	205.87±16.46	169.0±34.95
Total area occupied by the xylem (cm²)	64.43± 3.69	48.31±18.53
Number of vessels*	131.33±5.36	51.33±8.99
Diameter of vessels (µm)*	41.21± 0.18	61.11± 3.03
Total area of xylem / total area of functional root portion ratio (%)	33.16**	31.55**

*Variable with significant difference ($p<0.05$)

**Median for non-parametric datas

Table 4. Morphometric data of roots of *Eucalyptus grandis* with $1 < Ø \leq 2$mm diameter from superficial (0-50cm) and deep (12-16m) layers of the soil from individuals under rainfall reduction (TR) (mean ± s.e).

Morphological traits	Superficial TR	Deep TR
Diameter of the functional portion of the roots (mm)	1.5± 0.19	1.57± 0.16
Total area of the functional portion of the roots (cm²)	169.0±34.95	220.70±37.04
Total area occupied by the xylem (cm²)*	48.31±18.53	120.93±17.98
Number of vessels	51.33±8.99	31.33±4.18
Diameter of vessels (µm)*	61.11± 3.03	136.42± 5.66
Total area of xylem / total area of functional root portion ratio (%)*	29.03± 2.99	55.21± 1.80

*Variable with significant difference ($p<0.05$)

Table 5. Morphometric data of roots of *Eucalyptus grandis* with Ø > 2mm diameter at superficial (0-50cm) layers of the soil from individuals under the control treatment (TO) and under rainfall reduction (TR) (mean ± s.e).

Morphological traits	Superficial TO	Superficial TR
Diameter of the functional portion of the roots (mm)	2.57 ± 0.27	2.12± 0.11
Total area of the functional portion of the roots (cm²)	511.35±83.81	331.21±45.30
Total area occupied by the xylem (cm²)	168.55±22.36	99.45±6.29
Number of vessels	103.67±17.95	71.67±12.25
Diameter of vessels (µm)	88.31±10.30	70.68±6.63
Total area of xylem / total area of functional root portion ratio (%)	33.49± 2.19	30.59± 2.10

*Variable with significant difference ($p<0.05$)

Table 6. Morphometric data of roots of *Eucalyptus grandis* with Ø > 2mm diameter at superficial (0-50cm) and deep (12-16m) layers of the soil from individuals under rainfall reduction (TR) (mean ± s.e).

Morphological traits	Superficial TR	Deep TR
Diameter of the functional portion of the roots (mm)	2.12± 0.11	2.71± 0.18
Total area of the functional portion of the roots (cm²)*	331.21±45.30	627.17±42.32
Total area occupied by the xylem (cm²)*	99.45±6.29	276.10±28.09
Number of vessels	71.76±12.25	48±2.89
Diameter of vessels (µm)*	70.68±6.63	162.39±10.35
Total area of xylem / total area of functional root portion ratio (%)*	30.59± 2.10	43.98±3.18

*Variable with significant difference ($p<0.05$)

