UNIVERSIDADE FEDERAL DO PAMPA

VANESSA ROSSETO

OMISSÃO DE NUTRIENTES, ADUBAÇÃO COM BORO E CARGA DE FRUTOS EM OLIVEIRAS: INFLUÊNCIA NOS PARÂMETROS MORFOMÉTRICOS, FISIOLÓGICOS E DE QUALIDADE DO AZEITE

São Gabriel
2023

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Tese apresentada ao Programa de Pós-Graduação *Stricto Sensu* em Ciências Biológicas da Universidade Federal do Pampa, como requisito parcial para obtenção do Título em Ciências Biológicas.

Orientador: Prof. Dr. Frederico Costa Beber Vieira

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RESUMO

A olivicultura no Brasil encontra-se em crescente expansão nas últimas décadas, no entanto para que a atividade prospere são primordiais pesquisas sobre o manejo, com destaque para a área de fertilidade e adubação. Outro aspecto importante na olivicultura se refere à bianualidade e produção heterogênea em olivais, sendo relevantes estudos que avaliem árvores com diferentes cargas de frutos. Com isso, o objetivo da Tese foi investigar o estado nutricional de oliveiras (*Olea europaea* L. cultivar Arbequina) sob condições de deficiência e adubação e a relação entre carga de frutos e qualidade físico-química do azeite. Deste modo, foram conduzidos três estudos independentes entre si, sendo que o primeiro avaliou a deficiência nutricional em oliveiras jovens sob omissão de nutrientes durante três anos em casa de vegetação, no município de São Gabriel (RS); o segundo abordou o efeito da adubação com boro (B) sobre oliveiras adultas em dois olivais comerciais em São Gabriel e Caçapava do Sul (RS); o terceiro focou na relação entre a carga de frutos e a qualidade do azeite, assim como a comparação entre dois olivais em Caçapava do Sul em relação aos parâmetros do fruto e azeite. No estudo 1 observou-se que a omissão de nitrogênio (N), fósforo (P), potássio (K) e B alterou pelo menos um dos parâmetros morfométricos analisados, assim como o conteúdo de nutrientes e compostos fenólicos. Oliveiras privadas de N exibiram elevado estresse nutricional, apresentando menor diâmetro do caule e matéria seca nos ramos, assim como maiores níveis de compostos fenólicos nas folhas e tendência oposta nas raízes. A omissão de P produziu sintomas tardios de deficiência, com aumento da maioria dos compostos fenólicos, especialmente oleuropeína e verbascosídeo. A privação de B e K induziu a sintomas mais atenuados, inclusive com o tratamento -K apresentando maior comprimento e densidade de raízes do que o tratamento completo. No estudo 2 verificou-se que a adubação via solo e foliar com B não alterou o conteúdo de B nas folhas e apenas o kaempferol exibiu maior concentração no tratamento com 100 g de ulexita/com foliar. Contudo, menores teores de B foram observados na floração, independente do tratamento, sugerindo mobilização de nutrientes das folhas para atender a demanda da floração. Com relação ao estudo 3, não foram observadas diferenças significativas entre cargas de frutos sobre o teor de lipídios dos frutos, assim como na maior parte dos parâmetros de qualidade. Entretanto, árvores com baixa produção exibiram maiores concentrações de oleuropeína e verbascosídeo

nos frutos, além de oleaceína e oleocantal no azeite. Também foi observado maior conteúdo de P nos frutos de baixa produção e de B em frutos de alta produção. A extração e qualidade do azeite diferiram entre os olivais, com maior rendimento e eficiência de azeite no olival colhido com frutos mais maduros, ademais os parâmetros de qualidade estiveram dentro das normas, apresentando maior conteúdo de ácido oleico, oleaceina e hidroxitorosol no azeite. Deste modo, frutos com índice de maturação próximo a 4 levaram a maior produção de azeite, sem prejudicar a qualidade do produto.

Palavras-Chave: Arbequina; deficiência nutricional; adubação boratada; compostos fenólicos; perfil de ácidos graxos; carga de frutos.

ABSTRACT

Oliviculture in Brazil has been expanding in recent decades, however for the activity to prosper, research on management is essential, with emphasis on the area of fertility and fertilization. Another important aspect in olive growing refers to the biannuality and heterogeneous production in olive groves, highlighting the relevance of studies about trees with different fruit loads. Thus, the aim of this Thesis was to investigate the nutritional status of olive trees (*Olea europaea* L. cultivar Arbequina) under conditions of deficiency and fertilization and the relationship between fruit load and oil physical-chemical quality. Thus, three independent studies were carried out, the first of which evaluated nutritional deficiency in young olive trees under nutrient omission for three years in a greenhouse, in the municipality of São Gabriel (RS); the second addressed the effect of boron (B) fertilization on adult olive trees in São Gabriel and Caçapava do Sul (RS); the third focused on the relationship between fruit load and oil quality, as well as the comparison between two olive groves in Caçapava do Sul in terms of fruit and oil parameters. The omission of nitrogen (N), phosphorus (P), potassium (K) and B altered at least one of the morphometric parameters analyzed, as well as the content of nutrients and phenolic compounds. Olive trees deprived of N exhibited high nutritional stress, with smaller stem diameter and dry matter in the branches, as well as higher levels of phenolic compounds in the leaves and the opposite trend in the roots. The omission of P produced late symptoms, with an increase in most phenolic compounds, especially oleuropein and verbascoside. The deprivation of B and K induced more attenuated symptoms, even with the -K treatment showing greater root length and density than the complete treatment. In study 2, it was verified that soil and foliar fertilization with B did not alter the B content in the leaves and only kaempferol showed a higher concentration in the treatment with 100 g ulexite/with foliar. However, lower levels of B were observed at flowering, regardless of treatment, suggesting mobilization of nutrients from leaves to attend flowering demand. Concerning study 3, no significant differences were observed between fruit loads on the lipid content of the fruits, as well as in most of the quality parameters. However, trees with low production showed a higher concentration of oleuropein and verbascoside in the fruits, in addition to oleacein and oleocanthal in the oil. A greater amount of P content was also observed in low fruit load and of B in high fruit load. The extraction and quality of the oil differed between the olive groves,

with higher yield and extractability of olive oil in the olive grove harvested with more mature fruits, in addition the quality parameters were within the norms, showing a higher content of oleic acid, oleacein and hydroxytyrosol in the olive oil. Thus, fruits with a maturation index close to 4 led to greater oil production, without impairing product quality.

Keywords: Arbequina; nutritional deficiency; borate fertilization; compounds phenolics; fatty acid profile; fruit load.

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LISTA DE SIGLAS

ADP – Adenosina Difosfato

ATP – Adenosina Trifosfato

BOR – *High Boron Requiring*

CQFS-RS/SC – Comissão de Química e Fertilidade do Solo – RS/SC

EEC– *European Economic Commission*

GLU– *β-glucosidase*

HDL – *High Density Lipoprotein*

IBRAOLIVA – Instituto Brasileiro de Olivicultura

IVALSA – *Istituto per la Valorizzazione del Legno e delle Specie Arboree*

IOC – *International Olive Council*

LOX– *Lipoxygenase*

MIP – *Major Intrinsic Proteins*

NIP – *Nodulin-26-like Intrinsic Protein*

PAL– *Phenylalanine Ammonia Lyase*

POD– *Peroxidase*

PPO– *Polyphenoloxidase*

p-HPEA– p-4-hidroxifeniletanol (Tirosol)

p-HPEA-EDA– Forma dialdeído do ácido elenólico ligado ao p-HPEA (Oleocantal)

3,4-DHPEA-EA– Isômero da oleuropeína agliconada

3,4-DHPEA– 3,4-diidroxifeniletanol (Hidroxitirosol)

3,4-DHPEA-EDA– Forma dialdeído do ácido elenólico ligado ao 3,4-DHPEA (Oleaceína)

SUMÁRIO

1 INTRODUÇÃO

A olivicultura é uma prática agrícola milenar na região mediterrânea, a qual abrange 98% da produção mundial, contudo tem-se observado uma crescente expansão em outras regiões do mundo, incluindo o Brasil (LUCENA *et al.,* 2017). O Brasil é o segundo maior importador de azeite e azeitona de mesa do mundo (IOC, 2023a), sendo que menos de 1% da demanda é suprida pela produção nacional, demonstrando assim o potencial de crescimento da olivicultura em nosso país. O estado do Rio Grande do Sul é o maior produtor de azeite nacional e possui cerca de 6.000 ha de área plantada, seguido da região da Serra da Mantiqueira, com 3.000 ha, destacando-se os estados de Minas Gerais, São Paulo e Rio de Janeiro (AMBROSINI *et al*., 2022; IBRAOLIVA, 2022).

Apesar de promissor, o desenvolvimento da olivicultura no Brasil depende da adequação às condições edafoclimáticas, as quais são discrepantes da sua região de origem, caracterizada pela baixa pluviosidade e pelos solos alcalinos (FERNÁNDEZ-ESCOBAR; MARÍN, 1999). Os programas de fertilização para oliveiras geralmente recomendam a utilização de nitrogênio (N), fósforo (P), potássio (K) e boro (B), sendo que os outros nutrientes devem ser aplicados apenas em situação de deficiência (LÓPEZ-GRANADOS *et al*., 2004; MESQUITA; GARCIA; COSTA, 2012; CQFS-RS/SC, 2016; TIECHER *et al*., 2020). A deficiência em N, P, K e B para oliveiras tem sido investigada, indicando uma série de alterações visuais e fisiológicas, como redução de crescimento aéreo e radicular ou ainda, crescimento anormal, modificações de parâmetros fotossintéticos e de regulação estomática, alterações nos níveis de outros nutrientes, além de diminuição na produtividade (HARTMANN; BROWN, 1953; ARQUERO; BARRANCO; BENLLOCH, 2006; BENLLOCH-GONZÁLEZ *et al.,* 2008; ARROBAS *et al.,* 2010; BOUSSADIA *et al*., 2010; CHATZISSAVVIDIS; THERIOS, 2010; NAIJA *et al*., 2014; FERNÁNDEZ-ESCOBAR *et al*., 2016; JIMÉNEZ-MORENO; FERNÁNDEZ-ESCOBAR, 2016; EREL, *et al*., 2017; HABERMAN *et al.* 2019; SOUZA *et al*., 2019).

A fim de evitar os efeitos negativos da deficiência nutricional, são necessários estudos que ajustem os sistemas de fertilização, garantindo um suprimento adequado de nutrientes e considerando as diferentes situações regionais. No caso do B, sua adubação é recomendada devido à alta demanda deste micronutriente para oliveiras, principalmente no período de diferenciação das gemas e da floração

(MESQUITA; GARCIA; COSTA, 2012; CQFS-RS/SC, 2016). Estudos com adubação com B têm observado uma série de alterações fisiológicas e produtivas após a fertilização, como aumento na concentração do nutriente nos tecidos vegetais e no percentual de flores perfeitas, modificações na concentração de metabólitos primários e secundários, elevação da produção de frutos e azeite (DELGADO; BENLLOCH; FERNÁNDEZ-ESCOBAR, 1994; PERICA *et al.,* 2001a; TALAIE; TAHERI, 2001; HEGAZI *et al.* 2018; PASKOVIĆ *et al.,* 2019; VISHEKAII *et al.* 2019). Contudo, a aplicação de adubação do micronutriente não se refletiu em aumento na produção e melhoria na qualidade do azeite em outros estudos (ATEYYEH; SHATAT 2006; LARBI *et al.,* 2011; RODRIGUES *et al.,* 2011; FERREIRA; RODRIGUES; ARROBAS, 2019), demonstrando assim a necessidade de pesquisas na área de fertilidade para oliveiras.

Destaca-se que para oliveiras ainda são escassos estudos internacionais que avaliaram o efeito da deficiência nutricional e adubação sobre metabólitos secundários, como os compostos fenólicos (LIAKOPOULOS; KARABOURNIOTIS, 2005; KARIOTI *et al.* 2006; HEGAZI *et al.,* 2018; PASKOVIĆ *et al.,* 2019), e são ainda mais escassos pesquisas nos olivais brasileiros. Tais compostos exibem elevada atividade antioxidante, proteção contra herbivoria e patógenos, sendo essenciais nos processos de defesa dos vegetais (ASSABGUI *et al.*, 1993; MEIR *et al.*, 1995; KONNO *et al.,* 1999; BENAVENTE-GARCÍA *et al.*, 2000; MEWIS *et al.*, 2012; PLUEMSAMRAN; ONKOKSOONG; PANICH, 2012; LIKIĆ *et al.*, 2014; YANG *et al.*, 2017; ISLAM *et al.*, 2019; LIU *et al.*, 2020). Os potenciais benefícios para a saúde humana advindos dos compostos fenólicos presentes nos tecidos de oliveiras e no azeite têm sido investigados extensivamente nos últimos anos (SERVILI *et al.*, 2004; HASSEN; CASABIANCA; HOSNI, 2015; TALHAOUI *et al.*, 2015; PEDRET *et al.*, 2018; MARKOVIĆ *et al.*, 2019; SCHWINGSHACKL *et al.*, 2019; TSARTSOU *et al.*, 2019; HOFER *et al.*, 2020; ROMANA-SOUZA *et al.*, 2020).

A caracterização do azeite em termos de parâmetros de qualidade e identidade é essencial para a garantia de consumo de produtos que atendam não apenas as necessidades alimentares, mas também que proporcionem os benefícios à saúde. Apesar do crescimento das pesquisas com azeites brasileiros, muitos estudos foram realizados com azeites comerciais (MELLO; PINHEIRO, 2012; SILVA *et al.*, 2012; BALLUS *et al.*, 2014; BALLUS *et al.*, 2015; BORGES *et al.*, 2017a; BORGES *et al.*, 2017b; BRUSCATTO *et al.*, 2017; RODRIGUES *et al.*, 2019; ZAGO

et al., 2019; CARVALHO *et al.*, 2020; CRIZEL *et al.*, 2020; PAZ *et al.*, 2020; SANTOS; SILVA; FANTE, 2021). Entretanto, ainda há pouca informação referente ao processamento do azeite como, por exemplo, rendimento e eficiência de extração, assim como determinadas análises químicas, como de compostos fenólicos individuais nos frutos e azeites.

Com isso, nosso grupo de pesquisa em “Fertilidade, Manejo e Conservação do Solo do Pampa”, tem conduzido estudos em olivais comerciais na região da Campanha Gaúcha e Serra do Sudeste, assim como experimentos em casa de vegetação. A presente tese de Doutorado incluiu três estudos. O primeiro estudo abordou o efeito da omissão de nutrientes (N, P, K ou B) em oliveiras jovens, através de estudo de longa duração (2019-2022) com mudas de oliveiras no porte para o plantio. Foram avaliados parâmetros morfométricos e de massa seca dos ramos e raízes, assim como o conteúdo de compostos fenólicos e nutrientes em folhas e raízes. Estudo anterior do grupo de pesquisa encontrou correlação positiva entre os teores de B no solo e a produção de frutos (BENDER; WEBER; VIEIRA, 2018) e um segundo estudo avaliou o efeito da adubação via solo e foliar com B sobre a produção de oliveiras adultas (FIGUEIREDO *et al*., 2022). A fim de fornecer mais subsídios ao tema em questão, o segundo estudo da Tese avaliou o efeito da adubação com B em oliveiras adultas em dois olivais, tendo como resposta o conteúdo de B e compostos fenólicos individuais em três períodos em 2019: pré-floração; floração e início da frutificação. A previsão inicial era avaliar o efeito de tal adubação sobre a extração e qualidade do azeite, entretanto a safra de 2020 apresentou baixíssima produção, inviabilizando a avaliação dos tratamentos com B. Mesmo sem correlação com os outros estudos, mas com aplicação nas áreas de Biologia, Agronomia e Tecnologia de Alimentos, o estudo três abordou o efeito da carga dos frutos e da localização dos olivais sobre parâmetros de extração e de qualidade físico-química do azeite. Estudos que abordaram o efeito da carga dos frutos sobre parâmetros dos frutos e azeite ainda são escassos para oliveiras (TOMBESI; BOCO; PILLI, 1999; BEN-GAL *et al.*, 2011; DAG *et al.*, 2011), com lacunas referentes à quantificação de compostos fenólicos individuais. Com isso, foram avaliados dois olivais no município de Caçapava do Sul na safra de 2020 e um olival na safra de 2021, considerando oliveiras com alta e baixa carga de frutos, de 19-40 kg frutos/árvore e 2-6 kg frutos/árvore, respectivamente.

2 OBJETIVOS E HIPÓTESES

A questão central do presente estudo, assim como do supracitado grupo de pesquisa, tem sido colaborar com o ajuste fino nas recomendações de fertilizantes para o sul do Brasil, visando à maximização da produção de frutos e a melhoria de processos fisiológicos para oliveiras. Acredita-se que os estudos sobre a questão nutricional e de carga de frutos possam colaborar com o manejo em olivais, a fim de diminuir os efeitos negativos da alternância de produção (anos *on* e *off*) e produção heterogênea em olivais, propiciando o aumento na qualidade do azeite, contribuindo assim para a segurança alimentar dos consumidores.

Deste modo, o objetivo geral do trabalho foi avaliar o estado nutricional de oliveiras sob condições de deficiência e adubação e a relação entre carga de frutos e a qualidade físico-química do azeite.

Hipótese: Em condições de deficiência nutricional os parâmetros morfométricos são prejudicados e há alteração nos níveis dos metabólitos analisados, enquanto que com a adubação há um aumento nos níveis do conteúdo de nutrientes. Oliveiras com diferentes cargas de frutos apresentam diferenças fisiológicas nos frutos que se refletem em diferenças na qualidade do azeite.

Os objetivos específicos e hipóteses associadas foram:

Estudo 1 – Investigar o efeito da omissão de nutrientes (N, P, K e B) sobre a morfometria, conteúdo de compostos fenólicos e de nutrientes em oliveiras jovens.

Hipótese: A omissão leva a variações nos níveis de estresse nutricional para cada tratamento (-N, -P, -K e -B), o que se reflete em diferenças em relação ao tratamento controle (com todos os nutrientes) sobre os parâmetros analisados e esta diferença é específica para cada nutriente omitido.

Estudo 2 – Analisar a resposta de oliveiras adultas à fertilização com B (via solo e foliar), considerando os teores de B e compostos fenólicos nas folhas.

Hipótese: A adubação propicia um aumento no conteúdo de B nas folhas. Cada composto fenólico apresenta resposta diferente de aumento ou diminuição da sua concentração em decorrência da adubação.

Estudo 3 – Avaliar o efeito da carga de frutos e do local sobre a extração do azeite e sobre parâmetros de qualidade do mesmo.

Hipótese: Azeites provenientes de oliveiras com diferentes cargas de frutos em um mesmo olival apresentam diferenças da qualidade do azeite, associadas às variações nas demandas metabólicas entre as classes analisadas, as quais se refletem nos parâmetros dos frutos e azeites. Em diferentes pomares no mesmo ano agrícola, as diferenças ambientais e de manejo promovem variações entre locais em relação a parâmetros de extração e qualidade do azeite.

3 REVISÃO DE LITERATURA

3.1 A oliveira: origem e taxonomia

A oliveira *(Olea europaea* L.) é uma árvore frutífera de origem mediterrânea, utilizada pelo Homem há milênios. Seu registro fóssil mais antigo foi encontrado na Jordânia e data de 780 mil anos a.C. (LANGGUT *et al.*, 2019). O cultivo da oliveira também é um dos mais antigos do mundo, com registros arqueológicos na região da Palestina, Israel, Jordânia e Síria de 6.500 anos a.C., seguidos da região de Creta na Grécia de 6.000-5.500 anos (LANGGUT *et al.*, 2019). A produção de azeite se difundiu nestas regiões principalmente na Idade do Bronze (2.000-1.000 anos a.C.) com registros arqueológicos de vasos de cerâmica, lâmpadas de azeite e instalações de processamento de azeitonas (LIPHSCHITZ *et al*., 1991; RILEY, 2002). Posteriormente, o cultivo da oliveira se expandiu para outras regiões do mediterrâneo e atualmente a olivicultura é praticada em cinco continentes, em locais bem distantes do seu centro de origem, como Austrália, China e Brasil (LUCENA; MANRIQUE, MÉNDEZ, 2017; TORRES *et al*., 2017).

A oliveira pertence à família Oleaceae, a qual apresenta 30 gêneros e 600 espécies (CRONQUIST, 1981). Sua distribuição natural abrange o sul da Europa, África e Ásia (KASSA *et al.*, 2019). A espécie possui seis subespécies: *europaea, laperrinei, cuspidata, guanchica, maroccana e cerasiformis. Olea europaea* subsp*. europaea* apresenta ainda duas variedades: *var. sylvestris* que é uma variedade selvagem, enquanto que a *var. europaea* é a oliveira cultivada. (GREEN, 2002; KASSA *et al.*, 2019).

A espécie cultivada possui porte mediano (4-8 m), com copa arredondada e densa. As folhas de oliveira são simples, opostas, lanceoladas, adaptadas a ambientes secos, de modo que a parte superior é verde escura com uma camada cerosa, enquanto a parte inferior é branco-prateada com tricomas, os quais protegem os estômatos, diminuindo as perdas de água pela transpiração (RAPOPORT; MORENO-ALÍAS, 2017). As flores de oliveiras desenvolvem-se a partir de gemas situadas em ramos do ano anterior (FABBRI; BENELLI, 2000), dispostas em inflorescências tipo racimo, com quatro sépalas verdes soldadas e quatro pétalas brancas também soldadas (VIEIRA NETO *et al.*, 2008). As flores podem ser hermafroditas ou estaminadas (LAVEE *et al.*, 1996). A espécie é

polinizada pelo vento e, além disso, vários cultivares necessitam de polinização cruzada para a fecundação (SÁNCHEZ-ESTRADA; CUEVAS, 2018).

Os frutos são denominados azeitonas, sendo drupas com forma e tamanho variado dependendo do cultivar, sendo utilizadas pelo Homem para a produção de azeite ou azeitona de mesa. A azeitona é composta por uma semente, envolvida por um endocarpo lenhoso, além de mesocarpo e epicarpo, que podem mudar de coloração de acordo com o estágio de maturação (VIEIRA NETO *et al.*, 2008).

Apesar de atualmente terem sido catalogados centenas de cultivares de oliveiras, apenas algumas dezenas são apropriadas para produção em larga escala (APARICIO; LUNA, 2002; IVALSA, 2020). De acordo com o Cadastro Olivícola do Rio Grande do Sul 2022, os cultivares mais plantados foram Arbequina, Koroneiki, Picual, Arbosana e Frantoio (AMBROSINI *et al*., 2022).

3.2 O cultivar Arbequina

O cultivar Arbequina possui origem espanhola, na região da Catalunha, na província de Lérida, sendo que seu nome provém de Arbeca, um município de tal localidade (CRIADO *et al*., 2004). Nos últimos anos o cultivo de Arbequina se estendeu a outras regiões da Espanha, como Andaluzia e Aragón, além de outros países do mundo, incluindo o Brasil (ROMERO *et al*., 1997). Tal cultivar é utilizado para a produção de azeite, altamente apreciado em todo o mundo, sendo frutado, doce, ligeiramente pungente, com coloração amarelada (APARICIO; LUNA, 2002) e com baixo índice de amargor (FRANCO *et al*., 2015).

