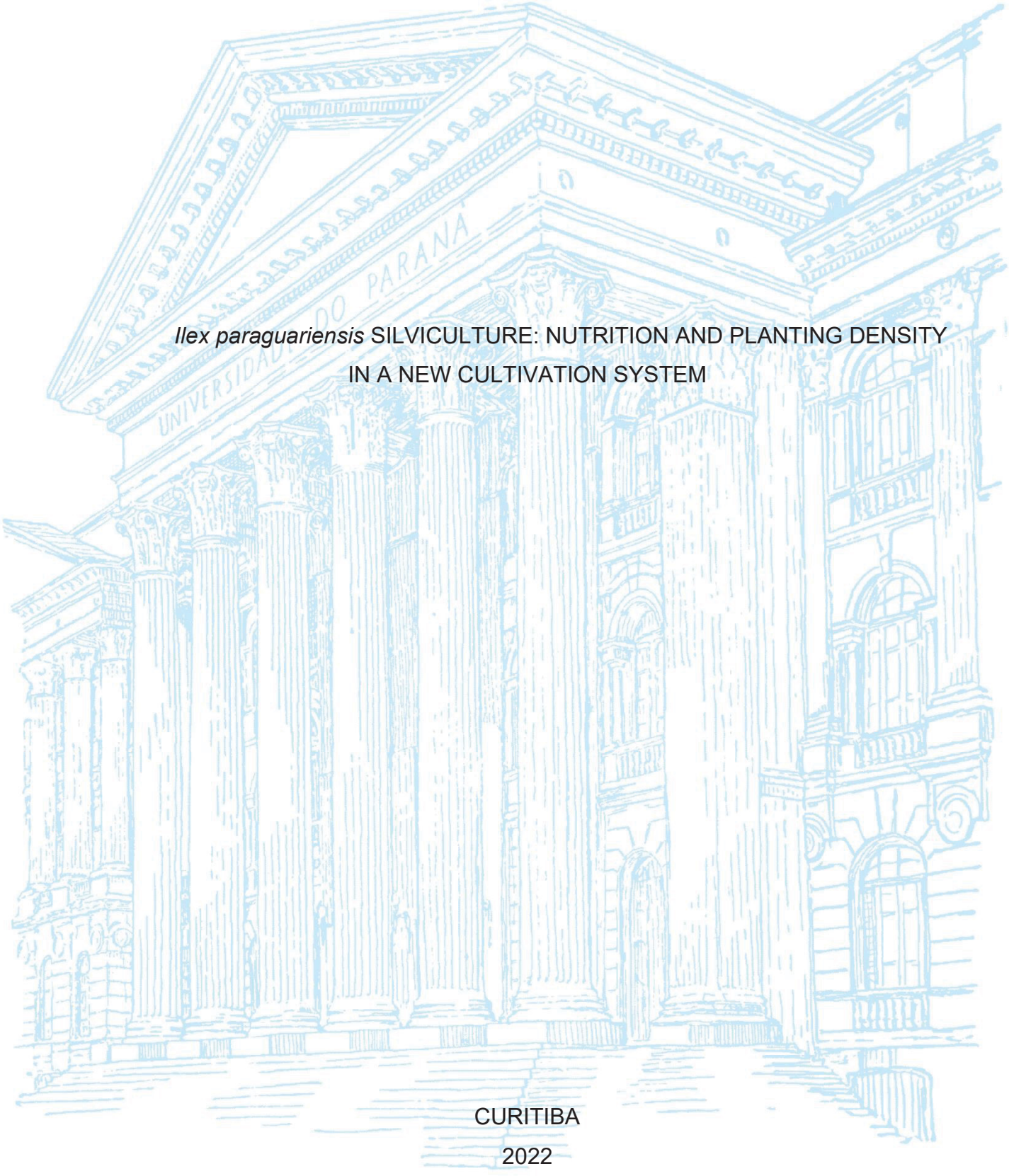
UNIVERSIDADE FEDERAL DO PARANÁ

MÔNICA MORENO GABIRA



Ilex paraguariensis SILVICULTURE: NUTRITION AND PLANTING DENSITY IN A NEW CULTIVATION SYSTEM

CURITIBA

2022

MÔNICA MORENO GABIRA

Ilex paraguariensis SILVICULTURE: NUTRITION AND PLANTING DENSITY IN A NEW CULTIVATION SYSTEM

Tese apresentada ao curso de Pós-Graduação em Engenharia Florestal, Setor de Ciências Agrárias, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Doutora em Engenharia Florestal.

Orientador: Prof. Dr. Ivar Wendling

Coorientadores: Profa. Dra. Dagma Kratz
Prof. Dr. Carlos André Stuepp

CURITIBA
2022

Ficha catalográfica elaborada pela
Biblioteca de Ciências Florestais e da Madeira - UFPR

Gabira, Mônica Moreno
Ilex paraguariensis silviculture: nutrition and planting density in a new cultivation system / Mônica Moreno Gabira. – Curitiba, 2022.
1 recurso on-line : PDF.

Orientador: Prof. Dr. Ivar Wendling
Coorientadores: Profa. Dra. Dagma Kratz
Prof. Dr. Carlos André Stuepp
Tese (Doutorado) - Universidade Federal do Paraná, Setor de Ciências Agrárias, Programa de Pós-Graduação em Engenharia Florestal. Defesa: Curitiba, 16/02/2022.
Área de concentração: Silvicultura.

1. Erva-mate - Cultivo - Paraná. 2. Erva-mate - Manejo. 3. Biomassa. 4. Sistemas silviculturais. 5. Erva-mate - Produtividade. 6. Teses. I. Wendling, Ivar. II. Kratz, Dagma. III. Stuepp, Carlos André. IV. Universidade Federaldo Paraná, Setor de Ciências Agrárias. V. Título

CDD – 634.9
CDU – 634.0.285(816.2)

Bibliotecária: Berenice Rodrigues Ferreira – CRB 9/1160

MINISTÉRIO DA EDUCAÇÃO
SETOR DE CIÊNCIAS AGRÁRIAS
UNIVERSIDADE FEDERAL DO PARANÁ
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO ENGENHARIA FLORESTAL - 40001016015P0

TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação ENGENHARIA FLORESTAL da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **MÔNICA MORENO GABIRA** intitulada: ***Ilex paraguariensis* SILVICULTURE: NUTRITION AND PLANTING DENSITY IN A NEW CULTIVATION SYSTEM**, sob orientação do Prof. Dr. IVAR WENDLING, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de doutora está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

CURITIBA, 16 de Fevereiro de 2022.

Assinatura Eletrônica
17/02/2022 08:51:04.0
IVAR WENDLING
Presidente da Banca Examinadora

Assinatura Eletrônica
17/02/2022 08:59:57.0
MÁRCIA TOFFANI SIMÃO SOARES
Avaliador Externo (EMBRAPA FLORESTAS)

Assinatura Eletrônica
17/02/2022 17:43:38.0
MAGALI RIBEIRO DA SILVA
Avaliador Externo (FACULDADE DE CIêNCIAS AGRONôMICAS)

Assinatura Eletrônica
17/02/2022 15:17:08.0
ALESSANDRO CAMARGO ANGELO
Avaliador Interno (UNIVERSIDADE FEDERAL DO PARANÁ)

Assinatura Eletrônica
17/02/2022 23:41:15.0
ROSÂNGELA ASSIS JACQUES
Avaliador Externo (UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL)

Documento assinado eletronicamente de acordo com o disposto na legislação federal Decreto 8539 de 08 de outubro de 2015.
Gerado e autenticado pelo SIGA-UFPR, com a seguinte identificação única: 151727
Para autenticar este documento/assinatura, acesse https://www.prppg.ufpr.br/siga/visitante/autenticacaoassinaturas.jsp e insira o codigo 151727

Aos meus pais, Maria Aparecida e Gilmar,
dedico.

AGRADECIMENTOS

À Deus, por guiar meus passos e me dar saúde e lucidez.

À Universidade Federal do Paraná, à Embrapa Florestas e ao Programa de Pós-Graduação em Engenharia Florestal da UFPR, pela oportunidade de desenvolver minha tese de doutorado, usufruindo de toda a infraestrutura e pessoal indispensáveis à conclusão deste trabalho.

À Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), pelo financiamento durante parte do doutorado. Tenho o privilégio de ter realizado toda a minha titulação acadêmica em instituições públicas de alta qualidade e honrarei o investimento que me foi concedido.

Ao meu orientador Dr. Ivar Wendling, pela oportunidade de expandir meus horizontes na ciência, pela orientação, suporte e conhecimentos. Foi uma grata surpresa ser sua orientada e ter a oportunidade de conviver e aprender tanto contigo.

À minha co-orientadora Profª. Drª. Dagma Kratz, pela oportunidade de estar na UFPR e desenvolver tantos trabalhos no LASF, por todas as conversas, conselhos e pela confiança que tem em mim. Você foi um dos motivos de eu ter escolhido continuar minha carreira acadêmica na UFPR e sou muito grata por esses anos.

Ao meu co-orientador Prof. Dr. Carlos Andre Stuepp, pela oportunidade de trabalhar contigo, por todos os conhecimentos, oportunidades e pela confiança.

A todos os integrantes do Laboratório de Sementes Florestais (LASF/UFPR) e do Grupo de Estudos em Propagação de Espécies Florestais (GEPEF): Dagma, Carlos, Simone, Manoela, Leticia, Francival, Daniele, Alexandre, Cleberson, Haron, Vinícius, Camila e Gabriel; por terem me acolhido, por todos os ensinamentos, oportunidades, cafés, conversas e conselhos. Apesar de não ter trabalhado com sementes em minha tese, foi no LASF em que passei grande parte do meu doutorado e aprendi demais sobre sementes, mudas, ciência e a vida. À Leticia, muito obrigada por estar comigo do início ao fim, desde o meu primeiro dia na UFPR até os ajustes finais da tese, você foi essencial no desenvolvimento deste trabalho e uma grande amiga que levarei deste período. À Manoela, obrigada por todas as nossas conversas, pela amizade e ajuda nos dias de campo e de secar amostras – e por me ensinar a fazer chimarrão!

Aos amigos que fiz na Embrapa, Natalia, Manoela, Jessica, Renata e Leandro, por toda a ajuda no campo e no laboratório, por todas as risadas, almoços na Propagação e discussões complicadas e engrandecedoras. Ter vocês por perto faz com que até as atividades mais chatas sejam divertidas! Em especial à Natália, obrigada por estar comigo do primeiro ao último dia de trabalho no campo e no laboratório, por me ajudar a pensar os experimentos e análises, por me lembrar de tantas coisas que deixo passar, por toda paciência, amizade e momentos bons.

À Drª. Cristiane Vieira Helm, pela oportunidade de fazer parte das minhas análises no LPFNM/Embrapa, por toda ajuda e disponibilidade durante esses anos e pela valiosa orientação no desenvolvimento e escrita do terceiro capítulo desta tese.

À Drª. Annie Desrochers e ao Dr. Miguel Montoro Girona, pelo auxílio no desenvolvimento, escrita e revisão do primeiro capítulo desta tese.

Ao Márcio Pizzatto, pela disponibilidade de espaço e apoio financeiro e logístico para o desenvolvimento do experimento desta tese.

Aos meus pais, Maria Aparecida e Gilmar, por todo o amor, apoio e incentivo que sempre tive, por respeitarem e confiarem em minhas escolhas e por estarem sempre presentes.

Ao César, por todo o amor, confiança e paciência que muito me ajudaram nesses anos.

A todos e todas que cruzaram meu caminho nos últimos anos e que, de alguma forma, fizeram parte desta tese, muito obrigada!

RESUMO

Apesar de sua importância econômica, ecológica e social, a silvicultura da erva-mate ainda carece de informações importantes e alternativas aos sistemas tradicionais de produção de biomassa. Neste trabalho, tivemos por objetivo desenvolver um novo sistema de cultivo de erva-mate com alta densidade de plantio e adequada fertilização com nitrogênio; dividimos nosso estudo em 3 capítulos, que estão descritos a seguir. O primeiro capítulo é uma revisão sistemática de estudos com densidade de plantio em espécies florestais em que discutimos e demos uma visão geral d os estudos nas últimas décadas para entender como a densidade de plantio pode influenciar o crescimento e a produtividade dos povoamentos florestais. Em nossas buscas, não encontramos estudos com povoamentos de alta densidade de erva-mate, indicando a falta de conhecimentos na área. No segundo e terceiro capítulos, expusemos os resultados de um experimento de campo implantado em 2018 em General Carneiro, PR. O ensaio de campo foi estabelecido em esquema fatorial duplo com 3 densidades de plantio (9.090, 13.333 e 25.000 plantas ha^{-1}) e 5 doses de nitrogênio (0%, 50%, 100%, 200% e 300% da dose recomendada de N) avaliado em 5 colheitas subsequentes realizadas de maio de 2019 a maio de 2021 com períodos de rotação reduzidos. No segundo capítulo, avaliamos a biomassa total (por hectare e por planta) de folhas jovens e maduras. No terceiro capítulo, avaliamos a atividade antioxidante e os teores de compostos fenólicos totais e metilxantinas (cafeína e teobromina) em folhas jovens e maduras. O aumento da densidade de plantio aumentou a biomassa total do povoamento, embora os teores de biomassa individual e compostos bioquímicos não tenham apresentado alterações significativas. O maior rendimento em biomassa total foi obtido com 25.000 plantas ha^{-1} e dose de 200% N – 1.792 kg ha^{-1} em janeiro de 2021. No tratamento de alta densidade (25.000 plantas ha^{-1}), observou-se uma demanda expressiva de fertilização com N, com maiores produtividades nos tratamentos com maior disponibilidade de N. Considerando os compostos bioquímicos, a densidade de plantio e as doses de N influenciaram a atividade antioxidante, os compostos fenólicos totais e os teores de metilxantinas, embora não tenha havido uma resposta linear. Houve influência significativa das épocas de colheita e da idade das folhas em nossos resultados bioquímicos, indicando que outros fatores ambientais podem influenciar os metabólitos secundários da erva-mate até 2 anos após o plantio. Nossos resultados demonstram o potencial da erva-mate para o manejo em povoamentos de alta densidade, com períodos de rotação reduzidos e manejo adequado de nutrientes para a produção de altos volumes de biomassa.

Palavras-chave: compostos bioquímicos, produtividade de biomassa, silvicultura, plantio de alta densidade, erva-mate

ABSTRACT

Despite its economic, ecological, and social importance, yerba mate silviculture still lacks important information and alternatives to the traditional leaves production systems. In this study, we aimed to develop a new cultivation system for yerba-mate with a high planting density system and adequate nitrogen fertilization; we divided our study into three chapters, which are described below. The first chapter is a systematic review of studies with planting density in forest species; we discussed and gave an overview of how far we have gone in the last decades to understand how planting density may influence the growth and yield of forest stands. In our searches, we did not find studies with high-density stands of yerba mate, indicating the lack of knowledge in this field. In the second and third chapters, we exposed the results of a field experiment established in 2018 in General Carneiro, PR, Brazil. The field trial was established in a double factorial scheme with 3 planting densities (9,090, 13,333, and 25,000 plants ha^{-1}) and 5 N doses (0 %, 50 %, 100 %, 200 %, and 300 % of the recommended N dose) evaluated over 5 subsequent harvests performed from May 2019 to May 2021 in a reduced rotation period. In the second chapter, we evaluated total biomass (per hectare and per plant) of young and mature leaves. In the third chapter, we evaluated the antioxidant activity and total phenolic compounds and methylxanthines (caffeine and theobromine) contents in young and mature leaves. Increasing planting density increased stand total biomass, although individual biomass and biochemical compounds contents did not present significant changes. The highest yield in total biomass was obtained with 25,000 plants ha^{-1} and 200 % N dose – 1,792 kg ha^{-1} in January 2021. In the high-density treatment (25,000 plants ha^{-1}), we observed an expressive demand of plants in N fertilization, with higher yields in treatments with higher N availability. Considering the biochemical compounds, planting density and N doses influenced antioxidant activity, total phenolic compounds, and methylxanthines contents, although there was not a linear response. There was a significant influence of harvest seasons and leaf age on our biochemical results, indicating that other environmental factors may influence yerba mate secondary metabolites until 2 years after planting. Our results demonstrate the potential of yerba mate to be managed in high-density stands, with reduced rotation periods and adequate nutrient management for high volume biomass production.

Keywords: biochemical compounds, biomass productivity, silviculture, high-density planting, yerba mate

LIST OF FIGURES

FIGURE 1. WORKFLOW DESCRIPTION OF THE PRISMA METHODOLOGY 23
FIGURE 2. NUMBER OF PUBLICATIONS ON THE SUBJECT OF PLANTING DENSITY FROM 2001 TO 2020. 25
FIGURE 3. NUMBER OF PUBLICATIONS ON THE SUBJECT OF PLANTING DENSITY AROUND THE WORLD FROM 2001 TO 2020 26
FIGURE 4. FREQUENCY OF GENUS IN STUDIES ON THE SUBJECT OF PLANTING DENSITY FROM 2001 TO 2020. 27
FIGURE 5. AVERAGE SPACINGS USED IN PLANTING DENSITY TRIALS FROM 2001 TO 2020. 28
FIGURE 6. GRAPHICAL DESCRIPTION OF THE PLANTING DENSITY TREATMENTS USED IN *Ilex paraguariensis* EXPERIMENT. 40
FIGURE 7. TOTAL FRESH BIOMASS (kg ha^{-1}) OF YERBA MATE SUBMITTED TO DIFFERENT COMBINATIONS OF PLANTING DENSITY AND N DOSES, EVALUATED IN FIVE SUBSEQUENT HARVESTS. MEANS FOLLOWED BY THE SAME CAPITAL LETTERS DO NOT DIFFER BETWEEN DENSITIES IN THE SAME N DOSE AND HARVEST, AND MEANS FOLLOWED BY THE SAME SMALL LETTERS DO NOT DIFFER BETWEEN N DOSES IN THE SAME PLANTING DENSITY AND HARVEST BY THE TUKEY TEST AT 5 % PROBABILITY.....43
FIGURE 8. TOTAL FRESH BIOMASS (g $plant^{-1}$) OF YERBA MATE SUBMITTED TO DIFFERENT COMBINATIONS OF PLANTING DENSITY AND N DOSES, EVALUATED IN FIVE SUBSEQUENT HARVESTS. MEANS FOLLOWED BY THE SAME CAPITAL LETTERS DO NOT DIFFER BETWEEN DENSITIES IN THE SAME N DOSE AND HARVEST, AND MEANS FOLLOWED BY THE SAME SMALL LETTERS DO NOT DIFFER BETWEEN N DOSES IN THE SAME PLANTING DENSITY AND HARVEST BY THE TUKEY TEST AT 5 % PROBABILITY.....44
FIGURE 9. YOUNG LEAVES FRESH BIOMASS (kg ha^{-1}) OF YERBA MATE SUBMITTED TO DIFFERENT COMBINATIONS OF PLANTING DENSITY AND N DOSES, EVALUATED IN FOUR SUBSEQUENT

HARVESTS. MEANS FOLLOWED BY THE SAME CAPITAL LETTERS DO NOT DIFFER BETWEEN DENSITIES IN THE SAME N DOSE AND HARVEST, AND MEANS FOLLOWED BY THE SAME SMALL LETTERS DO NOT DIFFER BETWEEN N DOSES IN THE SAME PLANTING DENSITY AND HARVEST BY THE TUKEY TEST AT 5 % PROBABILITY. 46
FIGURE 10. MATURE LEAVES FRESH BIOMASS (kg ha^{-1}) OF YERBA MATE SUBMITTED TO DIFFERENT COMBINATIONS OF PLANTING DENSITY AND N DOSES, EVALUATED IN FOUR SUBSEQUENT HARVESTS. MEANS FOLLOWED BY THE SAME CAPITAL LETTERS DO NOT DIFFER BETWEEN DENSITIES IN THE SAME N DOSE AND HARVEST, AND MEANS FOLLOWED BY THE SAME SMALL LETTERS DO NOT DIFFER BETWEEN N DOSES IN THE SAME PLANTING DENSITY AND HARVEST BY THE TUKEY TEST AT 5 % PROBABILITY. 47
FIGURE 11. PEARSON CORRELATION FOR TOTAL PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY BY ABTS AND DPPH RADICALS FROM YOUNG AND MATURE LEAVES OF YERBA MATE SUBMITTED TO DIFFERENT PLANTING DENSITY AND N DOSES, EVALUATED OVER FIVE SUBSEQUENT HARVEST PERIODS. 56
FIGURE 12. PRINCIPAL COMPONENT ANALYSIS FOR TOTAL PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY BY ABTS AND DPPH RADICALS OF YERBA MATE YOUNG AND MATURE LEAVES SUBMITTED TO DIFFERENT PLANTING DENSITY AND N DOSES, EVALUATED OVER FIVE SUBSEQUENT HARVEST PERIODS. 58
FIGURE 13. CORRELATION OF BIOCHEMICAL COMPOUNDS QUANTIFIED IN UFLC FROM YOUNG AND MATURE LEAVES OF YERBA MATE SUBMITTED TO DIFFERENT PLANTING DENSITIES AND N DOSES, EVALUATED OVER FIVE SUBSEQUENT HARVEST PERIODS. 60
FIGURE 14. PRINCIPAL COMPONENT ANALYSIS OF BIOCHEMICAL COMPOUNDS QUANTIFIED IN UFLC FROM YOUNG AND MATURE LEAVES OF YERBA MATE SUBMITTED TO DIFFERENT PLANTING

DENSITIES AND N DOSES, EVALUATED OVER FIVE SUBSEQUENT HARVEST PERIODS. 62

LIST OF TABLES

TABLE 1. SUMMARY OF STUDIES USING MORPHOLOGICAL MEASUREMENTS IN RESPONSE TO INCREASING PLANTING DENSITY FROM 2001 TO 2020. 29
TABLE 2. SUMMARY OF STUDIES USING PHYSIOLOGICAL MEASUREMENTS IN RESPONSE TO INCREASING PLANTING DENSITY FROM 2001 TO 2020. 34
TABLE 3. ORGANIC MATTER CONTENT (OM), SOIL AVAILABILITY OF PHOSPHORUS (P), POTASSIUM (K), CALCIUM (CA), AND MAGNESIUM (MG), AND SOIL PHYSICAL ASPECTS IN THE EXPERIMENTAL AREA BEFORE *Ilex paraguariensis* PLANTATION. 39
TABLE 4. ANNUAL DOSE OF FERTILIZER APPLIED IN THE EXPERIMENT. 40
TABLE 5. ANALYSIS OF VARIANCE (ANOVA) FOR TOTAL BIOMASS PRODUCTIVITY OF YERBA MATE SUBMITTED TO DIFFERENT COMBINATIONS OF N DOSE AND PLANTING DENSITY. 41
TABLE 6. DESCRIPTIVE STATISTICS OF TOTAL PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY BY ABTS AND DPPH RADICALS FROM YERBA MATE YOUNG AND MATURE LEAVES SUBMITTED TO DIFFERENT PLANTING DENSITIES AND N DOSES, EVALUATED OVER FIVE SUBSEQUENT HARVEST PERIODS. Y = YOUNG LEAVES; M = MATURE LEAVES. 55
TABLE 7. IMPORTANCE FOR EACH PRINCIPAL COMPONENT OF TOTAL PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY BY ABTS AND DPPH RADICALS IN YERBA MATE YOUNG AND MATURE LEAVES SUBMITTED TO DIFFERENT PLANTING DENSITY AND N DOSES, EVALUATED OVER FIVE SUBSEQUENT HARVEST PERIODS. 57
TABLE 8. DESCRIPTIVE STATISTICS OF CAFFEINE, THEOBROMINE, 3-CAFFEOYLQUINIC ACID (3-CQA), 4-CAFFEOYLQUINIC ACID (4-CQA), AND 5-CAFFEOYLQUINIC ACID (5-CQA) FROM YERBA MATE YOUNG AND MATURE LEAVES SUBMITTED TO DIFFERENT PLANTING DENSITIES AND N DOSES, EVALUATED

OVER FIVE SUBSEQUENT HARVEST PERIODS. Y = YOUNG LEAVES; M = MATURE LEAVES; SD = STANDARD DEVIATION; SEM = STANDARD ERROR OF THE MEAN; CV = COEFFICIENT OF VARIATION; MED = MEDIAN; VAR = VARIANCE. 59
TABLE 9. IMPORTANCE OF EACH PRINCIPAL COMPONENTS OF BIOCHEMICAL COMPOUNDS QUANTIFIED IN UFLC IN YOUNG AND MATURE LEAVES OF YERBA MATE SUBMITTED TO DIFFERENT PLANTING DENSITIES AND N DOSES, EVALUATED OVER FIVE SUBSEQUENT HARVEST PERIODS. SD: STANDARD DEVIATION; PV: PROPORTION OF VARIANCE; CP: CUMULATIVE PROPORTION 61

SUMARY

1 INTRODUCTION 16
1.1 REFERENCES 17
2 CHAPTER I – THE IMPACT OF PLANTING DENSITY ON FOREST PLANTATIONS: AN OVERVIEW 20
2.1 INTRODUCTION 20
2.2 MATERIAL AND METHODS 22
2.2.1 Criterion of literature Search 22
2.2.2 Data Analysis 24
2.2.2.1 Quantitative assessment 24
2.2.2.2 Qualitative assessment 24
2.3 RESULTS AND DISCUSSION 25
2.3.1 Quantitative analysis 25
2.3.2 Qualitative analysis 28
2.4 FINAL CONSIDERATIONS 36
3 CHAPTER II – PLANTING SPACING AND NITROGEN FERTILIZATION ON YERBA MATE: YIELD RESPONSES ON A HIGH-DENSITY PRODUCTION SYSTEM 37
3.1 INTRODUCTION 37
3.2 MATERIAL AND METHODS 39
3.3 RESULTS AND DISCUSSION 41
3.4 CONCLUSIONS 48
4 CHAPTER III – NEW METHOD TO PRODUCE YERBA MATE: DOES PLANTING DENSITY AND NITROGEN DOSES INFLUENCE ON PHENOLIC COMPOUNDS AND METHYLXANTHINES CONTENTS? 49
4.1 INTRODUCTION 49
4.2 MATERIAL AND METHODS 51
4.2.1 Experiment establishment and treatments 51
4.2.2 Plant material 52
4.2.3 Aqueous extracts preparation 52
4.2.4 Content of total phenolic compounds 52
4.2.5 Antioxidant activity (ABTS and DPPH radicals) 53
4.2.6 Caffeine, theobromine, and caffeoylquinic acids 53

4.2.7 Statistical and data analysis 54
4.3 RESULTS AND DISCUSSION 55
4.3.1 Total phenolic compounds and antioxidant activity 55
4.3.2 Caffeine, theobromine, and caffeoylquinic acids 58
4.4 CONCLUSIONS 63
5 FINAL CONSIDERATIONS 64
REFERENCES 65

1 INTRODUCTION

Yerba mate (*Ilex paraguariensis* A.St.-Hil.) is an arboreal species of the Aquifoliaceae family, native to the mixed rainforest of South America – in Brazil, Argentine, Paraguay, and Uruguay (Carvalho, 2006). In Brazil, the species occurs in the Southern States; mainly in Parana, Santa Catarina, and Rio Grande do Sul, associated with the “araucaria forest”. In these countries, yerba mate is traditionally consumed as hot or cold beverages, called “chimarrão” and “tereré”, respectively (Sturion & Resende, 2010). In the last decades, the tea made from yerba mate leaves has attracted the attention of the national and international markets, increasing the interest in species plantations.