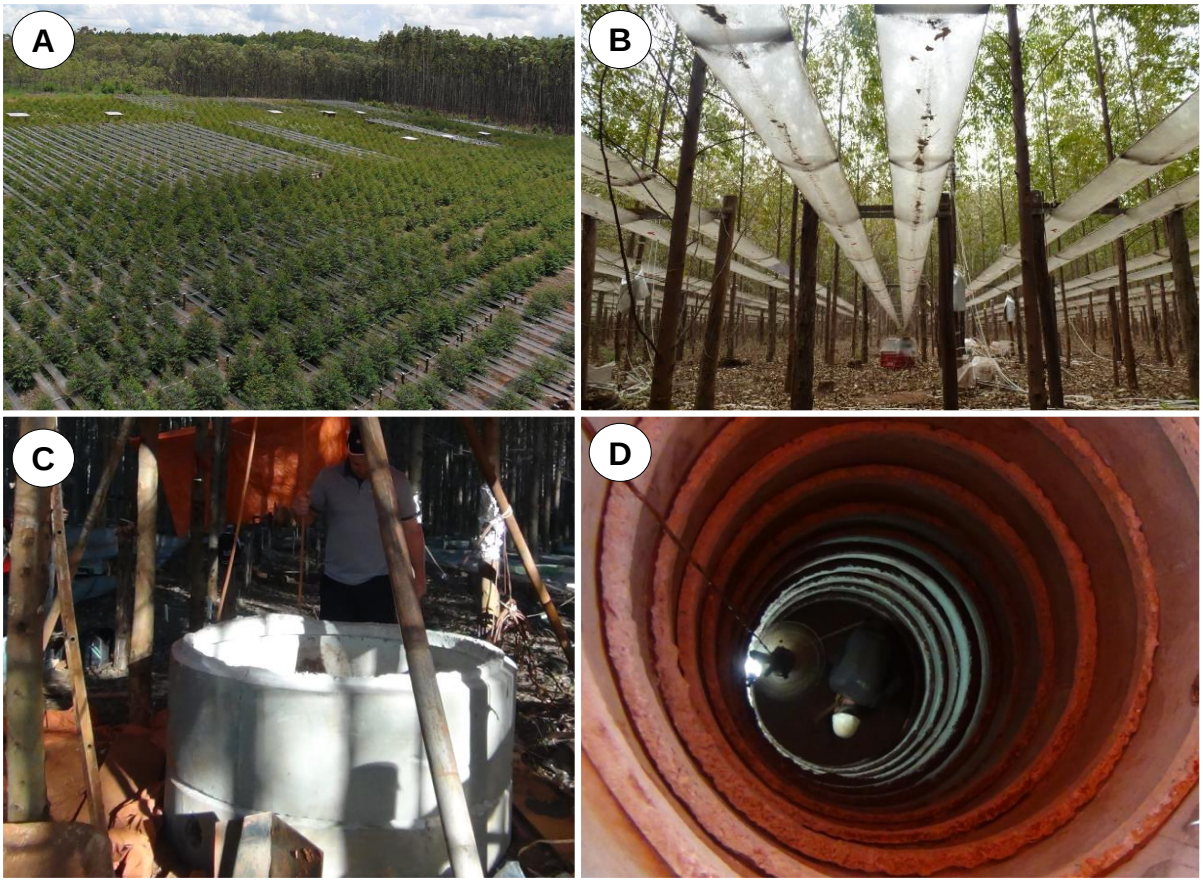

Figure 1. Studied area and experimental design. A, B. Rainfall interception experiment in eucalyptus plantation at 222 (A) and 855 (B) days after planting. C, D. Opening of trenches with 17 meters deep in the soil.