Em 2000 a área cultivada de Arbequina nas regiões de Lérida, Tarragona e Córdoba, na Espanha, era de 91.000 ha e sua utilização em olivais tem aumentado em função de sua precocidade produtiva, baixo vigor e alto conteúdo de azeite de boa qualidade (BARRANCO; RALLO, 2000). Como desvantagens estão a sensibilidade a certas pragas e doenças e a baixa estabilidade do óleo; com isso recomenda-se a mistura do azeite (blend) com outros cultivares e o plantio intercalado em linhas entre cultivares, devido a auto-incompatibilidade (SÁNCHEZ-ESTRADA; CUEVAS, 2018; IVALSA, 2020).

O plantio do cultivar Arbequina foi amplamente disseminado no Brasil, favorecido pela sua adaptação a diferentes condições ambientais, sendo o cultivar mais plantado no estado do Rio Grande do Sul, presente em 96% dos olivais

gaúchos (AMBROSINI *et al*., 2022) e com grande expressividade nos olivais da região da Serra da Mantiqueira em Minas Gerais (SILVA *et al,.* 2012). Os municípios de Caçapava do Sul e São Gabriel, localizados no estado do Rio Grande do Sul, apresentaram 176 ha (14 produtores) e 229 ha (cinco produtores) de olivicultura, respectivamente (AMBROSINI *et al*., 2022).

3.3 Olivicultura no Brasil

O cultivo de oliveiras no país remonta ao período colonial, contudo apenas nas últimas décadas tem se observado o aumento da olivicultura para a produção de azeite e azeitona, em especial nas regiões Sul e Sudeste (MESQUITA; GARCIA; COSTA*,* 2012). O estado do Rio Grande do Sul é o maior produtor de azeite e possui cerca de 6.000 ha de área plantada, seguido da região da Serra da Mantiqueira, com 3.000 ha, a qual engloba os estados de Minas Gerais, São Paulo e Rio de Janeiro (IBRAOLIVA, 2022). O crescimento da olivicultura no país tem sido motivado pela criação de indústrias para obtenção do azeite (lagares), pela produção de mudas nacionais de qualidade, pelas recentes premiações dos azeites produzidos nacionalmente e pelos incentivos de financiamentos governamentais (IBRAOLIVA, 2020; AMBROSINI *et al*., 2022; JOÃO, 2022).

A expansão da olivicultura no Rio Grande do Sul concentra-se principalmente na metade centro-sul do estado. Tal expansão vem de encontro à necessidade de diversificação da produção agrícola da região, aliando geração de renda, aumento da oferta de empregos e sustentabilidade no uso dos recursos naturais do Pampa, fortalecendo assim o desenvolvimento regional. De acordo com o Zoneamento Agroclimático e Edafoclimático, o Rio Grande do Sul possui cerca de 7,4 milhões de hectares como área recomendável para a olivicultura, sendo que parte da região centro-sul do estado se enquadra em tal categoria (WREGE *et al.*, 2009; ALBA; FLORES; WREGE, 2013).

Apesar das perspectivas de aumento do cultivo da oliveira, as diferenças de clima e solo do sul do Brasil em comparação à região do Mediterrâneo, fazem com que haja uma grande necessidade de pesquisas em relação ao manejo da cultura, com o intuito de adaptar as práticas culturais às nossas condições (cultivares adaptados, controle de pragas e doenças, podas, adubação e calagem, entre outras).

3.4 Fatores climáticos, fenologia e bianualidade

A oliveira é uma espécie de clima temperado, presente em regiões áridas ou semi-áridas em seus locais de origem, com baixa pluviosidade, ao contrário das regiões subtropicais e tropicais de altitude do Brasil. Ressalta-se que para o desenvolvimento adequado e boa produtividade na olivicultura é necessário atentar às suas exigências climáticas. Algumas pesquisas indicaram que oliveiras requerem um determinado número de horas de frio para a quebra de dormência das gemas e/ou indução da floração (RALLO; MARTINS, 1991; HABERMAN *et al.*, 2017). Estudo desenvolvido por dez anos em olivais no Uruguai revelou grande variabilidade da temperatura interanual, além de elevada umidade e precipitação na primavera, prejudicando a floração, sendo fatores limitantes para a olivicultura em regiões temperadas úmidas (CONDE-INNAMORATO *et al.*, 2019). No Hemisfério Sul observou-se que em vários locais não há um número suficiente de horas de frio no inverno para a floração de alguns cultivares, como Frantoio e Leccino (TORRES *et al.*, 2017).

A avaliação da fenologia, por meio do estudo dos períodos de desenvolvimento vegetativo (brotação), floração e frutificação está intrinsecamente associada com a produção, sendo norteador para as medidas de manejo, como podas e adubações. Estudos sobre a fenologia de oliveiras ainda são escassos no Brasil. Os cultivares Arbequina e Koroneiki foram avaliados em 2008 e 2009 no Rio Grande do Sul e identificou-se o início da floração entre 16-20 de outubro e término entre 09-11 de novembro, com duração de 27 e 15 dias, respectivamente, exibindo sincronia entre os dois cultivares e pequena variação intra-anual, associada à pluviosidade (CAPPELLARO, 2010). Já no Uruguai pesquisas com seis cultivares de oliveiras, durante dez anos, identificaram que o período de brotação ocorreu no inverno, entre a primeira semana de agosto e a segunda semana de setembro, enquanto que a floração ocorreu entre o final de setembro e final de novembro, com variação ao longo dos anos, associada principalmente a diferenças de temperatura (CONDE-INNAMORATO *et al.*, 2019).

A oliveira apresenta bianualidade com alternância entre anos de alta produção (*on*) e baixa produção (*off*). A alternância apresenta grande variabilidade entre cultivares, como alguns cultivares sicilianos na Itália e Barnea cv. no Uruguai,

os quais exibiram índice de alternância muito elevado, podendo até inviabilizar sua produção em larga escala (MARINO *et al*., 2017; CONDE-INNAMORATO *et al.*, 2019). Em função da bianualidade a produção de azeitonas pode variar de 5-30 t/ha e a produção em um olival com alternância de produção é 10-20 % menor do que um olival com uniformidade de produção, considerando um período de dois anos (LAVEE, 2007).

Os mecanismos associados à alternância de produção a nível individual são regulados por fatores endógenos, enquanto a bianualidade é sincronizada a nível populacional ou regional em função de fatores ambientais (LAVEE, 2007). Com relação aos fatores endógenos, observou-se redução de fitoquímicos, diminuição da ação de enzimas antioxidantes e hormônios (ácido abscísico) em oliveiras no ano *off*, além de menor produção, comparado com o ano *on* (BENJEDDOU; AHMED; ROUINA, 2019). Haberman *et al.* (2017) sugerem a existência de uma 'memória bioquímica', em que a carga de frutos do verão pode afetar a expressão de genes relacionados à indução floral na primavera seguinte. Condições ambientais extremas, como estresse térmico e hídrico podem induzir a alternância de produção, como por exemplo, a alta precipitação, a qual pode diminuir a frutificação e estimular o crescimento vegetativo, resultando em uma superprodução no ano seguinte (LAVEE, 2007).

Em geral, oliveiras jovens nos primeiros três a quatro anos de produção não exibem alternância de produção em sistemas produtivos bem manejados, contudo após este período elas passam a alternar a produção, principalmente em sistemas que não realizem manejos periódicos. Dentre as condições que auxiliam na produção mais uniforme de azeitonas incluem-se as podas, desbaste de frutos em anos *on*, aplicação de hormônios sintéticos, evitar a colheita tardia, assim como garantir o suprimento adequado de nutrientes e irrigação (LAVEE, 2007). A adubação também influenciou a alternância produtiva, de modo que oliveiras com menores níveis de N foram mais suscetíveis à bianualidade (HABERMAN *et al.*, 2019).

3.5 Solo e fertilidade

Além dos fatores climáticos, as características da maioria dos solos brasileiros são discrepantes da região de origem da oliveira. Na região mediterrânea em geral

os solos são alcalinos (FERNÁNDEZ-ESCOBAR; MORENO; GARCIA-CREUS, 1999). Já no Brasil, são comuns os solos ácidos sendo, portanto recomendada a calagem antes do plantio para que o pH se mantenha próximo a 6,5 (MESQUITA; GARCIA; COSTA, 2012; TIECHER *et al*., 2020).

Os nutrientes utilizados convencionalmente em programas de fertilização para oliveiras são o N, P, K e B (LÓPEZ-GRANADOS *et al*., 2004), além do cálcio (Ca) e magnésio (Mg) fornecidos através da calagem (TIECHER *et al*., 2020). Os primeiros estudos publicados sobre nutrição de oliveiras datam dos anos 40 e 50, com avaliação dos efeitos da adubação e identificação de sintomas de deficiência nutricional de N, P, K, Mg e B em olivais na Califórnia (HARTMANN; BROWN, 1953). A partir dos anos 1960-90, com base em análises foliares, seguindo critérios agronômicos tradicionais, foram determinados os níveis de nutrientes para nortear programas de fertilização (FERNÁNDEZ-ESCOBAR; MORENO; GARCIA-CREUS, 1999). Tais níveis de nutrientes também têm sido utilizados como valores de referência no Brasil (MESQUITA; GARCIA; COSTA, 2012; CQFS-RS/SC, 2016; TIECHER *et al*., 2020) (Tabela 1).

Tabela 1. Níveis de nutrientes na matéria seca de folhas de oliveiras amostradas em janeiro (verão), referência para o Brasil. Adaptado de MESQUITA; GARCIA; COSTA (2012); CQFS-RS/SC (2016).

Elemento	Deficiente	Adequado	Tóxico
Nitrogênio (%)	<1,4	1,5-2,0	-
Fósforo (%)	<0,05	0,1-0,3	-
Potássio (%)	<0,4	0,8-1,2	-
Cálcio (%)	<0,3	> 1	-
Magnésio (%)	<0,08	0,1-0,3	-
Boro (ppm)	<14	19-150	> 185
Manganês (ppm)	-	> 20	-
Zinco (ppm)	-	> 10	-
Cobre (ppm)	-	> 4	-
Sódio (%)	-	-	> 0,2
Cloro (%)	-	-	> 0,5

A seguir são apresentadas informações mais detalhadas sobre os nutrientes N, P, K e B, comentando sobre suas funções nos vegetais, assim como os efeitos da deficiência, excesso e adubação para oliveiras.

3.5.1 Nitrogênio

O N é componente de aminoácidos, nucleotídeos, clorofila entre outras moléculas (RAVEN; EVERT; EICHHORN, 2001). Os íons nitrato ($NO3^-$) e amônio ($NH4^+$), presentes na solução do solo são absorvidos pelas raízes (LAWLOR; LEMAIRE; GASTAL., 2001). Nas plantas o íon nitrato após a passagem na membrana plasmática tem seu metabolismo regulado pelas mitocôndrias e cloroplastos, interagindo com os processos de fotossíntese e respiração celular (SZAL; PODGÓRSKA, 2012). O N é componente de proteínas estruturais e enzimas, em especial em regiões de crescimento, regulando a expansão e divisão celular, que se reflete em maior escala em aumento de biomassa e crescimento vegetal, assim como na produção dos cultivos (LAWLOR; LEMAIRE; GASTAL., 2001).

Em estudos que avaliaram a omissão de nutrientes para oliveiras, em geral o N é um dos primeiros nutrientes a apresentar sintomas visuais de deficiência. As folhas velhas adquirem um tom pálido, seguido de clorose (amarelamento) por toda a folha. Posteriormente, observou-se uma redução do crescimento dos sistemas aéreo e radicular (BOUSSADIA *et al*., 2010; NAIJA *et al*., 2014; FERNÁNDEZ-ESCOBAR *et al*., 2016; SOUZA *et al*., 2019). Baixos níveis de N foliar afetaram negativamente a fotossíntese, como a redução dos níveis de clorofila e da taxa máxima foliar de assimilação de luminosidade (BOUSSADIA *et al*., 2010). A deficiência de N também foi associada ao aumento nos níveis de carboidratos como amido, glucose, manitol e sucrose, na parte aérea e radicular (BOUSSADIA *et al*., 2010; NAIJA *et al*., 2014). A ausência de adubação nitrogenada pode levar uma redução significativa na produção, sendo recomendada a aplicação anual de nutrientes móveis em olivais sem irrigação e que realizam adubação via solo, a fim de manter os níveis adequados de tais nutrientes para o suprimento do cultivo (RODRIGUES *et al.*, 2011).

Para oliveiras a adubação com N, dependendo das doses do nutriente, pode ser vantajosa para o crescimento vegetativo, desenvolvimento reprodutivo e produção de azeite (JASROTIA *et al*., 1999; ROSATI; CAPORALI; PAOLETTI, 2015). A resposta a adubação com N em termos do aumento da produtividade dos frutos pode estar associada com a menor disponibilidade de N mineral no solo (BOUHAFA *et al*., 2014). Haberman *et al.* (2019) identificaram que oliveiras com

deficiência em N apresentaram menor crescimento, intensidade de floração, carga de frutos, percentual de flores perfeitas e produtividade dos frutos em quatro safras, comparado com os tratamentos com maiores doses de N, destacando-se o tratamento com aplicação de 150 kg N. ha^{-1}. Já Connell *et al.* (2002) não observaram influência da adubação com nitrogênio foliar para o crescimento vegetativo, tamanho dos frutos e produção.

Assim como a escassez de N acarreta prejuízos para a oliveira, o excesso deste nutriente pode ser tão ou mais danoso ainda, promovendo perdas qualitativas e quantitativas na produção. A adubação com N em doses intermediárias podem favorecer a produção de frutos, contudo em doses mais elevadas podem levar a uma redução da floração, diminuindo a frutificação em 50% e carga de frutos (EREL *et al.* 2008, 2013). A adubação em excesso com N pode causar impacto negativo no crescimento radicular, morfometria dos ramos e na floração, além dos aspectos produtivos dos frutos e azeite (FERNÁNDEZ-ESCOBAR; MARÍN, 1999; FERNÁNDEZ-ESCOBAR *et al*., 2012; FERNÁNDEZ-ESCOBAR *et al*., 2014; OTHMAN; LESKOVAR, 2019). O maior crescimento vegetativo pelo excesso de N implica em maior exigência de podas, aumentando os gastos econômicos. Elevadas doses de N podem afetar negativamente a qualidade do azeite, com a redução dos níveis de compostos fenólicos (CENTENO *et al.*, 2017) e aumento dos índices de peróxidos (BOUHAFA *et al*., 2014).

Na Espanha, o excesso na fertilização com N é reportado como sendo recorrente em olivais, com a aplicação de 80-200 kg $N.ha^{-1}$, muitas vezes sem considerar as reservas de N no solo e a avaliação foliar (FERNÁNDEZ-ESCOBAR, 2011). Além disso, com base em uma série de estudos em olivais no país, tem se proposto alterações nos índices de referência de nitrogênio foliar, de modo que a deficiência estaria em 1,22-1,35% ao invés de 1,4%, enquanto que o excesso de nitrogênio estaria acima de 1,7 % em vez de 2,0% (MOLINA-SORIA; FERNÁNDEZ-ESCOBAR, 2012).

Considerando a situação dos olivais brasileiros, mais especificamente no estado do Rio Grande do Sul, Bender *et al.* (2018) avaliaram 13 olivais e verificaram que 58% das amostras de folhas apresentavam níveis de N altos. Como a maior parte destes olivais apresentou solo com baixo teor de matéria orgânica (64%), os autores recomendaram que a adubação nitrogenada seja realizada com mais parcimônia, com maiores doses apenas em condições de deficiência. Figueiredo *et*

al. (2022) em estudo na mesma região encontraram níveis altos de N nas folhas em 30 % e 58 % das amostras, no inverno e verão, respectivamente, e sugere o monitoramento periódico no teor de N para evitar adubação excessiva, a qual poderia comprometer os processos reprodutivos e de produção.

Os manuais de recomendação de adubação para oliveiras para o sul do Brasil indicam a realização de adubação de manutenção de 16 kg N. ha^{-1} para cada tonelada de fruto colhido, em função da exportação de nutrientes pelos frutos e pelos resíduos das podas, assim como as perdas por lixiviação e volatilização (CQFS-RS/SC, 2016; TIECHER *et al*., 2020). Estudos na Espanha indicaram que a exportação anual média de nitrogênio pelos frutos e poda foi de 54,4 Kg. ha^{-1} (FERNÁNDEZ-ESCOBAR *et al*., 2015). Sugere-se que a adubação com nitrogênio seja realizada em três etapas, sendo a primeira (50%) no período da floração; a segunda (25%) cerca de 40 dias após a floração plena e a terceira (25%) após a colheita (TIECHER *et al*., 2020). No entanto, nas condições edafoclimáticas do RS, as épocas de aplicação de N, as doses recomendadas e os limites ideais dos teores de N foliar carecem de mais trabalhos de pesquisa para o ajuste fino do sistema de recomendação.

3.5.2 Fósforo

O P é um elemento essencial na fotossíntese, respiração e divisão celular, compondo moléculas importantes como o ATP e ADP, ácidos nucleicos, coenzimas e fosfolipídios (RAVEN; EVERT; EICHHORN, 2001; RAUSCH; BUSCHER, 2002). Também colabora no desenvolvimento dos tecidos meristemáticos e outras atividades metabólicas do vegetal (MESQUITA; GARCIA; COSTA, 2012).

A deficiência de P em oliveiras pode ser identificada visualmente através de redução do tamanho foliar, enrugamento das folhas mais velhas juntamente com leve clorose, que se torna gradualmente avermelhada até a queda das folhas. Também se observou perda de crescimento dos ramos, crescimento em altura reduzido, além de baixa relação parte aérea/raízes em comparação com plantas nutridas (HARTMANN; BROWN, 1953; JIMÉNEZ-MORENO; FERNÁNDEZ-ESCOBAR, 2016; SOUZA *et al*., 2019). Mesmo o P sendo determinante para o crescimento radicular, o impacto da carência deste elemento pode variar de acordo com o cultivar, como demonstrado por Naija *et al.* (2014), em que Chetoui cv. exibiu

crescimento radicular similar ao controle, enquanto Meski cv. apresentou apenas 10,67 % de crescimento das raízes em relação ao controle.

Apesar de sua importância para as plantas, deve-se considerar que as reservas de P, as quais se concentram em rochas de fosfato, é um recurso não renovável, com longevidade das reservas estimada em 300 anos e localização restrita, já que cerca de 81% das reservas se concentram em apenas dois países (Marrocos e China) (VAN KAUWENBERGH *et al*., 2013; CHOWDHURY *et al.,* 2017). O uso excessivo de adubação fosfatada pelo setor agropecuário tem gerado uma série de problemas ambientais, impactando não apenas os locais de cultivo e criação de animais, mas outros sistemas, sendo seus efeitos documentos em vários locais do mundo, destacando-se a eutrofização, a qual impacta negativamente ecossistemas aquáticos (CHOWDHURY *et al.,* 2017).

A aplicação de adubação fosfatada em oliveiras propiciou aumento da intensidade de floração, assim como da carga de frutos e número de frutos/árvore, (EREL *et al.* 2008). Também foi observado aumento da produção de frutos com adubação foliar com P para Arbequina cv., mas não para Picual cv. (CENTENO; GÓMEZ-DEL-CAMPO, 2011). A aplicação de H_3PO_4 promoveu aumento do conteúdo de P especialmente nas raízes, em oliveiras em casa de vegetação, entretanto os autores sugeriram que o baixo requerimento de P para oliveiras pode ser conseqüência da habilidade que plantas perenes lenhosas possuem em reusar o P e a baixa remoção do mesmo (JIMÉNEZ-MORENO; FERNÁNDEZ-ESCOBAR, 2016). Outro estudo não verificou efeito da adubação com P para oliveiras, em relação à produção de frutos, parâmetros biométricos dos frutos, assim como crescimento do tronco e copa (FERREIRA *et al*., 2018a) .

A simbiose entre o sistema radicular e fungos arbusculares micorrízicos (FAM) tem sido identificada como um dos fatores que aumentaram a eficiência na captação de P para oliveiras, de modo que plantas inoculadas exibiram 173% maior crescimento aéreo e 136% maior peso das raízes, comparadas com plantas não inoculadas (GANZ; KAILIS; ABBOTT, 2002). Oliveiras inoculadas também apresentaram maior conteúdo de P nas raízes e sistema aéreo (DAG *et al*., 2009). O estudo e posterior utilização de espécies nativas de FAM encontradas em olivais são promissores na produção de inoculantes para mudas comerciais (CALVENTE *et al*., 2004).

No caso de olivais no sul do Brasil, Bender *et al.* (2018) encontraram baixos teores de P no solo (49%), contudo a maior parte das amostras de folhas (76%) se enquadraram no nível adequado. Os autores sugerem que a oliveira seja pouco exigente ao P, sendo neste caso mais similar a essências florestais do que as frutíferas em geral. Em olival em Portugal, Ferreira *et al.* (2018a) também observaram teores de P no solo abaixo do nível crítico (87%), entretanto 83% das amostras de folhas exibiram níveis adequados de tal nutriente. Em ambos os trabalhos, os autores comentam a necessidade de ajustes do método analítico de amostragem de P no solo, que pode estar sendo subestimado.

A aplicação de P é recorrente em programas de fertilização, contudo sua quantidade tem sido questionada, em função da baixa resposta de oliveiras com tal adubação (JIMÉNEZ-MORENO; FERNÁNDEZ-ESCOBAR, 2016; FERREIRA *et al*., 2018a; FERNÁNDEZ-ESCOBAR, 2019). No Brasil a recomendação de adubação de manutenção com P é de 4 kg P_2O_5 para cada tonelada de frutos colhidos, bem mais moderada do que a adubação recomendada para Portugal, que é de 40-60 kg P_2O_5. ha^{-1}. ano (FERREIRA *et al*., 2018a). A adubação fosfatada proposta no Brasil visa suprir a exportação nos frutos e podas, avaliada em 6,87 kg. ha^{-1}. ano em olivais na Espanha e 1,75 kg. ha^{-1}. ano em olival em Portugal (RODRIGUES *et al.*, 2012; FERNÁNDEZ-ESCOBAR *et al*., 2015). Especula-se que o suprimento de P pelo solo em condições do sul do Brasil seja beneficiada pela maior disponibilidade hídrica, justificando que nos plantios gaúchos a necessidade de fertilização com P seja menor do que na região Mediterrânea. No entanto, pela baixa mobilidade do P no solo, a recomendação é de que seja feita a adubação de correção deste elemento na implantação do pomar de oliveiras, incorporando-o na camada arável junto à prática da calagem (CQFS-RS/SC, 2016).

3.5.3 Potássio

O K é essencial na regulação metabólica em plantas. Tal elemento participa dos processos de osmose, balanço iônico, controle da polarização da membrana, regulação do pH citoplasmático, ativador de diversas enzimas, colabora na síntese de proteínas e amido, atua no funcionamento e estrutura de cloroplastos, abertura e fechamento dos estômatos, tropismos, assim como no transporte de solutos pelo floema (RAVEN; EVERT; EICHHORN, 2001; SHABALA; POTTOSIN, 2014). O K

também é fundamental no aumento da tolerância ao estresse abiótico, em especial em situações de estresse hídrico (seca e alagamento), salinidade, estresse pelo frio e congelamento, elevada luminosidade, toxicidade por amônio, além de estresse biótico, participando da defesa contra patógenos (SHABALA; POTTOSIN, 2014; ZÖRB; SENBAYRAM; PEITER, 2014).