In Brazil, yerba mate is explored in native forests or cultivated in the forest understory, in agroforestry systems, or in monostands (Penteado Junior & Goulart, 2019; Westphalen et al., 2020); in 2020, yerba mate production in Brazil was 527,546 tons (IBGE, 2021). Traditionally, yerba mate monostands are established with low planting density (2,222 – 2,500 plants ha^{-1}) and harvested each 18-24 months (Santin et al., 2017; Santin et al., 2019). In this system, harvested biomass is composed mainly by mature leaves and thin branches (Santin et al., 2014). However, young leaves have a higher concentration of bioactive compounds, which is interesting for tea industry (Yin et al., 2015). There is an increasing demand of yerba mate leaves with higher morphological and biochemical quality, which can be obtained through breeding programs associated with differentiated silvicultural traits, e.g., reducing rotation periods.

Yerba mate infusions have polyphenols and methylxanthines contents comparable to those of coffee (*Coffea arabica*) and green tea (*Camellia sinensis*), increasing the interest of pharmaceutical and food industries searching for its health benefits (Lima & Farah, 2019; Meinhart et al., 2019). To meet the increasing demand for differentiated yerba mate products, several studies have been developed as part of breeding programs searching for genotypes with specific chemical and sensorial characteristics, and processing methods to keep these characteristics in the final products (Duarte et al., 2019; Duarte et al., 2020; Gabira et al., 2020; Westphalen et al., 2020; Tomasi et al., 2021; Vieira et al., 2021). Apart of genetic factors, silvicultural traits can also change leaves characteristics, since polyphenols and methylxanthines are secondary metabolites and their contents in leaves change according to

environmental conditions, such as temperature, water and nutrients availability, and pest or diseases attacks (Zhu et al., 2019; Begon & Townsend, 2020; Erb & Kliebenstein, 2020); However, no studies have been developed aiming to manage yerba mate young leaves yield and biochemical characteristics to produce differentiated raw material for pharmaceutical and food industries.

Chapter I of this study is a systematic review about planting density in forest plantations worldwide. Our objective was to discuss and give an overview of how far we have gone in the last decades to understand how planting density influences plant morphological and physiological characteristics. In our searches, we did not obtained studies about yerba mate, indicating the importance of the study we performed. Chapters II and III present the results of a field trial established in 2018 with yerba mate submitted to different planting densities and nitrogen doses. From this trial, we measured total productivity, mature and young leaves productivity, these data are exposed and discussed in Chapter II. We also evaluated how planting density and N doses influenced on biochemical compounds contents in mature and young leaves, which is exposed and discussed in Chapter III.

1.1 REFERENCES

BEGON, M.; TOWNSEND, C.R. **Ecology : from individuals to ecosystems**. Fifth ed. Hoboken, New Jersey: John Wiley & Sons, inc., 2020. 864p.

CARVALHO, P.E.R. **Espécies Arbóreas Brasileiras. Vol. II**. Colombo: Embrapa Florestas, 2006. 627p.

DUARTE, M.M.; MIRESKI, M.C.; OLISZESKI, A.; WENDLING, I.; STUEPP, C.A. Rooting of yerba mate cuttings with different lenghts. **Revista Eletrônica Científica da UERGS**, v.5, p.5–11, 2019. DOI: 10.21674/2448-0479.51.05-11.

DUARTE, M.M.; TOMASI, J.D.C.; HELM, C.V.; AMANO, E.; LAZZAROTTO, M.; GODOY, R.C.B. DE; NOGUEIRA, A.C.; WENDLING, I. Caffeinated and decaffeinated mate tea: Effect of toasting on bioactive compounds and consumer acceptance. **Revista Brasileira de Ciências Agrarias**, v.15, p.e8513, 2020. DOI: 10.5039/agraria.v15i3a8513.

ERB, M.; KLIEBENSTEIN, D.J. Plant secondary metabolites as defenses, regulators, and primary metabolites: the blurred functional trichotomy. **Plant Physiology**, v.184, p.39–52, 2020. DOI: 10.1104/pp.20.00433.

GABIRA, M.M.; WALTER, L.S.; BRITO, G.S.; SCHNEIDER, C.R.; WENDLING, I.; KRATZ, D. Alternative substrates and containers for *Ilex paraguariensis* seedlings production. **Acta Iguazu**, v.9, p.78–87, 2020. DOI: 10.48075/actaiguaz.v9i4.25492.

IBGE - Instituto Brasileiro de Geografia e Estatística. **Dados PAM e PEVS.** Disponível em: <https://sidra.ibge.gov.br/pesquisa/pam/tabelas>. Acesso em: 10/12/2021

LIMA, J. DE P.; FARAH, A. Methylxantines in stimulant foods and beverages commonly consumed in Brazil. **Journal of Food Composition and Analysis**, v.78, p.75–85, 2019. DOI: 10.1016/j.jfca.2019.02.001.

MEINHART, A.D.; DAMIN, F.M.; CALDEIRÃO, L.; GODOY, H.T. Methylxanthines in 100 Brazilian herbs and infusions: Determination and consumption. **Emirates Journal of Food and Agriculture**, v.31, p.125–133, 2019. DOI: 10.9755/ejfa.2019.v31.i2.1916.

PENTEADO JUNIOR, J.F.; GOULART, I.C.G. DOS R. **Erva 20: sistema de produção de erva-mate**. Brasília, DF: Embrapa, 2019. 152p.

SANTIN, D.; BENEDETTI, E.L.; BARROS, N.F. DE; ALMEIDA, I.C. DE; LEAL, G.P.; FONTES, L.; NEVES, J.C.L.; WENDLING, I.; REISSMANN, C.B. Effect of potassium fertilization on yeld and nutrition of yerba mate (*Ilex paraguariensis*). **Revista Brasileira de Ciência do Solo**, v.38, p.1469–1477, 2014.

SANTIN, D.; BENEDETTI, E.L.; BARROS, N.F. DE; ALMEIDA, I.C. DE; SIMIQUELI, G.F.; NEVES, J.C.L.; WENDLING, I.; REISSMANN, C.B. Adubação nitrogenada e intervalos de colheita na produtividade e nutrição da erva-mate e em frações de carbono e nitrogênio do solo. **Ciência Florestal**, v.29, p.1199–1214, 2019. DOI: 10.5902/1980509810843.

SANTIN, D.; BENEDETTI, E.L.; BARROS, N.F. DE; FONTES, L.L.; ALMEIDA, I.C. DE; NEVES, J.C.L.; WENDLING, I. Manejo de colheita e adubação fosfatada na cultura da erva-mate (*Ilex paraguariensis*) em fase de produção. **Ciência Florestal**, v.27, p.783–797, 2017.

STURION, J.A.; RESENDE, M.D. V. **Melhoramento genético da erva-mate**. Colombo: Embrapa Florestas - CNPF, 2010. 274p.

TOMASI, J. DE C.; LIMA, G.G. DE; WENDLING, I.; HELM, C.V.; HANSEL, F.A.; GODOY, R.C.B. DE; GRUNENNVALDT, R.L.; MELO, T.O. DE; TOMAZZOLI, M.M.; DESCHAMPS, C. Effects of different drying methods on the chemical, nutritional and colour of yerba mate (*Ilex paraguariensis*) leaves. **International Journal of Food Engineering**, v.0, 2021. DOI: 10.1515/ijfe-2020-0312.

VIEIRA, L.M.; MAGGIONI, R.D.A.; TOMASI, J.D.C.; NUNES GOMES, E.; WENDLING, I.; HELM, C.V.; KOEHLER, H.S.; ZUFFELLATO-RIBAS, K.C. Vegetative propagation, chemical composition and antioxidant activity of yerba mate genotypes. **Plant Genetic Resources: Characterization and Utilization**, v.19, p.112–121, 2021. DOI: 10.1017/S1479262121000150.

WESTPHALEN, D.J.; ANGELO, A.C.; ROSSA, Ü.B.; BOGNOLA, I.A.; MARTINS, C.E.N. Impact of different silvicultural techniques on the productive efficiency of *Ilex paraguariensis* A.St. Hill. **Agroforestry Systems**, v.94, p.791–798, 2020. DOI: 10.1007/s10457-019-00451-y.

YIN, Y.; KATAHIRA, R.; ASHIHARA, H. Metabolism of purine alkaloids and xanthine in leaves of maté (*Ilex paraguariensis*). **Natural Product Communications**, v.10, p.707–712, 2015. DOI: 10.1177/1934578X1501000503.

ZHU, B.; CHEN, L.B.; LU, M.; ZHANG, J.; HAN, J.; DENG, W.W.; ZHANG, Z.Z. Caffeine content and related gene expression: novel insight into caffeine metabolism in *Camellia* plants containing low, normal, and high caffeine concentrations. **Journal of Agricultural and Food Chemistry**, v.67, p.3400–3411, 2019. DOI: 10.1021/acs.jafc.9b00240.

2 CHAPTER I – THE IMPACT OF PLANTING DENSITY ON FOREST PLANTATIONS: AN OVERVIEW

Knowledge on tree morphological and physiological responses to planting density may influence plantation establishment practices according to desired size and characteristics of wood products. In this review, we discuss and give an overview of how far we have gone in the last decades to understand how planting density may influence growth and yield of forest stands. We used PRISMA methodology to select papers published in the last two decades that evaluated how planting density effected morphological and physiological characteristics of planted trees. We observed an increase in publications during the last few years, mainly assessing species for timber production (*Eucalyptus* and *Pinus*) in research mostly concentrated in USA, China, and Brazil. Planting density was found to have a major impact on stem diameter and volume, resulting in a greater stand productivity of high-density stands, but with trees of smaller individual volume. We found few studies evaluating physiological responses of trees to planting density, where species light and water requirements were most significant. Knowledge gaps were identified for tree species grown for leaves, fruits, or seeds. Information compiled in this review can serve as a basis for the establishment of studies aiming at better using of forest areas and developing plantations with optimal spacing design.

Key-words: growth and yield, forestry, forest stands, silviculture, stand density, tree spacing

2.1 INTRODUCTION

The forest-based industry is a key player in the socioeconomic development of countries around the world, offering several raw products to fiber, timber, energy, and non-wood products industries. Forest plantations for industrial purposes cover about 131 million ha, which corresponds to 2 % of the total world forest cover, according to the Global Forest Resources Assessment (2020). The same report points that the highest ratio of introduced species in planted forests is in South America – 97 % of planted areas corresponds to non-native species. In general, plantation forests are established with a design defined to provide the best conditions for trees growth,

to maximize productivity, and to manage timber shape focusing on several final products.

Competition between neighbor trees for light, nutrients, and water is the main aspect that silviculture aims to manage in forests and plantations. It is defined as a demand for a resource required by individuals that may exceed the immediate supply; as a result, some trees can become deprived from that resource (Begon and Townsend, 2020). Light is considered the primary source of competition and leads several physiological and morphological changes also called shade avoidance responses, that include hyponasty, stem and petiole elongation, apical dominance, leaf positioning, and life cycle adjustments. Shade avoidance responses caused by light signaling were explored in physiological, biochemical, and genetic aspects by Huber et al. (2020), who demonstrated that light competition could have consequences for plant community such as canopy structure, species composition, and individuals' size. Space is also a limiting factor for trees growth because it influences on resource consumption per area and on the access of individuals to environmental factors; though, it is not considered a resource for which plants compete because it is probably never limiting in itself in terrestrial ecosystems, like what happens for water and other resources (Wilson et al., 2007). Competition has direct effects on plant physiology, mediating individual growth by regulating resource acquisition (Benomar et al., 2012); therefore, it is fundamental to understand species responses to temporal and spatial variations in resources to provide theoretical guidance to stand management. Planted forests are straight-forward cases of competition because trees are even-aged and of the same species, regularly spaced, and with uniform resources availability.

Despite this focus on competition from silviculturists, we must consider that trees are not always competing; facilitation relationships can also take place in forest stands and silvicultural practices aiming to reduce tree density are not always a good approach. In general, facilitation relationships create a buffer zone that mitigate variations in soil humidity, atmospheric temperature, and light interception, which ease plant establishment and growth (Bravo-Oviedo et al., 2018). In plantations with wider spacings or subjected to thinning, wind penetrates more easily, increasing mechanical stresses in remained trees (Scott and Mitchell, 2005). Natural root grafting has also been reported as a means for facilitation relationships between jack pine trees to occur, allowing for a better use of resources (Tarroux and Desrochers, 2011). When these stands are thinned, however, the root system of removed trees increase the carbon

demand on remnant trees, that then decrease in growth compared to non-grafted trees even though they benefit from more resources (Benomar et al., 2011). This is an example showing that tree growth is not only dependent on competition but also on facilitation relationships (Begon and Townsend, 2020); these relationships should therefore be better understood in forest stands as a way to increase the efficiency of resources use.

Foresters take tree-spacing in plantations seriously because it affects profitability – close spacing requires more seedlings – and productivity, because closer spacings can improve tree form and timber quality for some industries, e.g., the lumber industry (Charlton et al., 2020; Glencross et al., 2012; Naji et al., 2016; Tonini et al., 2018). Tree growth tends to reach a maximum at a certain density and declines with additional trees, but this relation also depends on stand age, site quality, and environmental conditions (Will et al., 2001). As a key to understand density-related patterns, Weiner & Freckleton (2010) reviewed the empirical and mathematical basis of constant final yield, concluding that constant final yield appears to be the most robust pattern in ecology and classified it as essential to understand and predict plant community behavior. In general, there is no competition for resources at very low densities, which leads to a regular increase in biomass accumulation up to a certain point where biomass accumulation reduces because natural resources become limiting for growth. Despite its importance, field studies on tree density in planted forest stands are scarce and still lacks on overall information about how each species responds to increasing planting density to provide high-quality products. In this review, we discuss how planting density affects plantation forest growth and yield in temperate and tropical regions; and give an overview of how far we have gone in the last decades to understand how planting density is used as a silvicultural practice, providing relevant information to guide future research in this field.

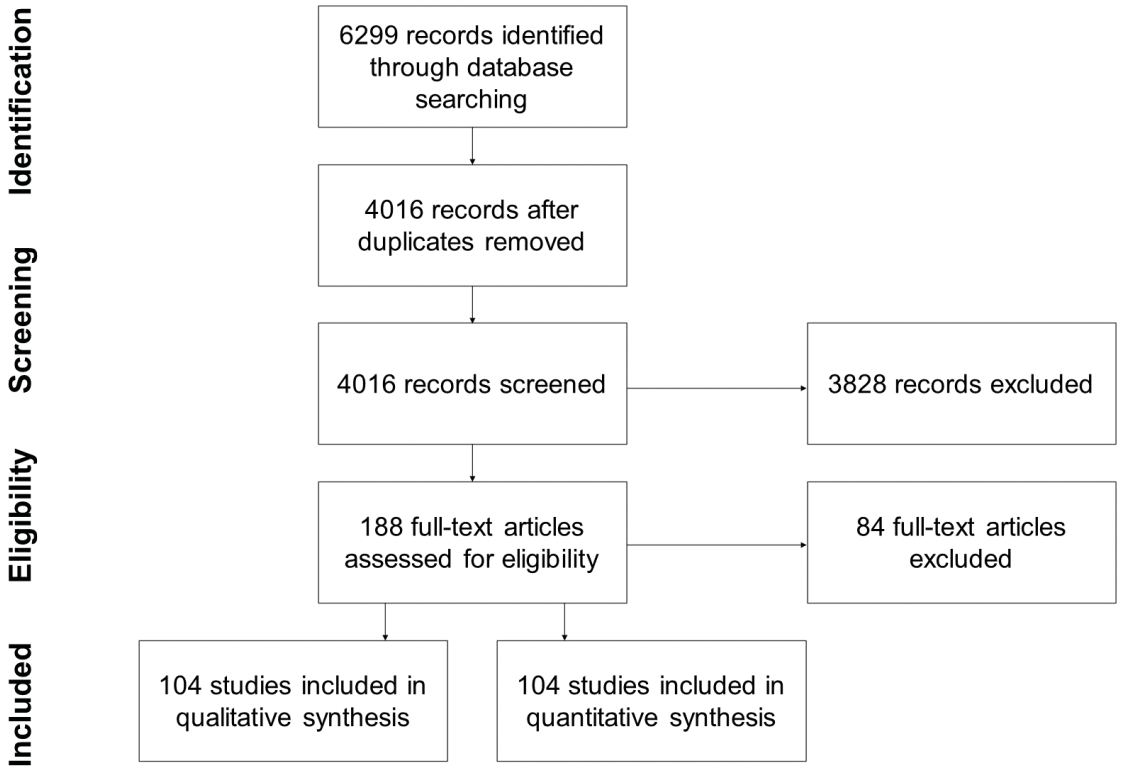
2.2 MATERIAL AND METHODS

2.2.1 Criterion of literature Search

We searched literature relating plant morphological and physiological responses to planting density in forest stands, published from 2001 to 2020. This period was chosen to select the most up-to-date advances in the field of planting

density. The literature review was conducted according to the PRISMA methodology (Moher et al., 2009) and its process is explained in Figure 1.

FIGURE 1. WORKFLOW DESCRIPTION OF THE PRISMA METHODOLOGY



SOURCE: Adapted from Moher et al. (2009).

We used two indexing electronic databases to identify literature available worldwide and covering a wide variety of high-quality journals: Web of Science and Scopus. Title, abstract, and keywords were searched through combinations of the words “forest”, “planting”, “plant”, “plantation”, “stem”, “stand”, “spacing”, and “density”, using Boolean operators (OR, AND). Search was limited to research papers published between 2001-2020, in English-language, and in scientific journals, excluding review articles, event papers, technical reports, and short communications. The database search returned 2986 papers in Web of Science and 3313 papers in Scopus. A total of 4016 papers remained after removing duplicates.

In the screening process, we analyzed and excluded papers that did not meet our criteria to be included in this review. In this process, we excluded papers that (a) focused on agricultural crop species as cotton, wheat, maize, and soy; (b) were based in field trials established in drylands and wetlands; (c) focused on the development of

GIS and remote sensing technologies; (d) were based on modelling or mathematical models, not on field trials. We included in our research papers focusing in both temperate and tropical species, considering that the most important studies in the field of planting density occurs in both regions. After the exclusion of these papers, 188 papers remained for the eligibility analysis: These papers were evaluated regarding clarity of information and the application of stand density on field trials. We only included in our study papers that (a) were based in data collected in planted forests, with clear information about spacing and evaluations; (b) used tree density as a factor affecting growth, not as a variable in natural forest stands; (c) evaluated planted tree species responses, not natural understory regeneration, shrub or herbaceous species; (d) evaluated tree morphological and physiological responses to planting density, excluding papers investigating wood quality or environmental aspects. After this analysis, a total of 104 papers were used in this review.

2.2.2 Data Analysis

2.2.2.1 Quantitative assessment

We used descriptive statistics to analyze general trends concerning frequency of publication, regions where these studies were developed, species most studied, and range of spacings applied. All papers selected for the review were evaluated and important information was extracted and analyzed in a Microsoft Excel spreadsheet.

2.2.2.2 Qualitative assessment

In the qualitative analysis, we evaluated relationships between growth variables and planting density. For this we considered the most used morphological and physiological characteristics in selected papers: Total height, diameter at breast height (DBH), individual and stand basal area, individual and stand timber volume, leaf biomass, leaf area index, crown height and diameter, intercepted radiation, carbon assimilation, transpiration, respiration, water use efficiency, leaf water potential, and chlorophyll content. All important information related to these variables were extracted and analyzed in a Microsoft Excel spreadsheet. Results were classified as a neutral (*), positive (+) or negative (-) response to spacing in each paper, which resulted in

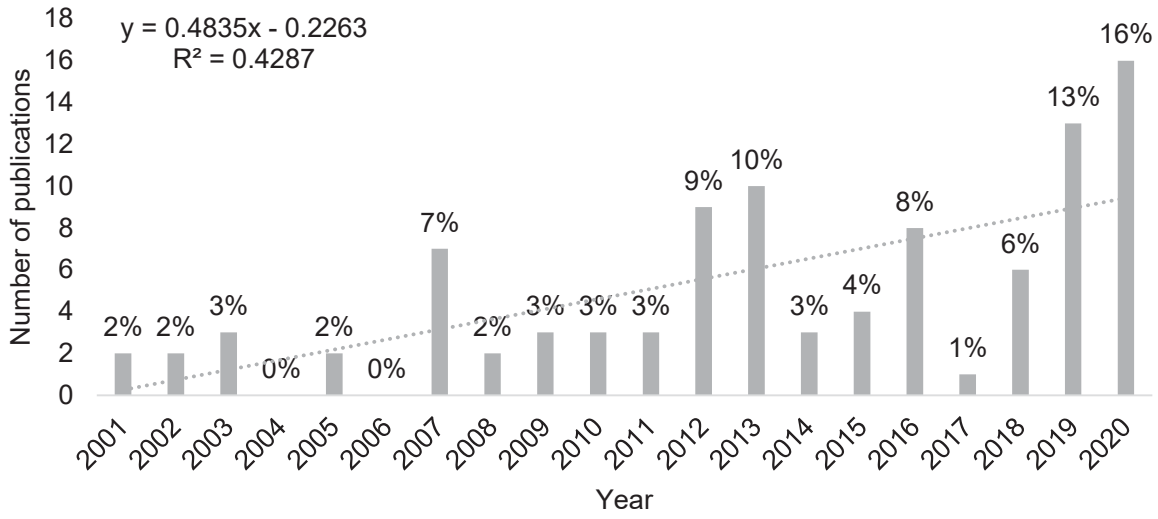
tables with species and the summary response of morphological or physiological growth characteristics to planting spacing.

2.3 RESULTS AND DISCUSSION

2.3.1 Quantitative analysis

We found an increasing trend in the number of publications about planting density (Figure 2). The number of publications fluctuated along the years, with the highest peaks in 2019 and 2020 while there were no publications on the subject in 2004 and 2006. Concerns about increasing timber quality and reducing rotation time drove the increase in planting density studies; although cost and environmental aspects were also considered (Bentancor et al., 2019; Charlton et al., 2020; Duan et al., 2019; Smith et al., 2016). Increasing planting density is an important alternative to increase forest production, especially in countries where areas for forest plantations are scarce due to competing crops or environmental aspects.

FIGURE 2. NUMBER OF PUBLICATIONS ON THE SUBJECT OF PLANTING DENSITY FROM 2001 TO 2020.

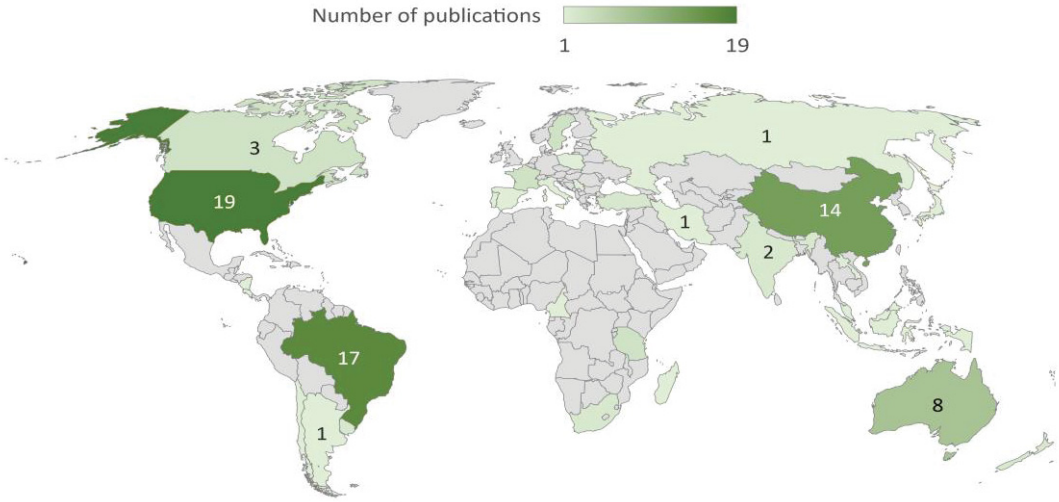


SOURCE: The author.