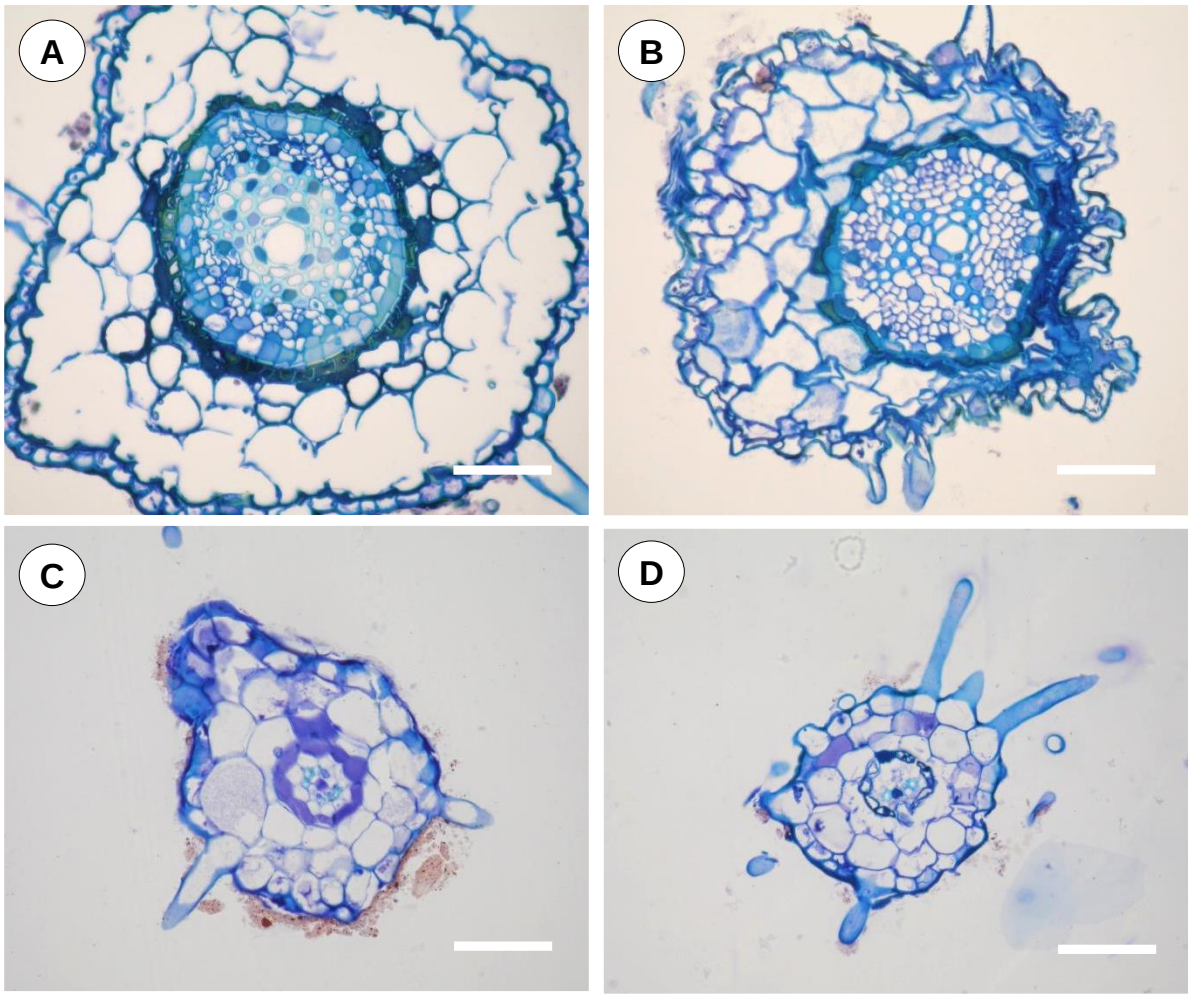

Figure 2. Cross sections of roots with diameter Ø ≤ 0.5mm of *Eucalyptus grandis*. A. Root from 0-50 cm depth in the soil from TO. B. Root from 0-50 cm depth in the soil from TR. C. Root from 12-15m depth in the soil from TO. D. Root from 12-15m depth in the soil from TR. Observe uniseriate epidermis with root hairs, parenchyma cortex and central vascular system. Scale bars: A-D = 50µm.