A escassez de potássio em oliveiras pode ser percebida visualmente nas folhas mais velhas, com sintomas de clorose no ápice e margem das folhas. Quando tal deficiência nutricional torna-se mais severa, principalmente aliada com períodos de seca, a clorose se torna necrose (FERNÁNDEZ-ESCOBAR *et al*., 2016; EREL, *et al*., 2017; SOUZA *et al*., 2019). A privação de K também pode ocasionar um desenvolvimento reduzido do sistema radicular, limitando a absorção de água e nutrientes (NAIJA *et al*., 2014). Estudos com oliveiras submetidas à omissão de K, juntamente com déficit de irrigação, indicaram danos no mecanismo de regulação dos estômatos (ARQUERO; BARRANCO; BENLLOCH, 2006; BENLLOCH-GONZÁLEZ *et al.,* 2008). Oliveiras jovens irrigadas e supridas adequadamente com K exibiram maior crescimento radicular e aéreo, do que plantas com menor adubação com K e com restrição hídrica. Tais plantas também exibiram prejuízo no mecanismo de fechamento estomático, sendo mais suscetíveis à desidratação (ARQUERO; BARRANCO; BENLLOCH, 2006). Oliveiras sob estresse hídrico e nutricional pela omissão de K apresentaram menor comprimento e peso fresco dos ramos, menor transpiração foliar, menor condutância dos estômatos do que plantas controles, sendo observadas diferenças entre cultivares com relação a tolerância sob tais condições restritivas (BENLLOCH-GONZÁLEZ *et al*., 2008).

A deficiência de K é recorrente em olivais, contudo a adubação potássica freqüentemente tem sido negligenciada pelos produtores (FERNÁNDEZ-ESCOBAR, 2019), uma vez que a resposta a adubação com K pode ser reduzida em oliveiras (FERREIRA *et al*., 2018b). Em um estudo, a aplicação de 500 g K_2O por oliveira garantiu um valor ótimo de produção (JASROTIA *et al.,* 1999), enquanto que em outro trabalho a adubação com K aumentou a intensidade de floração, mas não foi correlacionada com a carga de frutos e o número de frutos/árvore (EREL *et al*., 2008). A aplicação de KCl propiciou um aumento da concentração de K no solo e nos tecidos, assim como na relação parte aérea/raízes, indicando que o sistema aéreo é prioritário no armazenamento de tal macronutriente. Contudo, a matéria seca dos tecidos, assim como parâmetros fotossintéticos, como condutância dos

estômatos não diferiram entre plantas adubadas e não adubadas (FERREIRA *et al.*, 2018b).

A utilização de diferentes fontes de K na adubação pode gerar diferentes respostas em oliveiras. Em estudo com adubação utilizando KCl, KNO_3 e K_2SO_4 observou-se os níveis de N nas folhas foram maiores no tratamento com K_2SO_4, enquanto que a aplicação de KNO_3 propiciou aumento dos níveis de K em diferentes tecidos, principalmente com três aplicações. Já o peso fresco e seco das plantas não diferiu significativamente com os tratamentos, assim como o conteúdo de cloro (Cl), ferro (Fe) e zinco (Zn) (SAYKHUL *et al.*, 2014). Em outro trabalho observou-se que adubação com KCl e K_2SO_4 propiciaram aumento nos níveis de K nas folhas e nos frutos, mas não foram observadas diferenças entre a aplicação no solo e foliar (RESTREPO-DIAZ *et al.*, 2008).

Em oliveiras tem se verificado elevada exportação anual de K pelos frutos e podas, avaliada em 4,42 g. kg^{-1} MF e 1,98 g. kg^{-1} MF, respectivamente, totalizando 45,5 kg. ha^{-1}. Ano, para Picual cv. (FERNÁNDEZ-ESCOBAR *et al*., 2015). Em geral os estudos sobre exportação de nutrientes apresentam os resultados em massa seca (MS), de modo que para comparação utilizou-se os valores médios de umidade dos frutos de 56,03 % para o cultivar Picual, em estudo conduzido na Espanha (FRANCO *et al.*, 2015). Com isso, supõe-se que os valores de exportação de K obtidos por Fernández-Escobar *et al*. (2015) foram de 1,94 g. kg^{-1} MS de fruto. Rodrigues *et al.* (2012) encontraram valores de exportação de K de 6,83 g. kg^{-1} MS para a polpa e de 2,03 g. kg^{-1} MS para o caroço de oliveiras do cultivar Cobrançosa em Portugal.

No sul do Brasil, Bender *et al.* (2018) encontraram exportação de K de 12,8 g. kg^{-1} MS de fruto. Nos estados do sul do Brasil recomenda-se a aplicação de 20 kg de K_2O para cada tonelada de frutos colhidos (CQFS-RS/SC, 2016). Deve-se levar em conta as análises foliares, assim como o tipo de solo, já que vários solos onde atualmente se encontram os olivais do RS foram formados a partir de rochas ricas em K, portanto nesses casos a necessidade de adubação potássica é naturalmente menor (TIECHER *et al*., 2020). Além disso, deve-se considerar que os níveis de pluviosidade no sul do Brasil são bem maiores do que nas regiões mediterrâneas, caracterizadas por clima semi-árido a árido. Sendo assim, há maior disponibilidade de água para compor a solução do solo e o suprimento nutricional, de modo que em

alguns casos apenas a adubação do solo satisfaz a demanda de tal nutriente, sem a necessidade de adubação foliar, tornando a fertilização mais econômica.

3.5.4 Boro

O B atua no metabolismo primário e secundário em plantas, participando da estrutura da parede celular, manutenção da integridade da membrana celular, influenciando a utilização de íons Ca^{2+} e a síntese de ácidos nucléicos (RAVEN; EVERT; EICHHORN, 2001). Além da sua importância primária na composição da parede celular, atuando no crescimento dos tecidos, tem se verificado que o B desempenha um papel fundamental na germinação do pólen para uma série de espécies vegetais, assim como na formação do tubo polínico e frutificação (BLEVINS; LUKASZEWSKI, 1998). Supõe-se que para a formação do tubo polínico seja necessária a utilização de moléculas com material proveniente tanto das partes florais femininas, como de proteínas dos grãos de pólen, ressaltando a importância de complexos de borato com resíduos de açúcares, os quais propiciam o rápido crescimento do tubo polínico (BLEVINS; LUKASZEWSKI, 1998). Um gradiente de B nos tubos polínicos foi observado para *Petunia*, com aumento na concentração do estilete para o ovário, de modo que o microelemento pode agir como um agente quimiotático, afetando o crescimento de tecidos reprodutivos (ROBBERTSE *et al.,*1990).

Devido à ênfase da presente tese em relação à nutrição de oliveiras com o elemento B, maiores detalhes serão apresentados em relação à dinâmica deste nutriente nas seções abaixo.

3.5.4.1 Mobilidade e armazenamento

Em função de sua importância na formação da estrutura da parede celular, reprodução e outros processos fisiológicos em plantas, o B necessita ser continuamente absorvido pelas raízes e translocado para os tecidos vegetais através do sistema vascular. Na solução do solo o B é encontrado predominantemente como ácido bórico (H_3BO_3), portanto em uma forma não iônica. Em condições de pH acima de 7,0, há também a presença do ânion borato $B(OH)_4^-$, o qual passa a predominar

em pH alcalino (KEREN; BINGHAM, 1985). Inicialmente se acreditava que o transporte passivo do ácido bórico através da membrana celular era o único mecanismo de entrada de B pelas raízes das plantas (RAVEN, 1980). Contudo, posteriormente verificou-se que ácido bórico apresentava baixa permeabilidade pela membrana (DORDAS; BROWN*,* 2000; STANGOULIS *et al.,* 2001), sendo necessário um sistema de transporte ativo, principalmente em situações de deficiência do micronutriente (DORDAS; BROWN*,* 2001; DANNEL; PFEFFER; RÖMHELD*.,* 2002). Análises de plantas mutantes de *Arabidopsis*, *BOR1-1 (High Boron Requiring)*, sensíveis à deficiência de B, permitiram identificar a presença de *BOR1*, molécula transportadora de B localizada nas células do periciclo, mediando o carregamento para o xilema de tal nutriente (NOGUCHI *et al.,*1997; TAKANO *et al.,* 2002).

Posteriormente identificou-se a proteína *NIP5;1*, a qual atua como um canal seletivo na membrana plasmática, seletivo principalmente ao ácido bórico e à água em menor escala, exibindo maior expressão do gene que traduz tal proteína, em situações de deficiência de B (TAKANO *et al.*, 2006). Também identificou-se a proteína *NIP6;1,* transportadora de ácido bórico, mas impermeável à água, presente em ramos, principalmente jovens (TANAKA *et al.,* 2008). Tanto *NIP5;1* como NIP6;1 pertencem à família *Major Intrinsic Proteins (MIP)* e sub-família *Nodulin-26-like Intrinsic Protein (NIP)*, cuja função é atuar como canais seletivos de moléculas pequenas sem carga e água, no caso de *NIP5;1* (TYERMAN; NIEMIETZ; BRAMLEY, 2002). As *NIPs* se constituem em um grupo altamente conservado, sendo único para plantas (WALLACE; CHOI; ROBERTS, 2006). Em estudos com clonagem do gene *CiNIP5* em cítricos avaliou-se a expressão gênica sob condição de estresse nutricional de B, concluindo que o gene atuou de forma similar ao *AtNIP5;1*, sendo expresso principalmente nas raízes, induzida sob a deficiência com B, mas reduzida em condições de toxidez, sugerindo-se a homologia entre os genes citados (AN *et al.*, 2012).

Uma vez no xilema, o B é translocado para o floema, se acumulando em pontos de crescimento nos sistemas vegetativos e reprodutivos (BLEVINS; LUKASZEWSKI, 1998). Para oliveiras observou-se maior concentração de B em órgãos reprodutivos do que em vegetativos (PERICA *et al.,* 2001b; HEGAZI *et al.,* 2018), estando de acordo com a suposição de que a reprodução demanda mais B do que o crescimento vegetativo, ou ainda de que as estruturas reprodutivas armazenam maior quantidade do micronutriente (DELL; HUANG, 1997). Também

verificou-se que durante o período de antese o conteúdo de B em folhas jovens de oliveiras diminuiu drasticamente, evidenciando mobilização de B das folhas para atender a demanda deste nutriente para flores e frutos (DELGADO; BENLLOCH; FERNÁNDEZ-ESCOBAR, 1994). A retranslocação de B pelo floema tem sido associada à formação de um complexo de B com moléculas transportadoras de açúcares ou polióis (BROWN; SHELP, 1997; BLEVINS; LUKASZEWSKI, 1998). Para oliveiras, o manitol e a glicose são os principais carboidratos translocados pelo floema, sendo produtos primários da fotossíntese (CATALDI *et al.*, 2000). Além disso, o manitol tem sido identificado como o carboidrato com maior acúmulo nos tecidos em resposta ao estresse hídrico e salino, seguido do myo-inositol e do sorbitol (CONDE *et al*., 2007; DICHIO *et al*., 2009; MECHRI *et al*., 2015).

3.5.4.2 Deficiência e toxidez

A oliveira é considerada uma espécie indicadora de solos com baixa disponibilidade de B, apresentando sintomas visuais de deficiência quando o requerimento de tal micronutriente não é adequado, principalmente em solos ácidos, rasos e erodidos (TSADILAS; CHARTZOULAKIS, 1999). Em regiões tropicais e chuvosas, o solo tende a apresentar menores teores de B em função da grande mobilidade do nutriente no solo, sendo acentuado em solos altamente intemperizados, arenosos e com baixo teor de matéria orgânica (MESQUITA; GARCIA; COSTA, 2012).

Os sintomas visuais de deficiência de B em oliveiras, em geral são observados em folhas jovens e incluem formação de folhas pequenas, distorcidas, com clorose apical; queda das folhas e morte nos pontos de crescimento; folhas novas em formato de rosetas; crescimento lateral e ramificações múltiplas; espessamento e crescimento irregular do sistema radicular ou prejuízos na emissão de novas raízes; necrose do câmbio; frutos com desenvolvimento irregular com partes marrons, conhecidas como cara-de-macaco e queda prematura dos mesmos (FERNÁNDEZ-ESCOBAR *et al.,* 2016; SOUZA *et al.,* 2019; HAIFA, 2020). A deficiência de B também tem sido associada à deficiência de K, Ca e Zn, tornando mais difícil a distinção dos sintomas visuais (TSADILAS; CHARTZOULAKIS, 1999; FERNÁNDEZ-ESCOBAR *et al.,* 2016). Em estudo com omissão de nutrientes com

oliveiras jovens observou-se que B, Ca e N foram os nutrientes que mais afetaram negativamente o crescimento e biomassa das plantas (SOUZA *et al.,* 2019).

A deficiência de B tem sido relacionada com o acúmulo e aumento de compostos fenólicos (LIAKOPOULOS; KARABOURNIOTIS, 2005). Por outro lado, níveis de B acima do recomendado foram associados com a diminuição dos níveis de N e Zn nas folhas, observando-se ainda uma correlação negativa da concentração de B com o número de folhas, diminuição da altura total e comprimento dos ramos laterais, além de declínio da taxa fotossintética para algumas concentrações e cultivares analisados na Grécia (CHATZISSAVVIDIS; THERIOS, 2010).

3.5.4.3 Adubação com boro

Oliveiras possuem alta demanda de B, principalmente no período de diferenciação das gemas e da floração, de modo que no Brasil recomenda-se a aplicação de adubação boratada, caso sejam detectados níveis de deficiência após análise foliares (MESQUITA; GARCIA; COSTA, 2012). Os fertilizantes podem ser granulados ou líquidos a serem aplicados via solo, foliar, na fertirrigação, na forma de nano-quelatos, entre outros (VISHEKAII *et al.*, 2019; HAIFA, 2020). No nosso país tem sido recomendada a adubação em oliveiras com deficiência de B, de 25-40 g B/planta ou adubação foliar de 0,1 % B antes da floração (MESQUITA; GARCIA; COSTA, 2012). Antes da implantação do olival se sugere a aplicação de 2-4 kg B. ha^{-1} (CQFS-RS/SC, 2016).

Estudos que avaliaram a adubação com B para oliveiras têm evidenciado efeitos significativos sobre a fisiologia desta espécie e a produção de frutos e azeite para diferentes cultivares, em diversas regiões do mundo. A aplicação de B (‘Solubor’, 20,5% B foliar, três dias antes da antese) aumentou o conteúdo do nutriente em tecidos vegetativos e reprodutivos para Manzanillo cv. (DELGADO; BENLLOCH; FERNÁNDEZ-ESCOBAR, 1994). Observou-se também que a adubação foliar com B (‘Solubor’ 0-737 mg B. L^{-1}, três semanas antes da antese) propiciou aumento nos níveis de B principalmente nas estruturas reprodutivas, além de acréscimo no percentual de flores perfeitas e carga dos frutos, mas não houve efeito na germinação do pólen e na produção final (PERICA *et al*., 2001a). A adubação com B (‘Solubor’ 300 mg B. L^{-1}, pré-floração e frutificação) em oliveiras

jovens (Arbequina cv.) propiciou maior produção apenas no ano *off*, em resposta a tal adubação, não sendo observadas modificações no rendimento e qualidade do azeite (LARBI *et al.*, 2011).

A aplicação de B (ácido bórico 0,5%, uma semana antes da antese) juntamente com sulfato de zinco em oliveiras adultas (Zard cv.) possibilitou acréscimo de carga de frutos final e na colheita, sendo tal resultado associado principalmente com o efeito da diminuição da abscisão dos frutos (TALAIE; TAHERI, 2001). A adubação com B foliar (ácido bórico, 0-500 mg B. L^{-1}; três aplicações durante a floração) em oliveiras adultas (Frantoio cv.) gerou um aumento da concentração de B, principalmente nas gemas, seguida dos frutos e folhas; clorofila e açúcares solúveis totais; ácido giberélico. Por outro lado, houve diminuição da concentração de fenóis totais, auxina e ácido abscísico (HEGAZI *et al*., 2018). A aplicação de nano-quelatos e ácido bórico (180 e 270 mg B L^{-1}) em oliveiras adultas (Zard cv.) em três períodos (no estágio de inchaço das gemas, antes da floração e após a colheita) propiciou aumento número de flores perfeitas, percentual de carga de frutos, crescimento vegetativo, rendimento do fruto e do azeite, fenóis totais, atividade antioxidante e modificações no perfil de ácidos graxos, principalmente nos tratamentos com ácido bórico (VISHEKAII *et al.*, 2019).

Entretanto, nem sempre tem se observado efeito significativo em oliveiras com a aplicação de B. Ateyyeh e Shatat (2006) avaliaram oliveiras adultas (Rasie cv.), que receberam B foliar (‘Borax’, 0-600 ppm) em quatro períodos: colheita, desenvolvimento de gemas florais, estágio das gemas verdes e antes da plena floração. Os autores encontraram maiores níveis de B em flores e frutos em plantas adubadas, mas não houve diferença entre oliveiras adubadas e não adubadas quanto número de flores perfeitas e pólen, assim como no percentual de germinação e carga de frutos. Em estudo com omissão de B por um período de quatro anos com oliveiras jovens (Verdeal Transmontana cv.) observou-se uma redução gradual nos níveis do micronutriente no solo e folhas, contudo a produção não diferiu com relação às árvores que receberam adubação (‘Borax’, 11% B) (RODRIGUES *et al.*, 2011). A aplicação de B foliar (‘Tradebor’, 11% m/m B-etanolamina) e via solo (‘Borax’, 11% B) aumentou significativamente a concentração de B em todos os experimentos, mas não produziu uma significativa resposta em termos de produtividade (FERREIRA; RODRIGUES; ARROBAS, 2019). Também verificou-se que para Arbequina cv. as folhas mais velhas apresentaram maior concentração de

B do que nas folhas mais jovens no tratamento com B foliar, enquanto que para o Cobrançosa cv. diferenças não foram observadas, indicando que a mobilidade de B em oliveiras depende do cultivar (FERREIRA; RODRIGUES; ARROBAS, 2019).

Com relação ao nível crítico de B para oliveiras observou-se alta correlação entre o teor do nutriente nas folhas e produção, indicando que concentração de 40,8 mg B kg^{-1} nas folhas como o nível crítico deste micronutriente, o que equivale a mais que o dobro do teor mínimo foliar de 19 mg B kg^{-1}, considerado adequado para oliveiras (HEGAZI *et al*., 2018). Esta diferença não necessariamente implica em descrédito ao que vem sendo empregado atualmente, mas pode demonstrar que os níveis críticos tradicionais podem não ser adequados para todas as situações edafoclimáticas e/ou para todas os cultivares de oliveira. Incongruências também podem ser encontradas para níveis críticos de B no solo. Para o sul do Brasil, Bender *et al.* (2018) apontaram um valor de nível crítico de 1,6 mg B. kg^{-1} solo para que as oliveiras pudessem atingir produção de frutos acima de 90% do rendimento relativo, enquanto 0,3 mg B. kg^{-1} solo já é suficiente para a maioria das culturas (CQFS-RS/SC, 2016). Isto evidencia como a oliveira apresenta exigências peculiares e ressalta a necessidade de ajustes no sistema de recomendação de adubação para esta cultura no Brasil.

Em decorrência das evidências sobre a relação entre status nutricional e os níveis de compostos fenólicos em plantas, na seção abaixo são apresentadas informações sobre os polifenóis, sua importância para as oliveiras e para os seus produtos, em especial folhas, azeitonas e azeite.

3.6 Compostos fenólicos

Os compostos fenólicos ou polifenóis são moléculas derivadas do metabolismo secundário de plantas, caracterizadas por um grupo hidroxila ligado a um anel aromático (RAVEN; EVERT; EICHHORN, 2001). As classes de compostos fenólicos presentes em oliveiras incluem ácidos fenólicos, álcoois fenólicos, ligninas, flavonóides e secoiridóides (SERVILI *et al.,* 2004; CHAROENPRASERT; MITCHELL, 2012; BAKHOUCHE *et al.,* 2013). Na Figura 1 é apresentado um esquema resumido da rota metabólica dos compostos fenólicos para oliveiras.

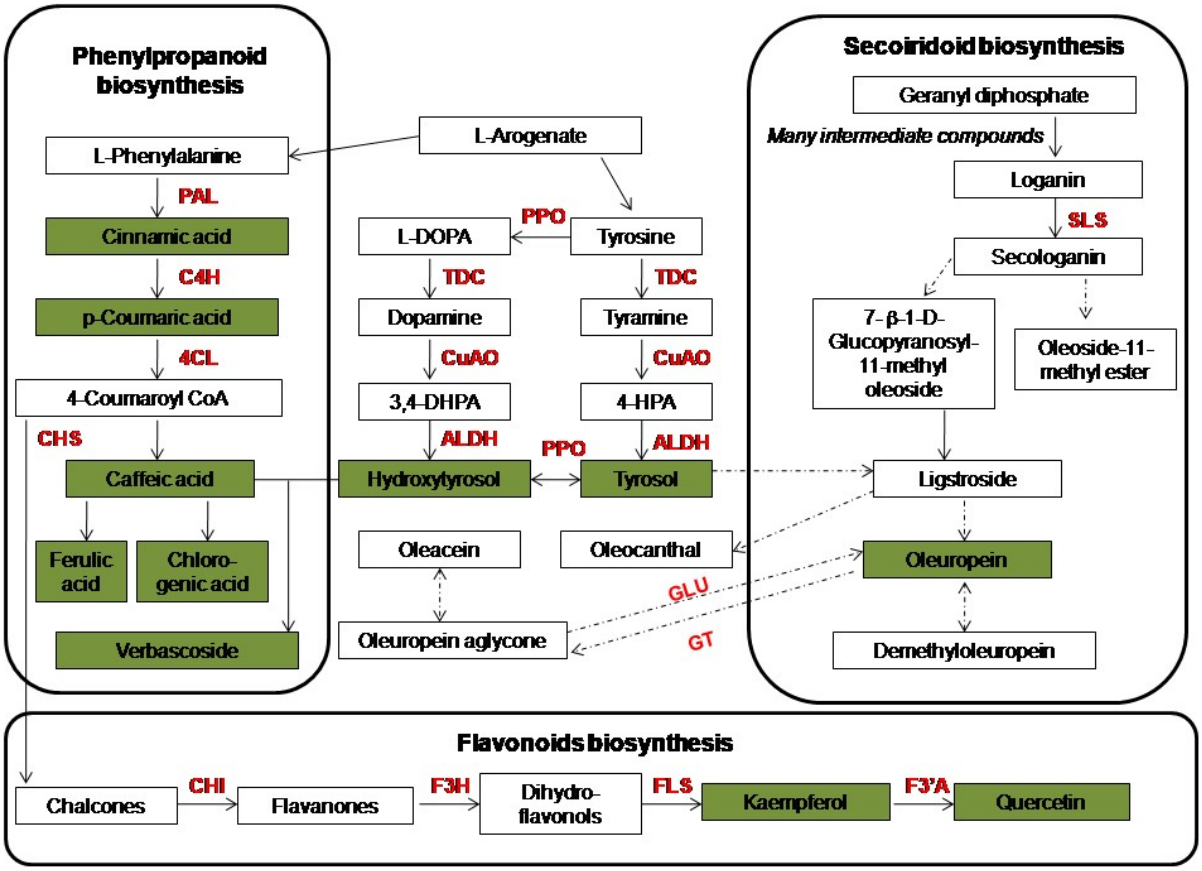


Figura 1. Rota metabólica de compostos fenólicos para oliveiras. Setas tracejadas indicam etapas ainda não elucidadas. Compostos fenólicos em verde foram avaliados na tese. Em vermelho são apresentadas algumas enzimas associadas à formação dos compostos fenólicos. Adaptado de: OBIED *et al.*, 2008; SAIMARU; ORIHARA, 2010; AGATI *et al.*, 2012; ALAGNA *et al.*, 2012, 2016; MOUGIOU *et al.*, 2018.