Publications on planting density were widespread worldwide, with many countries having only one publication (Figure 3). The subject of planting density was more frequent in trials located in United States of America (USA – 19), followed by Brazil (17) and China (14). These are countries with a strong tradition of plantation

forestry, although species and products generated from these forests focus on different industries – mainly timber, pulp and paper, or biomass for energy production. USA and China are located in temperate climates, with mainly natural and planted forests of coniferous species (Groot and Cortini, 2016; Harrington et al., 2009; Will et al., 2005, 2001; Zhao et al., 2020, 2011), while Brazil is located in the tropical zone, with planted forests of different broad-leaved species (da Páscoa et al., 2019; Narducci et al., 2016; Schwerz et al., 2020a, 2020b; Silva et al., 2012; Tonini et al., 2018). We thus observed more studies on *Pinus* species in USA and China, and more studies on *Eucalyptus* species in Brazil. Other countries with a strong forest industry did not present a high number of studies in the subject of planting density because trials were located in natural forests, without a regular spacing between plants, and explored thinning management and ecological aspects other than growth parameters; those studies were removed from this review in the screening process.

FIGURE 3. NUMBER OF PUBLICATIONS ON THE SUBJECT OF PLANTING DENSITY AROUND THE WORLD FROM 2001 TO 2020

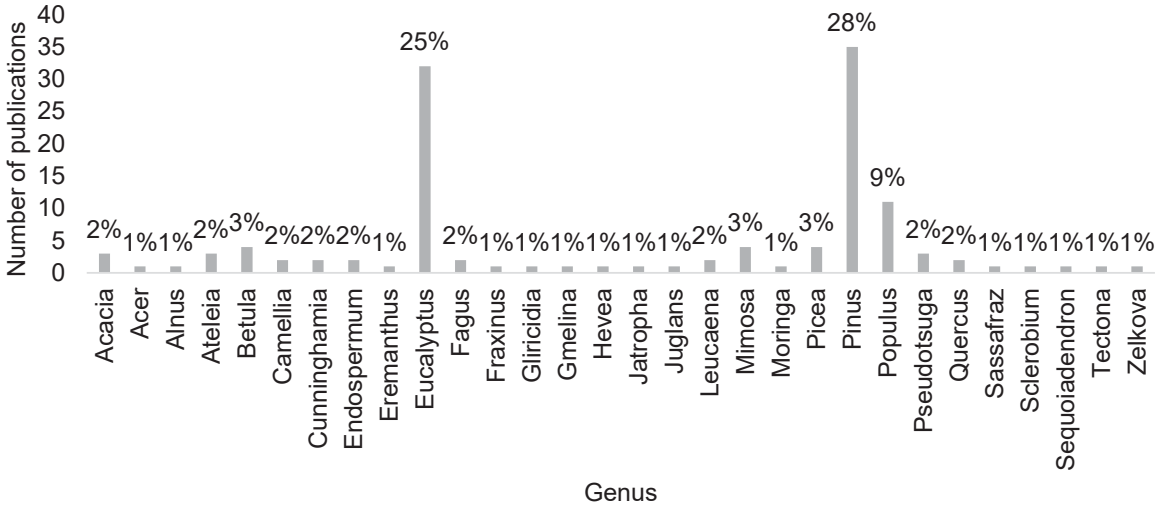


SOURCE: The author.

Species of the genus *Pinus* and *Eucalyptus* are the most studied in planting density trials, representing 28 % and 25 % of the analyzed studies (Figure 4). Among *Pinus* species, the most used in trials was *Pinus taeda*, corresponding to 14 studies; for *Eucalyptus* species, the most used was the *Eucalyptus grandis*, with 7 studies. These are the most important species for plantations in several countries, used by the timber, pulp and paper, and energy industries. The lack of information on tree species cultivated for products other than wood becomes obvious; the only species found for

other products were *Camellia sinensis* (Kigalu, 2007a, 2007b), *Hevea brasiliensis* (Naji et al., 2014), *Fraxinus angustifolia* (Özbayram and Çiçek, 2020), *Gliricidia sepium* (Silva et al., 2012), *Jatropha curcas* (Andrianirina et al., 2019), and *Moringa oleifera* (Mendieta-Araica et al., 2013).

FIGURE 4. FREQUENCY OF GENUS IN STUDIES ON THE SUBJECT OF PLANTING DENSITY FROM 2001 TO 2020.

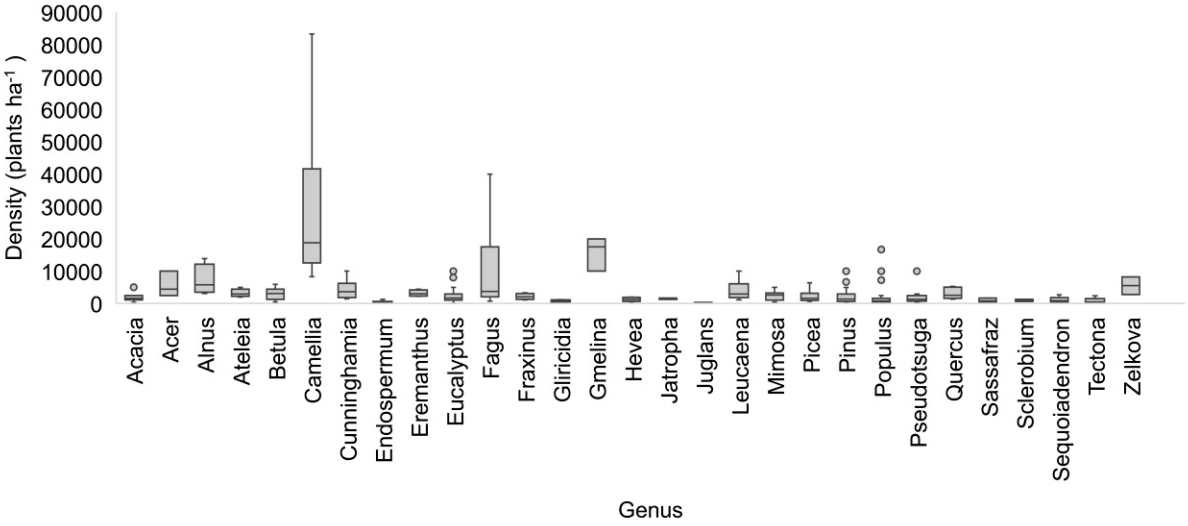


SOURCE: The author.

We observed that planting densities were different for coniferous and broad-leaved species (Figure 5). Planting density varied among species and went from 100 stems ha^{-1} for *Eucalyptus globulus* (Henskens et al., 2001) to 250,000 stems ha^{-1} for *Populus* spp. and *Salix* spp. (Mamashita et al., 2015). Differences in planting density were mainly due to the planned end products from these trials. *Pinus* species, the biggest fraction of coniferous species used in the studies, are mainly used for timber production, especially for the sawmill industry; for this it is necessary to produce timber with greater DBHs that cannot be achieved in high planting densities. Contrasting, *Eucalyptus* and *Populus*, among other broad-leaved species, are used for pulp and paper or energy industries, that can use boles of smaller DBH; these are reached in shorter rotation periods and can be planted in higher densities. Studies with *Camellia sinensis* also use higher densities because the main products of these plantations are the leaves used in the tea industry (Kigalu, 2007a, 2007b). It must be noted that we removed from Figure 5 densities used in Mamashita et al. (2015) because they were

testing very high planting densities in a short-term study and their values were outliers in our summary.

FIGURE 5. AVERAGE SPACINGS USED IN PLANTING DENSITY TRIALS FROM 2001 TO 2020.



SOURCE: The author.

2.3.2 Qualitative analysis

Studies on tree density in planted forest stands mainly address biomass production and timber quality, which reflects on the variables measured (Table 1). The most commonly measured morphological variables were diameter at breast height (DBH; 74 % of papers), height (H; 64 % of papers), and stand volume (SV; 48 % of papers). Table 1 shows growth responses of trees to increasing planting density for different tree species. In general, there was a tradeoff between tighter spacing and biomass production per area, and initial spacing strongly influenced plant growth in different periods during stand growth. Higher planting densities reduced stem and/or crown diameter and, in most studies, there was a linear relationship among these growth variables (Benomar et al., 2012; da Páscoa et al., 2019; Dassot et al., 2015; Han et al., 2020; Harrington et al., 2009; Silva et al., 2012). Height growth tended to increase with planting density, but this trend was not consistent and did not occur in many experiments. Height is not always a density-dependent variable and soil fertility is said to be the most important factor for height growth (Henskens et al., 2001; Woodruff et al., 2002); therefore, competition for soil nutrients could be the main cause for reduced height growth for trees in high-density stands.

TABLE 1. SUMMARY OF STUDIES USING MORPHOLOGICAL MEASUREMENTS IN RESPONSE TO INCREASING PLANTING DENSITY FROM 2001 TO 2020.

Species	S	H	DBH	CH	CD	IV	SV	IBA	SBA	LB	LAI	Reference
Acacia mangium	*/-	*/+	-				+					(Nirsatmanto, 2016; Tonini et al., 2018)
Acacia mearnsii							+			+		(Eloy et al., 2018)
Acer velutinum			-									(Naji et al., 2016)
Alnus rubra	+	-	-									(DeBell and Harrington, 2002)
Ateleia glazioviana		*	-				+/-		+	+	+	(Eloy et al., 2018; Schwerz et al., 2020b, 2020a)
Betula alba			-				+					(Fernández-Núñez et al., 2010)
Betula alnoides	-		*	+	*							(Wang et al., 2016)
Betula pendula	*	*	-		-			*			+	(Johansson, 2007)
Betula pubescens	*	*	-		-			*			+	(Johansson, 2007)
Camellia sinensis										+		(Kigalu, 2007b)
Cunninghamia lanceolata	-	*	-			-			+	+	+	(Farooq et al., 2020; Liu et al., 2019)
Endospermum medullosum	+	*	-	*/+					+			(Glencross et al., 2012; Smith et al., 2016)
Eremanthus erythropappus		*	-		-							(da Páscoa et al., 2019)
Eucalyptus benthamii	*					-	+					(Resquin et al., 2019)
Eucalyptus camaldulensis	+	*/-	-	*/-		-	+		+			(Horner et al., 2009; Nagar et al., 2015; Paula et al., 2013)
Eucalyptus cloeziana		*	-	*								(Alcorn et al., 2007)
Eucalyptus dunnii	*	*	-			-	*/+		+			(Bentancor et al., 2019; Resquin et al., 2019)
Eucalyptus globulus	-	*	-			-	+/-	-	+		+	(Forrester et al., 2013; Henskens et al., 2001; Miranda et al., 2015)
Eucalyptus grandis	*	*	-			-	+/-	*	+	+	+	(Eloy et al., 2018, 2016; Forrester and Smith, 2012; Resquin et al., 2019; Schwerz et al., 2020b, 2020a)
E. grandis x E. camaldulensis						-	+				+	(Hakamada et al., 2017, 2020a)
E. grandis x E. urophylla		-	*/-			*/-	+	-	+		+	(Hakamada et al., 2017, 2020a; Ramalho et al., 2019; Silva et al., 2019; Tavares et al., 2020)
Eucalyptus marginata		*	-						+		+	(Grigg et al., 2008)
Eucalyptus pilularis		*/+	*/-	*/-			-	*				(Alcorn et al., 2007; Cassidy et al., 2013; Forrester and Smith, 2012; West and Smith, 2020)
Eucalyptus urophylla						-	+				+	(Hakamada et al., 2017, 2020a)
Fagus orientalis			-				*		+			(Guner et al., 2010)
Fagus sylvatica		*	-	*	-							(Dassot et al., 2015)
Fraxinus angustifolia		-	*	+		*						(Özbayram and Çiçek, 2020)
Gliricidia sepium		-	-		-							(Silva et al., 2012)
Gmelina arborea	-	*	*			-	+				+	(Tenorio et al., 2019)
Hevea brasiliensis		*	-	+								(Naji et al., 2014)
Jatropha curcas		*										(Andrianirina et al., 2019)
Juglans nigra			-	+	-							(Bohanek and Groninger, 2003)
Leucaena leucocephala		*/-	*/-	-	*/-	-	+					(Liu et al., 2018; Prasad et al., 2011)
Mimosa caesalpiinifolia		-	-		-							(Silva et al., 2012)
Mimosa scabrella		*	-				+/-		+	+	+	(Eloy et al., 2018; Schwerz et al., 2020b, 2020a)
Moringa oleifera		*								+		(Mendieta-Araica et al., 2013)
Picea glauca	-	*	-						+			(Metsaranta, 2019)

Picea mariana	+/-	*	-				+		+			(Groot and Cortini, 2016; Metsaranta, 2019)
Pinus banksiana		*	-	+		-						(Hébert et al., 2016)
Pinus contorta		+	-			-	+		+			(Liziniewicz et al., 2012)
Pinus elliottii	-	*	-			*	+			+	+	(Barron-Gafford et al., 2003; Burkes et al., 2003; Gadow and Kotze, 2014; Will et al., 2001)
Pinus massoniana		*	-	+	-						*	(Yan et al., 2019)
Pinus patula	+/-	*/+	-			*						(Charlton et al., 2020; Gadow and Kotze, 2014)
Pinus pinea		+	-									(Muñoz et al., 2020)
Pinus ponderosa			-			-	*					(Gyenge et al., 2010)
Pinus radiata	-	*	-	+			+					(Fernández-Núñez et al., 2010; Lasserre et al., 2005; Lin et al., 2013; Waghorn et al., 2007)
Pinus resinosa	-	*	-						+			(Metsaranta, 2019)
Pinus sibirica			-									(Milyutina et al., 2013)
Pinus sylvestris	*	*	*/-			-	*		*	*		(Egbäck et al., 2012; Milyutina et al., 2013; Nan et al., 2020; Wegiel and Polowy, 2020)
Pinus taeda	-	*/-	-	+/-		-	*/+		+/-	*/+	+	(Akers et al., 2013; Barron-Gafford et al., 2003; Bose et al., 2018; Burkes et al., 2003; Cardoso et al., 2013; Clark et al., 2008; Egbäck et al., 2015; Roth et al., 2007; Russell et al., 2009; Torreano et al., 2012; Will et al., 2005, 2001; Zhao et al., 2020, 2011)
P. balsamifera x P. trichocarpa	+	*	-	+	-			-				(Benomar et al., 2012)
P. balsamifera x P. maximowiczii	+	*	-	+	-			-				(Benomar et al., 2012)
Populus deltoides	*	-	-				+					(Fang et al., 2007; Rončević et al., 2013)
P. deltoides × P. ×euramericana		*	-				-				*	(Tun et al., 2018)
P. deltoides × P. nigra		+	-									(Toillon et al., 2013)
P. simonii x P. nigra		-	-			-	+					(Ahmed et al., 2020)
Populus tomentosa	*	*/-	-	*	-	-	+					(Han et al., 2020; Huijuan et al., 2020)
Populus xeuramericana		*	-			-	+					(Fang et al., 2007; Xue et al., 2012)
Pseudotsuga menziesii	-	*/+	-	+	-	-	*/+		*/+			(Harrington et al., 2009; Marziliano et al., 2015; Woodruff et al., 2002)
Quercus acutissima	*	-	-				+		+			(Cheng et al., 2020)
Quercus petraea		*	-									(Colin et al., 2012)
Sassafraz tzumu	-						+					(Sun et al., 2018)
Sclerobium paniculatum	-	+	-									(Narducci et al., 2016)
Sequoiadendron giganteum		-	-	+	-						+	(O'Hara and York, 2014)
Tectona grandis		+	-	+	-	-	+					(Pachas et al., 2019)
Zelkova serrata	-	-	-									(Tanabe et al., 2019)

S = survival; H = total height; DBH = diameter at breast height; CH = crown height; CD = crown diameter; IV = individual volume; SV = stand volume; IBA = individual basal area; SBA = stand basal area; LB = leaf biomass; LAI = leaf area index. * = neutral influence of increasing planting density; + = positive influence of increasing planting density; - = negative influence of increasing planting density.
SOURCE: The author.

In the first years after planting, interaction between trees is limited and competition may not be observed until later, when trees reach above- and below-ground space of neighbors. Studies analyzed in this review evaluated trees from one (Schwerz et al., 2019; Silva et al., 2019) to 90 (Wegiel and Polowy, 2020) years after planting and results on morphological characteristics were found to be highly heterogenous. In a *Eucalyptus* plantation, Silva et al. (2019) observed differences in DBH and volume per tree between densities as soon as 24 months after planting. The authors attributed these differences to the higher light interception by tree crowns, which lead to a higher production of photoassimilates. For *Acacia mearnsii*, Schwerz et al. (2019) observed a greater biomass allocation in branches and leaves in the first year after planting; at 7 years after planting, leaf biomass significantly reduced while total stand timber biomass increased, regardless of planting density. Studies that examined biomass allocation along gradients of light, water, and nutrients availability support the hypothesis that plants tend to allocate biomass to the organs that acquires the more limiting resources (Ma et al., 2021; McCarthy and Enquist, 2007; Rodríguez-García and Bravo, 2013); therefore, plants competing for resources in a high-density stand tend to change allocation patterns according to stand age and the planting density. The greater allocation in growth of branches and leaves in the first years form the canopy, which is important to regulate light interception and determine forest productivity (Barbier et al., 2010); and changes in tree growth after canopy closure are often related to competition with neighbor trees (Will et al., 2001).

Several shade-avoidance responses related to the production and transport of the phytohormones auxin and cytokinin drive trees growth and development in high-density stands. The auxin is known to steer plant growth and development through the elongation of hypocotyl and petiole, and upward leaf movements, enhancing access to light (Galvão and Fankhauser, 2015). Auxin biosynthesis and transport is related to photoreceptors signaling (Küpers et al., 2020), and in high density stands it leads to growth in heigh instead of stem lateral growth and branches development in the lower portion of the trees, defining plant shape; its metabolism and how it influences on plant growth and development was extensively reviewed by Casanova-Sáez and Voß (2019). The influence of cytokinin on plants growth and on acclimation to the light gradient in high-density stands is related to the differences in transpiration rate in shaded and sun exposed leaves; the reduced influx of cytokinin in shaded leaves as a result of a lower transpiration rate reduces protein synthesis and amino acids export,

which induces a reallocation of resources to sun-exposed leaves – with higher sink strength (Pons et al., 2001). As a result of changes induced by these phytohormones, specific leaf area is reduced in trees subjected to high-density spacings, although leaf area index can increase in these circumstances.

Generally, there is a reduction of leaf expansion in shaded leaves and a stimulation in the unshaded ones, and it directly influences on leaf area index (LAI) and on specific leaf area (SLA) (Pons et al., 2001). As a measure of canopy closure, LAI is an indicative of stand productivity and biomass accumulation, and it can be an alternative measure of growing space and stocking control in plantations (O'Hara and York, 2014). Few studies have, however, considered this index among measured growth variables. Only 13 studies (12.5 %) considered leaf area index as a response variable to planting density and all of them revealed a neutral or positive relation with increasing density (Akers et al., 2013; Farooq et al., 2020; Grigg et al., 2008; Hakamada et al., 2020a; Henskens et al., 2001; Johansson, 2007; O'Hara and York, 2014; Schwerz et al., 2020a; Tenorio et al., 2019; Tun et al., 2018; Will et al., 2005, 2001; Yan et al., 2019). LAI is often used as an indicator for canopy closure and light interception in agricultural crops (Sharma-Natu and Ghildiyal, 2005), but we must consider that in forest plantation, tree density is largely different from agricultural plantations and so is light interception. Hakamada et al. (2020a) indicated that increasing LAI in clonal *Eucalyptus* plantations was directly related to light interception and, consequently, to a more efficient biomass production. The authors observed that genotype related characteristics also influenced on clone responses to planting density, which is an important factor to be considered on breeding programs. Akers et al. (2013) obtained similar results for *Pinus taeda* plantation under different densities and authors attributed this increase in LAI and light interception to an increase in light-use efficiency in high-density stands when compared to lower-densities stands. It must be noted that an increase in LAI or SLA is not always accompanied by an increase in photosynthesis efficiency, considering that increasing leaf expansion can result in thicker leaves with lower stomatal density, and consequently, reduced gas exchange potential (Mcelwain et al., 2016; Thyroff et al., 2019).

Competition for resources cannot always be managed with water and nutrient additions or other silvicultural treatments and the effects of resources management are more likely to be obtained for leaf, fruit, and seed yield than for wood (Kigalu, 2007a, 2007b; Mendieta-Araica et al., 2013; Narducci et al., 2016). For other species, fruit and

seed yields are not influenced or negatively influenced by density, independently of other resources management, as for *Pinus pinea* (Muñoz et al., 2020). It indicates a potential to manage spacing arrangements in interaction with other silvicultural treatments to increase yield even when other resources become scarce. Establishing high-density plantations in sites with high availability of water and nutrients is a strategy to increase productivity by reducing competition between trees without increasing plantation coasts (Harrington et al., 2009; Sun et al., 2018; Toillon et al., 2013; Zhao et al., 2020).

The increase in total plantation biomass with a decrease in individual tree biomass is an important aspect to consider for forest producers. There is a potential of producing wood for energy in high density stands; although for more valuable wood products, such as lumber and veneer, these stands should be submitted to thinning managements to produce larger boles. At high initial densities, thinning may be the best option for producers to guarantee high valuable wood products at the end of rotation, regulating timber form and volume (Charlton et al., 2020). Smith et al. (2016) highlight the need to evaluate economical aspects and use possibilities of thinned stems, and Glencross et al. (2012) also suggest the use of high planting density with thinning treatments to obtain different products at different times to guarantee both stand and individual high volume. Adapted management strategies guarantee both wood and energy products in forest plantations, including increasing tree density, additional thinning, and reducing rotation length, resulting in financial gains (Scott and Tiarks, 2008).

Although we consider the constant final yield as an index of maximum planting density in a stand, most studies evaluated in this review did not reach a planting density that would be considered limiting for tree growth. A possibility is that many researchers focused on density ranges to obtain an increase in stand biomass, but still considering the production of high-quality wood products. In some studies, both individual and stand volume did not present linear responses to increasing stand density, indicating that increasing planting density not always results in increasing biomass productivity (Gadow and Kotze, 2014; Guner et al., 2010; Marziliano et al., 2015; Özbayram and Çiçek, 2020; Tavares et al., 2020; Wegiel and Polowy, 2020). It is important to note that most plantations are not fertilized during the whole rotation, so a decrease in planting density would lower soil nutrient extraction and an economy in fertilization (Medeiros et al., 2020). High planting density can also improve resources use

efficiency through physiological mechanisms, which we will discuss in the next paragraphs.

Physiological evaluations in response to tree density are less numerous in the literature, probably because they are less easily done in the field compared to dendrological measurements (Table 2). Physiological studies mainly focus on *Eucalyptus* and *Pinus* species and are addressed by a restricted number of research groups. The number of measured variables was also reduced compared to morphological variables, focusing especially on light and water influence on trees.

TABLE 2. SUMMARY OF STUDIES USING PHYSIOLOGICAL MEASUREMENTS IN RESPONSE TO INCREASING PLANTING DENSITY FROM 2001 TO 2020.

Species	IR	A	E	R	WUE	LWP	Cl	Reference
Ateleia glazioviana	*							(Schwerz et al., 2020a)
Camellia sinensis			-		+			(Kigalu, 2007a)
Eucalyptus globulus		*						(Henskens et al., 2001)
Eucalyptus grandis	*							(Schwerz et al., 2020a)
Eucalyptus grandis × E. camaldulensis			+		*	-		(Hakamada et al., 2017, 2020a, 2020b)
Eucalyptus grandis x E. urophylla			+		*	-		(Hakamada et al., 2017, 2020a, 2020b)
Eucalyptus urophylla			+		*	-		(Hakamada et al., 2017, 2020a, 2020b)
Fagus orientalis		*						(Guner et al., 2010)
Mimosa scabrella	*							(Schwerz et al., 2020a)
Pinus elliottii	-	*		+				(Will et al., 2001)
Pinus sibirica							+	(Milyutina et al., 2013)
Pinus sylvestris							+	(Milyutina et al., 2013)
Pinus taeda	-	*		+	-			(Akers et al., 2013; Will et al., 2005, 2001)
Populus deltoides × P. nigra					-			(Toillon et al., 2013)

IR = intercepted radiation; A = carbon assimilation; E = transpiration; R = respiration; WUE = water use efficiency; LWP = leaf water potential; Cl = chlorophyll content. * = neutral influence of increasing planting density; + = positive influence of increasing planting density; - = negative influence of increasing planting density. SOURCE: The author.

The first and most obvious influence of closer planting spacing is a reduction in light interception, which directly influences plants physiological functions. In a study with *Pinus* plantations at different densities, Will et al. (2001) observed that tree growth correlated with intercepted radiation; as stand density increased, the amount of radiation intercepted per unit of leaf area decreased due to self-shading. The authors observed that differences in net photosynthesis and respiration rates were minimal and did not correlate with growth at the different stand densities, possibly because

resources necessary for plants physiological activities were not limiting in any treatment. Toillon et al. (2013) also observed that high planting densities accentuated competition for light in poplar plantations, which led to increased height growth instead of stem diameter. As trees are closer together, there was a reduction in R:FR ratio, indicating an upcoming competition for light and a decrease of total light intensity in the stand (Huber et al., 2020); however, only Benomar et al. (2011) observed differences in C assimilation related to planting density among the studies used in this review. In the first stages of seedlings growth, low R:FR ratio promotes auxin synthesis, increasing hypocotyl elongation, this process induces seedlings to grow searching for light (Casanova-Sáez and Voß, 2019).