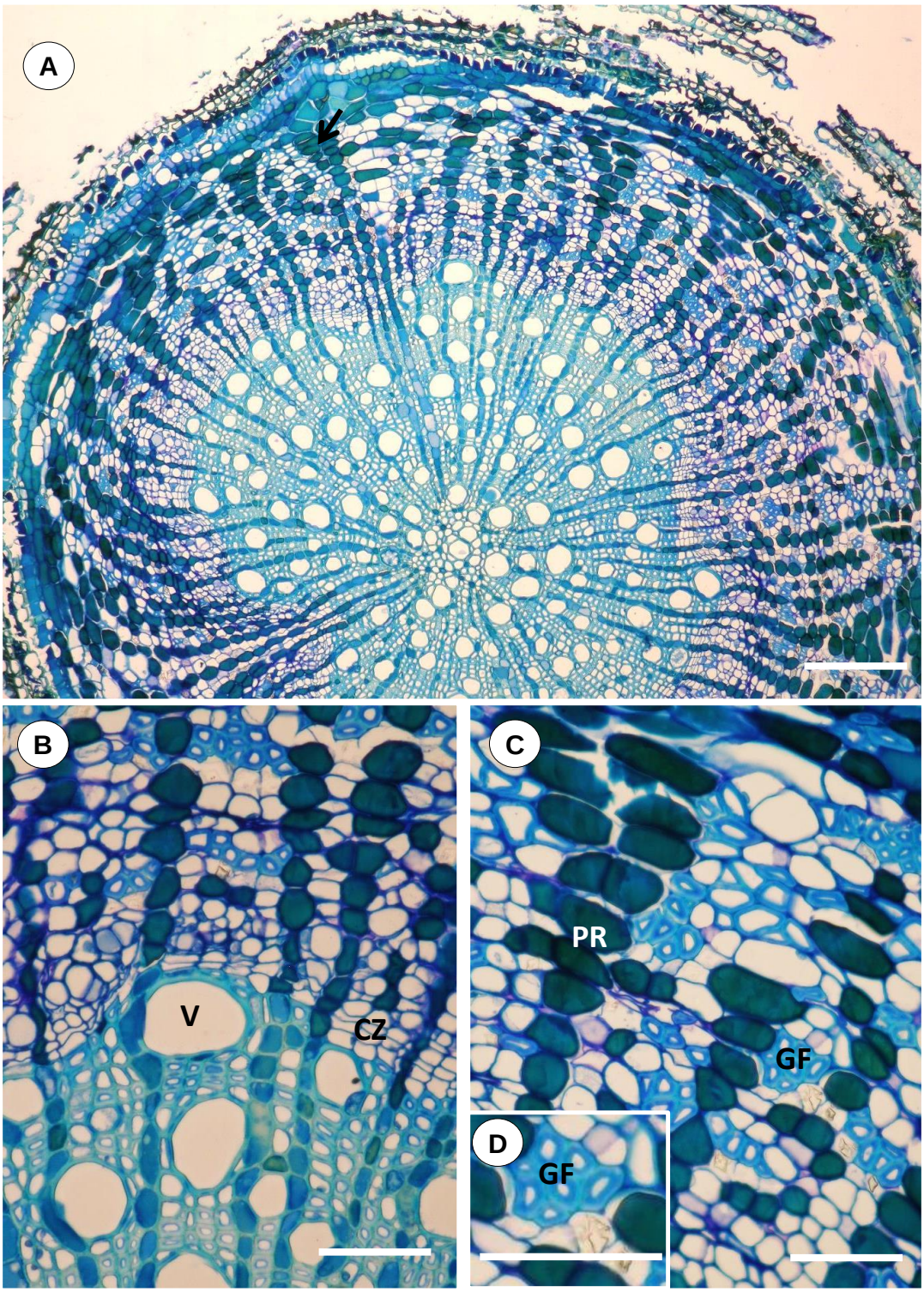

Figure 3. Cross sections of roots of *Eucalyptus grandis* with diameter 1mm < Ø ≤ 2mm from 0-50 cm depth in the soil from reduction rainfall treatment. A. Root constituted by secondary xylem and phloem and periderm. Observe dilated rays in the outer portion of the phloem (arrow). B. Detail showing secondary xylem, cambial zone (CZ) and phloem. C. Detail of phloem showing parenchyma rays (PR) and gelatinous fibers (GF). D. Insert showing gelatinous fibers. Scale bars: A = 150 µm; B-D = 50 µm.