Os secoiridóides são uma classe de iridóides, presentes na família Oleaceae (OBIED *et al.,* 2008), destacando-se em oliveiras a oleuropeína, ligstrosídeo, dimetiloleuropeína e verbascosídeo (acteosídeo). Também destacam-se outros compostos como oleuropeína agliconada, hidroxitirosol, tirosol, 3,4-di-hidroxifenilglicol, oleosídeo 11-metil éster, entre outras moléculas (Figura 2) (AMIOT; FLEURIE.; MACHEIX, 1989; BIANCHI; POZZI, 1994; SAIMARU; ORIHARA, 2010; CHAROENPRASERT; MITCHELL, 2012).

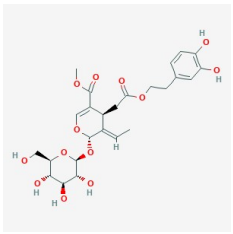
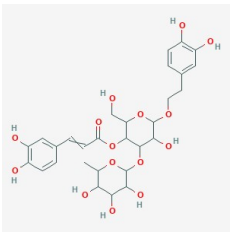
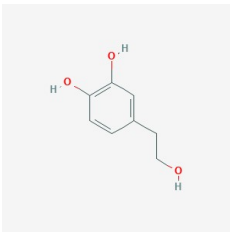
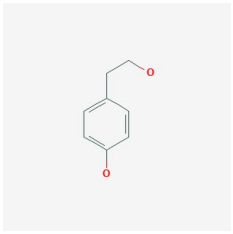
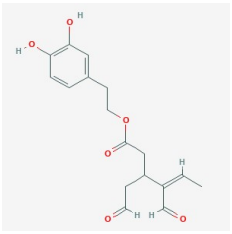
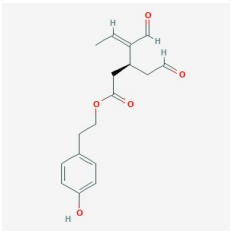
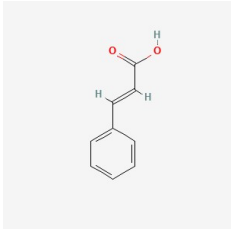
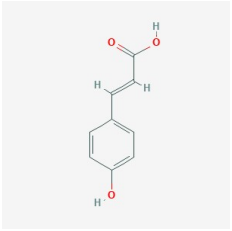
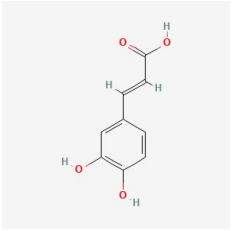
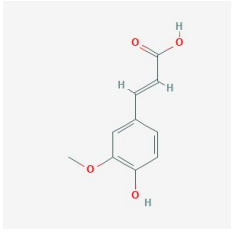
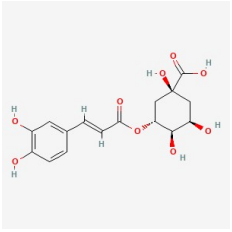
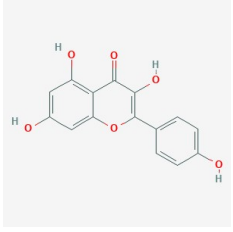
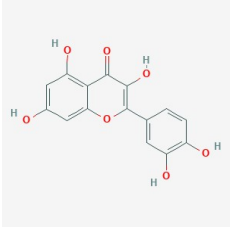
Oleuropeína 	**Verbascosídeo** 	**Hidroxitirosol** 
Tirosol 	**Oleaceína** 	**Oleocantal** 
Ácido cinâmico 	**Ácido p-cumárico** 	**Ácido cafeico** 
Ácido ferúlico 	**Ácido clorogênico** 	
Kaempferol 	**Quercetina** 	

Figura 2. Estrutura química de alguns polifenóis presentes em oliveiras. Fonte: NCBI (2023).

A biossíntese dos compostos fenólicos para *Olea europaea* envolve uma série de etapas com a atuação de diversas enzimas, sendo que alguns passos ainda não

foram totalmente compreendidos como, por exemplo, a biossíntese da oleuropeína (ALAGNA *et al.,* 2016; MOUGIOU *et al.,* 2018). Estudos moleculares abordando a expressão gênica de possíveis enzimas associadas com a produção de compostos fenólicos para oliveiras têm sido desenvolvidos nos últimos 15 anos, com análises transcriptoma e expressão gênica associada à rota de secoiridóides em frutos em diferentes estágios de maturação (ALAGNA *et al.,* 2009, 2012, 2016). Também avaliou-se a expressão de transcritos associados à rota de fenilpropanóides e precursores de flavonóides sob condições de estresse salino (ROSSI *et al.*, 2016). A expressão gênica de enzimas possivelmente associadas com a biossíntese de secologanina (precursor da oleuropeína), hidroxitirosol e tirosol foi avaliada durante a ontogenia dos frutos (MOUGIOU *et al.,* 2018).

3.6.1 Polifenóis no estresse ambiental e defesa das plantas

O estresse abiótico, biótico e nutricional induz a uma série de modificações no metabolismo primário, assim como no secundário em plantas, influenciando os níveis dos compostos fenólicos, os quais atuam na tolerância a condições adversas e proteção contra pestes e patógenos (CARETTO *et al,* 2015; SHARMA *et al.,* 2019; KHARE *et al*., 2020). Em oliveiras foram observadas alterações na concentração de compostos fenólicos em função de estresse hídrico (ROMERO *et al.*, 2002), estresse pelo frio (ORTEGA-GARCÍA; PERAGÓN, 2009), estresse salino (PETRIDIS *et al.*, 2012. Em estudo com oliveiras jovens submetidas ao déficit hídrico por 30 dias observou-se nas raízes um aumento da concentração de fenóis totais em 60% em comparação com plantas controle; aumento nos níveis de oleuropeína e os flavonóides luteolina-7-glucosídeo e apigenina-7-glucosídeo; diminuição nos níveis de hidroxitirosol, tirosol, verbascosídeo e dos flavonóides luteolina-7-rutinosídeo e quercetina, indicando que os compostos que tiveram aumento podem atuar nos mecanismos de proteção contra danos causados por estresse ambiental (MECHRI *et al.*, 2019).

Os flavonóides se constituem em um mecanismo de proteção fisiológica para organismos expostos ao estresse oxidativo de diferentes origens, sendo de grande importância na interação de plantas com o ambiente (AGATI *et al.,* 2012). A exposição a altos níveis de raios solares induz o aumento de flavonóides, os quais

protegem as estruturas fotossintéticas, ácidos nucléicos e proteínas contra a radiação ultravioleta (RAVEN; EVERT; EICHHORN, 2001).

O estresse nutricional pode influenciar a produção de compostos fenólicos induzindo a rota metabólica de fenilpropanóides (DIXON; PAIVA, 1995). A deficiência nutricional de P foi associada com a produção de antocianinas, enquanto que baixos níveis de Fe induziram a produção de ácidos fenólicos em plantas (DIXON; PAIVA, 1995). A aplicação de N em diferentes formas, como nitrato e uréia em tremoço-branco (*Lupinus albus* L.) influenciou a exsudação e acumulação de compostos fenólicos, em especial isoflavonóides (WOJTASZEKI *et al.*, 1993). Em condições de deficiência de B também se observou um aumento da atuação de enzimas envolvidas com o metabolismo de polifenóis, como a *phenylalanine ammonia lyase* (PAL), *β-glucosidase* (GLU) e *polyphenoloxidase* (PPO) (BLEVINS; LUKASZEWSKI, 1998; CAMACHO-CRISTÓBAL; ANZELLOTTI; GONZÁLEZ-FONTES *et al.*, 2002).

Estudos sobre estresse nutricional por B conduzidos em oliveiras também tem indicado alterações nos níveis dos polifenóis. Maiores níveis de fenóis totais nas folhas foram observados em oliveiras crescendo em câmaras climáticas, mas o mesmo padrão não foi detectado em experimento de campo com árvores de 20 anos de idade. Além disso, o conteúdo de oleuropeína foi menor em plantas com deficiência de B do que as plantas nutridas (LIAKOPOULOS; KARABOURNIOTIS, 2005). Oliveiras jovens com deficiência de B apresentaram dois secoiridóides glicosídeos, os quais estavam ausentes em plantas controle, indicando que os polifenóis possuem um papel importante na adaptação à deficiência de B (KARIOTI *et al.*, 2006). Estudo com adubação foliar boratada em oliveiras adultas indicou correlação negativa entre os níveis de fenóis totais nas folhas e quantidade de fertilizante (HEGAZI *et al.*, 2018). Entretanto, outro estudo com adubação foliar com B em oliveiras jovens observou um aumento nos níveis de fenóis totais e oleuropeína em plantas adubadas (PASKOVIĆ *et al.*, 2019) Em função da discrepância dos resultados são essenciais estudos futuros que avaliem a relação entre adubação com B e os níveis de compostos fenólicos, dada a importância do B para a nutrição de oliveiras e dos polifenóis nos mecanismos de defesa e adaptação ao estresse.

Os compostos fenólicos atuam em mecanismos de defesa química da planta contra herbivoria e resistência a doenças, além de proteção contra injúrias físicas e

inibição à entrada e ocupação de patógenos (BECKMAN, 2000; MUMM; HILKER, 2006). Fenilpropanóides como coumestrol e coumarinas podem ser tóxicos a herbívoros, produzindo efeito anticoagulante (DIXON; PAIVA, 1995). Além de função repelente de insetos, os fenilpropanóides e seus derivados atuam na síntese de fitoalexinas; produção de protetores contra a radiação ultravioleta; formação de compostos que atuam como barreiras celulares como ligninas, suberinas e outros polifenóis, dentre outras funções (HAHLBROCK; SCHEEL, 1989).

Em oliveiras também se observou a atuação de compostos fenólicos em mecanismos de defesa. O ácido elenólico, por exemplo, está presente em grande quantidade na superfície das folhas, atuando como barreira física, a qual inibe a germinação de esporos de patógenos, enquanto que os secoiridóides como o ligstrosídeo e a oleuropeína apresentaram efeito antimicrobiano e estimularam a produção de fitoalexinas (KUBO; MATSUMOTO; TAKASE, 1985; UCCELLA, 2001). Neste sentido, a oleuropeína contribui com um mecanismo de defesa química por intermédio da GLU, que degrada parte da molécula. Posteriormente a oleuropeína agliconada atua como um agente alquilante, que participa de ligações cruzadas com aminoácidos, produzindo um complexo de alto peso molecular e diminuindo os teores de lisina, reduzindo assim o valor nutricional dos tecidos predados/infectados (KONNO *et al.,* 1999). Koudounas *et al.* (2015) avaliaram a atuação da enzima GLU e seu transcrito (OeGLU) em oliveiras e verificaram maior expressão gênica em regiões de crescimento como folhas jovens, células meristemáticas de ramos e raízes; gemas florais, ovários desenvolvidos e células em anteras. Os autores também observaram que a expressão gênica de OeGLu coincidiu com os padrões de acumulação/degradação de oleuropeína.

3.6.2 Polifenóis: presença em frutos e azeite e benefícios à saúde

A composição e níveis de compostos fenólicos em oliveiras estão associados com sua localização nos tecidos e órgãos vegetais; fase de desenvolvimento; período fenológico; cultivar; localização geográfica; processamento do azeite e das azeitonas de mesa; fatores ambientais e interações entre as espécies (MARSILIO; CAMPESTRE; LANZA, 2001; SERVILI *et al.,* 2004; CHAROENPRASERT; MITCHELL, 2012; TALHAOUI *et al.*, 2015; MARTINS; BRONZE; VENTURA, 2022). As técnicas de extração também influenciam na composição e principalmente na

quantificação dos compostos fenólicos e incluem os métodos de maceração, ultrassom, microondas, fluido super/subcrítico, entre outros, ou ainda a mescla de mais de um método (JAPÓN-LUJÁN; CASTRO, 2007; XYNOS *et al.*, 2012; ROSA *et al.,* 2019).

Dentre os compostos fenólicos com maior expressividade em oliveiras destaca-se a oleuropeína. Este secoiridóide é sintetizado em vários tecidos e órgãos, contudo ocorre em maior quantidade em gemas vegetativas, flores completas, folhas e frutos imaturos (MALIK; BRADFORD, 2006; ORTEGA-GARCÍA *et al.,* 2008; ALAGNA *et al.,* 2012; HASSEN; CASABIANCA; HOSNI, 2015). Durante a ontogenia dos frutos de oliveira, a fase inicial é caracterizada pelo progressivo crescimento dos frutos, além do acúmulo de oleuropeína e clorofila. Conforme aumenta a maturação, há uma redução drástica nos níveis destes compostos e na fase final a oleuropeína atinge níveis mais baixos; em frutos escuros há uma elevação da atividade de citocininas e posterior acúmulo de antocianinas (SHULMAN; LAVEE, 1976, AMIOT; FLEURIE.; MACHEIX, 1989). Além disso, há a ação da enzima PPO, que oxida os fenóis formando outros compostos (ORTEGA-GARCÍA *et al.,* 2008).

Durante o processamento do azeite há uma redução nos níveis de alguns flavonóides e compostos glicosídeos como a oleuropeína, enquanto há um aumento nos teores de hidroxitirosol (3,4-diidroxifeniletanol ou 3,4-DHPEA) e tirosol (p-4-hidroxifeniletanol ou p-HPEA). Além destes álcoois fenólicos, o azeite extra virgem apresenta derivados dos secoiridóides, como a oleaceína (3,4-DHPEA-EDA), forma dialdeído do ácido elenólico ligado ao 3,4-DHPEA, o qual é um isômero da oleuropeína agliconada (3,4-DHPEA-EA). Também há formação do oleocantal (p-HPEA-EDA), forma dialdeídodo ácido elenólico ligado ao p-HPEA, assim como um aumento de flavonóides agliconados, ligninas e ácidos fenólicos (MORELLÓ *et al.,* 2005; BAKHOUCHE *et al.,* 2013; MIHO *et al.,* 2020; MARTINS; BRONZE; VENTURA, 2022) (Figura 2).

Com relação às propriedades sensoriais da azeitona de mesa, a oleuropeína e suas formas agliconadas são os principais responsáveis pelo sabor amargo e juntamente com outros polifenóis propiciam notas sensoriais características (CAPONIO; GOMES; PASQUALONE, 2001; BRENES *et al.,* 2002). Para o azeite tem se sugerido que compostos derivados da oleuropeína, ligstrosídeo e dimetiloleuropeína estão associadas com o amargor e as notas pungentes (SERVILI,

et al., 2004). O sabor amargo é proporcionado pela composição dos ácidos benzóico e cinâmico, assim como de secoiridóides, enquanto que o sabor picante é proveniente de outros fenóis (APARICIO; LUNA, 2002). Os polifenóis também contribuem com a cor e textura em azeitonas de mesa (MARSILIO; CAMPESTRE; LANZA, 2001). Por fim, os compostos fenólicos colaboram com a estabilidade oxidativa do azeite, em especial a oleaceína, composto predominante no azeite extra virgem (ROMERO *et al*., 2002; SERVILI *et al.,* 2004).

Polifenóis presentes em oliveiras e seus produtos apresentam potenciais benefícios para a saúde humana, em especial a oleuropeína, hidroxitirosol e oleaceína (SERVILI *et al.,* 2004; CHAROENPRASERT; MITCHELL, 2012; TALHAOUI *et al.,* 2015; HASSEN; CASABIANCA; HOSNI, 2015). Estudos *in vitro* e *in vivo* com oliveiras e seus produtos ou ainda, com seus compostos fenólicos sintéticos, indicaram uma série de benefícios como ação antioxidante (LEE; LEE, 2010); antimicrobiana (YAKHLEF *et al.,* 2018); antiviral (LEILA *et al.,* 2019); proteção contra o câncer de mama (ELAMIN *et al.,* 2013); anti-hiperglicemia (HADRICH *et al.,* 2015); redução de peso e esteatose hepática (LEPORE *et al.,* 2015); prevenção contra a arteriosclerose (MONTSERRAT-DE LA PAZ, *et al.,* 2016); influência nos parâmetros hematológicos (FERDOUSI *et al.,* 2019); proteção na espermatogênese com administração de medicações anti-câncer (ROSTAMZADEH *et al.,* 2020).

Além da presença de polifenóis e outros compostos com atividade antioxidante, a presença de ácidos graxos monoinsaturados no azeite tem sido associado com benefícios à saúde, como favorecer o aumento do colesterol *High Density Lipoprotein* (HDL), proteção cardiovascular, aumento da atividade antioxidante, regulação no metabolismo da glicose, reduzindo risco de diabetes, entre outros benefícios (PEDRET *et al*., 2018; SCHWINGSHACKL *et al*., 2019; TSARTSOU *et al*., 2019). Entretanto, os estudos divergem na interpretação dos resultados, sendo que alguns sugerem que azeites de oliva com alto teor de compostos fenólicos são mais efetivos (PEDRET *et al*., 2018; SCHWINGSHACKL *et al*., 2019), enquanto que outro estudo sugere que azeite de oliva com baixos teores de polifenóis pode ser suficiente para os benefícios mencionados acima, enfatizando a importância da fração lipídica na redução das doenças comentadas (TSARTSOU *et al*., 2019).

3.7 Azeite de oliva

Nos últimos anos tem se observado que o setor oleícola está em crescente expansão mundial. Os maiores produtores na safra 2020/2021 foram os países: Espanha (1º), Tunísia (2º), Itália (3º), Portugal (4º), Argentina (5º), Turquia (6º), Grécia (7º), Chile (8º), Marrocos (9º) (IOC, 2023a). A olivicultura é praticada em cerca de 40 países em 10,3 milhões de hectares, sendo que 97,9% dos olivais se localizam na região mediterrânea, 1,3% nas Américas, 0,4% na Oceania e 0,4% em outras regiões da Ásia (LUCENA *et al.,* 2017).

O consumo de azeite no Brasil tem aumentado nos últimos anos e atualmente o país é o segundo maior importador de azeite e azeitona de mesa do mundo, de modo que o Brasil tem chamado atenção do mercado internacional (IOC, 2020). Na safra de 2020/2021 o país importou 106.500 t de azeite extra virgem. Levando em conta o preço de aproximadamente 286,2 euros/100 kg de um azeite extra virgem espanhol, por exemplo, o custo seria de 304,8 milhões de euros apenas com este tipo de azeite, equivalente a mais de 1,6 bilhões de reais (Dados de IOC, 2023b). Na referida safra o estado do Rio Grande do Sul produziu 202,000 l de azeite (JOÃO, 2022). Considerando a densidade do azeite 0,9 kg. L^{-1} (MELLO; PINHEIRO, 2012), a quantidade de azeite produzido no estado na referida safra foi de 181,8 t, o que equivale apenas 0,17 % do azeite importado. Com isso, observa-se o elevado potencial de crescimento da cadeia produtiva da oliveira no país.

3.7.1 Processamento do azeite

A produção do azeite é um processo que visa separar o azeite dos demais constituintes dos frutos. O processamento se inicia com a moagem das azeitonas formando uma pasta, que propicia o rompimento das células e liberação do óleo; sendo posteriormente realizada a malaxagem, na qual há mistura da pasta, a fim de favorecer a coalescência (aglutinamento) do óleo e por último a separação do azeite da fase sólida e da água (TAMBORRINO *et al*., 2017).

A etapa da moagem pode ser realizada utilizando diversos equipamentos, como moinhos de pedra (mais antigos), martelo, disco ou de facas (CAPONIO *et al*., 2003). Na etapa seguinte, a malaxagem, há mistura contínua da pasta, fundindo as gotas de óleo em gotas maiores e quebrando a emulsão com água, além de expor células oleosas que porventura não tenham sido rompidas durante o processo de

moagem (CLODOVEO, 2012). Para aumentar a coalescência das gotas de óleo, a temperatura da pasta é aumentada a fim de diminuir a viscosidade da mistura (KALUA *et al*., 2006). Durante a malaxagem ocorre ativação de uma série de enzimas presentes no fruto, como *lipoxygenase* (LOX), *peroxidase* (POD), GLU PPO, entre outras, as quais favorecem reações de hidrólise e oxidoredução nos ácidos graxos e compostos fenólicos (CLODOVEO, 2012; TATICCHI *et al*., 2013).

Após a malaxagem, a etapa seguinte consiste na separação do azeite da pasta, utilizando prensagem (método mais antigo), percolação ou sistemas de centrifugação com decantadores de duas ou três fases (GIOVACCHINO; SOLINAS; MICCOLI *et al*., 1994). Os decantadores de três fases necessitam adicionar água à pasta, possibilitando a separação da matéria sólida da fração líquida e posteriormente a separação do azeite e água por centrifugação (BIANCHI; TAMBORRINO; SANTORO, 2013). Já os decantadores de duas fases realizam a separação do azeite da pasta úmida, sem necessidade de adição de água, sendo mais vantajoso do que os decantadores de três fases, uma vez que há geração de menor quantidade de efluente, redução no consumo de água e energia, menor perda dos compostos fenólicos, devido ao seu caráter hidrofílico, além de variações nas características organolépticas. Na última etapa de produção usa-se uma centrífuga vertical para limpeza do azeite (CAPONIO *et al*., 2018).

3.7.2 Composição físico-química e classificação do azeite

O azeite de oliva é constituído predominantemente por uma fração saponificável e insaponificável. A fração saponificável é representada por ácidos graxos (98%), compondo a estrutura celular dos frutos, na forma de mono-, di- e tri-acilgliceróis e fosfolipídios com ligações éster (TENA *et al.,* 2015). A fração insaponificável (2%) é representada por compostos minoritários, que incluem principalmente alcoóis alifáticos e triterpenos, esteróis, hidrocarbonos, compostos voláteis, pigmentos e compostos fenólicos (SERVILI *et al.*, 2004). Em relação aos componentes do fruto, a fração lipídica corresponde a 30% do mesocarpo, 1% do endocarpo e 27% das sementes (CONDE; DELROT; GERÓS, 2008).

A maioria dos ácidos graxos presentes no azeite são cadeias de 14-24 carbonos, destacando-se o monoinsaturado ácido oléico como principal composto, seguido do poliinsaturado ácido linoléico e o saturado ácido palmítico (IOC, 2019a).

A biossíntese de ácidos graxos ocorre predominantemente nas células do mesocarpo, envolvendo muitas organelas e uma série de enzimas, tendo como precursor a molécula acetil- CoA (CONDE; DELROT; GERÓS, 2008).

O *International Olive Council* (IOC) estabelece critérios para o agrupamento do azeite de oliva baseado em padrões de identidade e qualidade de tal produto (IOC, 2019a). Já no Brasil, os critérios são estabelecidos pela Instrução Normativa n° 1, de 30 de janeiro de 2012 do MAPA (BRASIL, 2012). De acordo com a normativa brasileira o azeite é classificado como:

a) azeite de oliva virgem: o produto extraído do fruto da oliveira unicamente por processos mecânicos ou outros meios físicos, sob controle de temperatura adequada, mantendo-se a natureza original do produto; o azeite assim obtido pode, ainda, ser submetido aos tratamentos de lavagem, decantação, centrifugação e filtração;
b) azeite de oliva refinado: o produto proveniente de azeite de oliva do grupo azeite de oliva virgem mediante técnicas de refino que não provoquem alteração na estrutura glicerídica inicial;
c) óleo de bagaço de oliva: o produto constituído pela mistura de óleo de bagaço de oliva refinado com azeite de oliva virgem ou com azeite de oliva extra virgem;
d) óleo de bagaço de oliva refinado: o produto proveniente do bagaço do fruto da oliveira mediante técnica de refino que não provoque alteração na estrutura glicerídica inicial.