Few data are available on direct measurements of gradients in transpiration rates in forest canopies, although there is evidence that this physiological parameter vary parallel to light gradient (Pons et al., 2001). Except for *Camellia sinensis* (Kigalu, 2007a), it was observed that transpiration and respiration increased with increasing planting density (Hakamada et al., 2017, 2020a, 2020b; Will et al., 2001). These results are closely related to water use efficiency – for *Camellia sinensis*, the reduction in transpiration rates increased water use efficiency in high-density stands, while for *Eucalyptus* clones' responses varied according to genotype (Hakamada et al., 2020a, 2020b; Kigalu, 2007a). Differences in plant physiological responses could be linked to species characteristics such as drought resistance, number of stomata, and leaf type; however, we found a lack of information on the mechanisms related to changes in water use with plantation density and its influence on tree growth.

Understanding the dynamics of physiological responses to increasing stand density is fundamental to plan adequate management scenarios and to obtain high-valuable products. Increasing planting density may also have implications for ecosystem water balance since high density stands have a higher water use from a landscape perspective (Forrester, 2015). It is known that tree physiology responses in high density stands are mediated by competition for resource acquisition (Resquin et al., 2019; Will et al., 2005), indicating the importance of site quality and the knowledge about species requirements to establish a monospecific forest plantation. Several studies selected in this review indicate that site characteristics have a major impact on tree growth and physiological responses to planting density, indicating the importance of physiological evaluations in field trials to develop management programs for each

species (Akers et al., 2013; Guner et al., 2010; Toillon et al., 2013; Will et al., 2005, 2001).

2.4 FINAL CONSIDERATIONS

We observed an increasing trend in studies on plant morphological and physiological responses to planting density in forest plantations. Most studies are in countries with a well-established forest market for timber, pulp and paper, and energy, which explains the high number of studies focusing on *Pinus* and *Eucalyptus* species. The main variables evaluated in field trials are DBH and timber volume, illustrating a study field especially focused on timber production; therefore, we highlight the need to develop research on the production of other forest products such as leaves, fruits, and seeds. We also highlight the need to consider the use of physiological evaluations in field trials, which could give more accurate responses of plants to competition for resources.

Increasing planting density of forest plantations is important for regions where forest plantations compete with other high-valuable crops or where space is a limiting factor for the development of the forest industry. High-density forest stands can represent an increase in value and yield for a variety of products, but we still need to improve knowledge about plants growth focusing on products other than timber. Our results can be a basis for the establishment of forest plantations aiming at the better use of forest stands and the development of new silviculture studies.

3 CHAPTER II – PLANTING SPACING AND NITROGEN FERTILIZATION ON YERBA MATE: YIELD RESPONSES ON A HIGH-DENSITY PRODUCTION SYSTEM

We studied the effects of planting density and N doses in yerba mate biomass production. In September 2018 we established a field trial in a factorial scheme with 3 planting densities (9,090, 13,333, and 25,000 plants ha^{-1}) and 5 N doses (0 %, 50 %, 100 %, 200 %, and 300 % of the recommended N dose). We evaluated total biomass (per hectare and per plant), young and mature leaves biomass in 5 subsequent harvests. Increasing planting density increased stand total biomass, although individual biomass did not present significant changes. The highest yield in total biomass was obtained with 25,000 plants ha^{-1} and 200 % N dose – 1,792 kg ha^{-1} in January 2021. In the high-density treatment (25,000 plants ha^{-1}) we observed an expressive demand of plants in N fertilization, with higher yields in treatments with higher N availability. Young and mature leaves biomass yield reflected total biomass yield, with more productivity in high-density planting with higher availability of N. Yield per plant did not reduce in high-density planting, indicating no competition between neighbors. Our results demonstrate the potential of yerba mate to be managed in high-density stands, with reduced rotation periods and the adequate nutrient management for high biomass production.

Keywords: biomass productivity, *Ilex paraguariensis*, plant nutrition, planting density, stand density, silviculture

3.1 INTRODUCTION

Yerba mate (*Ilex paraguariensis* A. St.-Hill.) is an arboreal species native to the South American Humid Subtropical Forest and it is cultivated in monospecific stands or in agroforestry systems in Brazil, Argentine, and Paraguay (Carvalho, 2003). Its leaves are social and economically important for small producers in the regions where the species occurs because they are used to produce traditional beverages as chimarrão, tereré, and tea (Lima & Farah, 2019). In traditional silvicultural systems (monospecific stands), yerba mate is produced in low density planting spacings (2,222 – 2,800 plants ha^{-1}), with a rotation period of 18-24 months and producers still lack

information about silvicultural traits to increase leaves quality and yield (Santin et al., 2015; Santin et al., 2016).

In recent years, several studies have demonstrated that yerba mate leaves can be a source of caffeine, theobromine, and other bioactive compounds for food, cosmetic, and pharmaceutical industries (Riachi & Maria, 2017; Valduga et al., 2019; Duarte et al., 2020). Reducing rotation period is an alternative to increase leaves quality, since biochemical compounds contents are higher in young leaves and smaller periods between harvests reduce damages caused by environmental conditions and pest attacks (Blum-Silva et al., 2015; Yin et al., 2015). The interest in using yerba mate as a raw material for health-promoting and functional products require studies aiming at increasing yield and quality of leaves produced in plantations.

Increasing planting density is a silvicultural alternative to increase yield in several agricultural and forestry plantations (Leite et al., 2011; Charlton et al., 2020; Hakamada et al., 2020; Schwerz et al., 2020). In forest stands, studies indicate that individual biomass yield tends to reduce while stand yield increases until a limit when competition between neighbor plants causes significant changes in plant morphological and physiological aspects (Huber et al., 2020). *Camellia sinensis*, used to produce the most common tea worldwide, is planted in high-density stands (8600 – 180,000 plants ha^{-1}) and its leaves are harvested in regular intervals to guarantee a high yield and specific characteristics expected by industries (Kigalu, 2007a; Kigalu, 2007b; Rawat et al., 2017; Du et al., 2021). The use of high-density stands is an alternative to reduce rotation periods and to increase yield in yerba mate plantations, but the species still lacks knowledge on silvicultural traits that would enable high-density plantations establishment.

It is known that planting density alters resources availability, which includes nutrients, demanding adequate management practices to guarantee a regular yield over time (Leite et al., 2011; Hakamada et al., 2020). Yerba mate occurs naturally in low fertility soils, but studies reported that in intense silvicultural systems it responds positively to fertilization, indicating that biomass productivity may be related to nutrients availability (Santin et al., 2015; Santin et al., 2016; Barbosa et al., 2018; Toppel et al., 2018). As nitrogen (N) is a main component of several molecules in plants, it is essential to regularly manage this nutrient in high-density plantations to guarantee a regular yield.

There are no records of yerba mate cultivated in high-density silvicultural systems; therefore, this study aimed to assess the effects of planting density and N doses on biomass yield of yerba mate. For this, we consider the following hypothesis: (a) Increasing planting density would increase biomass yield; (b) Increasing planting density would demand a higher N dose to provide the expected yield.

3.2 MATERIAL AND METHODS

The experiment was established in September 2018, in a private property in General Carneiro – PR, Brazil (26º25'39'' S, 51º18'56'' W, 983 m). Region's climate is Cfb type (humid subtropical), with mild temperatures in summer and the occurrence of frosts in winter, without a defined dry season (Alvares et al., 2013). Temperature averages do not exceed 22 ºC during summer and 18 ºC during winter. Soil chemical and physical analysis are exposed in Table 3.

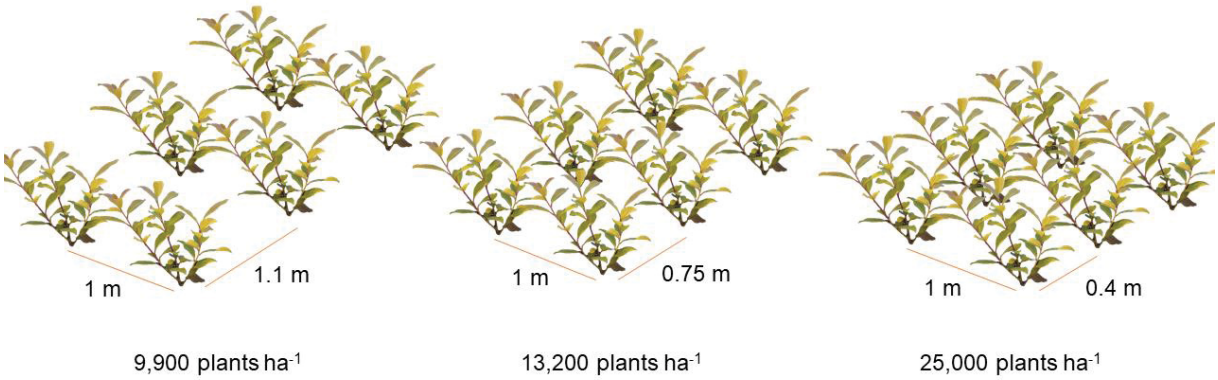
TABLE 3. ORGANIC MATTER CONTENT (OM), SOIL AVAILABILITY OF PHOSPHORUS (P), POTASSIUM (K), CALCIUM (CA), AND MAGNESIUM (MG), AND SOIL PHYSICAL ASPECTS IN THE EXPERIMENTAL AREA BEFORE *Ilex paraguariensis* PLANTATION.

	OM	P	K	Ca	Mg	Clay	Silt	Sand
	%	- - - - mg/dm³ - - - -		$cmol_c/dm^3$		- - - - - - $g.kg^{-1}$ - - - - - -		
Soil content	5.65	1.05	70.40	0.20	0.20	745	125	88
Analysis level	High	Very low	Very low	Low	Low			

SOURCE: The author.

Three planting densities were used: 9,090, 13,333, and 25,000 plants ha^{-1}, all of them above the commonly applied in commercial yerba mate plantations. Spacing between rows was fixed in 1.00 m and spacing between plants in the row varied accordingly to planting density treatment – 1.10 m, 0.75 m, and 0.40 m for 9,090, 13,333, and 25,000 plants ha^{-1}, respectively (Figure 6).

FIGURE 6. GRAPHICAL DESCRIPTION OF THE PLANTING DENSITY TREATMENTS USED IN *Ilex paraguariensis* EXPERIMENT.



SOURCE: The author.

At experiment establishment, in September 2018, we applied fertilization according to soil analysis – 44.0 kg N ha^{-1}, 153.9 kg P ha^{-1}, and 42.5 kg K ha^{-1}, which represented the annual recommended dose of P and half the dose of N and K (Wendling & Santin, 2015). After plantation, fertilization was applied every January and September, considering the different N doses for each treatment: 0 %, 50 %, 100 %, 200 %, and 300 % of the recommended N dose (Table 4). In each fertilization, we applied the half annual dose of N and K to reduce nutrient losses, and every September we applied total annual P dose. Fertilization was not applied in May harvest to avoid plants growth during winter, when they are subjected to frosts and new branches are deeply affected.

TABLE 4. ANNUAL DOSE OF FERTILIZER APPLIED IN THE EXPERIMENT.

Fertilizer	Dose (kg ha^{-1})				
	0 %	50 %	100 %	200 %	300 %
Urea (45 % N)	0.0	48.8	97.0	194.0	291.0
Single superphosphate (21 % P_2O_5)	733.0	733.0	733.0	733.0	733.0
Potassium chloride (58 % K_2O)	73.3	73.3	73.3	73.3	73.3

SOURCE: The author.

Four months after planting (January 2019), we pruned seedlings, removing the apical meristem to induce the formation of lateral shoots. We evaluated total fresh biomass (leaves and branches) and the productivity of young and mature leaves in five harvests every January and May, from May 2019 until May 2021. In every harvest we kept 20-30 % leaves in the plant to maintain physiological activity. After weighting total biomass in a precision scale, we separated leaves in young and mature. Young leaves

were membranous and light green or reddish colored, located in the upper branches' region; those thicker and darker, located in the lower region of the branches were classified as mature leaves. The separated leaves were also weighted in a precision scale. Data was expressed in kg ha^{-1}, considering the planting density of each treatment, and individual total yield was expressed in g $plant^{-1}$.

The experiment was established in a completely randomized blocks design in a double factorial arrangement, with three planting densities and five nitrogen doses evaluated over five harvests in four blocks. Each treatment repetition consisted in 30 plants and was separated from other treatments by a double plant row (border) to avoid the influence of other treatments. From analysis of variance (ANOVA) results, we performed Tukey's range test at 5 % probability. For the analysis and graphics, we used the R software version 3.6.3 (R Core Team, 2020).

3.3 RESULTS AND DISCUSSION

Analysis of variance indicated that planting density and N dose had significant effects on total biomass productivity; however, we did not observe a significant interaction of these factors with harvests (Table 5). It is expected that subsequent harvests will have increased leaves production since plants are more acclimated to field conditions, tending to grow and produce more biomass. To avoid diluting the effect of planting density and N dose, we separated statistical analysis for each harvest.

TABLE 5. ANALYSIS OF VARIANCE (ANOVA) FOR TOTAL BIOMASS PRODUCTIVITY OF YERBA MATE SUBMITTED TO DIFFERENT COMBINATIONS OF N DOSE AND PLANTING DENSITY.

Variation	GL	Mean Square	Pr(>F)
Block	3	1160438	1.03^{-03} ***
Harvest	4	535737	2^{-04} ***
Planting density	2	11351893	2^{-16} ***
N dose	4	1111044	5.57^{-09} ***
Planting density x N dose	8	476708	5.21^{-06} ***
Harvest x planting density	8	102855	0.34
Harvest x N dose	16	170245	0.024
Harvest x N dose x planting density	32	46485	0.98
Residue	222	91093	
CV (%)		47.70	

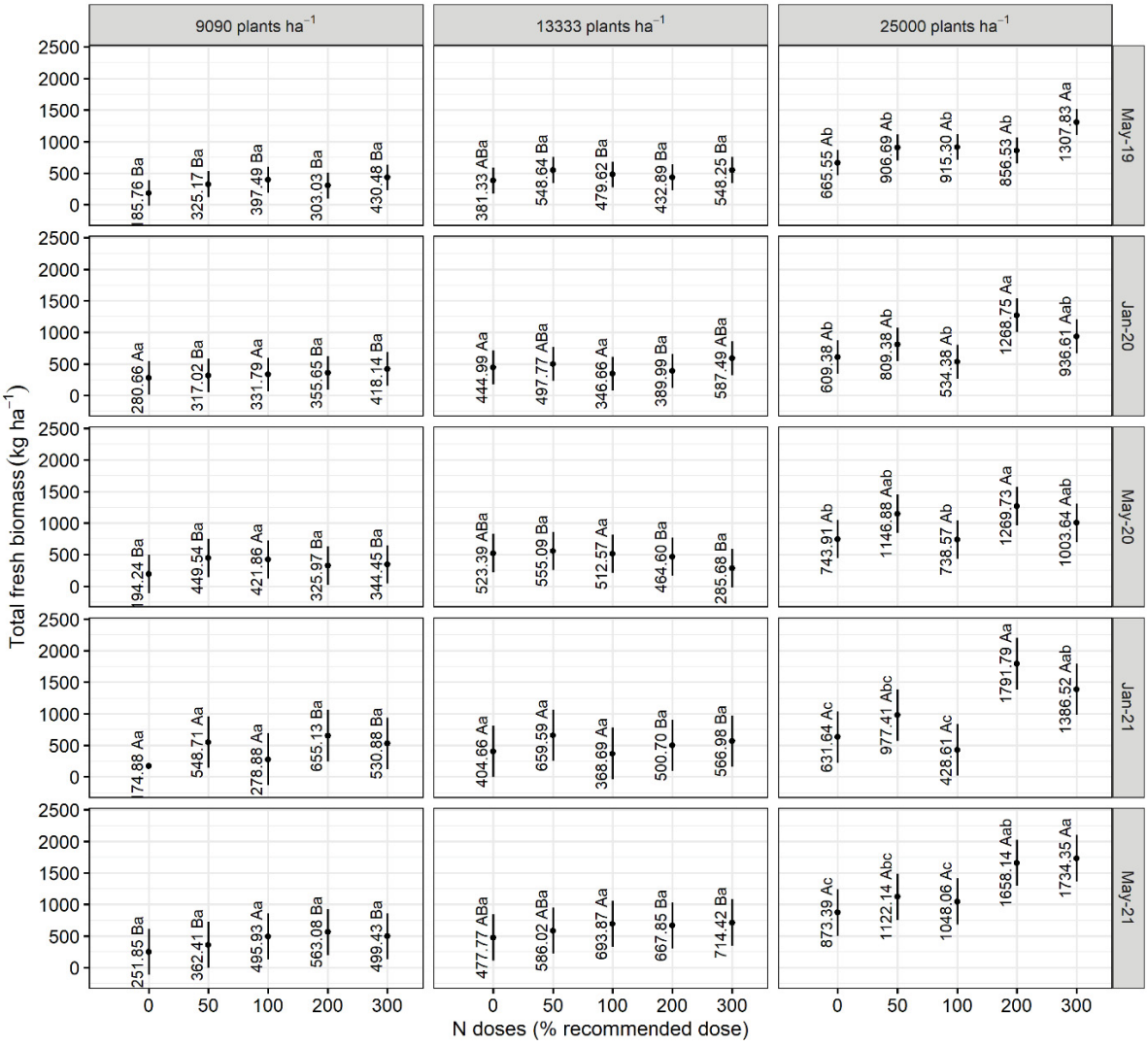
Signif. codes: *** = 0.00; ** = 0.01; * = 0.05. SOURCE: The author.

The use of high-density stands with reduced rotation periods is an innovative silvicultural system for yerba mate. High-density stands are already used for tea plants

(*Camellia sinensis*) production in several countries, with the production of high-quality raw material for the pharmaceutical and food industries (Kigalu, 2007a; Tfouni et al., 2018; Valduga et al., 2019). Our results indicate that yerba mate plants also have the potential to be managed as tea plants, although this silvicultural system requires more accurate resources management to guarantee the expected yield. Santin et al. (2016) also highlight the importance of an adequate nutritional management for yerba mate plantations searching to reduce rotation periods and slightly increasing planting density.

Total biomass differed among treatments, with the highest productivity ha^{-1} with 25,000 plants ha^{-1}, regardless nutrition treatments (Figure 7). The highest biomass production – 1,792 kg ha^{-1} – was obtained in January 2021 with 25,000 plants ha^{-1} and the addition of 200 % of the recommended N dose. The same planting density had greater differences among N doses. Although we observed differences in total biomass production between harvests, it is clear that in planting density of 25,000 plants ha^{-1} we had higher productivity when applying 200 % or 300 % of the recommended N dose for yerba mate.

FIGURE 7. TOTAL FRESH BIOMASS (kg ha^{-1}) OF YERBA MATE SUBMITTED TO DIFFERENT COMBINATIONS OF PLANTING DENSITY AND N DOSES, EVALUATED IN FIVE SUBSEQUENT HARVESTS. MEANS FOLLOWED BY THE SAME CAPITAL LETTERS DO NOT DIFFER BETWEEN DENSITIES IN THE SAME N DOSE AND HARVEST, AND MEANS FOLLOWED BY THE SAME SMALL LETTERS DO NOT DIFFER BETWEEN N DOSES IN THE SAME PLANTING DENSITY AND HARVEST BY THE TUKEY TEST AT 5 % PROBABILITY.

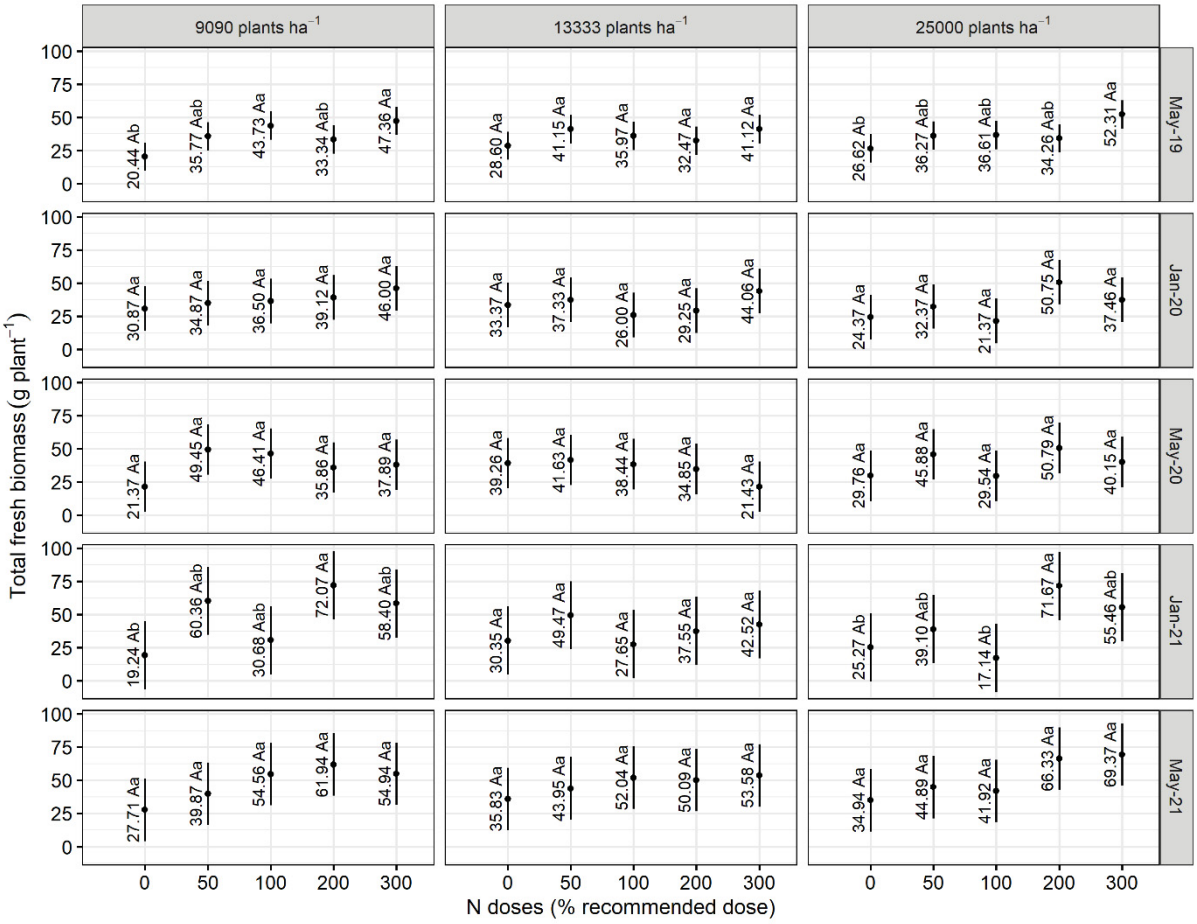


SOURCE: The author.

In the first three years after planting, we observed an increasing general trend in total biomass production in all treatments, varying from 155 kg ha^{-1} in May 2019 to 1,792 kg ha^{-1} in January 2021. The significant differences in productivity in stands with 25,000 plant ha^{-1} indicate that the availability of N may be a limiting factor for plants growth and biomass accumulation in high-density stands. Contrarily, in low-density stands the availability of resources for a small number of plants did not promote its growth, indicating that increasing N availability will not reflect in higher productivity per plant.

Leaves yield per plant differed only for 9,090 and 25,000 plants ha^{-1} in May 2019 and in January 2021 (Figure 8). Although there were no significant differences, we observe an increasing trend in individual biomass over time in treatments with 100 % or more N dose. Plants submitted to 0 % N dose did not present an increasing trend in biomass over harvests. The highest yields per plant were obtained in January 2021, with 72.07 g plant^{-1} in 9,090 plants ha^{-1} submitted to 200 % N dose; and 71.67 g plant^{-1} in 25,000 plants ha^{-1} submitted to 200 % N dose. The smallest yield per plant was obtained in the same harvest in 9,090 plants ha^{-1} submitted to 0 % N dose.

FIGURE 8. TOTAL FRESH BIOMASS (g plant^{-1}) OF YERBA MATE SUBMITTED TO DIFFERENT COMBINATIONS OF PLANTING DENSITY AND N DOSES, EVALUATED IN FIVE SUBSEQUENT HARVESTS. MEANS FOLLOWED BY THE SAME CAPITAL LETTERS DO NOT DIFFER BETWEEN DENSITIES IN THE SAME N DOSE AND HARVEST, AND MEANS FOLLOWED BY THE SAME SMALL LETTERS DO NOT DIFFER BETWEEN N DOSES IN THE SAME PLANTING DENSITY AND HARVEST BY THE TUKEY TEST AT 5 % PROBABILITY.