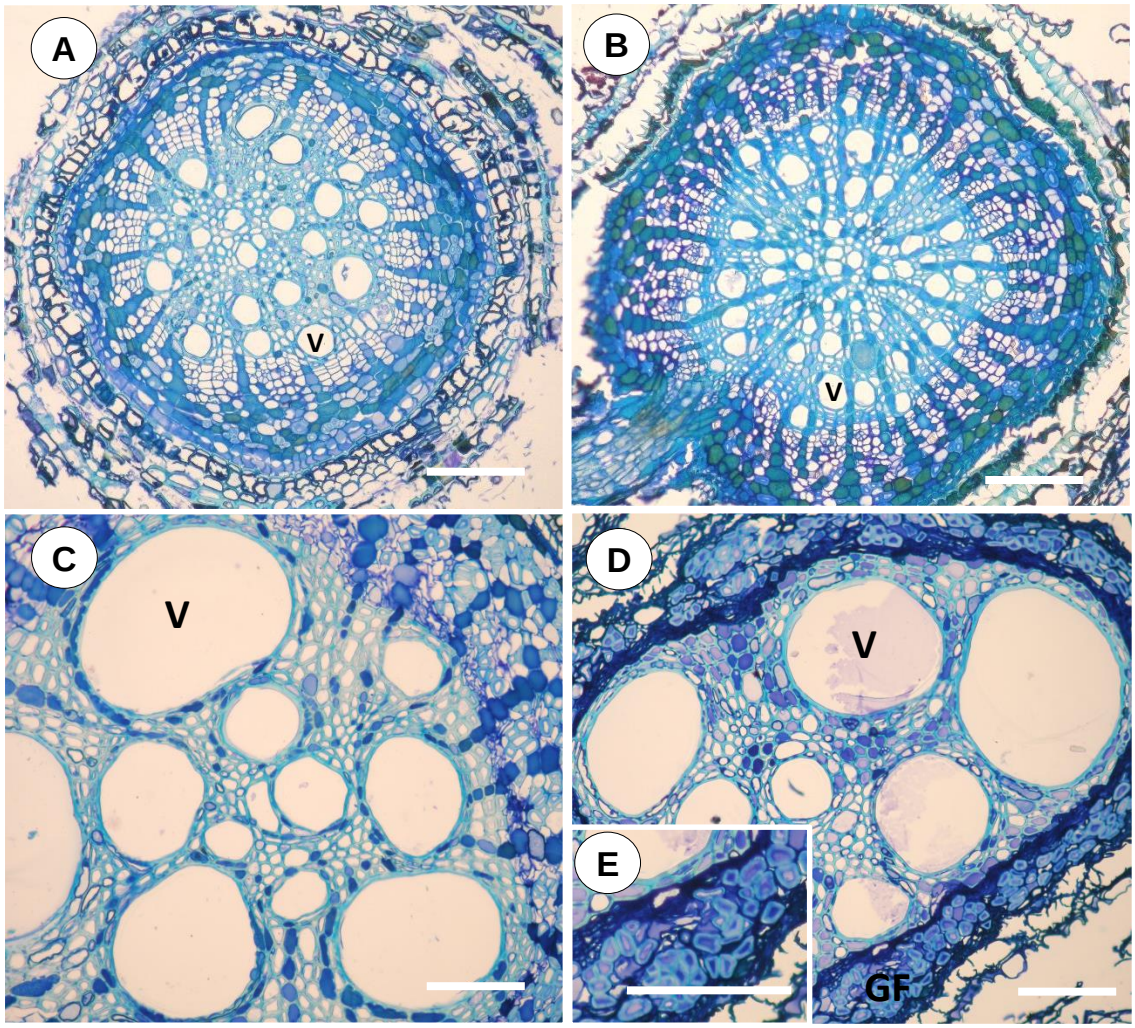

Figure 4. Cross sections of roots with diameter 0.5 < Ø ≤ 1mm of *Eucalyptus grandis*. A, B. Superficial roots (0-50cm). C-E. Deep roots (12-16m). Roots from control treatment (A, C) and roots from rainfall reduction treatment (B, D, E). Observe the wider vessels (V) in the xylem of deep roots (C ,D) and abundant gelatinous fibers (GF) in D and E. Scale bars: 100 µm.