3.7.3 Parâmetros de qualidade

A determinação dos padrões de qualidade do azeite para sua comercialização é regulada e normatizada por uma série de órgãos internacionais, como IOC, *European Economic Commission* (EEC), entre outros (TENA et al., 2015). No Brasil a regulação dos padrões de qualidade do azeite de oliva e óleo de bagaço de oliva é determinada pela Instrução Normativa IN 01/2012 (BRASIL, 2012).

A classificação do azeite pode ser definida em termos de qualidade e identidade. A qualidade do azeite é avaliada em função do percentual de acidez livre, índice de peróxidos e extinção específica no ultravioleta. Já a identidade do

azeite considera a matéria-prima e os métodos de obtenção do mesmo (BRASIL, 2012).

A acidez livre é definida como a quantidade de ácidos graxos livres, os quais não estão mais ligados aos triacilgliceróis originais, sendo expressa em percentual de ácido oleico (GROSSI *et al*., 2019). As enzimas LOX catalisam a hidrólise dos ácidos graxos, de modo que a degradação enzimática pode ser acelerada em função da luz, umidade e calor durante o processamento dos frutos, assim como pela ação da microbiota, tais como leveduras (CIAFARDINI; ZULLO, 2015; TENA *et al*., 2015). O percentual de acidez livre indica o frescor, manuseio e processamento correto das azeitonas (TENA *et al*., 2015).

O índice de peróxido indica a presença de peróxidos e outros produtos similares, formados por meio da oxidação dos ácidos graxos insaturados, expresso em miliequivalente de oxigênio ativo por kilograma de azeite ($mEqO_2$. kg^{-1}) (BRASIL, 2012). Tal índice avalia a oxidação primária do azeite e está relacionado às condições de armazenamento após a produção, sob influência da exposição à luz, temperatura e oxigênio (GROSSI *et al*., 2015). Novamente cita-se a atividade das enzimas LOX, que oxidam pigmentos e ácidos graxos insaturados de azeitonas e azeites (AMANPOUR *et al*., 2019).

Na extinção específica no ultravioleta utiliza-se um espectrofotômetro em comprimentos de onda ultravioleta, o qual fornece informações sobre a qualidade do produto, assim como o estado de preservação e modificações em função dos processos tecnológicos (IOC, 2019b). Com a oxidação do azeite ou práticas de refino são formados radicais peróxidos e hidroperóxidos, produzindo uma mudança na configuração das ligações químicas. Deste modo, são formados compostos oxidativos secundários, denominados dienos e trienos conjugados, os quais são absorvidos em comprimentos de onda específicos, de 232 nm e 270 nm respectivamente, diluídos em ciclohexano (TENA *et al*., 2015; IOC, 2019b). Já o ΔK avalia a variação da extinção no comprimento de onda máximo de 270 nm (IOC, 2019b).

O perfil de ácidos graxos avalia a proporção dos diferentes ácidos graxos, sendo expresso em percentual relativo (BRASIL, 2012; IOC, 2017a). Na tabela 2 são apresentados os limites estabelecidos para que um azeite seja considerado extra virgem ou virgem, considerando os parâmetros de qualidade e identidade (percentual de ácidos graxos). Os limites de tolerância dos parâmetros de qualidade

seguem as normas nacionais e internacionais (BRASIL, 2012; EEC, 2013; IOC, 2019a) enquanto que o perfil de ácidos graxos para o azeite extra virgem segue a regulamentação de IOC, em função de ser uma recomendação mais atual.

Tabela 2. Limites de tolerância dos padrões de qualidade dos azeites extra virgem e virgem e do perfil de ácidos graxos para o azeite extra virgem, de acordo com normas nacionais e internacionais (BRASIL, 2012; EEC, 2013; IOC, 2019a).

Parâmetro	**Azeite extra virgem**	**Azeite virgem**
Acidez livre (% de ácido oleico)	≤ 0,8	≤ 2,0
Índice de peróxidos (mEq. kg^{-1})	≤ 20	≤ 20
Extinção específica no ultravioleta		
270 nm	≤ 0,22	≤ 0,25
Δ K	≤ 0,01	≤ 0,01
232 nm	≤ 2,50	≤ 2,60
Ácidos graxos (%)		
Ácido mirístico (C14:0)	≤ 0,03	
Ácido palmítico (C16:0)	7,5-20,0	
Ácido palmitoleico (C16:1)	0,3-3,5	
Ácido heptadecanoico (C17:0)	≤ 0,4	
Ácido heptadecenoico (C17:1)	≤ 0,6	
Ácido esteárico (C18:0)	0,5-5,0	
Ácido oleico (C18:1)	55,0-83,0	
Ácido linoleico (C18:2)	2,5-21,0	
Ácido linolênico (C18:3)	≤ 1,0	
Ácido araquídico (C20:0)	≤ 0,6	
Ácido gadoleico/eicosenoico (C20:1)	≤ 0,5	
Ácido behênico (C22:0)	≤ 0,2	
Ácido lignocérico (C24:0)	≤ 0,2	

A composição de ácidos graxos no azeite é variável sendo dependente do cultivar, localização geográfica, condições climáticas, adubação, entre outros fatores (GARCÍA-INZA *et al.*, 2014; FRANCO *et al.*, 2015; TORRES *et al.*, 2017; ALOWAIESH *et al.*, 2018; KRITIOTI; MENEXES; DROUZA, 2018; LÉMOLE; WEIBEL; TRENTACOSTE, 2018; WANG *et al.*, 2018; VISHEKAII *et al.*, 2019; YU *et al.*, 2021). Com relação aos cultivares, observa-se que Arbequina é caracterizada

pela baixa quantidade de ácido oleico e alta quantidade de ácido linoleico (APARICIO; LUNA, 2002).

Destaca-se também a avaliação do índice de maturação (IM) das azeitonas, a fim de se determinar o período ideal de colheita para garantia de maior rendimento do azeite associado à melhor qualidade. De acordo com IOC para a maior parte dos cultivares que exibem mudança da coloração dos frutos, se obtêm azeite de melhor qualidade quando os índices de maturação dos frutos estão entre 3 e 4. O índice 3 abrange azeitonas com mais da metade do epicarpo tornando-se avermelhado ou roxo. Já o índice 4 se refere à azeitonas com epicarpo roxo e mesocarpo branco (IOC, 2011).

4 RESULTADOS

Os resultados da Tese serão apresentados na forma de artigos.

Estudo 1 – **Artigo: "Nutrient scarcity in young olive trees: effects on shoot and root growth and phenolic compounds".** A ser submetido para a revista "Scientia Horticulturae".

No caso do estudo 2 foram produzidos dois artigos. O primeiro avaliou todos os tratamentos com B, como variável resposta os teores de B nas folhas. O segundo abordou os tratamentos contrastantes e sua relação com o conteúdo de compostos fenólicos e B nas folhas.

Artigo: "Effect of boron fertilization and phenological period on boron content in olive leaves". Publicado na Revista "Research, Society and Development" em 15 de maio de 2023.

Artigo: "Boron fertilization and its relationship with boron and phenolic compounds content in olive leaves". A ser submetido para a revista "Scientia Horticulturae".

Estudo 3 – **Artigo: "Chemical composition of fruits and oils from olive trees with different fruit loads in southern Brazil".** A ser submetido para a revista "Anais da Academia Brasileira de Ciências".

Nutrient scarcity in young olive trees: effects on shoot and root growth, phenolic compounds and genic expression

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Abstract

Researches on nutritional deficiency in olive trees are voluminous, however there are few studies that evaluated its effect on the levels of individual phenolic compounds and genic expression. Therefore, the study aimed to investigate the effect of nitrogen (N), phosphorus (F), potassium (K) and boron (B) omission on the concentration of phenolic compounds, expression of three genes associated with the formation of polyphenols, morphometric parameters and nutritional content in shoots and roots. Five treatments were evaluated in a greenhouse for three years, which included the complete treatment (C) and treatments with nutrient omission (-N, -P, -K, -B). Plants with nitrogen omission exhibited smaller diameter and dry matter (DM) in the shoots, reduction of N in the soil and leaves, and increase in the phenolic compounds in the leaves, while the opposite tendency occurred in the roots. The omission of P generated late symptoms of deficiency, with a reduction of DM in the shoots, a decrease in P in the soil and roots, as well as an increase in the levels of phenolic compounds, especially oleuropein and verbascoside. Potassium, on the other hand, showed more attenuated symptoms, with length and root density greater than C, even with a reduction of K in the soil and tissues, besides to most phenolic compounds exhibiting levels similar to C. Boron deprivation did not cause changes in the morphometric parameters, showing adequate foliar levels of B, suggesting that the seedlings reserves were sufficient to supply B, even after a long period. It is concluded that young olive trees deprived of N showed more pronounced nutritional stress and that phenolic compounds showed different levels according to nutritional omission and organ/tissue. Up-regulated of PAL transcripts and down-regulated of CuAO and PPO transcripts with potassium deprivation provide evidence of nutritional stress at the molecular level.

Keywords: Nutritional stress, Mineral deficiency, Arbequina, Oleuropein, Verbascoside

1. Introduction

Olive tree (*Olea europaea* L.) cultivation is an ancient agricultural practice, having emerged in the Middle East 6500 years ago and later in Greece 6000-5500 years (Langgut et al., 2019). With the increase in olive oil consumption, oliviculture has expanded to several countries in the world, with edaphoclimatic conditions distinct from the Mediterranean base (Torres et al., 2017). Fertility programs of olive groves around the world generally include fertilization with the macronutrients nitrogen (N), phosphorus (P) and potassium (K), however in many cases there is no adequate assessment of the real need for fertilization, which can generate economic losses and environmental contamination problems (Fernández-Escobar et al., 2013; Erel et al., 2017). Boron (B) highlights as an important micronutrient, since its deficiency is recurrent in olive groves (Tsadilas and Chartzoulakis, 1999; Sibbett and Ferguson, 2002; Soyergin et al., 2002; Bender et al., 2013).

Nutritional deficiency studies for olive trees indicated that under such conditions a series of morphological, morphometric and physiological changes occur, as reduced aerial and root growth or even abnormal growth, changes in photosynthetic parameters and stomatal regulation, changes in the levels of other nutrients, increase in the concentration of carbohydrates, besides to a decrease in productivity (Hartmann and Brown, 1953; Arquero et al., 2006; Benlloch-González et al., 2008; Arrobas et al., 2010; Boussadia et al., 2010; Chatzissavvidis and Therios, 2010; Naija et al., 2014; Fernández-Escobar et al., 2016; Jiménez-Moreno and Fernández-Escobar, 2016; Erel et al., 2013; Haberman et al., 2019; Souza et al., 2019).

Abiotic, biotic and nutritional stress in plants induces alterations in the primary metabolism, as well as in the secondary one. In this sense, phenolic compounds stand out, produced in different metabolic routes, with broad functions, operated on tolerance to environmental stress conditions, as well as protection against pests and pathogens (Caretto et al., 2015; Sharma et al., 2019; Khare et al., 2020). Phenolic compounds present in olive trees and olive oil include phenolic acids, phenolic alcohols, lignins, flavonoids, secoiridoids and derivatives (Servili et al., 2004; Charoenprasert and Mitchell, 2012; Talhaoui et al., 2015; Martins et al., 2022). Researches with olive trees indicated changes in the levels of specific phenolic compounds in situations of drought stress (Mechri et al. 2019; Mechri et al. 2020),

cold stress (Ortega-García and Peragón, 2009), salt stress (Petridis et al., 2012) and nutritional stress (Liakopoulos and Karabourniotis, 2005; Karioti et al., 2006). The aforementioned works showed that specific phenolic compounds respond differently to stress, so that important compounds in olive trees, such as oleuropein and hydroxytyrosol, presented higher or lower concentrations than control plants depending on the type of stress and plant tissue.

The biosynthesis of phenolic compounds in olive trees involves a series of steps with the action of several enzymes, and some steps have not yet been fully understood such as the biosynthesis of oleuropein (Alagna et al., 2016; Mougiou et al., 2018). Studies with olive trees have also evaluated the effect of salt stress on the expression of transcripts associated with the phenylpropanoid route and flavonoid precursors (Rossi et al., 2016), in addition to the relationship between biotic stress and transcript related to phenylpropanoids (Gouvinhas et al., 2019).

Research on nutritional stress in young olive trees in general has been carried out with olive tree cuttings, with fertirrigation using Hoagland-Arnon nutrient solution (Hartmann and Brown, 1953; Boussadia et al., 2010; Naija et al., 2014; Fernández-Escobar et al., 2016; Souza et al., 2019). The present study aimed to analyze the nutritional deficiency in olive trees using the absent nutrient method, evaluating seedlings of the appropriate size for planting, with via soil fertilizers, which are conditions similar to those that occur in many rainfed young olive groves. The study was conducted for three years in order to ensure the condition of severe nutritional deficiency and its effect on morphometric parameters, nutritional content, phenolic compounds and gene expression in the aerial and root systems.

2. Material and Methods

2.1 Plant material and experimental design

The experiment was carried out in a greenhouse at Universidade Federal do Pampa - Campus São Gabriel, Brazil (30°20'S, 54°21'O). Olive seedlings (cultivar Arbequina) of one year old were cultivated for three years, from May 2019 to May 2022. Initially, the young olive trees (0.8-1.0 m height) were kept in the greenhouse for acclimatization for 30 days until the experiment was set up. Then, the plants were transferred to a pot filled with 11 kg of a Quartzipsamments (Soil Taxonomy) or Neossolo Quartzarênico in the Brazilian classification (Santos et al., 2018) taken from the subsoil (20-40 cm depth). The soil was characterized by acidity (pH 5.1), low clay

content (13%), poor organic matter (0.3%) and low fertility, with 4.6 mg.dm^3 of exchangeable P; 19 mg.dm^3 of exchangeable K; and 0.1 mg.dm^3 of available B.

The experiment was arranged in a completely randomized design with five treatments and four replicates: (C) Complete, with addition of all essential macronutrients and micronutrients; (-N) omission of N; (-P) omission of P; (-K) omission of K; (-B) omission of B. The complete treatment consisted of the addition of the following nutrients, expressed in mg of nutrient per kg of soil: N=100; P=300; K=150; Ca=200; Mg=91; S=40; B=1; Cu=1.5; Fe=5.0; Mn=5; Zn=5; Mo=0.15. (Silva et al., 2007). The omitted nutrient treatments had fertilizers with equal concentrations, except for the absent nutrient.

The nutrient sources used were: Urea ($CO(NH_2)_2$); Simple Superphosphate - SFS (18% P_2O_5 CNA+H_2O; 18% Ca; 10% S); Triple Superphosphate - SFT (41% P_2O_5 CNA+H_2O and 13% Ca); KCl; Ulexite (6% B; 1% P_2O; 3.2% S-SO_4); $CuSO_4$; $FeSO_4.7H_2O$; $MnCl_2.4H_2O$; $ZnSO_4.7H_2O$; $(NH_4)_6Mo_7O_{24}.4H_2O$. The S-SO_4 was obtained from the different sources previously mentioned. For treatment with omission of P, without addition of SFS, which is also a source of S, $MgSO_4.7H_2O$ was added in order to obtain adequate source of S. In the case of Ca and Mg, the source of such nutrients was obtained through liming, using dolomitic filler limestone (26% CaO and 14% MgO). All treatments received limestone application (pH adjustment to 7.5) and the corresponding nutrients. The exception was the dosage of N and K, which was split into three equal doses: the first at the time of transplanting mixed with the soil and the remaining two were broadcasted on coverage, after 15 and 30 days after transplanting (CQFS-RS/SC, 2016). Due to the longer duration of the experiment, fertilization with sources of N, P, K and B was repeated, with the exception of the omitted nutrient, every six months.

2.2 Morphometric measurements of the shoots and roots

Vegetative growth was measured monthly on each experiment. The shoot height was evaluated, considered from surface level to the apical meristem, as well as the stem diameter, about 10 cm above the surface in two measurements, using the average value for the analyzes.

At the end of the experiment, the shoots and roots were separated and the roots were washed with a weak water jet. Half of the roots were used for

morphometric analysis and the other half for chemical analysis. The morphometric data of the root system were determined by scanning, using a Scanner (Epson Expression 1000 XL) and The LA2400 Scanner for WinRHIZO software (Regent Instruments Inc.). From the scan were determined the total length (m), surface area (m^2), volume (m^3), and density (cm/cm^3) of the roots. Shoot and root dry matter were determined after oven dried at 50°C until constant weight.

2.3 Nutrient concentration

Dried samples of leaf and root tissues were ground (60 mesh). Total N, P, and K were determined after wet digestion with sulfuric acid and hydrogen peroxide, according to the methodology of Tedesco et al. (1995). Nitrogen levels were determined by distillation using a Kjeldahl steam distiller, adding sodium hydroxide to the digestion product, followed by titration with sulfuric acid and boric acid as indicator. Phosphorus contents were evaluated with an aliquot of the digestion extract and analyzed by the colorimetric method using UV-Vis spectrophotometer (Murphy and Riley, 1962). Potassium concentrations were determined by flame spectrometry, using an aliquot of the digestion product, diluted in distilled water (1:10). Boron contents were determined by the dry-ash at 600°C in the muffle furnace and evaluated colorimetrically by the Azomethine-H method (Tedesco et al., 1995).

2.4 Phenolic compounds

Dried and ground samples of roots and leaves were used as described in sections 2.2 and 2.3. Extraction of phenolic compounds of the samples was carried out by macerating 0.5 g of matter mixed in 25 ml methanol. The mixture was agitated in a horizontal shaker at 200 rpm for 1 h. After centrifugation (10 min at 4000 rpm) the supernatant was filtered through a 0.45 mm syringe filter. Identification and quantification of individual phenolic compounds from methanolic extracts were analyzed using a high performance liquid chromatography (HPLC) based on a modification of the International Olive Council Method (IOC, 2017).

HPLC analyses were performed using an Agilent 64 (Agilent Technologies, Santa Clara, USA) equipped with a quaternary pump (1200 Series) and diode array detector (DAD) (Agilent 1260 Series Photo Diode Array Detector). The separation was conducted at 30°C using a reversed phase LC Column Eclipse Plus C18 (4.6 x 150 mm, 5 µm) (Supelco, Bellefonte, PA, USA). A ternary elution gradient was

established at a flow rate of 1.0 mL.min^{-1} with (A) water 0.2 % acetic acid (v/v), (B) methanol and (C) acetonitrile, as follows (A/B/C): 0 min (96:2:2 %); 5 min (80:10:10 %); 10 min (70:15:15 %); 20 min (50:25:25 %); 30 min (96:2:2 %). The injection volume was set at 20 µL and detection of phenolic compounds was performed at 280 nm. Phenolic standards (Sigma-Aldrich®) of oleuropein, verbascoside, hydroxytyrosol, tyrosol, quercetin, kaempferol, caffeic, chlorogenic, *trans*-cinnamic, *p*-coumaric and *trans*-ferulic acids were used to quantify the phenolic compounds (Table 1S).

2.5 Molecular analysis

RNA from roots samples frozen in liquid nitrogen was extracted using the PureLink® RNA Mini Kit (Ambion, USA), following the protocol provided by the manufacturer. For cDNA synthesis, the Platus Transcriber RNase H – cDNA First Strand kit (Sinapse Inc, Brazil) with oligo $(dT)_{18}$ primer was used, for total RNA of 0.1 ng- 5 µg. The amount of RNA and cDNA was determined with Qubit Fluorometer and RNA and ssDNA kits (Thermo Fisher Scientific, USA). The primers evaluated to measure gene expression were obtained from the literature: phenylalanine ammonia-lyase (PAL) (Rossi et al., 2016), copper methylamine oxidase (CuAO) and polyphenol oxidase (PPO) (Mougiou et al., 2018) (Table 2S). The real-time PCR reactions were executed on Droplet Digital™ PCR and QX200™ ddPCR™ EvaGreen® Supermix (Bio-Rad, CA, USA) which provides absolute quantification of transcripts. The cycling conditions for ddPCR were 95°C/5 min (enzyme activation); 95°C/30 s in 40 cycles (denaturation); 60°C/1 min in 40 cycles (annealing/extension); 4°C/5 min and 90°C/5 min (signal stabilization). The ddPCR data were analyzed using the QuantaSoft™ analysis software (Bio-Rad, CA, USA). Analyzes were performed in triplicate for each nutritional treatment.

2.6 Statistical analysis

The morphometric parameters of the aerial and root systems, dry mass, nutrient and phenolic compounds were compared between treatments using analysis of variance (ANOVA) and Dunnett's test ($p \leq 0.05$), performed in the SigmaPlot program v.11.0.

3. Results

3.1 Morphometry and dry matter

Visual symptoms of morphological alterations of shoots and leaves were not frequent and clear for each nutrient deprivation (data not shown). Scanning of the root system allowed the identification of differences among treatments regarding the morphometric parameters analyzed (Figure 1).

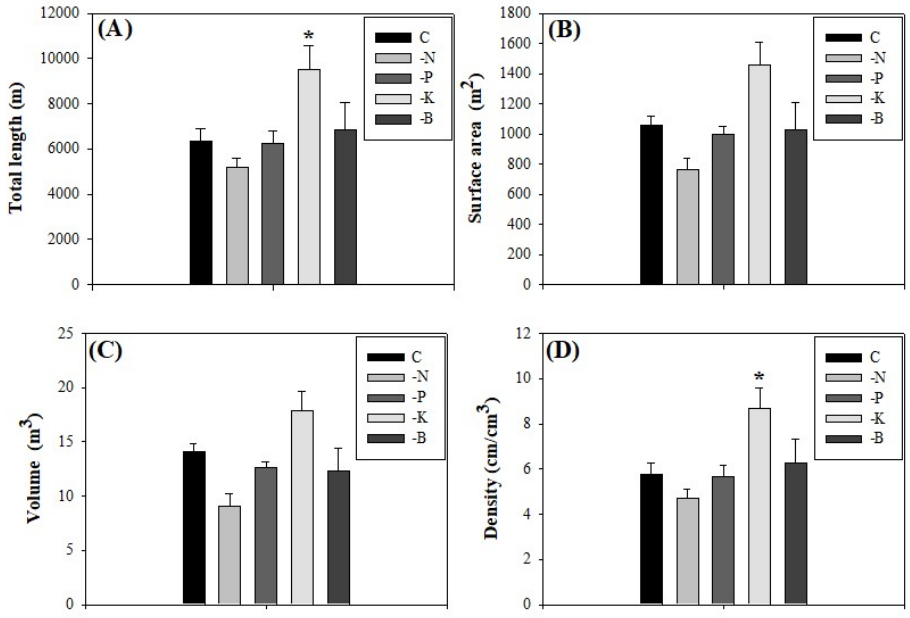

Figure 1 – Morphometry of roots of young olive trees, considering the following nutritional treatments: complete (C-all nutrients) and omission of nitrogen (-N), phosphorus (-P), potassium (-K) and boron (-B). Asterisk indicates significant difference from the control treatment, according to Dunnett's test ($p \leq 0.05$).