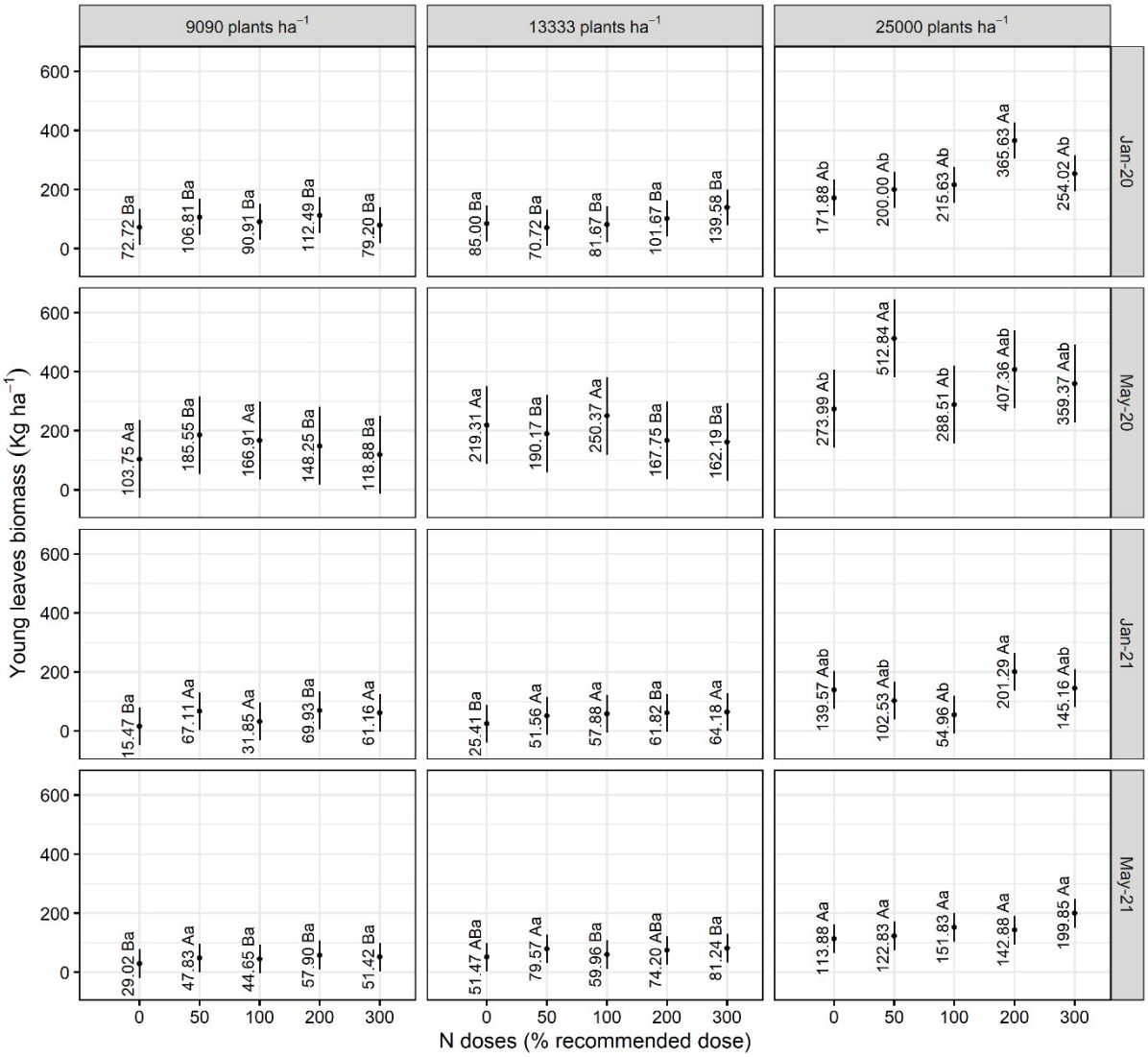


SOURCE: The author.

Although we obtained high productivity per area in 25,000 plants ha^{-1} planting density, we did not observe a reduction in biomass productivity per plant, as observed in planting density studies with other species, such as *Eucalyptus* (Resquin et al., 2019; Hakamada et al., 2020). It indicates that during the experimental period we did not reach a limiting planting density, which would cause a reduction in individual growth due to competition with neighbors for light, water, and nutrients (Huber et al., 2020). Competition between plants in monospecific stands depends highly on species requirements and environmental resources availability, which explains why high-density stands require a more accurate management to provide the expected biomass yield (Leite et al., 2011). The effects of planting density will be of mainly importance after canopy closure in the next years, when we expect to have more accurate responses on yerba mate management in this new silvicultural system.

As for total biomass, young leaves had more biomass productivity in higher planting density (25,000 plants ha^{-1}) (Figure 9). Also, this planting density had significant differences among N doses while planting densities of 9,090 and 13,333 plants ha^{-1} did not have any significant difference for this factor. For harvests in January, the highest productivity was obtained with 200 % of the recommended N dose in 25,000 plant ha^{-1} – 365 kg ha^{-1} in 2020 and 201 kg ha^{-1} in 2021; although in January 2021 there was no significant difference from other N doses. In May 2020 the highest young leaves productivity was obtained with 50 % N dose, but it did not differ from 200 % and 300 % N dose. In May 2021 there was no difference between N doses, indicating that N availability was not a limiting factor for plants growth.

FIGURE 9. YOUNG LEAVES FRESH BIOMASS (kg ha^{-1}) OF YERBA MATE SUBMITTED TO DIFFERENT COMBINATIONS OF PLANTING DENSITY AND N DOSES, EVALUATED IN FOUR SUBSEQUENT HARVESTS. MEANS FOLLOWED BY THE SAME CAPITAL LETTERS DO NOT DIFFER BETWEEN DENSITIES IN THE SAME N DOSE AND HARVEST, AND MEANS FOLLOWED BY THE SAME SMALL LETTERS DO NOT DIFFER BETWEEN N DOSES IN THE SAME PLANTING DENSITY AND HARVEST BY THE TUKEY TEST AT 5 % PROBABILITY.

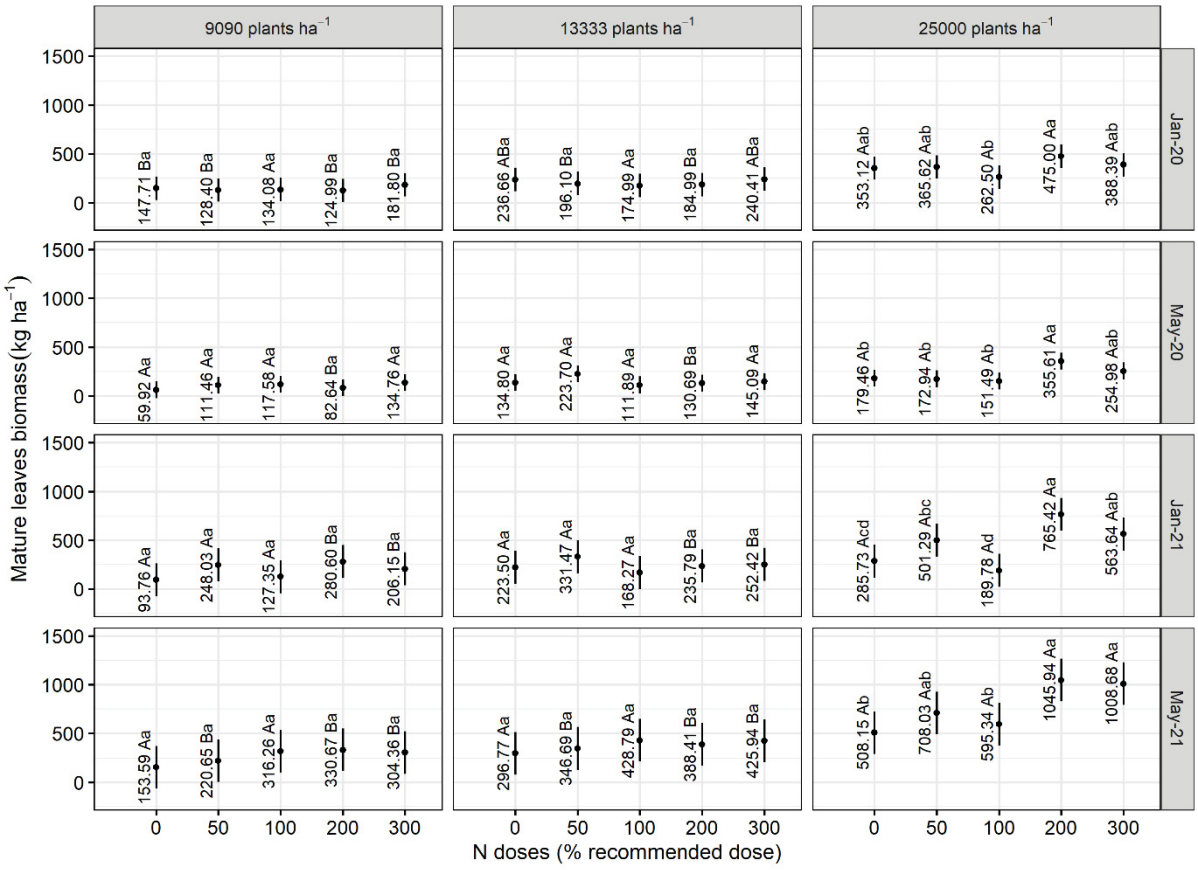


SOURCE: The author.

The production of mature leaves increased in the last harvests, indicating that plants are growing and accumulating more biomass in all treatments (Figure 10). Following the same trend of total biomass productivity, the highest biomass productivity of mature leaves was obtained in 25,000 plant ha^{-1} planting density – 1,046 kg ha^{-1} with 200 % N dose in May 2021 (Figure 10). For this fraction of biomass productivity, we observed higher production in stands with 200 % or 300 % of the recommended N dose – 475 kg ha^{-1} with 200 % N dose in January 2020; 356 and 255 kg ha^{-1} with 200 % and 300 % N dose in May 2020; 765 and 564 kg ha^{-1} with 200 % and 300 % N dose in January

2021; 1,046 and 1,009 kg ha^{-1} with 200 % and 300 % N dose in May 2021 – although it did not differ from other treatments in the harvest of January 2020.

FIGURE 10. MATURE LEAVES FRESH BIOMASS (kg ha^{-1}) OF YERBA MATE SUBMITTED TO DIFFERENT COMBINATIONS OF PLANTING DENSITY AND N DOSES, EVALUATED IN FOUR SUBSEQUENT HARVESTS. MEANS FOLLOWED BY THE SAME CAPITAL LETTERS DO NOT DIFFER BETWEEN DENSITIES IN THE SAME N DOSE AND HARVEST, AND MEANS FOLLOWED BY THE SAME SMALL LETTERS DO NOT DIFFER BETWEEN N DOSES IN THE SAME PLANTING DENSITY AND HARVEST BY THE TUKEY TEST AT 5 % PROBABILITY.



SOURCE: The author.

In traditional production systems of yerba mate, the first harvest occurs only 24-36 months after plantation and subsequent harvests occur each 18-24 months (Wendling & Santin, 2015); while in our experimental system, the first harvest occurred 8 months after planting and rotation period reduced to 4-8 months. Santin et al. (2015) obtained an average yield of 1.7 ton ha^{-1} in the first harvest of a yerba mate plantation, 36 months after planting; considering the sum of total biomass obtained in all harvests performed in our experiment until May 2021 (32 months after planting), we obtained higher yields in all treatments. In addition to yield, it is important to highlight that reducing periods between harvest increases the possibility to have a high-quality raw

material, considering that bio compounds concentration is higher in young leaves than in mature ones (Blum-Silva et al., 2015; Yin et al., 2015).

Since we did not achieve a plateau or a decrease in biomass production at any N dose used in this study, we assume that even the highest dose applied did not cause toxicity. Differences in productivity at 25,000 plants ha^{-1} are related to an increasing nutrients demand since the consumption of resources per area is higher; this relation was also observed in planting density studies with other species, such as *Eucalyptus dunnii* and *Cunninghamia laceolata* (Bentancor et al., 2019; Duan et al., 2019). Still, the absence of differences in biomass productivity at 9,090 and 13,333 plants ha^{-1} may be due to the high availability of N in the soil, which was not totally consumed by plants during the experimental period even in treatments without N fertilization. Soil high fertility also explains why we did not observe any symptoms of chlorosis in yerba mate leaves, which would indicate a high remobilization due to a limitation in N absorption.

The findings from this study have important implications to increase growth and yield of yerba mate biomass production. High-density stands increase leaves production per area and have potential implications in increasing resources use efficiency and a sustainable intensification of this agricultural system. The classification of leaves in young and mature during harvests create new possibilities to the establishment of yerba mate in industrial markets other than the traditional beverages; therefore, making it necessary to reduce periods between harvests and increase the quality of raw material.

3.4 CONCLUSIONS

Higher yerba mate biomass production per hectare was obtained with increasing planting densities, although no differences were found in yield per plant. 25,000 plants ha^{-1} planting density increased responses to nitrogen application and 200 % or 300 % of the recommended nitrogen dose increased biomass production in the first three years after planting.

4 CHAPTER III – NEW METHOD TO PRODUCE YERBA MATE: DOES PLANTING DENSITY AND NITROGEN DOSES INFLUENCE ON PHENOLIC COMPOUNDS AND METHYLXANTHINES CONTENTS?

Yerba mate is a tree species native to South America; its leaves and thin branches are increasingly consumed by different forms of infusion (in hot or cold water) and other products, because of its beneficial effects to human health. Recently, its biochemical composition has attracted the attention of pharmaceutical and cosmetic industries, increasing the need to establish plantations for these purposes. This study aimed to evaluate how planting density and nitrogen (N) doses influence yerba mate leaves biochemical compounds. We evaluated the antioxidant activity and total phenolic compounds contents, methylxanthines (caffeine and theobromine), and caffeoylquinic acids in yerba mate subjected to high planting densities (9,090, 13,333, and 25,000 plants ha^{-1}) and N doses (0 %, 50 %, 100 %, 200 %, and 300 % of the recommended N dose) over five harvests performed during the first two years after planting. Planting density and N doses influenced on biochemical compounds, mainly on caffeine and theobromine contents while planting density did not influence on methylxanthines and caffeoylquinic acids contents, indicating that environmental factors may influence on yerba mate secondary metabolites.

Keywords – Antioxidant activity, caffeine, caffeoylquinic acids, *Ilex paraguariensis*, nutrient management, secondary metabolism, silviculture, theobromine

4.1 INTRODUCTION

Yerba mate (*Ilex paraguariensis* A. St-Hill) is a native tree species from South America, occurring in South Brazil, Argentine, and Paraguay (Carvalho, 2003); its leaves are widely consumed as hot or cold beverages in South America, with an average annual consumption around 3-10 kg per person (Gan et al., 2018). The species is exploited in natural areas or planted in agroforestry systems and monospecific stands with low-density planting spacings (2,222-2,800 plants ha^{-1}) harvested each 18-24 months (Wendling and Santin, 2015), and producers are still resistant to applying differentiated silvicultural traits aimed at improving productivity

and quality of obtained products. Despite its economic and social importance, yerba mate plantations still lack on management regarding the production of high-value raw material for other industries than the traditional beverages (Vieira et al., 2021; Wendling et al., 2018).

Recently, yerba mate biochemical characteristics have attracted the attention of pharmaceutical and food industries due to its caffeine, theobromine, and phenolic compounds contents (de Lima et al., 2019; Negrin et al., 2019). Caffeine is a well-known methylxanthine stimulant of the central nervous system and occurs naturally in plants used to make beverages as coffee and tea (Gan et al., 2018; Lima and Farah, 2019). Recent studies have identified high caffeine genotypes in yerba mate plantations, indicating its high potential to be used as a source of this secondary metabolite (Cardozo Junior et al., 2010; Duarte et al., 2020; Vieira et al., 2021). Apart of its stimulant properties, other health benefits related to methylxanthines and phenolic compounds have been reported for yerba mate, such as antioxidant and diuretic activity, hypocholesterolemic and hepatoprotective activity, protector of DNA oxidation, and protector of the cardiovascular system (Alkhatib and Atcheson, 2017; Bracesco, 2019; Pozebon et al., 2015; Riachi and Maria, 2017; Santos et al., 2015); however, the production of yerba mate still lacks information on how to increase leaves production with high contents of these compounds.

Although methylxanthines and phenolic compounds benefits human health, their primary purpose is to support plant ecological functions. The biosynthesis of secondary metabolites is highly susceptible to and regulated by environmental conditions as temperature, luminosity, water and nutrients availability, and pest or disease attacks (Borges et al., 2017; Li et al., 2020); Also, secondary metabolites influence on plant growth and development, guaranteeing its survival through stressful environments. Several silvicultural practices can alter environmental conditions and consequently influence on secondary metabolism, leading to changes on its biosynthesis. Among silvicultural traits, increasing planting density and nitrogen (N) fertilization are indicated to increase stand productivity and leaves yield; also, these practices can influence on secondary metabolites contents. N is on the pathway basis for secondary metabolites biosynthesis as a key component of amino-acids and, as nitrogen-based alkaloids, methylxanthines contents have been related to N availability (Tzortzakis et al., 2020).

Given the importance of nitrogen in the biosynthesis of secondary metabolites, as well as the impacts that planting density may have on resources availability, this study was based on the hypothesis that a) yerba mate plants submitted to increasing N doses have different contents of methylxanthines and phenolic compounds; b) planting density influences on methylxanthines and phenolic compounds contents on yerba mate plants. Therefore, we evaluated how planting density and N doses influence on methylxanthines and phenolic compounds contents on yerba mate plants.

4.2 MATERIAL AND METHODS

4.2.1 Experiment establishment and treatments

In September 2018 we established the experiment in General Carneiro – PR, Brazil (26º25'39'' S, 51º18'56'' W, 983 m). Region's climate is Cfb type (humid subtropical), with mild temperatures in summer and the occurrence of frosts in winter, without a defined dry season (Alvares et al., 2013). Maximum temperature averages do not exceed 22 ºC during summer and 18 ºC during winter, while minimum reach -3 ºC in winter. Soil chemical and physical analysis are exposed in Table 3 from Chapter II.

Three high planting densities were evaluated: 9,090, 13,333, and 25,000 plants ha^{-1}, all of them above the planting density commonly applied in commercial yerba mate plantations – 2,222 plants ha^{-1}. Spacing between rows was fixed in 1.00 m and between plants in the row varied accordingly to planting density treatment – 1.10 m, 0.75 m, and 0.40 m for 9,090, 13,333, and 25,000 plants ha^{-1}, respectively.

At experiment establishment, we applied fertilization according to soil analysis – 44.0 kg N ha^{-1}, 153.9 kg P ha^{-1}, and 42.5 kg K ha^{-1}, which represented the annual recommended dose of phosphorus (P) and half the dose of nitrogen (N) and potassium (K) as recommended by Wendling and Santin (2015) for yerba mate. After plantation, fertilization was applied every January and September, considering the different N doses for each treatment: 0 %, 50 %, 100 %, 200 %, and 300 % of the recommended N dose (Table 4, Chapter II). In each fertilization we applied the half annual dose of N and K to reduce nutrient losses, and every September we applied total annual P dose. Fertilization was not applied in May to avoid plants growth during winter, when new branches could be deeply affected by frosts.

4.2.2 Plant material

Eight-months-old seedlings produced from seeds harvested in the region were used. Four months after planting (January 2019), seedlings were pruned, removing the apical meristem to induce the formation of lateral shoots. Harvests occurred every January and May from 2019 until 2021. In every harvest, we kept 20-30 % leaves in the plant to maintain physiological activity. We harvested young and mature leaves, free of injuries and stored them in Styrofoam boxes until processing, within 24 hours after harvest to avoid leaves oxidation. Leaves samples were microwave-dried for 5 minutes following the methodology described by Tomasi et al. (2021), grounded, and stored at -4 ºC until biochemical analysis.

4.2.3 Aqueous extracts preparation

Samples were used to prepare an aqueous extract with 0.50 g ± 0.01 g of leaves that were added to 50 mL of boiling water (98 ºC) and vortexed for 30 seconds. The solutions were subjected to ultrasound at 30 ºC for 30 minutes and then filtered through a qualitative paper and made up to 100 mL. For Liquid Cromatography (UFLC) analysis, extracts were filtered through a 0.22 µm nylon filter. Samples moistening contents were determined to calculate the actual weight of samples and consequent extract concentration. For this, 0.50 g of grounded samples were weighted, dried in a forced-air oven at 100 ºC for 24 hours and weighted again; both weights were used to determine the real weight of each sample.

4.2.4 Content of total phenolic compounds

Total phenolic compounds were quantified by Folin-Ciocalteau spectrophotometric method (Singleton and Rossi, 1965) with minor modifications. Briefly, in a 10 mL volumetric flask, we added 0.1 mL of the extract, 6.0 mL of distilled water, and 0.5 mL of Folin–Ciocalteau reagent, followed by 1 min of vortex mixing. We added 2 mL of 15 % aqueous Na_2CO_3 in water (w/v) solution, vortex mixing for 30 s, and the final volume was adjusted with distilled water. After 2 h of reaction in a dark room at 23 ºC, the samples absorbances were measured at 760 nm in a Shimadzu-1800 UV/VIS (Shimadzu Corp., Japan) spectrophotometer. As a reference, an

analytical curve was performed with gallic acid within the range of 0.25–13 mg L^{-1}. Results were expressed in mg gallic acid equivalent per gram of sample (mg GAE g^{-1}) on a dry basis.

4.2.5 Antioxidant activity (ABTS and DPPH radicals)

Antioxidant activity of extracts by free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) (Brand-Williams et al., 1995) was determined with minor modifications. Initially, 0.1 mL of sample was added to 3.9 mL of DPPH methanolic solution (0.06 mmol L^{-1}). This reaction occurred in a dark environment at 23 ºC for 30 min, and the absorbance was measured at 515 nm. Antioxidant activity by free radical ABTS [2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] (Re et al., 1999) was determined by reacting 10 mL of ABTS (7 mmol L^{-1}) with 176 µL of potassium persulfate (140 mmol L^{-1}) in a dark room at 23 ºC for 16 h. An aliquot of 1 mL of ABTS solution was added in 100 mL of sodium acetate buffer (48.5 mmol L^{-1}) pH 4.5, with adjustment of absorbance to 0.7 ± 0.05. Then, 3 mL of ABTS buffered solution was added to 30 µL of the extract. After 2 h the absorbance was measured at 734 nm.

All antioxidant activities assays were performed in a spectrophotometer UV–VIS Shimadzu-1800 UV/VIS (Shimadzu Corp., Japan). Results were compared with a standard curve (Trolox 0–1000 µmol L^{-1} for DPPH and 0–2500 µmol L^{-1} for ABTS) and expressed in µmol Trolox equivalent per gram of sample (TEAC µmol g^{-1}) on a dry basis.

4.2.6 Caffeine, theobromine, and caffeoylquinic acids

The analysis were carried out using an ultra-fast liquid chromatography (UFLC) (Shimadzu, Japan) controlled by LC solution software and equipped with an automatic injector and UV detector (SPD-20A). Compounds separation was performed using a Shim-Pack CLC-ODS (M) C18 column (250 × 4.6 mm i.d., 5 µm particle size), protected by a Shim-Pack CLC G-ODS precolumn (100 × 4.0 mm id) both Shimadzu (Kyoto, Japan). Separation of compounds present in aqueous extract (20 µL injection) was conducted at 30 °C with a flow rate of 0.5 mL min^{-1}. The mobile phases consisted of a gradient elution of water with acetic acid (99.9:0.1, v/v) (solvent A) and acetonitrile (solvent B). Compound detection was performed at a fixed wavelength of 280 nm. The

gradient elution program was: 0–15 min (3–3 % B), 15–20 min (3–20 % B), 20–40min (20–20 % B), 40–45min (20–30 % B), 45–55 min (30– 100 % B), 55–75 (100–100 % B), 75–80 (100–3 % B) and 80–95 (3–3 % B).

The identification and quantification of 1,3,7-trimethylxanthine (caffeine) and 3,7-dimethylxanthine (theobromine) was performed with an analytical curve of caffeine and theobromine standards at the range of 0–1.0 and 0–0.5 mg mL^{-1} respectively. Identification of the caffeoylquinic acids (3-CQA, 4-CQA, 5-CQA) and semi-quantification was performed with an analytical curve at the range of 0–10 mg mL^{-1} of 3-CQA Sigma®. Results were expressed as mg of compound per g sample (mg g^{-1}) on a dry basis.

4.2.7 Statistical and data analysis

The experiment was established in a completely randomized blocks design in a double factorial arrangement, with 3 planting densities and 5 N doses evaluated over five harvests. In each block, treatments repetitions were composed of 30 plants. During harvests, leaves from 10 plants of each repetition were collected and pooled to one composite sample. The analysis of total phenolic compounds and antioxidant activity were performed in triplicate, while the UFLC analysis was performed in duplicate.

Due to the number of observations, we performed the statistical analysis in two distinct groups: phenolic compounds and antioxidant activity; and caffeine, theobromine, and caffeoylquinic acids. For both groups we performed a Principal Component Analysis (PCA), building linear combinations with the original variables, reducing their dimensions and the number of variables to be evaluated and interpreted through the principal components created. For this, we performed a Pearson correlation between variables, a scree plot to define the components with major importance to explain our data, and a biplot graphic. For the analysis and figures we used the R software version 3.6.3 (R Core Team, 2020).

4.3 RESULTS AND DISCUSSION

4.3.1 Total phenolic compounds and antioxidant activity

The phenolic compounds and antioxidant activity data present high amplitude, variance, and standard deviation (Table 6); however, it is important to consider that the measurement unit of these compounds is very small (mg g^{-1}) and, therefore, the variation in dataset does not necessarily represent a significant difference at a biochemical level. Despite the expressive differences in values, we obtained for phenolic compounds means of 116.96 and 91.90 mg GAE g^{-1} for young and mature leaves; for antioxidant activity means of 1982.67 and 1650.25 TEAC µmol g^{-1} for ABTS radical, 818.00 and 650.01 TEAC µmol g^{-1} for DPPH radical, in young and mature leaves, respectively.

TABLE 6. DESCRIPTIVE STATISTICS OF TOTAL PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY BY ABTS AND DPPH RADICALS FROM YERBA MATE YOUNG AND MATURE LEAVES SUBMITTED TO DIFFERENT PLANTING DENSITIES AND N DOSES, EVALUATED OVER FIVE SUBSEQUENT HARVEST PERIODS. Y = YOUNG LEAVES; M = MATURE LEAVES.