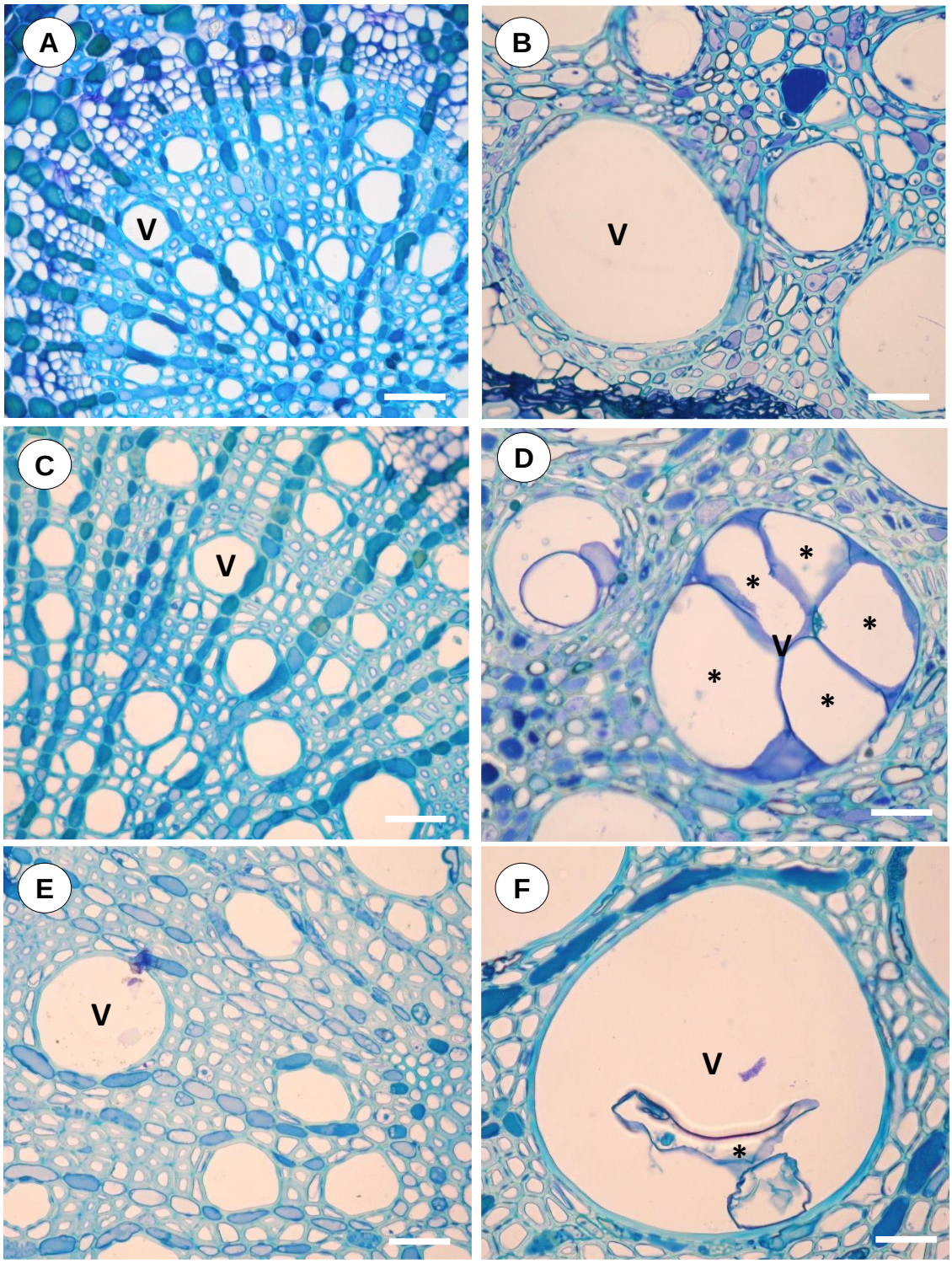

Figure 5. Cross sections of roots of *Eucalyptus grandis* from reduction rainfall treatment. A, C, E. Roots from 0-50cm depth in the soil. B, D, F. Roots from 12-16 m depth in the soil. Diameter of roots : $0.5 < Ø \leq 1$mm (A,B); $1 < Ø \leq 2$mm (C,D); $Ø > 2$mm (E,F). Observe the larger vessels in B, D and F and tyloses (*) in D and F. Scale bars: 50 µm.

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