The -K treatment exhibited greater root length and density than the control (Figures 1A, 1D). No significant differences were observed for surface area and volume of roots among treatments compared to C (Figures 1B, 1C). However, treatment -N stands out, as its roots exhibited, respectively, average volume and surface area, 36% and 28% lower than C (Figures 1B, 1C).

The shoot height of olive trees showed a similar trend between treatments, not differing significantly after one, two and three years of trial (Figure 2). In the first year, all seedlings showed greater growth, while in the second year, the treatments

decreased their growth rate, evidencing a steady stabilization trend for all treatments in the third year (Figure 2).

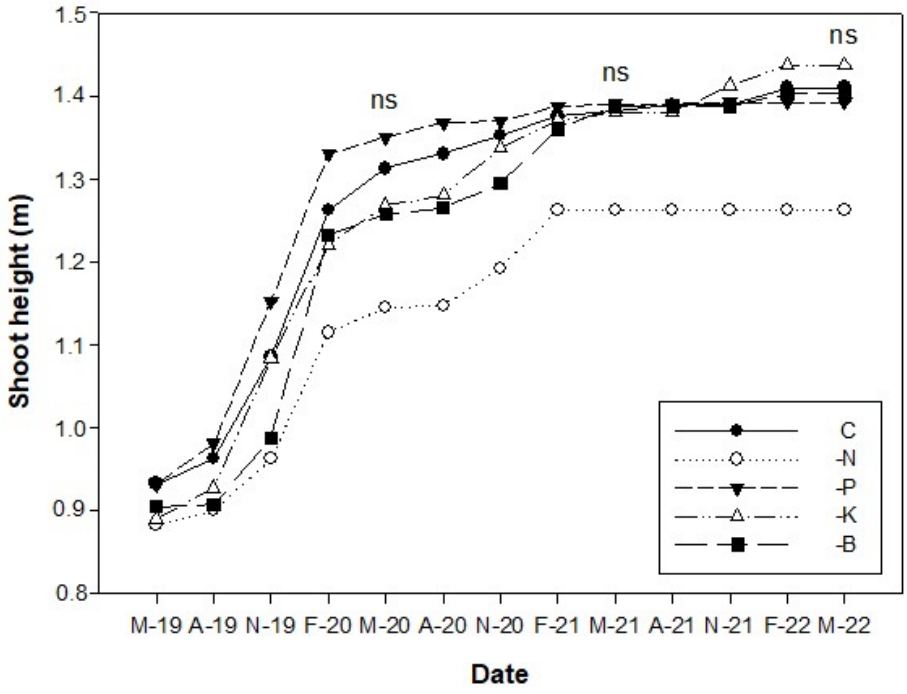

Figure 2 – Shoot height (m) of young olive trees, considering the following nutritional treatments: complete (C-all nutrients) and omission of nitrogen (-N), phosphorus (-P), potassium (-K) and boron (-B). ns = non-significant statistical difference (ANOVA).

The -K treatment started to show higher values of height from Sep./21, however later the plants showed stabilization in shoot height (Figure 2). Even though it did not differ statistically from the other treatments, the -N treatment showed a mean value in May/22 about 12% lower than the -K treatment (Figure 2). It is noteworthy that from Sep./20 the olive trees began to show a greater increase in lateral growth to the detriment of apical growth, regardless of treatment (data not shown).

Contrarily to the stabilization observed in the plant height, the diameter of the stem showed variations throughout the assay. With one year of experiment the treatment -P showed higher values than -K, -B and C and differed significantly from -N (Figure 3). From Feb./21, the -K treatment started to show higher mean values and in the second year (May/21) all treatments differed statistically from -N (Figure 3). After three years of experiment, there was a significant difference between -N and

the others, indicating that nitrogen deficiency generated olive trees with thinner stems (Figure 3).

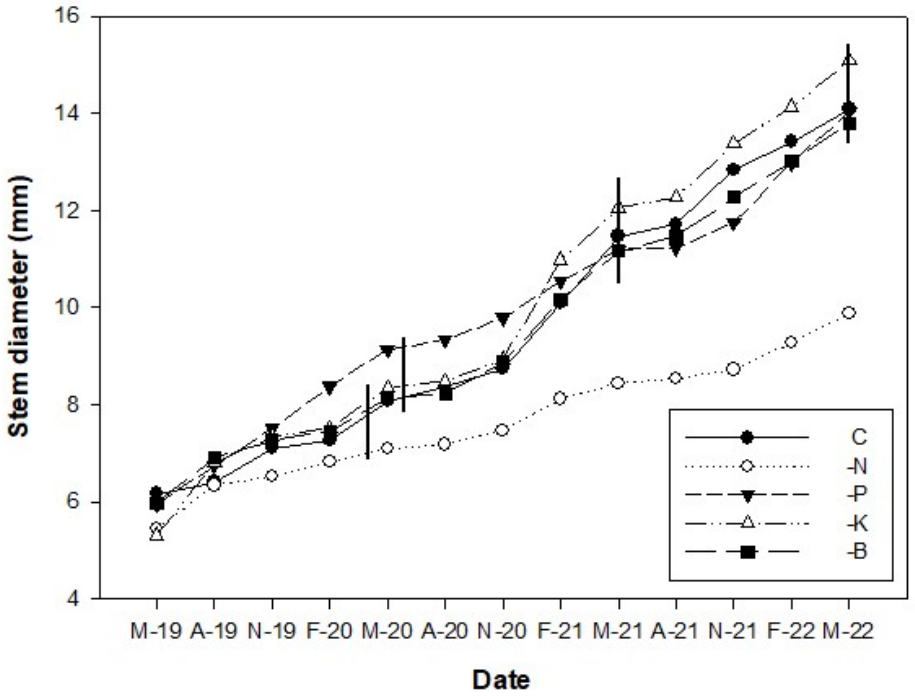

Figure 3 – Stem diameter (mm) of young olive trees, considering the following nutritional treatments: complete (C-all nutrients) and omission of nitrogen (-N), phosphorus (-P), potassium (-K) and boron (-B). Vertical bar indicates least significant difference by Tukey's test with $p<0.05$ (n=4).

The -K treatment stands out, whose average value at the end of the test was about 34% higher than -N (Figure 3). Stem growth occurred in all the experiments, contrary to total height, especially in the warmer months (November to May) (Figures 2, 3).

For dry matter, no significant differences were observed between treatments considering the root system (Figure 4A). Nonetheless, the -N treatment is again emphasized, which presented a mean value 61 % lower than the complete treatment (Figure 4A). In the shoot dry matter, significant differences were observed for the -N and -P treatments, which exhibited lower matter compared to C (Figure 4B).

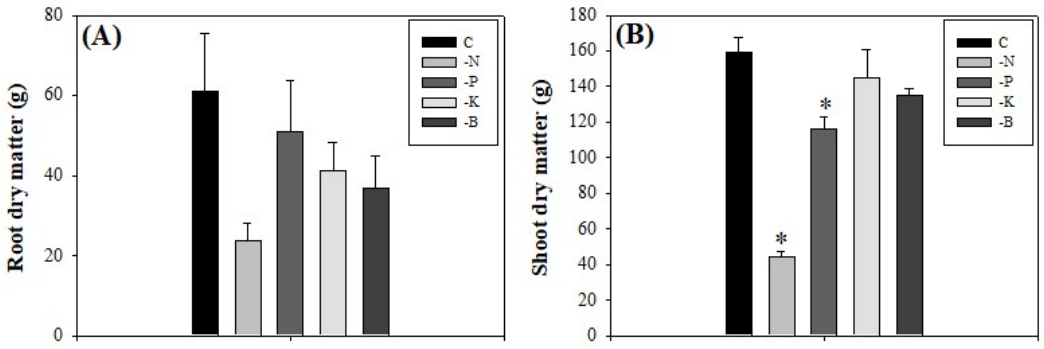

Figure 4 – Dry matter of roots and shoots (g) of young olive trees, considering the following nutritional treatments: complete (C-all nutrients) and omission of nitrogen (-N), phosphorus (-P), potassium (-K) and boron (-B). Asterisk indicates significant difference from the control treatment, according to Dunnett's test ($p \leq 0.05$).

3.2 Nutrient content

All treatments with nutritional deficiency differed in the content of minerals in relation to the complete treatment, in at least one of the tissues and/or in the soil of the pots (Figure 5). The -N treatment exhibited lower N concentration in the soil and leaves, as well as lower B content in the soil (Figures 5A, D, E). The content of minerals evaluated in the roots did not differ from C (Figures I-L).

The omission of P affected mainly soil nutrients, with P, K and B having lower contents than the complete treatment (Figures 5B-D). In the roots, P concentration was also lower, while the N content was higher than C (Figures 5I, J). Although the -P treatment did not differ statistically from the full treatment, the P content in leaves in -P was 51% lower than in C. (Figure 5F).

The -K treatment resulted in lower K content in soil, leaves and roots, as well as B content in leaves (Figures 5C, G, H, K). Potassium concentration in -K was 52%, 68% and 74% lower, respectively, in the soil, leaves and roots compared to the complete treatment. Induced B deficiency produced lower levels of the micronutrient in soil and leaves, as well as N (Figures 5A, D, E, H). B content did not differ from C in roots (Figures 5I-L).

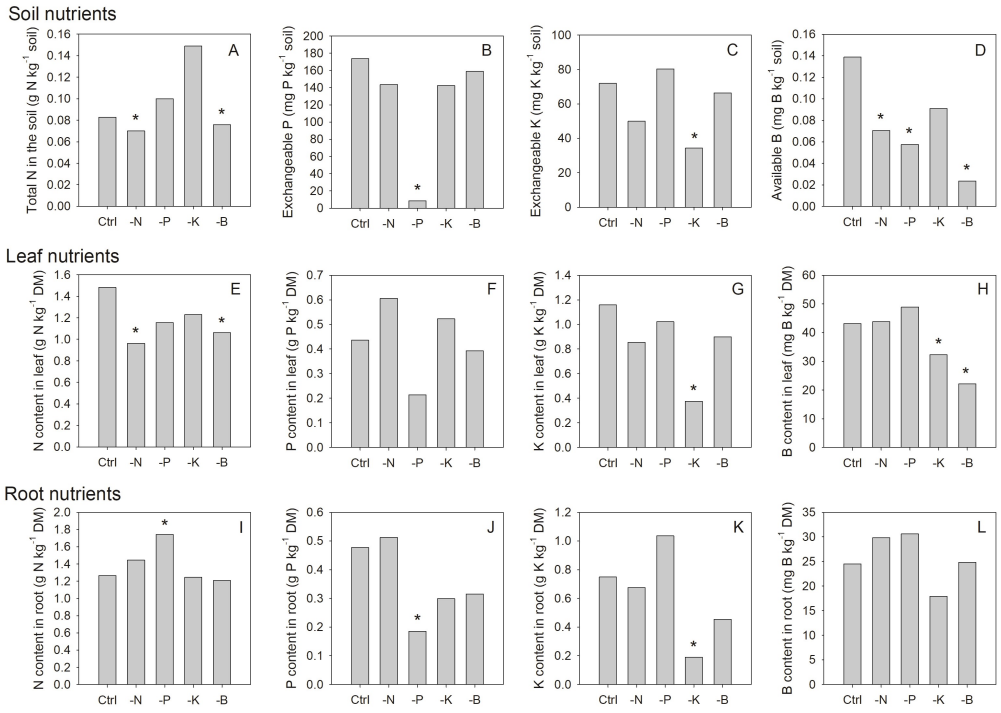

Figure 5 – Content of nitrogen, phosphorus, potassium and boron in the soil (A-D), leaves (E-H) and roots (I-L) of young olive trees, considering the following nutritional treatments: complete (C-all nutrients) and omission of nitrogen (-N), phosphorus (-P), potassium (-K) and boron (-B). Asterisk indicates significant difference from the control treatment, according to Dunnett's test ($p \leq 0.05$).

3.3 Phenolic compounds

The HPLC analyzes allowed the identification and quantification of 11 phenolic compounds in the leaves and 10 compounds in the roots, since *p*-coumaric acid was not identified (Tables 1, 2). The treatments exhibited different responses in relation to the content of phenolic compounds in the roots and leaves.

For the -N treatment, there was a discrepant pattern between roots and leaves. The roots showed lower concentration of some of the main phenolic compounds, while the opposite trend was observed in the leaves, with higher levels of phenolic alcohols, flavonoids and chlorogenic acid in relation to the complete treatment (Tables 1, 2). Hydroxytyrosol and kaempferol were the compounds that showed the most divergent patterns, with lower levels in the roots of 4% and 9%, respectively, as opposed to higher concentration of 32% and 15% in the leaves, compared to C (Tables 1, 2).

Table 1. Concentration of phenolic compounds (mg.100g^{-1} DW) in roots of young olive trees, considering the following nutritional treatments: complete (C-all nutrients) and omission of nitrogen (-N), phosphorus (-P), potassium (-K) and boron (-B).

Phenolic compound/ Treatment	**C**	**-N**	**-P**	**-K**	**-B**
Oleuropein	1016.04 (16.18)	689.37* (1.45)	1097.49* (2.31)	660.26* (3.01)	1090.89* (8.97)
Verbascoside	327.63 (31.92)	222.62* (13.95)	449.34* (5.29)	200.39* (17.00)	219.67* (10.01)
Hydroxytyrosol	8.83 (0.04)	8.46* (0.01)	8.79 (0.04)	8.69 (0.04)	8.74 (0.05)
Tyrosol	11.52 (0.25)	11.79 (0.41)	11.34 (0.33)	11.13 (0.27)	11.71 (0.27)
Quercetin	24.85 (0.10)	24.92 (0.47)	24.43 (0.06)	24.34 (0.04)	24.79 (0.27)
Kaempferol	21.66 (0.50)	19.68* (0.25)	20.22 (0.46)	20.94 (0.52)	23.58* (0.33)
Caffeic acid	2.53 (0.11)	2.41 (0.11)	2.65 (0.14)	2.44 (0.10)	2.43 (0.02)
Chlorogenic acid	6.02 (0.11)	5.80 (0.06)	6.03 (0.14)	5.94 (0.09)	6.34 (0.13)
p-Coumaric acid	-	-	-	-	-
trans-Cinnamic acid	2.59 (0.11)	1.85 (0.15)	2.20 (0.30)	2.44 (0.22)	3.06 (0.08)
trans-Ferulic acid	11.52 (0.69)	10.85 (0.25)	10.38 (0.34)	12.14 (0.36)	17.42* (0.85)

Mean values ± SE are presented (n = 3). Asterisk indicates significant difference between each treatment with omission and control (complete treatment), according to Dunnett's test ($p \leq 0.05$).

Phosphorus nutritional stress led to a higher concentration of several phenolic compounds, especially in the leaves (Tables 2). Verbascoside, for example, showed a higher content of 27% and 40% in roots and leaves, respectively (Tables 1, 2). It is also noteworthy that the average values of hydroxytyrosol and cinnamic acid in the leaves were double and 94 % higher to oleuropein, compared to the levels of these compounds in the C treatment (Table 2).

The treatment -K did not differ the concentration of most phenolic compounds, and the compounds that statistically differed showed lower levels in roots and leaves in relation to C (Tables 1, 2). Oleuropein and verbascoside are highlighted, which, respectively, presented values 35% and 40% lower in the roots based on the complete treatment (Table 1).

Table 2. Concentration of phenolic compounds (mg.100g^{-1} DW) in leaves of young olive trees, considering the following nutritional treatments: complete (C-all nutrients) and omission of nitrogen (-N), phosphorus (-P), potassium (-K) and boron (-B).

Phenolic compound/ Treatment	**C**	**-N**	**-P**	**-K**	**-B**
Oleuropein	1386.99 (154.42)	728.81 (86.53)	2686.39* (275.97)	849.00 (78.60)	1361.53 (26.42)
Verbascoside	381.70 (15.31)	336.06 (27.05)	630.91* (33.80)	318.79 (9.76)	562.16* (51.00)
Hydroxytyrosol	35.92 (1.47)	52.68* (4.56)	75.87* (4.57)	32.81 (1.07)	36.40 (0.53)
Tyrosol	19.97 (0.38)	27.95* (1.23)	23.58* (0.51)	18.92 (0.30)	21.55 (0.03)
Quercetin	37.39 (2.06)	56.73* (1.58)	38.81 (2.09)	36.74 (2.38)	34.49 (0.91)
Kaempferol	23.55 (0.77)	27.68* (0.65)	36.02* (1.46)	21.49 (0.11)	23.87 (0.35)
Caffeic acid	7.09 (0.18)	6.35 (0.35)	7.42 (0.20)	5.72* (0.15)	7.21 (0.11)
Chlorogenic acid	6.62 (0.17)	7.20* (0.16)	7.24* (0.08)	7.02 (0.15)	6.55 (0.00)
p-Coumaric acid	13.70 (0.35)	14.14 (0.56)	13.55 (0.28)	11.68* (0.18)	13.20 (0.10)
trans-Cinnamic acid	4.80 (0.27)	3.70 (0.38)	10.11* (0.78)	4.06 (0.27)	5.85 (0.68)
trans-Ferulic acid	24.32 (1.65)	26.91 (1.74)	23.14 (1.23)	22.66 (1.11)	23.87 (0.58)

Mean values ± SE are presented (n = 3). Asterisk indicates significant difference between each treatment with omission and control (complete treatment), according to Dunnett's test ($p \leq 0.05$).

In the case of the -B treatment, no significant differences were observed for most of the analyzed compounds, especially in the leaves (Tables 1, 2). Most of the phenolic compounds that differed showed a higher concentration than the complete treatment, however verbascoside showed the opposite tendency in the roots, with an average value 33% lower than C (Table 1). However, in the leaves the verbascoside content was 47% higher than the complete treatment (Table 2).

The concentration of phenolic compounds in general was higher in leaves than in roots for all treatments (Tables 1, 2). Hydroxytyrosol exhibited the greatest differences between roots and leaves, since the -P and -N treatments showed concentrations of the compound nine and six times higher in the leaves compared to the roots.

3.4 Molecular analysis

The expression of the genes evaluated in the roots showed different trends according to the nutritional omission. Levels of PAL gene transcripts increased in treatments with K and B omission (Figure 6A). As for CuAO, treatment with K deprivation showed the opposite pattern with a decrease in expression, while the omission of P induced higher levels of CuAO gene transcripts (Figure 6B). Considering PPO gene expression, there was a decrease in the number of transcripts in the treatment with omission of K (Figure 6C).

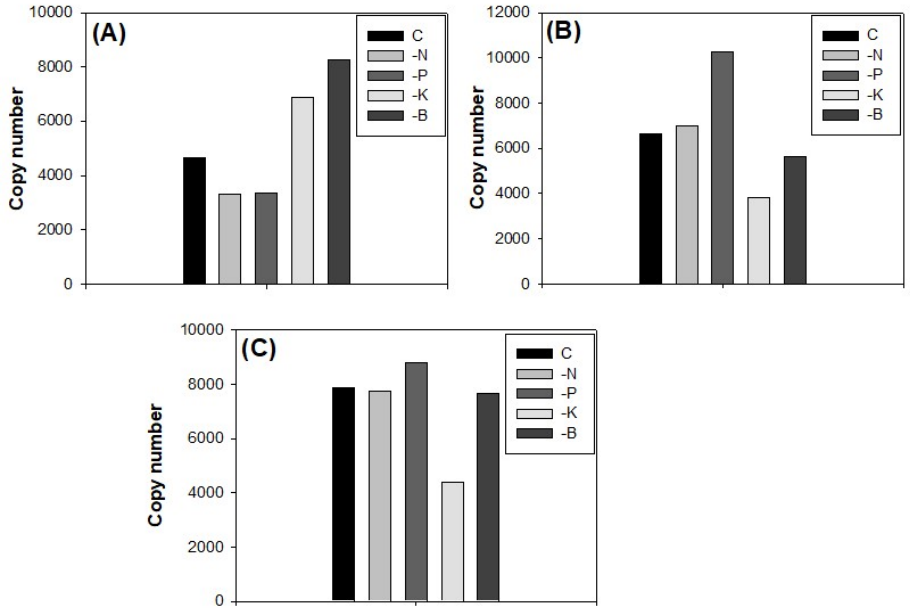

Figure 6. Copy number of transcripts in roots of young olive trees, considering the following nutritional treatments: complete (C-all nutrients) and omission of nitrogen (-N), phosphorus (-P), potassium (-K) and boron (-B). (A) phenylalanine ammonia-lyase (PAL), (B) copper methylamine oxidase (CuAO) and (C) polyphenol oxidase (PPO).

4. Discussion

4.1 Nitrogen

Nitrogen deficiency caused the most severe nutritional stress for young olive trees, considering the analyzed parameters.The reduction in morphometric and dry matter parameters, compared to the complete treatment, especially in the aerial system, indicates that the absence of N in the long term compromises the growth of

young olive trees. Nitrogen is an element present in molecules essential for the functioning of organisms, such as amino acids, nucleotides, chlorophyll, among others (Raven et al., 2004). Nitrogen is a component of structural proteins and enzymes, especially in growth regions and regulates cell expansion and division, which is reflected on a larger scale in increased biomass, plant growth and productivity (Lawlor et al., 2001). Decreased growth of the aerial and root systems were also reported in other studies with olive trees subject to N omission (Boussadia et al., 2010; Naija et al., 2014; Souza et al., 2019).

Leaf N content (<1.4 g. $100g^{-1}$) present in all treatments with nutrient omission, is considered insufficient to attend the nitrogen demands of olive trees (CQFS-RS/SC, 2016). The omission of N in young olive trees caused lower N concentration, besides a marked reduction in B in the leaves and roots (Souza et al., 2019). Naija et al. (2014) found lower levels of N in treatment with N deprivation in olive trees, and at the end of the experiment the macronutrient content was not detected in roots in the -N trial, as well as in the -P and -K treatments, indicating that the root system was negatively affected. Such an outcome was contrary to the trend of the present study, which did not indicate variations in the N content in the roots, considering all treatments with nutrient deprivation.

The harms impaired by the N deficiency on morphological and morphometric alterations in the first year of deprivation and on the olive metabolism after three years of omission reinforce the importance of adequate annual nitrogen fertilization. It should be taken into account that both the deficiency and excess of nitrogen can cause damage to plant development, as well as negatively interfere in the quality of the olive oil, in addition to economic and environmental damage (Erel et al., 2008; Fernández-Escobar et al., 2012; Erel et al., 2013; Bouhafa et al, 2014; Fernández-Escobar, et al., 2014; Centeno et al., 2017; Othman and Leskovar, 2019).

Since the growth of the aerial system was damaged by the absence of N, it is suggested that there is a trade-offs between growth and defense, according to the resource allocation theory, which assumes that in situations of limited supply there is a direction of the resources for certain physiological processes, limiting others (Caretto et al., 2015). The increase in the levels of phenolic compounds in the leaves corroborates with this theory, as some compounds such as phenolic alcohols can enhance the tolerance to nutritional stress. However, an opposite trend was observed in the roots, as four phenolic compounds exhibited lower concentrations than the

complete treatment. In this case, it is proposed that in the root system there is a gradual decline of the metabolic processes involved with growth and defense, indicating that the plant tissues of olive trees can develop different metabolic strategies in a situation of severe nitrogen shortage.