	Phenolic compounds		ABTS		DPPH	
	Y	M	Y	M	Y	M
Mean	116.96	91.90	1,982.67	1,650.26	818.00	650.02
Maximum	277.60	202.02	4,468.28	7,667.27	2,112.91	2,042.91
Minimum	42.50	33.51	146.79	394.46	34.68	55.92
Range	235.10	168.51	4,321.49	7,272.81	2,078.23	1,986.99
Median	113.49	91.78	1,911.10	1,568.29	797.42	663.97
Variance	842.13	307.23	336,139.10	242,583.20	64,213.60	56,826.95
Standard deviation	29.02	17.53	579.78	492.53	253.40	238.38
Standard error of the mean	0.97	0.58	19.32	16.42	8.45	7.95
Coefficient of variation (%)	24.81	19.07	29.24	29.84	30.98	36.67

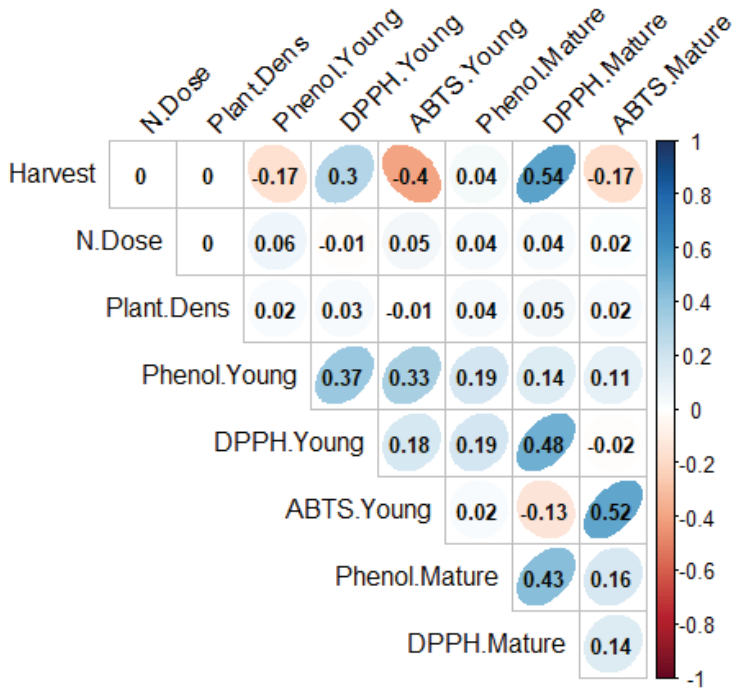
SOURCE: The author.

Both young and mature leaves of yerba mate presented total phenolic compounds content within the range expected for the species, comparable to those obtained by Blum-Silva et al. (2015), with 1-6 months-old leaves, and by Duarte et al. (2020) in green and toasted yerba mate leaves from adult plants. We observed that young leaves present higher averages of total phenolic compounds, which is expected since the biosynthesis of secondary metabolites occurs mainly in young leaves (Blum-Silva et al., 2015). The higher variation of total phenolic compounds in young leaves is

explained by different environmental conditions throughout the year, since these leaves are more susceptible to climate and resources availability changes (du Jardin, 2015); it was also observed by Schubert (2006) evaluating yerba mate leaves harvested in different periods of the year.

The highest value obtained from the Pearson correlation analysis was 0.54, corresponding to the correlation between DPPH in mature leaves and harvest periods (Figure 11). For phenolic compounds and antioxidant activity correlations, N doses and planting density were always near zero. As expected, antioxidant radicals ABTS and DPPH presented a high correlation between young and mature leaves – 0.52 for ABTS and 0.48 for DPPH.

FIGURE 11. PEARSON CORRELATION FOR TOTAL PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY BY ABTS AND DPPH RADICALS FROM YOUNG AND MATURE LEAVES OF YERBA MATE SUBMITTED TO DIFFERENT PLANTING DENSITY AND N DOSES, EVALUATED OVER FIVE SUBSEQUENT HARVEST PERIODS.



SOURCE: The author.

For Principal Component Analysis, we observed that the first component corresponds to 24 % of data, while the second one to 22 %; together, these components explain 46 % of data variation (Table 7). Our results also indicate that PC3, PC4, and PC5 contributes to 11.48 %, 11.14 %, and 11.04 % of data variation, respectively; making these components also important to explain our data.

TABLE 7. IMPORTANCE FOR EACH PRINCIPAL COMPONENT OF TOTAL PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY BY ABTS AND DPPH RADICALS IN YERBA MATE YOUNG AND MATURE LEAVES SUBMITTED TO DIFFERENT PLANTING DENSITY AND N DOSES, EVALUATED OVER FIVE SUBSEQUENT HARVEST PERIODS.

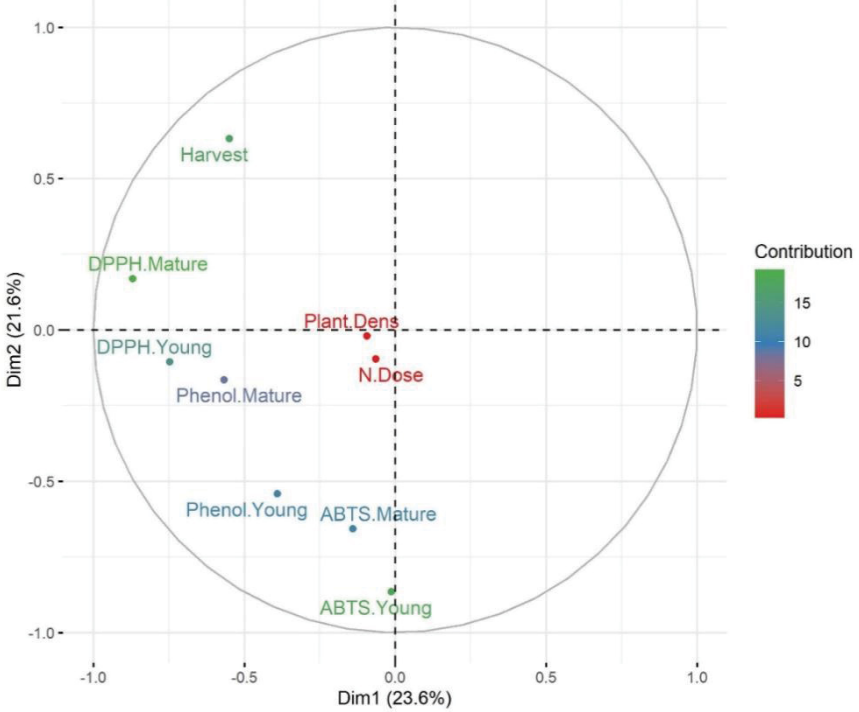
	Standard deviation	Proportion of Variance (%)	Cumulative Proportion (%)
PC1	1.4572	23.59	23.59
PC2	1.3955	21.64	45.23
PC3	1.0165	11.48	56.71
PC4	1.0012	11.14	67.85
PC5	0.9969	11.04	78.89
PC6	0.9218	9.44	88.33
PC7	0.6957	5.37	93.71
PC8	0.5630	3.52	97.23
PC9	0.4989	2.76	100.00

SOURCE: The author.

For the principal component 1 (PC1), DPPH in young and mature leaves has a high contribution and phenolic compounds in mature leaves is also important for its high influence (Figure 12). Principal component 2 (PC2) has a high influence on phenolic compounds and ABTS in young and mature leaves. Harvest periods had a high contribution for both principal components, although it is not close to the other variables in Figure 12. Planting densities and N doses did not contribute to explain our data in PCA, indicating that these factors did not influence on our results.

Phenolic compounds are the main secondary metabolites present in yerba mate; its contents can be related to heritability, but also to stress responses since secondary metabolites reflect plant answers to variations in environmental conditions, resources availability, and pest and disease attacks (Borges et al., 2017; Verma and Shukla, 2015). During the experiment, in summer, we observed attacks of *Gyropsylla spegazziniana*, a pest that causes serious damage to young leaves and is commonly found in yerba mate plantations during this period (Formentini et al., 2016), which may have increased secondary metabolites synthesis on those leaves. Also, total phenolic compounds contents directly account to the antioxidant activity and for both variables we did not obtain high correlations with increasing planting density and N dose. Yerba mate seems to have high intraspecific genetic variability, as observed by Streit et al. (2007) evaluating yerba mate populations from different regions; therefore, part of the variation observed in our results may be related to genetic diversity, masking the effect of our treatments.

FIGURE 12. PRINCIPAL COMPONENT ANALYSIS FOR TOTAL PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY BY ABTS AND DPPH RADICALS OF YERBA MATE YOUNG AND MATURE LEAVES SUBMITTED TO DIFFERENT PLANTING DENSITY AND N DOSES, EVALUATED OVER FIVE SUBSEQUENT HARVEST PERIODS.



SOURCE: The author.

4.3.2 Caffeine, theobromine, and caffeoylquinic acids

All UFLC data presented low mean standard error, even when we observed high coefficient of variation and standard deviation (Table 8). 5-CQA in young and mature leaves presented the highest mean values among caffeoylquinic acids – 92.80 and 83.28 mg g^{-1}, respectively; also, this compound presented higher amplitudes and standard deviations – 119.08 and 36.99 for young leaves, 117.76 and 32.92 for mature leaves. Caffeine values varied from 0.15 to 4.73 mg g^{-1} in young leaves and from 0.23 to 2.65 mg g^{-1} in mature leaves, with a higher mean content of this compound in young leaves – 1.32 mg g^{-1}. Theobromine had higher contents than caffeine, with mean 4.93 mg g^{-1} in young leaves and 5.18 mg g^{-1} in mature leaves.

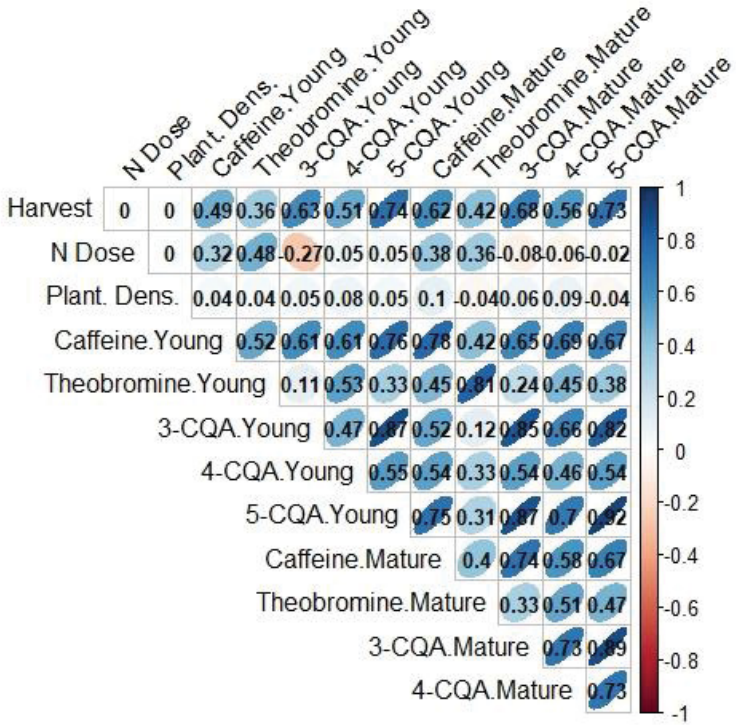
TABLE 8. DESCRIPTIVE STATISTICS OF CAFFEINE, THEOBROMINE, 3-CAFFEOYLQUINIC ACID (3-CQA), 4-CAFFEOYLQUINIC ACID (4-CQA), AND 5-CAFFEOYLQUINIC ACID (5-CQA) FROM YERBA MATE YOUNG AND MATURE LEAVES SUBMITTED TO DIFFERENT PLANTING DENSITIES AND N DOSES, EVALUATED OVER FIVE SUBSEQUENT HARVEST PERIODS. Y = YOUNG LEAVES; M = MATURE LEAVES; SD = STANDARD DEVIATION; SEM = STANDARD ERROR OF THE MEAN; CV = COEFFICIENT OF VARIATION; MED = MEDIAN; VAR = VARIANCE.

	Caffeine		Theobromine		3- CQA		4-CQA		5-CQA	
	Y	M	Y	M	Y	M	Y	M	Y	M
Mean	1.32	0.95	4.93	5.18	37.01	32.51	22.45	28.21	92.80	83.28
Max	4.73	2.65	30.29	30.11	68.42	59.21	59.84	60.26	163.80	157.35
Min	0.15	0.23	0.56	0.11	18.07	17.98	12.41	17.89	44.72	39.58
Med	1.12	0.78	3.94	3.67	31.58	27.03	20.58	25.40	72.30	67.17
Range	4.57	2.42	29.72	30.00	50.35	41.22	47.42	42.36	119.08	117.76
Var	0.56	0.32	23.42	35.10	155.87	123.63	52.38	80.72	1368.61	1083.78
SD	0.75	0.56	4.84	5.92	12.48	11.11	7.23	8.98	36.99	32.92
SEM	0.05	0.03	0.32	0.39	0.83	0.74	0.48	0.59	2.46	2.19
CV (%)	56.81	59.55	98.09	114.28	33.72	34.19	32.23	31.84	39.86	39.52

SOURCE: The author.

Phenolic compounds contents and antioxidant activity, caffeine, theobromine, and caffeoylquinic acids were influenced by harvest periods (Figure 13). Specially for caffeoylquinic acids, there was a high correlation with harvest periods for both young and mature leaves, i.e. 0.63 and 0.68 for 3-caffeoylquinic acid (3-CQA); 0,51 and 0,56 for 4-caffeoylquinic acid (4-CQA); and 0.74 and 0.73 for 5-caffeoylquinic acid (5-CQA). Planting density did not have a high influence in results, as observed for total phenolic compounds and antioxidant activity. Caffeine in young leaves had a high correlation (above 0.5) with all the other variables, except for theobromine in mature leaves.

FIGURE 13. CORRELATION OF BIOCHEMICAL COMPOUNDS QUANTIFIED IN UFLC FROM YOUNG AND MATURE LEAVES OF YERBA MATE SUBMITTED TO DIFFERENT PLANTING DENSITIES AND N DOSES, EVALUATED OVER FIVE SUBSEQUENT HARVEST PERIODS.



SOURCE: The author.

As well as for phenolic compounds, harvest periods presented correlation with all compounds evaluated through UFLC analysis, in both young and mature leaves. Similar results for total phenolic compounds and caffeoylquinic acids were expected because caffeoylquinic acids are the largest portion of yerba mate total phenolic compounds (Mateos et al., 2018); in our study, 5-CQA was the most abundant, followed by 3-CQA and 4-CQA. As mentioned before, phenolic compounds biosynthesis is strongly related to environmental changes, what explains the high correlation of caffeoylquinic acids with harvests performed in different periods.

For the Principal Component Analysis (PCA), two principal components explain approximately 69 % of data variation, with PC1 explaining 53 % and PC2 explaining15 % of data variation (Table 9).

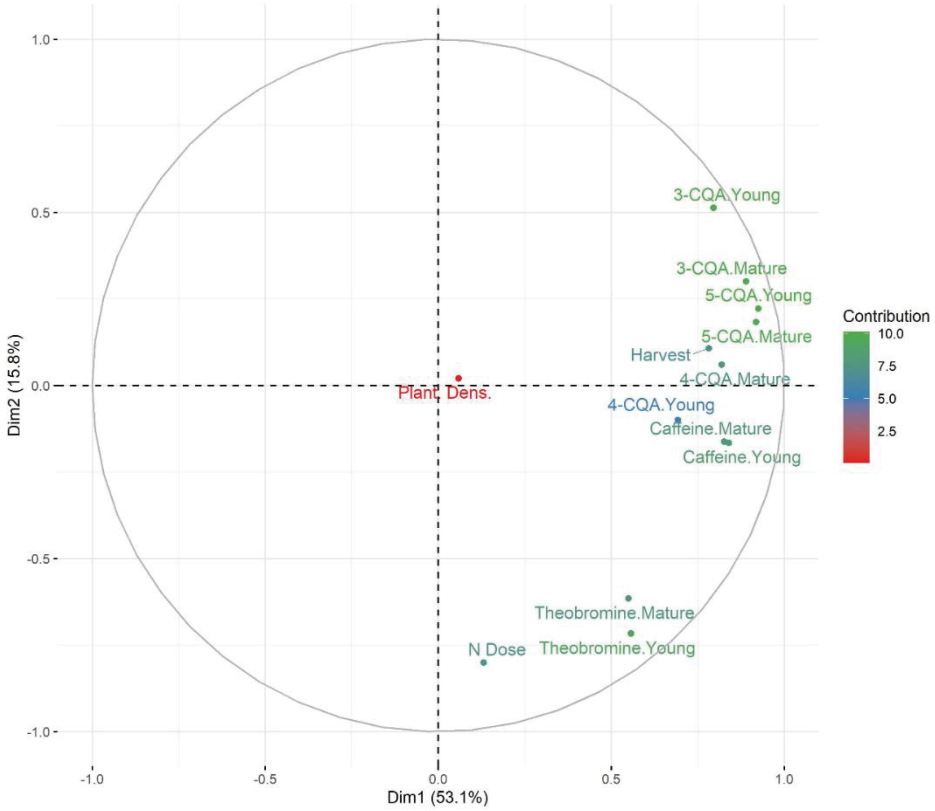
TABLE 9. IMPORTANCE OF EACH PRINCIPAL COMPONENTS OF BIOCHEMICAL COMPOUNDS QUANTIFIED IN UFLC IN YOUNG AND MATURE LEAVES OF YERBA MATE SUBMITTED TO DIFFERENT PLANTING DENSITIES AND N DOSES, EVALUATED OVER FIVE SUBSEQUENT HARVEST PERIODS. SD: STANDARD DEVIATION; PV: PROPORTION OF VARIANCE; CP: CUMULATIVE PROPORTION

	Standard Deviation	Proportion of Variance (%)	Cumulative Proportion (%)
PC1	2.6880	53.120	53.120
PC2	1.4312	15.760	68.880
PC3	1.0212	8.023	76.904
PC4	0.9306	6.662	83.566
PC5	0.7958	4.873	88.439
PC6	0.7201	3.989	92.428
PC7	0.5342	2.195	94.624
PC8	0.4966	1.988	96.521
PC9	0.3886	1.162	97.683
PC10	0.3573	0.982	98.666
PC11	0.3081	0.730	99.400
PC12	0.2130	0.349	99.745
PC13	0.1821	0.255	100.000

SOURCE: The author.

The biplot graphic indicates that planting density has a very low contribution for the data analyzed in this study – less than 2.5 (Figure 14). N doses, however, have a high contribution in PC2 results (5.0-7.5), and theobromine (7.5-10.0) and caffeoylquinic acids (10.0) highly contributes to explain responses of PC1. Harvest periods are also explained by PC1, but with low data variation. This data analysis indicates that planting density was not relevant for our results and N doses, even with 5.0 and 7.5 contribution, was not closely related to the compounds, except for theobromine. It confirms the results presented in Figure 12, which demonstrated that these variables did not significantly correlate with the biochemical compounds.

FIGURE 14. PRINCIPAL COMPONENT ANALYSIS OF BIOCHEMICAL COMPOUNDS QUANTIFIED IN UFLC FROM YOUNG AND MATURE LEAVES OF YERBA MATE SUBMITTED TO DIFFERENT PLANTING DENSITIES AND N DOSES, EVALUATED OVER FIVE SUBSEQUENT HARVEST PERIODS.



SOURCE: The author.

Caffeine and theobromine were the only biochemical compounds that presented correlation with N dose for both young and mature leaves, although it was not a strong correlation, while planting density did not correlate with any biochemical compounds evaluated in this study. It was expected that caffeine and theobromine presented a high correlation with N doses, since N is an important component of these molecules, as observed by Tomasi (2020) in yerba mate cultivated in a semi-hydroponic system; however, the soil analysis of our field trial indicated a high organic matter content, which may have reduced treatments effects, considering that even in the 0 % N dose plants were not necessarily subjected to N deficit. Other possibility for the low correlations is that N was preferably allocated to plant growth and other metabolites required in higher amounts by plants; a low correlation of caffeine to increasing N doses was also observed for *Coffea arabica* plants (Gonthier et al., 2011).

Plants cultivated in high-density plantations, as in this study, are subjected to stresses related to competition for light, water, and nutrients, and secondary metabolites biosynthesis would be a response of plants to these environmental

conditions (Begon and Townsend, 2020); however, the low influence of planting density in our results may be related to the age and size of plants. After two years of plantation, plants did not reach the neighbors above and belowground spaces, and competition is not occurring yet. In studies with *Camellia sinensis* cultivated in high-density stands, clones with higher productivity showed influences of planting density early than less productive ones, and other environmental factors such as water availability showed more significant influence on their growth and development (Kigalu, 2007a, 2007b). Future studies with adult yerba mate plants will confirm the morphophysiological and biochemical effects of competition due to planting density.

Secondary metabolites of yerba mate are of high interest for food and pharmaceutical industries and the benefits of these compounds to human health are extensively described, focusing mainly on phenolic compounds and methylxanthines (Bassani et al., 2014; Bojit et al., 2013; Cardozo Junior and Morand, 2016; Gullón et al., 2018; Riachi and Maria, 2017; Ribeiro et al., 2019). In our study, we observed that several factors influence on the biosynthesis of secondary metabolites in yerba mate cultivated in high-density plantations. Productivity results (Chapter II) indicate that a higher leaves yield is expected in high-density stands, increasing raw material availability to biochemical compounds extraction; therefore, we indicate that future studies must focus on developing silvicultural practices that will boost these metabolites' contents in plants, increasing its value for differentiated industries.

4.4 CONCLUSIONS

Nitrogen doses influence on yerba mate phenolic compounds and methylxanthines contents in field conditions, although there is not a clear response of plants to increasing fertilization. Planting density does not influence on these biochemical compounds' contents in the first three years after planting. Harvest periods influenced on biochemical compounds, indicating that environmental factors can have an important role in this aspect of yerba mate composition.

5 FINAL CONSIDERATIONS

Our review indicated an increasing trend in studies about high-density forestry stands, without any study including yerba mate and few studies on species cultivated in similar conditions, e.g., *Coffea arabica* and *Camellia sinensis*. The lack of information about differentiated cultivation systems for yerba mate influence on the quality and variety of species raw materials, also limiting its use by high-value industries, as the pharmaceutical and food industries.

In the study described in Chapters II and III, we observed that there is a high potential of yerba mate to be cultivated in high-density stands, with the adequate nutritional management and two harvests per year. The sum of biomass obtained in all harvests performed in our experiment was higher than that commonly obtained in the first harvest of a plantation with traditional planting density. We obtained higher productivity of young and mature leaves in the first years after planting, in a rotation period smaller than the used in traditional cultivation systems (18-24 months). Future studies will indicate how competition between individuals will influence on productivity and biochemical characteristics of yerba mate in high-density stands.

We indicate the need for future studies to evaluate other nutrients availability in high-density stands and the need to change nutritional management for this silvicultural system aiming at increasing leaves productivity and reducing rotation period. The interest of high-value industries in genotypes with specific characteristics also indicates the need to evaluate how clonal cultivars will be affected by high-density systems.

REFERENCES

AHMED, A.K.M.; FU, Z.; DING, C.; JIANG, L.; HAN, X.; YANG, A.; MA, Y.; ZHAO, X. Growth and wood properties of a 38-year-old *Populus simonii* × *P. nigra* plantation established with different densities in semi-arid areas of northeastern China. **Journal of Forestry Research**, v.31, p.497–506, 2020. DOI: 10.1007/s11676-019-00887-z.

AKERS, M.K.; KANE, M.; ZHAO, D.; TESKEY, R.O.; DANIELS, R.F. Effects of planting density and cultural intensity on stand and crown attributes of mid-rotation loblolly pine plantations. **Forest Ecology and Management**, v.310, p.468–475, 2013. DOI: 10.1016/j.foreco.2013.07.062.

ALCORN, P.J.; PYTTEL, P.; BAUHUS, J.; GEOFF, R.; SMITH, B.; THOMAS, D.; JAMES, R.; NICOTRA, A. Effects of initial planting density on branch development in 4-year-old plantation grown *Eucalyptus pilularis* and *Eucalyptus cloeziana* trees. **Forest Ecology and Management**, v.252, p.41–51, 2007. DOI: 10.1016/j.foreco.2007.06.021.

ALKHATIB, A.; ATCHESON, R. Yerba Maté (*Ilex paraguariensis*) metabolic, satiety, and mood state effects at rest and during prolonged exercise. **Nutrients**, v.9, p.882, 2017. DOI: 10.3390/nu9080882.

ALVARES, C.A.; STAPE, J.L.; SENTELHAS, P.C.; MORAES GONÇALVES, J.L. DE; SPAROVEK, G. Köppen's climate classification map for Brazil. **Meteorologische Zeitschrift**, v.22, p.711–728, 2013. DOI: 10.1127/0941-2948/2013/0507.

ANDRIANIRINA, Z.T.; MARTIN, M.; DONGMEZA, E.; SENGER, E. Effects of genotype, direct sowing and plant spacing on field performance of *Jatropha curcas* L. **Agronomy**, v.9, p.465, 2019. DOI: 10.3390/agronomy9080465.

BARBIER, N.; COUTERON, P.; PROISY, C.; MALHI, Y.; GASTELLU-ETCHEGORRY, J.P. The variation of apparent crown size and canopy heterogeneity across lowland Amazonian forests. **Global Ecology and Biogeography**, v.19, p.72–84, 2010. DOI: 10.1111/j.1466-8238.2009.00493.x.

BARBOSA, J.Z.; MOTTA, A.C.V.; CONSALTER, R.; POGGERE, G.C.; SANTIN, D.; WENDLING, I. Plant growth, nutrients and potentially toxic elements in leaves of yerba mate clones in response to phosphorus in acid soils. **Anais da Academia Brasileira de Ciências**, v.90, p.557–571, 2018. DOI: 10.1590/0001-3765201820160701.

BARRON-GAFFORD, G.A.; WILL, R.E.; BURKES, E.C.; SHIVER, B.; TESKEY, R.O. Nutrient concentrations and contents, and their relation to stem growth, of intensively managed *Pinus taeda* and *Pinus elliottii* stands of different planting densities. **Forest Science**, v.49, p.291–300, 2003. DOI: 10.1093/forestscience/49.2.291.

BASSANI, D.C.; NUNES, D.S.; GRANATO, D. Optimization of phenolics and flavonoids extraction conditions and antioxidant activity of roasted yerba-mate leaves (*Ilex paraguariensis* A. St.-Hil., Aquifoliaceae) using response surface methodology.