Among the phenolic compounds with discrepant patterns between roots and leaves, hydroxytyrosol stands out. In experiments with drought stress, higher levels of hydroxytyrosol and tyrosol were observed in olive tree leaves, while in the roots there was a reduction in the level of such compounds (Mechri et al. 2019; Mechri et al. 2020). The increased concentration of flavonoids (kaempferol and quercetin) in leaves under N deficiency may be associated with physiological protection for organisms exposed to oxidative stress from different sources, mediating the interaction of plants with the environment (Agati et al., 2012).

Phosphorus

Olive trees with omission of phosphorus showed later symptoms of nutritional stress, since up to 21 months the plants presented relatively greater height and diameter than other treatments. After this period, P scarcity seemed to accentuate, as visual symptoms began to appear more frequently, with a tendency towards total height stabilization and a smaller diameter increment. Young olive trees deprived of phosphorus showed reduced leaf size, wrinkling of older leaves along with mild chlorosis, which gradually turns reddish until the leaves fall, reduced height growth and low shoot-to-root ratio compared to nourished plants (Hartmann and Brown, 1953; Jiménez-Moreno and Fernández-Escobar, 2016; Souza et al., 2019). In the assay, it was observed that the parameters evaluated in the scanning of the roots did not differ from the control treatment. Even though phosphorus is crucial for root growth, the impact of P deficiency can vary according to the cultivar, as demonstrated by Naija et al. (2014), in which Chetoui cv. exhibited root growth similar to the control, while Meski cv. showed only 10.7% of root growth in relation to the control.

The phosphorus content for all treatments, with the exception of -P, was considered very high in the soil (> 60 mg. kg^{-1}) and high in the leaves (> 0.3 g. kg^{-1}) (CQFS-RS/SC, 2016), indicating that the periodic fertilizations along the experiment could have been carried out more sparingly. For the treatment with P omission, the nutrient content was very low in the soil (≤ 10 mg. kg^{-1}) and adequate in the leaves

(0.1-0.3 g. kg^{-1}) (CQFS-RS/SC, 2016). Such discrepancy evidences the relatively low requirement of P by the trees in the initial growth and suggests the use of the macronutrient accumulated in the tissues of seedling plants as an important pool during the period without nutrient exportation by fruit yield.

The application of phosphorus in olive groves is recurrent in fertilization programs, however its amount has been questioned, due to the low response of olive trees to such fertilization (Rodrigues et al., 2012; Jiménez-Moreno and Fernández-Escobar, 2016; Ferreira et al., 2018; Fernández-Escobar, 2019). The longer response time to the induction of nutritional deficiency by phosphorus presented in the study indicates that young olive trees used their own nutritional reserves for almost two years, without showing such severe symptoms. The low phosphorus requirement for olive trees may be a consequence of the ability of perennial woody plants to reuse P and low P removal (Jiménez-Moreno and Fernández-Escobar, 2016). Evaluating 13 olive orchards in southern Brazil, Bender et al. (2018) reported that most leaf samples (76%) presented appropriate level, although 49% of the soils exhibited low levels of exchangeable P. In an olive grove in Portugal, Ferreira et al. (2018) also observed P levels in the soil below the critical level (87%), however, 83% of the leaf samples showed adequate levels of this nutrient. In both studies, the authors comment on the need for adjustments in the interpretation of phosphorus in the soil for olive requirements.

Although P deprivation had a slight effect on shoot and root growth in our study, its scarcity markedly affected the biochemical compounds in young olive trees, mainly in leaf tissue. Phosphorus deprivation promoted the increase of most of the phenolic compounds analyzed in the leaves, besides oleuropein and verbascoside in the roots. Compounds such as hydroxytyrosol, verbascoside and oleuropein have antioxidant activity, due to the hydroxyl groups presented in the molecules, which can bind to free radicals (Hassen et al., 2015; Talhaoui et al., 2015; Marković et al., 2019). Oleuropein and verbascoside were the phenolic compounds present in the highest concentration in leaves and roots of olive trees. Higher concentrations of oleuropein compared to the control treatment were also observed in studies with induction of drought, cold and saline stress for olive trees (Ortega-García and Peragón, 2009; Petridis et al., 2012; Mechri et al. 2019; Mechri et al. 2020). In addition to the oxidizing activity, oleuropein also acts in the defense mechanism

against predation (Konno et al., 1999) and verbascoside presented fungicidal potential (Markakis et al., 2010), collaborating to the protection against biotic stress.

Phenylpropanoids such as cinnamic and chlorogenic acids were also produced at higher levels under P omission. Cinnamic acid contributes to abiotic stress tolerance through induction of ROS scavenging enzymes and reduction of lipid peroxidation (Sun et al., 2012; Singh et al., 2013). Chlorogenic acid also plays an important role in abiotic stress tolerance, protecting cells from oxidative stress (Soviguidi et al., 2022). Therefore, it is suggested that the stress generated by the deficiency of P, as well as of N, induces the production of chlorogenic acid, which protects olive tree leaves from the damage caused by the omission of such macronutrients.

Potassium

Potassium deficiency in olive trees generated more attenuated nutritional stress compared to other treatments. According to Naija et al. (2014) the omission of potassium induced a reduced development of the root system, limiting the absorption of water and nutrients. However, in the present study, the opposite trend was observed, as the length and root density of plants deprived of K were superior to the complete treatment.

The shortage of potassium in olive trees can be visually noticed in the older leaves, with symptoms of chlorosis at the apex and margin of the leaves. When such nutritional deficiency becomes more severe, mainly combined with periods of drought, chlorosis becomes necrosis (Fernández-Escobar et al., 2016; Erel et al., 2017; Souza et al., 2019). Olive trees submitted to K omission and drought presented damaged in the regulation mechanism of the stomata (Arquero et al., 2006; Benlloch-González et al., 2008). Arquero et al. (2006) identified that young olive trees irrigated and adequately supplied with K exhibited greater root and aerial growth than plants with less K fertilization and water restriction, so that the plants exhibited impairment in the stomatal closure mechanism, being more susceptible to dehydration. In the case of the present study, it was observed that K omission without water stress did not negatively affect growth parameters in the aerial and root systems.

Potassium deprivation caused a marked reduction in the concentration of the macroelement, exhibiting low concentration in the soil (31-60 $mg.kg^{-1}$), besides to insufficient content in leaves (<0.4 $g.kg^{-1}$) (CQFS-RS/SC, 2016) and reduced content

in roots. As for the other treatments, the K content in the soil was considered high and K concentration in the leaves was adequate (CQFS-RS/SC, 2016), suggesting that the potassium fertilization carried out in the assay was satisfactory. The reduction in K levels in soil and tissues was not accompanied by a decrease in morphometric parameters until the end of the experiment. These results indicate that the K requirement by the young olive trees was low. However, it is of utmost importance to consider that K is the main nutrient removed by fruit yield and pruning in olive orchards. For productive olive trees, annual exportation of K by fruits and pruning were estimated at 4.42 g. kg^{-1} MF and 1.98 g. kg^{-1} MF, respectively, totaling 45.5 kg. ha^{-1}.year (Fernández-Escobar et al., 2015). Potassium fertilization should take into account foliar analyzes and the type of soil, as some mother rocks are rich in K, so the need for this nutrient will be lower (Tiecher et al., 2020).

Most phenolic compounds did not change their levels due to K deficiency in the aerial system and roots, however in the latter there was a marked reduction in the levels of oleuropein and verbascoside. It is assumed that in the root system the allocation of resources has been directed towards growth, in detriment of stress tolerance and defense processes.

Boron

The omission of B did not produce changes in the morphometric parameters in the root and aerial systems, contrary to Souza et al. (2019) who observed a high reduction in dry mass, height and diameter of the stem in olive trees seedlings deprived of B, similarly to plants with N deficiency. Possibly, because it is a micronutrient, the olive trees evaluated in the present assay had initial reserves that could ensure long-term supply of B. Boron deficiency produced in other studies with olives trees, a several morphological alterations in the aerial system, such as the formation of small, distorted leaves, with apical chlorosis in young leaves; leaf drop and death at growing points; young leaves in the form of rosettes; lateral growth and multiple branches; malformed and parthenocarpic fruits (Arrobas et al., 2010; Fernández-Escobar et al., 2016; Souza et al., 2019).

Available boron concentration in soil was considered low for all treatments with nutrient omission (<0.1) (CQFS-RS/SC, 2016), especially in -B treatment, whose concentration was 86% lower than in C treatment. In addition to B uptake by the plants, it is probable that part of the boron from the fertilization was lost by leaching

along the experiment, causing the low soil content. However, B content in the leaves for all treatments was considered adequate (19-150 $mg.kg^{-1}$; CQFS-RS/SC, 2016), even for the -B treatment. The concentration of B observed in the treatment with deprivation of B in the leaves and roots of 22 $mg.kg^{-1}$ and 25 $mg.kg^{-1}$, respectively, was similar to those found by Souza et al. (2019) in olive seedlings under deprivation, of 19 $mg.kg^{-1}$ and 29 $mg.kg^{-1}$, for the aforementioned plant tissues.

It is noteworthy that the B deficiency negatively influenced the N content in the soil and leaves, indicating a possible relationship between the two nutrients. Studies with boron-deficient tobacco (*Nicotiana tabacum* L.) plants have observed a decrease in nitrate uptake and induced repression of some genes expression related to nitrate and ammonium metabolism (Camacho-Cristobal and Gonzáles-Fontes, 2007). It was also observed that the application of B at moderate levels contributes to the increment of N in amylacea maize (*Zea mays* L.) under salinity (Fuertes-Mendizábal et al., 2020).

The study provides evidence that seedlings with an excellent production pattern were able to store B in their tissues for a long period, thus reducing the effect of B omission. Fertilization with B should be carried out during the period of bud differentiation and flowering, when there is a greater metabolic demand for this micronutrient (CQFS-RS/SC, 2016).

As with the omission of K, the -B treatment did not alter the content of most phenolic compounds. Among the compounds that exhibited the highest content in the roots, ferulic acid stands out. Such phenolic acid was investigated in excess by B in wheat leaves (*Triticum aestivum*) and it was observed that the compound regulated antioxidant enzymes in nutritional stress (Yildiztugay et al., 2019). Oleuropein also exhibited higher levels of B in roots under B stress, contrary to what was found by Liakopoulos and Karabourniotis (2005), which observed higher levels of oleuropein in nourished adult olive trees, compared to plants with B deficiency.

Regarding gene expression, greater changes in the analyzed transcripts were observed in the treatment with omission of K, B and P. PAL is the first enzyme in the biosynthesis of phenylpropanoids, converting L-phenylalanine into cinnamic acid (Alagna et al., 2012). Up-regulated of PAL transcripts in treatments with K and B deprivation indicate that such nutritional stress caused activation of the phenylpropanoid pathway. Rossi et al. (2016) evaluated the effect of salt stress on gene expression and also observed an increase in the level of PAL gene transcripts

for Frantoio in the roots, with the opposite trend being observed for Leccino, indicating that gene expression levels vary according to cultivar and organ/tissue. Biotic stress caused by *Colletotrichum* spp., the causative agent of anthracnose, also influenced PAL gene expression levels, depending on the fungus inoculation rate and cultivar (Gouvinhas et al., 2019). CuAO and PPO enzymes are associated with the formation of hydroxytyrosol and tyrosol (Alagna et al., 2012; Mougiou et al., 2018). Down-regulated CuAO and PPO transcripts observed in potassium deprivation treatment suggest a decrease in the pathway of formation of such phenolic alcohols.

Conclusion

The nutritional stress caused by the deprivation of nutrients showed different effects according to the type of omission, and the shortage of nitrogen produced greater damage in young olive trees after three years. Nitrogen omission caused decrease in growth and mass mainly in the aerial system; reduction in N concentration in soil and leaves; alteration in levels of phenolic compounds in roots and leaves. Olive trees deprived of phosphorus showed later symptoms of nutritional stress. The omission of phosphorus caused a decrease in the shoots dry mass; lower P content in soil and roots; increase of most of the phenolic compounds analyzed in the leaves.

The deprivation of potassium produced the lowest response to nutritional stress. There was a greater length and density of the roots, in comparison with the complete treatment. The concentration of the macroelement was lower in the soil and tissues. The discrepant patterns in gene expression observed with the omission of potassium suggest greater activation in the phenylpropanoid pathway and reduced formation of hydroxytyrosol and tyrosol, indicating effects of nutritional stress at the molecular level. Nutritional stress with omission of boron did not generate morphometric changes in shoots and roots; B content was lower in soil and leaves; the concentration of most phenolic compounds did not differ from the complete treatment, as observed in plants with omission of K.

Phenolic compounds showed different patterns in relation to shortage for each nutrient, and in some cases opposite trends were observed to different plant tissues, within the same treatment, indicating that the same phenolic compound can present an increase in concentration in one tissue and a decrease in another, in order to attend the demands of the plant.

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Supplementary material

Table 1S. Parameters of the calibration curves obtained for the phenolic compounds in the HPLC-DAD.

Phenolic compounds	**Concen-tration (µg/mL)**	**Min***	**Standard curve**	**R^2**
Hydroxytyrosol	2.5 - 50	4.4	$1.489 + 2.70x10^{-5} \times A$	0.999
Tyrosol	2.5 - 50	5.8	$1.805 + 5.36x10^{-5} \times A$	0.999
Chlorogenic acid	0.5 - 50	6.5	$0.949 + 1.79x10^{-5} \times A$	0.998
Caffeic acid	0.1 - 50	7.2	$0.384 + 6.97x10^{-6} \times A$	0.999
p-Coumaric acid	2.5 - 50	9.2	$1.742 + 7.86x10^{-6} \times A$	1
trans-Ferulic acid	2.5 - 50	10.0	$1.673 + 1.59x10^{-5} \times A$	1
Verbascoside	2.5 - 500	11.1	$15.482 + 7.15x10^{-5} \times A$	0.992
Oleuropein	5.0 - 500	14.0	$17.089 + 9.22 \times 10^{-5} \times A$	0.994
trans-Cinnamic acid	0.1 - 50	15.8	$0.227 + 3.12x10^{-6} \times A$	1
Quercetin	2.5 - 50	16.8	$4.276 + 4.00 \times 10^{-5} \times A$	0.994
Kaempferol	2.5 - 50	19.3	$3.213 + 3.67 \times 10^{-5} \times A$	0.990

*Time retention

Table 2S. Primers used for quantitative real-time polymerase chain reaction assays. Gene annotation according to database from the NCBI (National Center for Biotechnology Information).

Gene Annotation	**Name**	**Sequence (5'->3')**	**Amplicon length (bp)**
Phenylalanine ammonia-lyase	PAL-F	CCTCCGTGGAACAATCAGTT	121
	PAL-R	CTCAGCCGTCAAGGATTCTC	
Copper methylamine oxidase	CuAO-F	CCTTACCTCCAGCTGATCCAT	291
	CuAO-R	GATCATTGGGATCTCCATAGG	
Polyphenol oxidase I, chloroplastic	PPO-F	CTATGAAAGAATATTGGGCAA ACTG	252
	PPO-R	ACGCTGCGAATCATTTCACTA TAT	

Effect of boron fertilization and phenological period on boron content in olive leaves

Efeito da adubação com boro e do período fenológico sobre o conteúdo de boro em folhas de oliveiras

Efecto de la fertilización con boro y el período fenológico sobre el contenido de boro en hojas de olivo

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Abstract
Boron (B) is one of the most important nutrients in olive growing, and in Brazil there is still a lack of studies that evaluated the effect of fertilization with B for olive trees. Thus, the aim of the study was to verify whether soil and foliar boron fertilization influenced the B leaves content, during the flowering period and the beginning of fruiting. Two olive groves were evaluated in the central region of the state of Rio Grande do Sul, in Caçapava do Sul (CS) and São Gabriel (SG), adopting a bifactorial experimental design with soil B doses (0, 25, 50 and 100 g) and presence/absence of foliar B. Boron contents were quantified before fertilization (T0), after soil fertilization (T1) and after foliar fertilization (T2). Fertilization with B in the soil and foliar did not increase the levels of foliar B. However, it was observed that B concentration decreased in T1 (flowering) compared to T0 (pre-flowering), with a reduction in mature leaves of CS and SG of approximately 45% and 34%, respectively. Boron concentration increased again in T2 (initial fruit development), regardless of fertilization, statistically differing from T1, in both olive groves. Higher B content was observed in CS in all evaluated periods, possibly being associated with higher concentration of B in the soil. The present study reinforces the hypothesis that in olive trees there is remobilization of B from the leaves to attend the metabolic needs of reproductive structures, especially in floral development.
Keywords: Granular fertilizer; Foliar fertilizer; Arbequina; Flowering; Initial fruit development.

Resumo
O boro (B) é um dos nutrientes mais importantes na olivicultura, sendo que no Brasil ainda uma escassez de estudos que avaliaram o efeito da adubação com B para oliveiras. Deste modo, o objetivo do trabalho foi verificar se a adubação do solo e foliar com boro influenciou os teores foliares de B, durante o período de floração e início da frutificação. Foram avaliados dois olivais na região central do estado do Rio Grande do Sul, em Caçapava do Sul (CS) e São Gabriel (SG), adotando-se um planejamento experimental bifatorial com doses de B solo (0, 25, 50 e 100 g) e presença/ausência de B

foliar. Foram quantificados os teores de B antes da adubação (T0), após a adubação no solo (T1) e após a adubação foliar (T2). A adubação com B no solo e foliar não aumentou os teores de B foliares. Entretanto, observou-se que os níveis de B diminuíram em T1 (floração) em comparação com T0 (pré-floração), com redução nas folhas maduras de CS e SG de aproximadamente 45% e 34%, respectivamente. A concentração de boro aumentou novamente em T2 (inicio da frutificação), independente da adubação, diferindo estatisticamente de T1 em ambos os olivais. Maior conteúdo de B foi observado em CS em todos os períodos avaliados, possivelmente sendo associados com maior concentração de B no solo. O presente estudo reforça a hipótese de que em oliveiras há remobilização de B das folhas para atender as necessidades metabólicas de estruturas reprodutivas, em especial no desenvolvimento floral.
Palavras-chave: Adubo granular; Adubo foliar; Arbequina; Floração; Desenvolvimento inicial do fruto.

Resumen
El boro (B) es uno de los nutrientes más importantes en la olivicultura, y en Brasil aún faltan estudios que evalúen el efecto de la fertilización con B para los olivos. Así, el objetivo de este estudio fue verificar si la fertilización foliar y del suelo con boro influyó en los niveles foliares de B, durante el período de floración y el inicio de la fructificación. Se evaluaron dos olivares en la región central del estado de Rio Grande do Sul, en Caçapava do Sul (CS) y São Gabriel (SG), adoptando un diseño experimental bifactorial con dosis de B al suelo (0, 25, 50 y 100 g) y presencia/ausencia de foliar B. Los contenidos de B se cuantificaron antes de la fertilización (T0), después de la fertilización al suelo (T1) y después de la fertilización foliar (T2). La fertilización con B en el suelo y foliar no incrementó los niveles de B foliar. Sin embargo, se observó que los niveles de B disminuyeron en T1 (floración) en comparación con T0 (prefloración), con una reducción en hojas maduras de CS y SG de aproximadamente 45% y 34%, respectivamente. La concentración de boro volvió a aumentar en T2 (inicio de la fructificación), independientemente de la fertilización, diferenciándose estadísticamente de T1 en ambos olivares. Se observó un mayor contenido de B en CS en todos los períodos evaluados, posiblemente asociado con una mayor concentración de B en el suelo. El presente estudio refuerza la hipótesis de que en el olivo se produce una removilización de B desde las hojas para cubrir las necesidades metabólicas de las estructuras reproductivas, especialmente en el desarrollo floral.
Palabras clave: Fertilizante granular; Fertilizante foliar; Arbequina; Floración; Desarrollo de frutos inmaduros.

1. Introduction

Olive cultivation is one of the oldest agricultural practices in the world, with archaeological records in the region of Palestine, Israel, Jordan and Syria from 6500 BP, followed by the Crete region of Greece from 6000-5500 BP (Langgut et al., 2019). Later, olive growing expanded to other regions of the Mediterranean and currently spread to the five continents, such as Australia, China and Brazil (Lucena et al., 2017). Rio Grande do Sul is the state with the largest olive oil producer in the country, followed by the Serra da Mantiqueira region, with emphasis on the states of Minas Gerais, São Paulo and Rio de Janeiro (IBRAOLIVA, 2022). From 2010 to 2022, olive oil production in Rio Grande do Sul followed an upward trend, with fluctuations in production due to climatic conditions and possibly alternation of production, and in 2022 a record harvest of 448,500 l of olive oil was reported (João, 2022).

According to the Agroclimatic and Edaphoclimatic Zoning, about 7.4 million hectares of the territory of Rio Grande do Sul has recommended edaphoclimatic conditions for the cultivation of olive trees (Alba et al., 2013). In 2022 the area with olive cultivation covered about 6000 ha in Rio Grande do Sul (Ambrosini et al., 2022). Most information on olive cultivation is based on the Mediterranean region, however, for the establishment of the culture in Brazil, research is fundamental with the aim of adapting cultural practices to regional conditions such as adapted cultivars, pest and disease control, pruning, fertilization and liming, among others.

Regarding fertilization, boron (B) stands out as one of the most important micronutrients for olive trees, whose deficiency is recurrent in olive groves (Bender et al., 2018). Boron contributes to the formation of cell

wall structure, reproduction and other processes of primary and secondary metabolism of plants (Raven et al., 2001). Due to its importance, boron needs to be continuously absorbed by roots and translocated to plant tissues through the vascular system (Blevins and Lukaszewski, 1998). For olive trees, a high demand for boron has been observed, especially during the period of bud differentiation and flowering, and the application of fertilizer based on B is recommended, with doses suggested in Brazil of 25-40 g B via soil/plant or foliar fertilization of 0.1% B before flowering (Mesquita et al., 2012).

Studies on the effect of boron fertilization on olive trees have shown an increase in B concentration in leaves after fertilization, as well as in buds, flowers and increase of fruit set (Delgado et al., 1994; Perica et al., 2001a; Ateyyeh & Shatat, 2006; Hegazi et al., 2018; Ferreira et al., 2019; Pascović et al., 2019). Despite the increasing number of publications considering national olive oil (Minuceli et al., 2021; Gonçalves et al., 2022) and its by-products (Ripoll et al., 2022; de Souza et al. 2022), there are few studies on nutrient recommendation and fertilization in Brazil for olive trees (Mesquita et al., 2012; CQFS-RS/SC, 2016, Bender et al., 2018; Tiecher et al., 2020, Figueiredo et al., 2022; Figueiredo, 2022). Thus, the aim of the study was to evaluate whether soil and foliar boron fertilization modified the levels of B in adult olive tree leaves, during the flowering period and the beginning of fruiting.

2. Methodology

2.1 Study area

The assay was conducted in two commercial olive orchards in the central region of the state of Rio Grande do Sul, in the municipalities of Caçapava do Sul (CS) (30°24'50”S, 53°27'55”W) and São Gabriel (SG) (30°05'10.6”S, 54°36'35.2”W), from July to November 2019. The climate is characterized as subtropical Cfa in both municipalities, according to the Köppen classification. Accumulated precipitation during the evaluated period was 716 mm and 701 mm, respectively for Caçapava do Sul and São Gabriel, while the minimum and maximum temperatures recorded were 8.4-25.9 °C and 7.9-28.5 °C for the aforementioned locations (Rio Grande do Sul, 2019). Olive trees (*Olea europaea* L.) of the Arbequina cultivar were selected, due to their great expressiveness in Brazilian olive orchards. The olive trees in CS were ten years old, planted 5 x 3 m apart in Regolithic Neosol. In SG, the olive trees were seven years old, spaced 7 x 5 m apart in Dystrophic Red Argisol. The olive groves received fertilizers in accordance with the olive fertilization recommendations for Brazil (CQFS-RS/SC, 2016; Tiecher et al., 2020), with the exception of the source of boron.