Anais da Academia Brasileira de Ciências, v.86, p.923–933, 2014. DOI: 10.1590/0001-3765201420130019.

BEGON, M.; TOWNSEND, C.R. **Ecology : from individuals to ecosystems**. Fifth ed. Hoboken, New Jersey: John Wiley & Sons, inc., 2020. 864p.

BENOMAR, L.; DESROCHERS, A.; LAROCQUE, G.R. Changes in specific leaf area and photosynthetic nitrogen-use efficiency associated with physiological acclimation of two hybrid poplar clones to intraclonal competition. **Canadian Journal of Forest Research**, v.41, p.1465–1476, 2011. DOI: 10.1139/X11-073.

BENOMAR, L.; DESROCHERS, A.; LAROCQUE, G.R. The effects of spacing on growth, morphology and biomass production and allocation in two hybrid poplar clones growing in the boreal region of Canada. **Trees - Structure and Function**, v.26, p.939–949, 2012. DOI: 10.1007/s00468-011-0671-6.

BENTANCOR, L.; HERNÁNDEZ, J.; PINO, A. DEL; CALIFRA, Á.; RESQUÍN, F.; GONZÁLEZ-BARRIOS, P. Evaluation of the biomass production, energy yield and nutrient removal of *Eucalyptus dunnii* Maiden grown in short rotation coppice under two initial planting densities and harvest systems. **Biomass and Bioenergy**, v.122, p.165–174, 2019. DOI: 10.1016/j.biombioe.2019.01.019.

BLUM-SILVA, C.H.; CHAVES, V.C.; SCHENKEL, E.P.; COELHO, G.C.; REGINATTO, F.H. The influence of leaf age on methylxanthines, total phenolic content, and free radical scavenging capacity of *Ilex paraguariensis* aqueous extracts. **Revista Brasileira de Farmacognosia**, v.25, p.1–6, 2015. DOI: 10.1016/J.BJP.2015.01.002.

BOHANEK, J.R.; GRONINGER, J.W. Impacts of intensive management on black walnut (*Juglans nigra* L.) growth and bole quality at mid-rotation. **Forest Science**, v.49, p.522–529, 2003. DOI: 10.1093/forestscience/49.4.522.

BOJIT, M.; HAAS, V.S.; ŠARIT, D.; MALEŠ, C. Determination of Flavonoids, Phenolic Acids, and Xanthines in Mate Tea (*Ilex paraguariensis* St.-Hil.). **Journal of Analytical Methods in Chemistry**, v.2013, p.658596, 2013. DOI: 10.1155/2013/658596.

BORGES, C.V.; MINATEL, I.O.; GOMEZ-GOMEZ, H.A.; LIMA, G.P.P. Medicinal Plants: Influence of Environmental Factors on the Content of Secondary Metabolites. **Medicinal Plants and Environmental Challenges**, p.259–277, 2017. DOI: 10.1007/978-3-319-68717-9_15.

BOSE, A.K.; NELSON, A.S.; KANE, M.; RIGLING, A. Density reduction in loblolly pine (*Pinus taeda* L.) stands to increase tree C assimilation: an approach with the dual δ13C and δ18O isotope signatures in needles. **Annals of Forest Science**, v.75, 2018. DOI: 10.1007/s13595-017-0687-1.

BRACESCO, N. *Ilex Paraguariensis* as a healthy food supplement for the future world. **Biomed Journal of Scientific & Technical Research**, v.16, p.1–3, 2019. DOI: 10.26717/BJSTR.2019.16.002808.

BRAND-WILLIAMS, W.; CUVELIER, M.E.; BERSET, C. **Use of a free radical method to evaluate antioxidant activityLWT - Food Science and Technology.** Academic Press, 1995.

BRAVO-OVIEDO, A.; PRETZCH, H.; RÍO, M. DEL. **Dynamics, silviculture and management of mixed forests**. [s.l.] Springer, 2018. 420p.DOI: 10.1007/978-3-319-91953-9.

BURKES, E.C.; WILL, R.E.; BARRON-GAFFORD, G.A.; TESKEY, R.O.; SHIVER, B. Biomass partitioning and growth efficiency of intensively managed *Pinus taeda* and *Pinus elliottii* stands of different planting densities. **Forest Science**, v.49, p.225–234, 2003.

CARDOSO, D.J.; LACERDA, A.E.B.; ROSOT, M.A.D.; GARRASTAZÚ, M.C.; LIMA, R.T. Influence of spacing regimes on the development of loblolly pine (*Pinus taeda* L.) in Southern Brazil. **Forest Ecology and Management**, v.310, p.761–769, 2013. DOI: 10.1016/j.foreco.2013.09.021.

CARDOZO JUNIOR, E.L.; DONADUZZI, C.M.; FERRARESE-FILHOO, O.; FRIEDRICH, J.C.; GONELA, A.; STURION, J.A. Quantitative genetic analysis of methylxanthines and phenolic compounds in mate progenies. **Pesquisa Agropecuaria Brasileira**, v.45, p.171–177, 2010. DOI: 10.1590/s0100-204x2010000200008.

CARDOZO JUNIOR, E.L.; MORAND, C. Interest of mate (*Ilex paraguariensis* A. St.-Hil.) as a new natural functional food to preserve human cardiovascular health - A review. **Journal of Functional Foods**, v.21, p.440–454, 2016. DOI: 10.1016/j.jff.2015.12.010.

CARVALHO, P.E.R. **Espécies arbóreas brasileiras**. Colombo: Brasília: Embrapa Informação tecnológica; Colombo: Embrapa Florestas, 2003. 1039p.

CASANOVA-SÁEZ, R.; VOSS, U. Auxin metabolism controls developmental decisions in land plants. **Trends in Plant Science**, v.24, p.741–754, 2019. DOI: 10.1016/j.tplants.2019.05.006.

CASSIDY, M.; PALMER, G.; SMITH, R.G.B. The effect of wide initial spacing on wood properties in plantation grown *Eucalyptus pilularis*. **New Forests**, v.44, p.919–936, 2013. DOI: 10.1007/s11056-013-9385-5.

CHARLTON, R.A.; NAGHIZADEH, Z.; HAM, C.; WESSELS, C.B. A value chain comparison of *Pinus patula* sawlog management regimes based on different initial planting densities and effect on wood quality. **Forest Policy and Economics**, v.111, p.102067, 2020. DOI: 10.1016/j.forpol.2019.102067.

CHENG, X.; YUAN, H.; XING, W.; WANG, Y.; YU, M. Effects of provenance and initial planting density on growth and wood properties in young sawtooth oak (*Quercus acutissima*) plantations. **European Journal of Forest Research**, v.139, p.1067–1078, 2020. DOI: 10.1007/s10342-020-01308-1.

CLARK, A.; LEWIS, I.; SCHIMLECK, J.L.; DANIELS, R.F. Effect of initial planting spacing on wood properties of unthinned loblolly pine at age 21. **Forest Products Journal**, v.58, p.78–83, 2008.

COLIN, F.; NINGRE, F.; FORTIN, M.; HUET, S. Quantification of *Quercus petraea* Liebl. forking based on a 23-year-long longitudinal survey. **Forest Ecology and Management**, v.282, p.133–141, 2012. DOI: 10.1016/j.foreco.2012.06.040.

DASSOT, M.; CONSTANT, T.; NINGRE, F.; FOURNIER, M. Impact of stand density on tree morphology and growth stresses in young beech (*Fagus sylvatica* L.) stands. **Trees - Structure and Function**, v.29, p.583–591, 2015. DOI: 10.1007/s00468-014-1137-4.

DEBELL, D.S.; HARRINGTON, C.A. Density and rectangularity of planting influence 20-year growth and development of red alder. **Canadian Journal of Forest Research**, v.32, p.1244–1253, 2002. DOI: 10.1139/x02-040.

DU, X.; SHEN, T.; ZHAO, L.; ZHANG, G.; HU, A.; FANG, S.; CAO, Y.; YAO, X. Design and experiment of the comb-brush harvesting machine with variable spacing for oil-tea camellia fruit. **International Journal of Agricultural and Biological Engineering**, v.14, p.172–177, 2021. DOI: 10.25165/j.ijabe.20211401.5703.

DUAN, A.; LEI, J.; HU, X.; ZHANG, J.; DU, H.; ZHANG, X.; GUO, W.; SUN, J. Effects of planting density on soil bulk density, pH and nutrients of unthinned Chinese fir mature stands in south subtropical region of China. **Forests**, v.10, 2019. DOI: 10.3390/f10040351.

DUARTE, M.M.; TOMASI, J.D.C.; HELM, C.V.; AMANO, E.; LAZZAROTTO, M.; GODOY, R.C.B. DE; NOGUEIRA, A.C.; WENDLING, I. Caffeinated and decaffeinated mate tea: Effect of toasting on bioactive compounds and consumer acceptance. **Revista Brasileira de Ciências Agrarias**, v.15, p.e8513, 2020. DOI: 10.5039/agraria.v15i3a8513.

EGBÄCK, S.; BULLOCK, B.P.; ISIK, F.; MCKEAND, S.E. Height-diameter relationships for different genetic planting stock of loblolly pine at age 6. **Forest Science**, v.61, p.424–428, 2015. DOI: 10.5849/forsci.14-015.

EGBÄCK, S.; LIZINIEWICZ, M.; HÖGBERG, K.A.; EKÖ, P.M.; NILSSON, U. Influence of progeny and initial stand density on growth and quality traits of 21 year old half-sib Scots pine (*Pinus sylvestris* L.). **Forest Ecology and Management**, v.286, p.1–7, 2012. DOI: 10.1016/j.foreco.2012.09.003.

ELOY, E.; SILVA, D.A. DA; CARON, B.O.; ELLI, E.F.; SCHWERZ, F. Effect of age and spacing on biomass production in forest plantations. **Revista Árvore**, v.42, p.e420214, 2018. DOI: 10.1590/1806-90882018000200014.

ELOY, E.; SILVA, D.A. DA; SCHMIDT, D.; TREVISAN, R.; CARON, B.O.; ELLI, E.F. Effect of planting age and spacing on energy properties of *Eucalyptus grandis* W. Hill ex Maiden. **Revista Árvore**, v.40, p.749–758, 2016. DOI: 10.1590/0100-67622016000400019.

FANG, S.; XUE, J.; TANG, L. Biomass production and carbon sequestration potential in poplar plantations with different management patterns. **Journal of Environmental Management**, v.85, p.672–679, 2007. DOI: 10.1016/j.jenvman.2006.09.014.

FAO. **Global Forest Resources Assessment 2020 – Key findings**. Rome.

FAROOQ, T.H.; YAN, W.; CHEN, X.; SHAKOOR, A.; RASHID, M.H.U.; GILANI, M.M.; HE, Z.; WU, P. Dynamics of canopy development of *Cunninghamia lanceolata* mid-age plantation in relation to foliar nitrogen and soil quality influenced by stand density. **Global Ecology and Conservation**, v.24, p.e01209, 2020. DOI: 10.1016/j.gecco.2020.e01209.

FERNÁNDEZ-NÚÑEZ, E.; RIGUEIRO-RODRÍGUEZ, A.; MOSQUERA-LOSADA, M.R. Carbon allocation dynamics one decade after afforestation with *Pinus radiata* D. Don and *Betula alba* L. under two stand densities in NW Spain. **Ecological Engineering**, v.36, p.876–890, 2010. DOI: 10.1016/j.ecoleng.2010.03.007.

FORMENTINI, M.A.; ALVES, L.F.A.; SCHAPOVALOFF, M.E. Insecticidal activity of neem oil against *Gyropsylla spegazziniana* (Hemiptera: Psyllidae) nymphs on Paraguay tea seedlings. **Brazilian Journal of Biology**, v.76, p.951–954, 2016. DOI: 10.1590/1519-6984.04915.

FORRESTER, D.I. Transpiration and water-use efficiency in mixed-species forests versus monocultures: Effects of tree size, stand density and season. **Tree Physiology**, v.35, p.289–304, 2015. DOI: 10.1093/treephys/tpv011.

FORRESTER, D.I.; SMITH, R.G.B. Faster growth of Eucalyptus grandis and *Eucalyptus pilularis* in mixed-species stands than monocultures. **Forest Ecology and Management**, v.286, p.81–86, 2012. DOI: 10.1016/j.foreco.2012.08.037.

FORRESTER, D.I.; WIEDEMANN, J.C.; FORRESTER, R.I.; BAKER, T.G. Effects of planting density and site quality on mean tree size and total stand growth of *Eucalyptus globulus* plantations. **Canadian Journal of Forest Research**, v.43, p.846–851, 2013. DOI: dx.doi.org/10.1139/cjfr-2013-0137.

GADOW, K. V.; KOTZE, H. Tree survival and maximum density of planted forests – Observations from South African spacing studies. **Forest Ecosystems**, v.1, p.21, 2014. DOI: 10.1186/s40663-014-0021-4.

GALVÃO, V.C.; FANKHAUSER, C. Sensing the light environment in plants: photoreceptors and early signaling steps. **Current Opinion in Neurobiology**, v.34, p.46–53, 2015. DOI: 10.1016/J.CONB.2015.01.013.

GAN, R.; ZHANG, D.; WANG, M.; CORKE, H. Health benefits of bioactive compounds from the genus Ilex, a source of traditional caffeinated beverages. **Nutrients**, v.10, p.1–17, 2018. DOI: 10.3390/nu10111682.

GLENCROSS, K.; NICHOLS, J.D.; GRANT, J.C.; SETHY, M.; SMITH, R.G.B. Spacing affects stem form, early growth and branching in young whitewood (*Endospermum medullosum*) plantations in Vanuatu. **International Forestry Review**, v.14, p.442–451, 2012. DOI: 10.1505/146554812804715865.

GRIGG, A.H.; MACFARLANE, C.; EVANGELISTA, C.; EAMUS, D.; ADAMS, M.A. Does initial spacing influence crown and hydraulic architecture of *Eucalyptus marginata*? **Tree Physiology**, v.28, p.753–760, 2008. DOI: 10.1093/treephys/28.5.753.

GROOT, A.; CORTINI, F. Effects of initial planting density on tree and stand development of planted black spruce up to age 30. **Forestry Chronicle**, v.92, p.200–209, 2016. DOI: 10.5558/tfc2016-039.

GULLÓN, B.; EIBES, G.; MOREIRA, M.T.; HERRERA, R.; LABIDI, J.; GULLÓN, P. Yerba mate waste: A sustainable resource of antioxidant compounds. **Industrial Crops and Products**, v.113, p.398–405, 2018. DOI: 10.1016/j.indcrop.2018.01.064.

GUNER, S.; YAGCI, V.; TILKI, F.; CELIK, N. The effects of initial planting density on above-and below-ground biomass in a 25-year-old *Fagus orientalis* Lipsky plantation in Hopa, Turkey. **Scientific Research and Essays**, v.5, p.1856–1860, 2010.

GYENGE, J.E.; FERNÁNDEZ, M.E.; SCHLICHTER, T.M. Effect of stand density and pruning on growth of ponderosa pines in NW Patagonia, Argentina. **Agroforestry Systems**, v.78, p.233–241, 2010. DOI: 10.1007/s10457-009-9240-z.

HAKAMADA, R.; HUBBARD, R.M.; FERRAZ, S.; STAPE, J.L.; LEMOS, C. Biomass production and potential water stress increase with planting density in four highly productive clonal *Eucalyptus* genotypes. **Southern Forests**, v.79, p.251–257, 2017. DOI: 10.2989/20702620.2016.1256041.

HAKAMADA, R.E.; HUBBARD, R.M.; MOREIRA, G.G.; STAPE, J.L.; CAMPOE, O.; FERRAZ, S.F. DE B. Influence of stand density on growth and water use efficiency in *Eucalyptus* clones. **Forest Ecology and Management**, v.466, p.118125, 2020a. DOI: 10.1016/j.foreco.2020.118125.

HAKAMADA, R.E.; HUBBARD, R.M.; STAPE, J.L.; LIMA, W. DE P.; MOREIRA, G.G.; FERRAZ, S.F. DE B. Stocking effects on seasonal tree transpiration and ecosystem water balance in a fast-growing Eucalyptus plantation in Brazil. **Forest Ecology and Management**, v.466, p.118149, 2020b. DOI: 10.1016/j.foreco.2020.118149.

HAN, Q.; ZHANG, Y.; GENG, X.; DU, K.; YANG, J.; KANG, X.-A.; KANG, X. Response of tree growth, crown, and branch development to planting density in four *Populus tomentosa* clones. **Austrian Journal of Forest Science**, v.3, p.179–198, 2020.

HAO, D.; GU, X.; XIAO, P.; LIANG, Z.; XU, L.; PENG, Y. Research progress in the phytochemistry and biology of Ilex pharmaceutical resources. **Acta Pharmaceutica Sinica B**, v.3, p.8–19, 2013. DOI: 10.1016/j.apsb.2012.12.008.

HARRINGTON, T.B.; HARRINGTON, C.A.; DEBELL, D.S. Effects of planting spacing and site quality on 25-year growth and mortality relationships of Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*). **Forest Ecology and Management**, v.258, p.18–25, 2009. DOI: 10.1016/j.foreco.2009.03.039.

HÉBERT, F.; KRAUSE, C.; PLOURDE, P.-Y.; ACHIM, A.; PRÉGENT, G.; MÉNÉTRIER, J. Effect of tree spacing on tree level volume growth, morphology, and wood properties in a 25-year-old *Pinus banksiana* plantation in the boreal forest of Quebec. **Forests**, v.7, p.276, 2016. DOI: 10.3390/f7110276.

HENSKENS, F.L.; BATTAGLIA, M.; CHERRY, M.L.; BEADLE, C.L. Physiological basis of spacing effects on tree growth and form in *Eucalyptus globulus*. **Trees - Structure and Function**, v.15, p.365–377, 2001. DOI: 10.1007/s004680100114.

HORNER, G.J.; BAKER, P.J.; NALLY, R. MAC; CUNNINGHAM, S.C.; THOMSON, J.R.; HAMILTON, F. Mortality of developing floodplain forests subjected to a drying climate and water extraction. **Global Change Biology**, v.15, p.2176–2186, 2009. DOI: 10.1111/j.1365-2486.2009.01915.x.

HUBER, M.; NIEUWENDIJK, N.M.; PANTAZOPOULOU, C.K.; PIERIK, R. Light signalling shapes plant–plant interactions in dense canopies. **Plant Cell and Environment**, 2020. DOI: 10.1111/pce.13912.

HUIJUAN, B.; JIALEI, Z.; RUNZHE, Z.; LISHUI, N.; LIANJUN, S. Effects of planting density and clone type on growth and wood volume of *Populus tomentosa* stands. **Journal of Forest Research**, p.1–6, 2020. DOI: 10.1080/13416979.2020.1809761.

JARDIN, P. DU. Plant biostimulants: Definition, concept, main categories and regulation. **Scientia Horticulturae**, v.196, p.3–14, 2015. DOI: 10.1016/J.SCIENTA.2015.09.021.

JOHANSSON, T. Biomass production and allometric above- and below-ground relations for young birch stands planted at four spacings on abandoned farmland. **Forestry**, v.80, p.41–52, 2007. DOI: 10.1093/forestry/cpl049.

KIGALU, J.M. Effects of planting density and drought on the productivity of tea clones (*Camellia sinensis* L.): Yield responses. **Physics and Chemistry of the Earth**, v.32, p.1098–1106, 2007a. DOI: 10.1016/j.pce.2007.07.022.

KIGALU, J.M. Effects of planting density on the productivity and water use of tea (*Camellia sinensis* L.) clones. I. Measurement of water use in young tea using sap flow meters with a stem heat balance method. **Agricultural Water Management**, v.90, p.224–232, 2007b. DOI: 10.1016/j.agwat.2007.03.005.

KÜPERS, J.J.; OSKAM, L.; PIERIK, R. Photoreceptors regulate plant developmental plasticity through auxin. **Plants**, v.9, p.940, 2020. DOI: 10.3390/PLANTS9080940.

LASSERRE, J.P.; MASON, E.G.; WATT, M.S. The effects of genotype and spacing on *Pinus radiata* [D. Don] corewood stiffness in an 11-year old experiment. **Forest Ecology and Management**, v.205, p.375–383, 2005. DOI: 10.1016/j.foreco.2004.10.037.

LEITE, F.P.; SILVA, I.R.; NOVAIS, R.F.; BARROS, N.F. DE; NEVES, J.C.L.; VILLANI, E.M. DE A. Nutrient relations during an eucalyptus cycle at different populations densities. **Revista Brasileira de Ciência do Solo**, v.35, p.949–959, 2011. DOI: 10.1590/S0100-06832011000300029.

LI, Y.; KONG, D.; FU, Y.; SUSSMAN, M.R.; WU, H. The effect of developmental and environmental factors on secondary metabolites in medicinal plants. **Plant Physiology and Biochemistry**, v.148, p.80–89, 2020. DOI: 10.1016/J.PLAPHY.2020.01.006.

LIMA, G.G. DE; RUIZ, H.Z.; MATOS, M.; HELM, C.V.; LIZ, M.V. DE; MAGALHÃES, W.L.E. Prediction of yerba mate caffeine content using near infrared spectroscopy. **Spectroscopy Letters**, v.52, p.282–287, 2019. DOI: 10.1080/00387010.2019.1622567.

LIMA, J. DE P.; FARAH, A. Methylxantines in stimulant foods and beverages commonly consumed in Brazil. **Journal of Food Composition and Analysis**, v.78, p.75–85, 2019. DOI: 10.1016/j.jfca.2019.02.001.

LIN, Y.; YANG, H.; IVKOVIĆ, M.; GAPARE, W.J.; COLIN MATHESON, A.; WU, H.X. Effect of genotype by spacing interaction on radiata pine genetic parameters for height and diameter growth. **Forest Ecology and Management**, v.304, p.204–211, 2013. DOI: 10.1016/j.foreco.2013.05.015.

LIU, F.Y.; GAO, C.J.; CHEN, M.; LI, K. Above- and below-ground biomass relationships of *Leucaena leucocephala* (Lam.) de Wit in different plant stands. **PLoS ONE**, v.13, p.e0207059, 2018. DOI: 10.1371/journal.pone.0207059.

LIU, L.; LI, Y.; ZHANG, J.; SUN, H. Impact of initial planting density on the optimal economic rotation of Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook) in an experimental forest plantation. **Forests**, v.10, p.713, 2019. DOI: 10.3390/f10090713.

LIZINIEWICZ, M.; EKÖ, P.M.; AGESTAM, E. Effect of spacing on 23-year-old lodgepole pine (*Pinus contorta* Dougl. var. *latifolia*) in southern Sweden. **Scandinavian Journal of Forest Research**, v.27, p.361–371, 2012. DOI: 10.1080/02827581.2011.639798.

MA, S.; WANG, X.; MIAO, W.; WANG, X.; SUN, H.; GUO, Z. Relative influence of environmental, stand factors and functional traits on allocation of forest productivity during the restoration of subtropical forests in central China. **Forest Ecology and Management**, v.482, p.118814, 2021. DOI: 10.1016/j.foreco.2020.118814.

MAMASHITA, T.; LAROCQUE, G.R.; DESROCHERS, A.; BEAULIEU, J.; THOMAS, B.R.; MOSSELER, A.; MAJOR, J.; SIDDERS, D. Short-term growth and morphological responses to nitrogen availability and plant density in hybrid poplars and willows. **Biomass and Bioenergy**, v.81, p.88–97, 2015. DOI: 10.1016/j.biombioe.2015.06.003.

MARZILIANO, P.A.; COLETTA, V.; MENGUZZATO, G.; NICOLACI, A.; PELLICONE, G.; VELTRI, A. Effects of planting density on the distribution of biomass in a douglas-fir plantation in Southern Italy. **IForest**, v.8, p.368–376, 2015. DOI: 10.3832/ifor1078-007.

MATEOS, R.; BAEZA, G.; SARRIÁ, B.; BRAVO, L. Improved LC-MSn characterization of hydroxycinnamic acid derivatives and flavonols in different commercial mate (*Ilex paraguariensis*) brands . Quantification of polyphenols, methylxanthines, and antioxidant activity. **Food Chemistry**, v.241, p.232–241, 2018. DOI: 10.1016/j.foodchem.2017.08.085.

MCCARTHY, M.C.; ENQUIST, B.J. Consistency between an allometric approach and optimal partitioning theory in global patterns of plant biomass allocation. **Functional Ecology**, v.21, p.713–720, 2007. DOI: 10.1111/J.1365-2435.2007.01276.X.

MCELWAIN, J.C.; YIOTIS, C.; LAWSON, T. Using modern plant trait relationships between observed and theoretical maximum stomatal conductance and vein density to examine patterns of plant macroevolution. **New Phytologist**, v.209, p.94–103, 2016. DOI: 10.1111/NPH.13579.

MEDEIROS, P.L.; SILVA, G.G.C.; OLIVEIRA, E.M.M.; RIBEIRO, C.O.; SILVA, J.M.S.; PIMENTA, A.S. Efficiency of nutrient use for biomass production of a *Eucalyptus* clone as a function of planting density in short-rotation cropping. **Australian Forestry**, v.83, p.66–74, 2020. DOI: 10.1080/00049158.2020.1774958.

MENDIETA-ARAICA, B.; SPÖRNDLY, E.; REYES-SÁNCHEZ, N.; SALMERÓN-MIRANDA, F.; HALLING, M. Biomass production and chemical composition of *Moringa oleifera* under different planting densities and levels of nitrogen fertilization. **Agroforestry Systems**, v.87, p.81–92, 2013. DOI: 10.1007/s10457-012-9525-5.

METSARANTA, J.M. Long-term tree-ring derived carbon dynamics of an experimental plantation in relation to species and density in Northwestern Ontario, Canada. **Forest Ecology and Management**, v.441, p.229–241, 2019. DOI: 10.1016/j.foreco.2019.03.038.

MILYUTINA, I.L.; SUDACHKOVA, N.E.; ROMANOVA, L.I. Response of the antioxidant system of light-demanding and shade-bearing pine species to phytocenotic stress. **Contemporary Problems of Ecology**, v.6, p.149–155, 2013. DOI: 10.1134/S199542551302011X.

MIRANDA, I.; GOMINHO, J.; PEREIRA, H. Heartwood, sapwood and bark variation in coppiced *Eucalyptus globulus* trees in 2nd rotation and comparison with the single-stem 1st rotation. **Silva Fennica**, v.49, 2015. DOI: 10.14214/sf.1141.

MOHER, D.; LIBERATI, A.; TETZLAFF, J.; ALTMAN, D.G. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. **PLoS Medicine**, v.6, p.e1000097, 2009. DOI: 10.1371/journal.pmed.1000097.
MUÑOZ, V.L.; RÍO, R. DEL; BALZARINI, M. Stone pine (*Pinus pinea*) growth and cone yield as a function of planting density in Chile. **Bosque**, v.41, p.381–386, 2020. DOI: 10.4067/S0717-92002020000300381.

NAGAR, B.; RAWAT, S.; RATHIESH, P.; SEKAR, I. Impact of initial spacing on growth and yield of *Eucalyptus camaldulensis* in arid region of India. **World Applied Sciences Journal**, v.33, p.1362–1368, 2015. DOI: 10.5829/idosi.wasj.2015.33.08.247.

NAJI, H.R.; BAKAR, E.S.; SOLTANI, M.; EBADI, S.E.; ABDUL-HAMID, H.; JAVAD, S.K.S.; SAHRI, M.H. Effect of initial planting density and tree features on growth, wood density, and anatomical properties from a *Hevea brasiliensis* trial plantation. **Forest Products Journal**, v.64, p.41–47, 2014. DOI: 10.13073/FPJ-D-13-00071.

NAJI, H.R.; NIA, M.F.; KIAEI, M.; ABDUL-HAMID, H.; SOLTANI, M.; FAGHIHI, A. Effect of intensive planting density on tree growth, wood density and fiber properties of maple (*Acer velutinum* Boiss.). **IForest**, v.9, p.325–329, 2016. DOI: 10.3832/ifor1333-008.

NAN, W.; TA, F.; MENG, X.; DONG, Z.; XIAO, N. Effects of age and density of *Pinus sylvestris* var. *mongolica* on soil moisture in the semiarid Mu Us Dunefield, northern China. **Forest Ecology and Management**, v.473, p.118313, 2020. DOI: 10.1016/j.foreco.2020.118313.

NARDUCCI, T.S.; YARED, J.A.G.; JÚNIOR, S.B. Growth and survival of *Sclerolobium paniculatum* vogel and the relationship between rainfall and the increment in diameter at different planting spacings. **Revista Árvore**, v.40, p.447–454, 2016. DOI: 10.1590/0100-67622016000300008.

NEGRIN, A.; LONG, C.; MOTLEY, T.J.; KENNELLY, E.J. LC-MS metabolomics and chemotaxonomy of caffeine-containing holly (*Ilex*) species and related taxa in the Aquifoliaceae. **Journal of Agricultural and Food Chemistry**, v.67, p.5687–5699, 2019. DOI: 10.1021/acs.jafc.8b07168.

NIRSATMANTO, A. Early growth of improved *Acacia mangium* at different planting densities. **Journal of Tropical Forest Management**, v.22, p.105–113, 2016. DOI: 10.7226/jtfm.22.2.105.

O'HARA, K.L.; YORK, R.A. Leaf area development and crown architecture in a giant sequoia spacing study. **Forest Science**, v.60, p.776–783, 2014. DOI: 10.5849/forsci.13-063.

ÖZBAYRAM, A.K.; ÇIÇEK, E. Initial planting density experiments of narrow-leaved ash in Turkey: Ten-year results. **Sumarski List**, v.144, p.269–278, 2020. DOI: 10.31298/SL.144.5-6.4.

PACHAS, A.N.A.; SAKANPHET, S.; SOUKKHY, O.; LAO, M.; SAVATHVONG, S.; NEWBY, J.C.; SOULIYASACK, B.; KEOBOUALAPHA, B.; DIETERS, M.J. Initial spacing of teak (*Tectona grandis*) in northern Lao PDR: Impacts on the growth of teak and companion crops. **Forest Ecology and Management**, v.435, p.77–88, 2019. DOI: 10.1016/j.foreco.2018.12.031.

PÁSCOA, K.J.V. DA; SCOLFORO, J.R.S.; FERRAZ FILHO, A.C.; ALTOÉ, T.F.; GOMIDE, L.R. Growth of *Eremanthus erythropappus* (DC.) MacLeish in different planting spacings. **Floresta e Ambiente**, v.26, 2019. DOI: 10.1590/2179-8087.083417.

PAULA, R.R.; REIS, G.G.; REIS, M.G.F.; OLIVEIRA NETO, S.N.; LEITE, H.G.; MELIDO, R.C.N.; LOPES, H.N.S.; SOUZA, F.C. Eucalypt growth in monoculture and silvopastoral systems with varied tree initial densities and spatial arrangements. **Agroforestry Systems**, v.87, p.1295–1307, 2013. DOI: 10.1007/s10457-013-9638-5.

PONS, T.L.; JORDI, W.; KUIPER, D. Acclimation of plants to light gradients in leaf canopies: evidence for a possible role for cytokinins transported in the transpiration stream. **Journal of Experimental Botany**, v.52, p.1563–1574, 2001. DOI: 10.1093/jexbot/52.360.1563.

POZEBON, D.; DRESSLER, V.L.; CAETANO, M.; MARCELO, A.; OLIVEIRA, T.C. DE; FERRÃO, M.F. Food Additives & Contaminants : Part B Toxic and nutrient elements in yerba mate (*Ilex paraguariensis*). **Food Additives & Contaminants: Part B**, v.8, p.215–220, 2015. DOI: 10.1080/19393210.2015.1053420.

PRASAD, J.V.N.S.; KORWAR, G.R.; RAO, K. V; MANDAL, U.K.; RAO, G.R.; SRINIVAS, I.; VENKATESWARLU, B.; RAO, S.N.; KULKARNI, H.D. Optimum stand density of *Leucaena leucocephala* for wood production in Andhra Pradesh, Southern India. **Biomass and Bioenergy**, v.35, p.227–235, 2011. DOI: 10.1016/j.biombioe.2010.08.012.

R CORE TEAM. **R: A language and environment for statistical computing**VienaR Foundation for Statistical Computing, , 2020. Disponível em: <https://www.r-project.org/>

RAMALHO, F.M.G.; PIMENTA, E.M.; GOULART, C.P.; ALMEIDA, M.N.F. DE; VIDAURRE, G.B.; HEIN, P.R.G. Effect of stand density on longitudinal variation of wood and bark growth in fast-growing *Eucalyptus* plantations. **IForest**, v.12, p.527–532, 2019. DOI: 10.3832/ifor3082-012.

RAWAT, J.M.; RAWAT, B.; TEWARI, A.; JOSHI, S.C.; NANDI, S.K.; PALNI, L.M.S.; PRAKASH, A. Alterations in growth, photosynthetic activity and tissue-water relations of tea clones in response to different soil moisture content. **Trees - Structure and Function**, v.31, p.941–952, 2017. DOI: 10.1007/s00468-016-1519-x.

RE, R.; PELLEGRINI, N.; PROTEGGENTE, A.; PANNALA, A.; YANG, M.; RICE-EVANS, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. **Free Radical Biology and Medicine**, v.26, p.1231–1237, 1999. DOI: 10.1016/S0891-5849(98)00315-3.

RESQUIN, F.; NAVARRO-CERRILLO, R.M.; CARRASCO-LETELIER, L.; CASNATI, C.R. Influence of contrasting stocking densities on the dynamics of above-ground biomass and wood density of *Eucalyptus benthamii*, *Eucalyptus dunnii*, and *Eucalyptus grandis* for bioenergy in Uruguay. **Forest Ecology and Management**, v.438, p.63–74, 2019. DOI: 10.1016/j.foreco.2019.02.007.

RIACHI, L.G.; MARIA, C.A.B. DE. Yerba mate: an overview of physiological effects in humans. **Journal of Functional Foods**, v.38, p.308–320, 2017. DOI: 10.1016/j.jff.2017.09.020.

RIBEIRO, V.R.; MACIEL, G.M.; FACHI, M.M.; PONTAROLO, R.; FERNANDES, I. DE A.A.; STAFUSSA, A.P.; HAMINIUK, C.W.I. Improvement of phenolic compound bioaccessibility from yerba mate (*Ilex paraguariensis*) extracts after biosorption on Saccharomyces cerevisiae. **Food Research International**, v.126, p.108623, 2019. DOI: 10.1016/j.foodres.2019.108623.

RODRÍGUEZ-GARCÍA, E.; BRAVO, F. Plasticity in Pinus pinaster populations of diverse origins: Comparative seedling responses to light and Nitrogen availability. **Forest Ecology and Management**, v.307, p.196–205, 2013. DOI: 10.1016/j.foreco.2013.06.046.

RONČEVIĆ, S.; ANDRAŠEV, S.; IVANIŠEVIĆ, P.; KOVAČEVIĆ, B.; KLAŠNJA, B. Biomass production and energy potential of some eastern cottonwood (*Populus deltoides* bartr. ex marsh.) clones in relation to planting spacing. **Sumarski List**, v.137, p.33–42, 2013.

ROTH, B.E.; JOKELA, E.J.; MARTIN, T.A.; HUBER, D.A.; WHITE, T.L. Genotype × environment interactions in selected loblolly and slash pine plantations in the Southeastern United States. **Forest Ecology and Management**, v.238, p.175–188, 2007. DOI: 10.1016/j.foreco.2006.10.010.

RUSSELL, M.B.; BURKHART, H.E.; AMATEIS, R.L. Biomass partitioning in a miniature-scale loblolly pine spacing trial. **Canadian Journal of Forest Research**, v.39, p.320–329, 2009. DOI: 10.1139/X08-178.

SANTIN, D.; BENEDETTI, E.L.; BARROS, N.F. DE; ALMEIDA, I.C. DE; WENDLING, I. Intervalos de colheita e adubação potássica influenciam a produtividade da erva-mate. **Floresta**, v.46, p.509–518, 2016. DOI: 10.5380/rf.v46i3.41551.

SANTIN, D.; WENDLING, I.; BENEDETTI, E.L.; MORANDI, D.; DOMINGOS, D.M. Sobrevivência, crescimento e produtividade de plantas de erva-mate produzidas por miniestacas juvenis e por sementes. **Ciência Florestal**, v.25, p.571–579, 2015. DOI: 10.5902/1980509819608.

SANTOS, E.C.S.; BICCA, M.A.; BLUM-SILVA, C.H.; COSTA, A.P.R.; SANTOS, A.A. DOS; SCHENKEL, E.P.; FARINA, M.; REGINATTO, F.H.; LIMA, T.C.M. DE. Anxiolytic-like, stimulant and neuroprotective effects of Ilex paraguariensis extracts in mice. **Neuroscience**, v.292, p.13–21, 2015. DOI: 10.1016/j.neuroscience.2015.02.004.

SCHUBERT, A.; ZANIN, F.F.; PEREIRA, D.F.; ATHAYDE, M.L. Variação anual de metilxantinas totais em amostras de *Ilex paraguariensis* A. St. - Hil. (erva-mate) em Ijuí e Santa Maria, Estado do Rio Grande do Sul. **Química Nova**, v.29, p.1233–1236, 2006. DOI: 10.1590/S0100-40422006000600016.

SCHWERZ, F.; ELOY, E.; ELLI, E.F.; CARON, B.O. Reduced planting spacing increase radiation use efficiency and biomass for energy in black wattle plantations: Towards sustainable production systems. **Biomass and Bioenergy**, v.120, p.229–239, 2019. DOI: 10.1016/j.biombioe.2018.11.034.

SCHWERZ, F.; NETO, D.D.; CARON, B.O.; NARDINI, C.; SGARBOSSA, J.; ELOY, E.; BEHLING, A.; ELLI, E.F.; REICHARDT, K. Biomass and potential energy yield of perennial woody energy crops under reduced planting spacing. **Renewable Energy**, v.153, p.1238–1250, 2020a. DOI: 10.1016/j.renene.2020.02.074.

SCHWERZ, F.; NETO, D.D.; CARON, B.O.; TIBOLLA, L.B.; SGARBOSSA, J.; ELOY, E.; ELLI, E.F.; CARVALHO, L.G. Carbon stocks, partitioning, and wood composition in short-rotation forestry system under reduced planting spacing. **Annals of Forest Science**, v.77, 2020b. DOI: 10.1007/s13595-020-00974-w.

SCOTT, D.A.; TIARKS, A. Dual-cropping loblolly pine for biomass energy and conventional wood products. **South Journal of Applied Forestry**, v.32, p.33–37, 2008.

SCOTT, R.E.; MITCHELL, S.J. Empirical modelling of windthrow risk in partially harvested stands using tree, neighbourhood, and stand attributes. **Forest Ecology and Management**, v.218, p.193–209, 2005. DOI: 10.1016/j.foreco.2005.07.012.

SHARMA-NATU, P.; GHILDIYAL, M.C. Potential targets for improving photosynthesis and crop yield. **Current Science**, v.88, p.1918–1928, 2005.

SILVA, P.S.L. E; HOLANDA, A.E.R.; PAIVA, H.N. DE; OLIVEIRA, F.H.T. DE; OLIVEIRA, O.F. DE. Planting density and initial growth of two tree species adapted to the semi-arid region. **Revista Árvore**, v.36, p.951–959, 2012. DOI: 10.1590/S0100-67622012000500017.

SILVA, W.G.; SANTOS, D.M. DA C.L. DOS; LIMA, A.P.L.; MATTOS, F.D.; LIMA, S.F.; PAULA, R.D.C.M. DE. Growth and volumetric production of eucalyptus clones in different planting spaces. **Journal of Neotropical Agriculture**, v.6, p.38–47, 2019. DOI: 10.32404/rean.v6i3.3386.

SINGLETON, V.L.; ROSSI, J.A. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. **American Journal of Enology and Viticulture**, v.16, 1965.

SMITH, R.G.B.; GLENCROSS, K.; NICHOLS, J.D.; PALMER, G.; VIRANAMANGGA, R. Effect of initial spacing on first thinning product recovery, and financial outcomes in whitewood (*Endospermum medullosum*) plantations in Vanuatu. **Small-scale Forestry**, v.15, p.45–59, 2016. DOI: 10.1007/s11842-015-9307-9.

STREIT, N.M.; HECKTHEUER, L.H.R.; CANTO, M.W. DO; MALLMANN, C.A.; STRECK, L.; PARODI, T.V.; CANTERLE, L.P. Relation among taste-related compounds (phenolics and caffeine) and sensory profile of erva-mate (*Ilex paraguariensis*). **Food Chemistry**, v.102, p.560–564, 2007. DOI: 10.1016/j.foodchem.2006.05.028.

SUN, H.; DIAO, S.; LIU, R.; FORRESTER, D.; SOARES, A.; SAITO, D.; DONG, R.; JIANG, J. Relationship between size inequality and stand productivity is modified by self-thinning, age, site and planting density in *Sassafras tzumu* plantations in central China. **Forest Ecology and Management**, v.422, p.199–206, 2018. DOI: 10.1016/j.foreco.2018.02.003.

TANABE, J.; ENDO, R.; KURODA, S.; ISHIGURI, F.; NARISAWA, T.; TAKASHIMA, Y. Variance components and parent–offspring correlations of growth traits vary among the initial planting spacings in *Zelkova serrata*. **Silvae Genetica**, v.68, p.45–50, 2019. DOI: 10.2478/sg-2019-0008.

TARROUX, E.; DESROCHERS, A. Effect of natural root grafting on growth response of jack pine (*Pinus banksiana*; Pinaceae). **American Journal of Botany**, v.98, p.967–974, 2011. DOI: 10.3732/ajb.1000261.

TAVARES, A.P.C.; SOARES, T.S.; CRUZ, E.S. Dendrometric characteristics and basic wood density of eucalyptus clones in different spacing. **Journal of Neotropical Agriculture**, v.7, p.104–108, 2020. DOI: 10.32404/rean.v7i3.4355.

TENORIO, C.; ROQUE, R.M.; CARLOS VALVERDE, J.; ARIAS-AGUILAR, D. Biomass production and characteristics of short rotation plantations of clones of *Gmelina arborea* in three spacings. **Silvae Genetica**, v.68, p.92–100, 2019. DOI: 10.2478/sg-2019-0017.

TFOUNI, S.A.V.; CAMARA, M.M.; KAMIKATA, K.; GOMES, F.M.L.; FURLANI, R.P.Z. Caffeine in teas: Levels, transference to infusion and estimated intake. **Food Science and Technology**, v.38, p.661–666, 2018. DOI: 10.1590/1678-457x.12217.

THYROFF, E.C.; BURNEY, O.T.; MICKELBART, M. V.; JACOBS, D.F. Unraveling shade tolerance and plasticity of semi-evergreen oaks: insights from maritime forest live oak restoration. **Frontiers in Plant Science**, v.10, p.1526, 2019. DOI: 10.3389/FPLS.2019.01526/BIBTEX.

TOILLON, J.; FICHOT, R.; DALLÉ, E.; BERTHELOT, A.; BRIGNOLAS, F.; MARRON, N. Planting density affects growth and water-use efficiency depending on site in *Populus deltoides* × *P. nigra*. **Forest Ecology and Management**, v.304, p.345–354, 2013. DOI: 10.1016/j.foreco.2013.05.017.

TOMASI, J.C. **Bioactive compounds of yerba mate according to genotype, nitrogen fertigation and drying methods and acceptance of mate tea by consumers**. 2020. 142p. Tese (doutorado em Agronomia: Produção Vegetal) – Setor de Ciências Agrárias, Universidade Federal do Paraná.

TOMASI, J. DE C.; LIMA, G.G. DE; WENDLING, I.; HELM, C.V.; HANSEL, F.A.; GODOY, R.C.B. DE; GRUNENNVALDT, R.L.; MELO, T.O. DE; TOMAZZOLI, M.M.; DESCHAMPS, C. Effects of different drying methods on the chemical, nutritional and colour of yerba mate (*Ilex paraguariensis*) leaves. **International Journal of Food Engineering**, v.0, 2021. DOI: 10.1515/ijfe-2020-0312.

TONINI, H.; SCHWENGBER, D.R.; MORALES, M.M.; MAGALHÃES, C.A. DE S.; OLIVEIRA, J.M.F. DE. Growth, biomass, and energy quality of *Acacia mangium* timber grown at different spacings. **Pesquisa Agropecuária Brasileira**, v.53, p.791–799, 2018. DOI: 10.1590/S0100-204X2018000700002.

TOPPEL, F.V.; JUNIOR, A.M.; MOTTA, A.C.V.; FRIGO, C.; MAGRI, E.; BARBOSA, J.Z. Soil chemical attributes and their influence on elemental composition of yerba mate leaves. **Floresta**, v.48, p.425–434, 2018. DOI: 10.5380/rf.v48.

TORREANO, S.J.; SMALLEY, G.W.; NUNLEY, A.N.; RENNIE, J.C. Structure and growth of 40-year-old unthinned loblolly pine planted at three spacings in south-central tennessee. **Southern Journal of Applied Forestry**, v.36, p.136–140, 2012. DOI: 10.5849/sjaf.11-002.

TUN, T.N.; GUO, J.; FANG, S.; TIAN, Y. Planting spacing affects canopy structure, biomass production and stem roundness in poplar plantations. **Scandinavian Journal of Forest Research**, v.33, p.464–474, 2018. DOI: 10.1080/02827581.2018.1457711.

TZORTZAKIS, N.; SGHERRI, C.; PEARSON, B.J.; ZHANG, M.; SHARMA, A.; LEÓN, F.; AVERY, B.; KJELGREN, R.; MCCURDY, C.R. Effects of nutrient fertility on growth and alkaloidal content in *Mitragyna speciosa* (Kratom). **Frontiers in Plant Science**, v.11, p.597696, 2020. DOI: 10.3389/fpls.2020.597696.

VALDUGA, A.T.; GONÇALVES, I.L.; MAGRI, E.; FINZER, J.R.D. Chemistry, pharmacology and new trends in traditional functional and medicinal beverages. **Food Research International**, v.120, p.478–503, 2019. DOI: 10.1016/j.foodres.2018.10.091.

VERMA, N.; SHUKLA, S. Impact of various factors responsible for fluctuation in plant secondary metabolites. **Journal of Applied Research on Medicinal and Aromatic Plants**, v.2, p.105–113, 2015. DOI: 10.1016/j.jarmap.2015.09.002.

VIEIRA, L.M.; MAGGIONI, R.D.A.; TOMASI, J.D.C.; NUNES GOMES, E.; WENDLING, I.; HELM, C.V.; KOEHLER, H.S.; ZUFFELLATO-RIBAS, K.C. Vegetative propagation, chemical composition and antioxidant activity of yerba mate genotypes. **Plant Genetic Resources: Characterization and Utilization**, v.19, p.112–121, 2021. DOI: 10.1017/S1479262121000150.

WAGHORN, M.J.; MASON, E.G.; WATT, M.S. Influence of initial stand density and genotype on longitudinal variation in modulus of elasticity for 17-year-old *Pinus radiata*. **Forest Ecology and Management**, v.252, p.67–72, 2007. DOI: 10.1016/j.foreco.2007.06.019.

WANG, C.S.; ZENG, J.; HEIN, S.; ZHAO, Z.G.; GUO, J.J.; ZENG, J. Crown and branch attributes of mid-aged *Betula alnoides* plantations in response to planting density. **Scandinavian Journal of Forest Research**, v.32, p.679–687, 2016. DOI: 10.1080/02827581.2016.1261936.

WEGIEL, A.; POLOWY, K. Aboveground carbon content and storage in mature scots pine stands of different densities. **Forests**, v.11, 2020. DOI: 10.3390/f11020240.
WEINER, J.; FRECKLETON, R.P. Constant final yield. **Annual Review of Ecology, Evolution, and Systematics**, v.41, p.173–192, 2010. DOI: 10.1146/annurev-ecolsys-102209-144642.

WENDLING, I.; SANTIN, D. **Propagação e nutrição de erva-mate**. Brasília: Embrapa, 2015. 195p.

WENDLING, I.; STURION, J.A.; STUEPP, C.A.; REIS, C.A.F.; RAMALHO, M.A.P.; RESENDE, M.D.V. DE. Early selection and classification of yerba mate progenies. **Pesquisa Agropecuaria Brasileira**, v.53, p.279–286, 2018. DOI: 10.1590/S0100-204X2018000300002.

WEST, P.W.; SMITH, R.G.B. Effects of tree spacing on branch-size development during early growth of an experimental plantation of *Eucalyptus pilularis* in subtropical Australia. **Australian Forestry**, v.83, p.39–45, 2020. DOI: 10.1080/00049158.2020.1715016.

WILL, R.E.; BARRON, G.A.; BURKES, E.C.; SHIVER, B.; TESKEY, R.O. Relationship between intercepted radiation, net photosynthesis, respiration, and rate of stem volume growth of *Pinus taeda* and *Pinus elliottii* stands of different densities. **Forest Ecology and Management**, v.154, p.155–163, 2001. DOI: 10.1016/S0378-1127(00)00625-3.

WILL, R.E.; NARAHARI, N. V.; SHIVER, B.D.; TESKEY, R.O. Effects of planting density on canopy dynamics and stem growth for intensively managed loblolly pine stands. **Forest Ecology and Management**, v.205, p.29–41, 2005. DOI: 10.1016/j.foreco.2004.10.002.

WILSON, J.B.; STEEL, J.B.; STEEL, S.L.K. Do plants ever compete for space? **Folia Geobotanica**, v.42, p.431–436, 2007. DOI: 10.1007/BF02861704.

WOODRUFF, D.R.; BOND, B.J.; RITCHIE, G.A.; SCOTT, W. Effects of stand density on the growth of young Douglas-fir trees. **Canadian Journal of Forest Research**, v.32, p.420–427, 2002. DOI: 10.1139/x01-213.

XUE, L.; JACOBS, D.F.; ZENG, S.; YANG, Z.; GUO, S.; LIU, B. Relationship between above-ground biomass allocation and stand density index in *Populus xeuramericana* stands. **Forestry**, v.85, p.611–619, 2012. DOI: 10.1093/forestry/cps071.

YAN, Z.; ZHOU, Q.; TENG, M.; JI, H.; ZHANG, J.; HE, W.; YE, Y.; WANG, B.; WANG, P. High planting density and leaf area index of masson pine forest reduce crown transmittance of photosynthetically active radiation. **Global Ecology and Conservation**, v.20, p.e00759, 2019. DOI: 10.1016/j.gecco.2019.e00759.

YIN, Y.; KATAHIRA, R.; ASHIHARA, H. Metabolism of purine alkaloids and xanthine in leaves of maté (*Ilex paraguariensis*). **Natural Product Communications**, v.10, p.707–712, 2015. DOI: 10.1177/1934578X1501000503.

ZHAO, D.; BULLOCK, B.P.; MONTES, C.R.; WANG, M.; WESTFALL, J.; COULSTON, J.W. Long-term dynamics of loblolly pine crown structure and aboveground net primary production as affected by site quality, planting density and cultural intensity. **Forest Ecology and Management**, v.472, p.118259, 2020. DOI: 10.1016/j.foreco.2020.118259.

ZHAO, D.; KANE, M.; BORDERS, B.E. Growth responses to planting density and management intensity in loblolly pine plantations in the southeastern USA Lower Coastal Plain. **Annals of Forest Science**, v.68, p.625–635, 2011. DOI: 10.1007/s13595.