2.2 Experimental design

The experiment was carried out in a two-factor design with randomized blocks and split plots (n=3). Factor A, in the main plots, consisted of the application of boron to the soil at rates of 0, 25, 50 and 100 g per tree of granular fertilizer, while factor B, in subplots, consisted of the presence and absence of application of foliar fertilizer. Each tree consisted of an experimental unit, totaling 24 trees in each field experiment. The same experimental design was replicated in the two olive orchards.

The fertilizer applied via soil was Ulexite, which presented 10% total B and 6% B soluble in citric acid. The fertilizer rates were broadcast around the trunk, in a square area of 4 m^2 (2 m x 2 m) below the canopy. For foliar B fertilizer, in turn, the commercial product Bortrac™ (10.9% B) was sprayed, in a single application, using 10 mL of Bortrac™ and 5 mL of adjuvant in 1.5 L of aqueous solution per tree, as indicated by the

manufacturer. Samples of mature leaves were collected in three periods: in pre-flowering, before application of B via soil (July) (T0); at flowering, 45 days after fertilization with Ulexite (August) (T1); at the beginning of fruit development, 18 days after application Bortrac™ (November) (T2). From each tree, 100 leaves were collected, washed with distilled water, dried at 50°C until constant weight and ground (18 mesh).

2.3 Chemical analysis

For the determination of boron in the leaves, B was initially extracted by burning in a muffle furnace at 600°C and was determined by colorimetry using azomethine-H (Tedesco et al., 1995). In total, 144 samples were evaluated, considering the two olive groves and the three times analyzed.

2.4 Statistical analysis

The boron content in leaves was statistically evaluated through factorial analysis of variance (blocks; treatments with B in the soil; presence of foliar fertilization; time sampling) with comparison of means by Tukey test ($p<0.05$). Analyzes were performed using the statistical program Sisvar version 5.8. Differences in leaf B concentration between olive groves were evaluated by Mann-Whitney, using the SigmaPlot version 11.0 program. Median values and quartiles were graphically represented by box-plot, with the aim of evaluating the amplitude of data distribution.

3. Results and Discussion

The boron content in leaves in both olive groves, in all treatments and sampling times was between 23.6 - 67.8 mg.kg^{-1} (average values; Table 1). The values fell within the threshold range of foliar levels considered adequate for olive trees, between 19 and150 mg.kg^{-1} (Mesquita et al., 2012; CQFS-RS/SC, 2016).

Table 1 – Boron concentration in mature olive leaves (mg.kg^{-1} DW) submitted to soil and foliar fertilization in Caçapava do Sul (CS) and São Gabriel (SG) olive groves, in three sampling time: T0 = before boron application/pre-flowering; T1 = after soil boron application/flowering; T2 = after foliar boron application/initial fruit development.

Fertilization/	CS			SG		
Time sampling	T0	T1	T2	T0	T1	T2
0 g [a]	58.6±2.9 [c]	29.2±1.3	65.2±4.1	34.7±1.5	24.3±1.4	42.7±2.9
25 g	57.8±5.0	32.1±1.7	67.8±3.1	36.0±1.5	25.4±1.6	43.4±2.4
50 g	58.0±3.3	32.3±1.2	63.9±3.5	41.0±3.0	24.6±1.4	48.5±2.8
100g	52.6±1.9	31.4±0.8	66.2±1.5	37.4±4.6	24.1±2.0	48.9±3.9
	NS	NS	NS	NS	NS	NS
No foliar	55.2±1.9	31.3±1.0	64.0±2.2	39.5±2.6	25.5±1.1	46.8±1.2
With foliar [b]	58.2±2.8	31.2±0.9	67.6±2.2	35.0±1.0	23.6±1.0	45.2±3.0
	NS	NS	NS	NS	NS	NS
Ulexite x Bortrac™	NS	NS	NS	NS	NS	NS

[a] Grams of ulexite per tree.
[b]Bortrac™ (10.9% B), in a rate of 10 mL of commercial productin 1.5 L of aqueous solution/tree.
[c]Mean values ± SE are presented (n = 9).

Source: Authors (2023).

Boron concentration in leaves did not differ statistically among soil and leaf B treatments (Table 1).

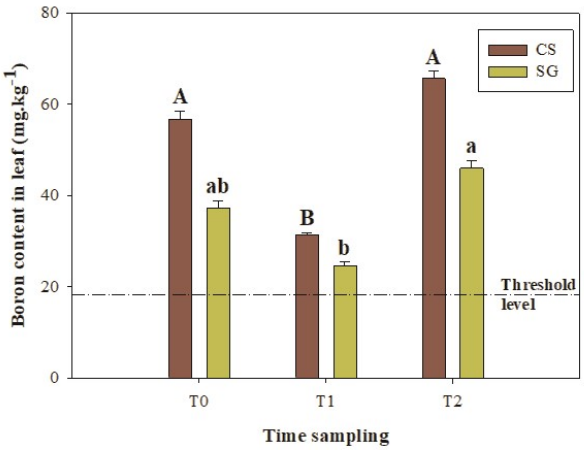
There was no significant difference between blocks, as well as the interaction of factors. It is important to highlight that before fertilization (T0), olive trees were nourished in relation to boron content, especially in CS (Table 1). In general, trials with fertilization for adult plants were conducted with olive trees with B deficiency or close to the limit of deficiency, as reported by Delgado et al. (1994) (19 ppm), Perica et al. (2001a) (17 ppm) and Hegazi et al. (2018) (13.4 ppm). In the present study, it was decided to carry out the experiments in olive trees without evident B deficiency in order to be connected to the reality of most olive growers currently in south of Brazil. It is worthy to note that no evidence of toxicity by B excess was noticed, even in the treatment with the largest rate of soil B fertilizer joined by foliar fertilization.

The absence of increase in leaf B content may be associated with the interval of time between the B fertilization and leaf sampling. The period of 45 days after broadcasting the non-soluble B-ulexite in the soil may be short to evidence increases in leaf B content because the nutrient uptake may be slow compared to the sink effect to the flowering demand. In the other side, the interval of 18 days to sprayed foliar B was within the period evaluated in the literature for olive trees, considering the effect of foliar fertilization with B, reported between 14-30 days (Perica et al., 2001a; Hegazi et al., 2018; Pasković et al., 2019).

Considering the same experimental design as in the present study, Figueiredo (2022) did not observe differences in the fruit yield in the 2020 harvest in the evaluated olive groves. However, in the following harvest, there was a greater residual effect for the treatment with 100g Ulexite/no foliar on fruit yield. Such result indicate that fertilization of B via soil, using a non-soluble source as ulexite, may supply the element in a very slowly manner. Contrarily to our results, Ateyyeh and Shatat (2006) observed an increase in the concentration of B in olive leaves and inflorescences after application of foliar B, however they did not observe increase in fruit yield between fertilized and non-fertilized plants. The authors suggest that olive trees with adequate levels of B may not respond significantly to the application of the micronutrient.

Regarding the sampling time of leaf tissue, a significant difference was observed in the two olive groves. In CS, T1 (flowering) differed from the other periods, with lower levels of B in the leaves. In SG, T1 exhibited lower B content than T2 (initial fruit development), but did not differ from T0 (pre-flowering) (Figure 1).

Figure 1 – Boron concentration in mature olive leaves ($mg.kg^{-1}$ DW) in Caçapava do Sul (CS) and São Gabriel (SG) olive groves, in three sampling time: T0 = before boron application/pre-flowering; T1 = after soil boron application/flowering; T2 = after foliar boron application/initial fruit development. Different letters, in the same olive grove, differ statistically according to Tukey's test with $p<0.05$ (n=9).



Source: Authors (2023).

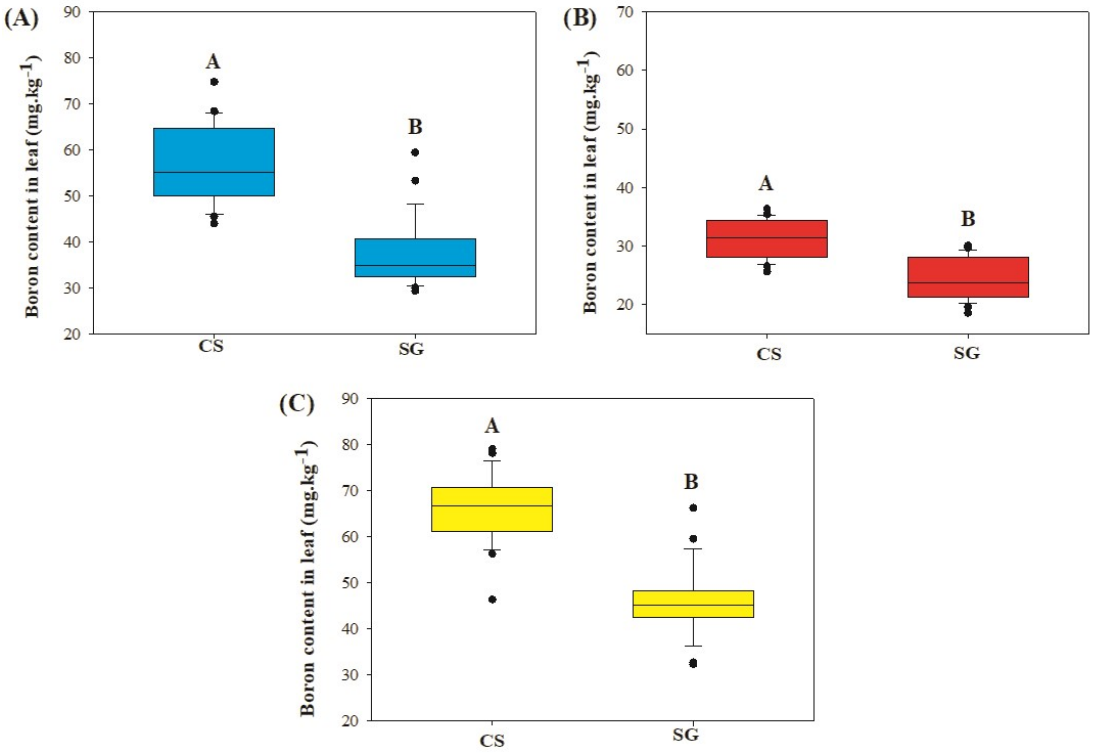
There was a reduction in boron concentration in mature leaves of CS and SG of approximately 45% and 34% respectively, at T1 compared to T0 (Figure 1). In the literature, it has been suggested that there is translocation of B from vegetative tissues to reproductive structures, especially flowers, so that such structures stock greater amounts of such micronutrient (Dell & Huang, 1997; Blevins & Lukaszewski, 1998). Studies with olive trees corroborate this hypothesis, since higher B contents were observed in reproductive structures, compared to vegetative structures (Perica et al., 2001a; Hegazi et al., 2018). In addition, a marked reduction in the levels of B in leaves during the anthesis period was reported (Delgado et al., 1994).

The retranslocation of B by the phloem has been associated with the formation of a complex of boron with transport molecules of sugars or polyols (Brown & Shelp, 1997; Blevins & Lukaszewski, 1998). Perica et al. (2001b) in assays with B fertilization in olive trees found evidence of B remobilization from leaves of different ages to inflorescences and fruits, through the phloem with transport mediated by sugars, such as mannitol and glucose. Liakopoulos et al. (2005) in studies with nutritional deficiency of B in olive trees found evidence of mobilization of the micronutrient from mature leaves to young leaves, in addition to an increase in the concentration of mannitol in plants with deficiency. Ferreira et al (2019) also suggest mobility of B in olive trees, and the retranslocation of B is dependent on the cultivar and must be taken into account in boron fertilization. Du et al. (2020) in studies with B foliar fertilization in Citrus trees showed long-distance mobilization of B, through a B-sucrose complex, from leaves to roots.

It stands out that B content in leaves at T2 practically doubled compared to T1. Thus, it is suggested that the amount of B in immature fruits is sufficient to attend their metabolic demands. Boron concentration differed between olive groves at the three sampling times, with CS showing higher values than SG (Figure 2). It was found that in T0 and T2 the SG olive grove showed a smaller range of data variation than CS.

Figure 2 – Box-plots of boron concentration in leaves of mature olive leaves (mg.kg^{-1} DW) in Caçapava do Sul (CS) and São Gabriel (SG), in three sampling time: T0 = before boron application/pre-flowering (A); T1 = after soil boron application/flowering (B); T2 = after foliar boron application/initial fruit development (C). Each box-plot is represented by the minimum, maximum, lower horizontal line of the box (1st quartile), intermediate line (median, 2nd quartile), upper horizontal line (3rd quartile) and 'outliers' (points). Different letters indicate

statistical differences according to the Mann-Whitney test with $p<0.05$ (n=27).



Source: Authors (2023).

Figueiredo (2022) analyzed the concentration of B in the soil at T0 and found values of 0.44 and 0.11 $mg.kg^{-1}$ in CS and SG, respectively. In addition, the author observed that the soil in SG is more sandy, with a lower clay content (4.8 $g.100g^{-1}$) than in CS (7.4 8 $g.100g^{-1}$), making it more difficult for the roots to absorb B, factors that may have influenced the differences found in the two olive groves.

4. Final Considerations

Fertilization for adult olive trees with soil fertilizer at doses from 0 to 100 g and foliar fertilizer did not increase B content in relation to non-fertilized trees. The micronutrient concentration before fertilization showed adequate levels, with a marked reduction being observed in the flowering period, regardless of the treatment with B. At the initial fruit development, exhibited B concentration similar to pre-flowering. It is suggested mobilization of B from leaves to flowers, corroborating results in the literature for olive trees. Boron content was higher in CS than in SG and may be associated with higher levels of B in the soil.

Further studies with a greater number of fertilizer applications and longer sampling times, from the end of a season's harvest to the beginning of the next season, will help to understand the effect of B fertilization on olive trees in different phenological periods. Future researches are also suggested to evaluate higher doses of foliar B, which do not lead to toxicity, but which provide more pronounced effects on micronutrient concentration in different tissues of olive trees.

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Boron fertilization and its relationship with boron and phenolic compounds content in olive leaves

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ABSTRACT

Boron (B) is an essential micronutrient for plants, but its effect on secondary metabolism of olive trees is not well known. The study investigated the B and phenolic compounds concentration in olive leaves before and after B fertilization. The experiment was carried out with mature olive trees in two olive groves, evaluating three treatments:(i) no B fertilization; (ii) B fertilization via soil; (iii) B fertilization via soil + foliar. Mature leaves were collected in three sampling times: pre-flowering, before soil B application (July); flowering (August); initial fruit development (November). Boron contents in leaf tissue were not significantly affected by B fertilization. However, the concentration decreased in the flowering period compared to pre-flowering, evidencing a mobilization of the micronutrient in order to attend to the demands of B by the floral structures. Regarding 11 phenolic compounds analyzed, only kaempferol exhibited significantly higher levels in the soil and foliar B treatment, but absolute values were predominantly larger in B-amended plants for most of the compounds. Oleuropein, verbascoside and hydroxytyrosol were the main compounds present in the leaves. Most of the phenolic compounds showed a higher concentration at the beginning of fruiting, compared to pre-flowering. It is concluded that in the analyzed reproductive period were observed significant differences in the content of metabolites evaluated and indicative of the mobilization of B from vegetative to reproductive structures.

Keywords: Arbequina, Plant nutrition, Reproductive phase; Phenols; Maceration

1. Introduction

The cultivation of olive trees (*Olea europaea* L.) is a millenary practice in the Mediterranean basin, which covers 98% of world production (Lucena et al., 2017). Nevertheless, in recent years, the cultivation has expanded to other regions of the world, such as South America, notably in Argentina, Chile, Peru, Uruguay and Brazil (Tapia et al. 2009; Torres et al. 2017; Conde-Innamorato et al. 2019). Humid tropical and subtropical regions have higher rainfall rates compared to the Mediterranean region, besides to areas with acidic, sandy or highly weathered soils, prone to leaching of nutrients (Mesquita et al., 2012). Thus, it is evident the need for assessments of adequate macro and micronutrients contents in olive groves, according to regional edaphoclimatic conditions.

Boron is an essential micronutrient in the primary and secondary metabolism in plants, constituting the cell wall and membrane structure, beyond impacts pollen germination, pollen tube formation and fruiting (Blevins and Lukaszewski, 1998; Raven et al., 2001). The B deficiency has been identified in several olive groves around the world (Tsadilas and Chartzoulakis, 1999; Sibbett and Ferguson, 2002; Soyergin et al., 2002; Bender et al., 2018). In this case, there is a suggestion of fertilization with B, and in Brazil the recommendation is 25-40 g of B via soil and 0.1% of foliar fertilizer (Mesquita et al., 2012), however in commercial olive groves it is customary to carry out fertilization even without deficiency before flowering.

The effect of B fertilization for olive trees has been associated with increase in the concentration of such micronutrient in reproductive and vegetative tissues; increase in the percentage of perfect flowers, fruit set, fruit size and weight; decrease in the percentage of fruit drop; increased content of other compounds, such as pigments, total soluble sugars, fructose, sucrose, glucose, gibberellic acid, volatile compounds, total phenols, antioxidant activity, macronutrients and others micronutrients, as well as increased fruit and olive oil yield and improved olive oil quality parameters (Delgado et al, 1994; Perica et al., 2001a; Gul et al., 2017; Hegazi et al., 2018; Ferreira et al., 2019; Pasković et al., 2019; Vishekaii et al., 2019; Genaidy et al., 2020; Rahmani et al. 2021).

Boron deficiency in olive trees has been associated with secondary metabolism, especially phenolic compounds (Liakopoulos and Karabourniotis, 2005; Karioti et al., 2006). Phenolic compounds are represented in olive trees by a series of

compounds such as phenolic acids and alcohols, lignans, flavonoids and secoiridoids (Servili et al., 2004; Charoenprasert and Mitchell, 2012; Bakhouche et al., 2013). In surveys with olive trees it was observed that foliar B fertilization in leaves was negatively correlated with the content of total phenols (Hegazi et al., 2018). In other studies, it was found an increase in the concentration of total phenols and oleuropein in leaves in foliar B fertilized plants in the greenhouse (Pasković et al., 2019) and total phenols in adult trees in olive trees under fertilization with boric acid and B nano-chelates (Vishekaii et al., 2019). Due to the discrepancy in the results and the gap of studies on the effect of B fertilization via soil on the levels of phenolic compounds, the main objective of this study was to analyze the response of olive trees to B fertilization (soil and foliar), considering the content of B and phenolic compounds in olive leaves. Increased levels of B and major phenolic compounds, such as oleuropein and verbascoside for adult olive trees are expected after fertilization.

2. Materials and methods

2.1 Experimental design and plant material

The study was carried out in two commercial olive orchards in Southern Brazil with adult olives trees (cultivar Arbequina) to July-November 2019.The climatic data are reported in Figure 1S (Supplementary material). All trees received recommended fertilizer (exception B) and no irrigation. Soil properties, plant age, spaced and coordinate of both olive orchards are presented in Table 1.

Table 1. Characterization of olive orchards in Rio Grande do Sul state, Brazil.

Parameters	**Caçapava do Sul (CS)**	**São Gabriel (SG)**
Localization	30°24'S, 53°26'W	30°05'S, 54°36'W
Age and spaced	10 years; 5 x 3 m	7 years; 7 x 5 m
Soil type	Regolithic Neosol	Dystrophic Red Argisol
Soil pH-H_20*	6.12	6.33
Soil B ($mg.kg^{-1}$)*	0.44	0.11

* Figueiredo, unpublished results.

Experiments were conducted followed by split plot randomized block design with three replicates. For the study, Ulexite (Produbor 10®, 10% total B, 6% soluble in citric acid) was used for fertilization with B in the soil and Bortrac™ (4.7% N; 10.9% B) was used as a source of foliar B. Three treatments were performed: 0 g Ulexite/no foliar B; 100 g Ulexite/no foliar B; 100 g Ulexite/with foliar B. Ulexite was broadcasted

on the soil surface within a square area of 4 m^2 (2 m x 2 m) around the olive trunk, only in July 2019. After two months from flower initiation, leaf fertilizer Bortrac™ was sprayed in the tree canopy, in a rate recommended by the manufacturer (10 mL Bortrac and 5 mL of adjuvant in 1.5 L of aqueous solution per tree, in a unique event of application). Neither B fertilization via soil nor foliar was repeated.

Mature leaves of olive trees were collected in three sampling times: pre-flowering, before soil B application (July 2019); flowering, 45 days after soil B fertilization (August 2019); initial fruit development, 18 days after foliar B application (November 2019). From each tree, 100 mature leaves were sampled on all sides of the crown, in the central part of the branches of the year, located at the height of the middle third of the crown. The leaves were washed with deionized water, oven dried at 50 ºC until constant mass, ground and sieved (particles with a diameter of less than 1000 µm were used).

2.2 Chemical analysis

Boron concentration was determined by the modified Azomethine dye method (Tedesco et al., 1995). Briefly, 500 mg of dried and ground leaves were burned in a muffle furnace at 600 ° C for 1 h. The ash was dissolved in five drops of distilled water and 10 mL of sulfuric acid solution with intermittent agitation for 1 h. After settling, in 4 mL aliquot of a supernatant sample solution was added 4 mL azomethine-H and buffer solution (3:1). The solution was kept in the dark for 30 min and the absorbance was determined in UV-VIS Spectrophotometer at 435 nm.

Extraction of phenolic compounds was carried out by maceration. Briefly, 500 mg of dried and ground leaves were mixed in 25 mL methanol. The mixture was agitated in a horizontal shaker at 90 rpm for 30 min. After centrifugation (10 min at 4000 rpm) the supernatant was filtered through a 0.45 mm syringe filter.Identification and quantification of individual phenolic compounds were analyzed using a high performance liquid chromatography (HPLC) based on a modification of the International Olive Council Method (IOC, 2017).

HPLC analyses were performed using an Agilent 64 (Agilent Technologies, Santa Clara, USA) equipped with a quaternary pump (1200 Series) and diode array detector (DAD) (Agilent 1260 Series Photo Diode Array Detector). The separation was conducted at 30°C using a reversed phase LC Column Eclipse Plus C18 (4.6 x 150 mm, 5 µm) (Supelco, Bellefonte, PA, USA). A ternary elution gradient was

established at a flow rate of 1.0 mL.min^{-1} with (A) water 0.2 % acetic acid (v/v), (B) methanol and (C) acetonitrile, as follows (A/B/C): 0 min (96:2:2 %); 5 min (80:10:10 %); 10 min (70:15:15 %); 20 min (50:25:25 %); 30 min (96:2:2%). The injection volume was set at 20 µL. Detection of phenolic compounds were performed at 280 nm and identified according to the retention times of pure standards Sigma-Aldrich® (Steineheim, Germany) and quantified using calibration curves presented in Table 1S (Supplementary material).

In the study, phenylpropanoids (phenolic acids and verbascoside), phenolic alcohols (hydroxytyrosol and tyrosol), flavonoids (kaempferol and quercetin) and secoiridoids (oleuropein) were evaluated, whose metabolic route is shown in Figure 1.

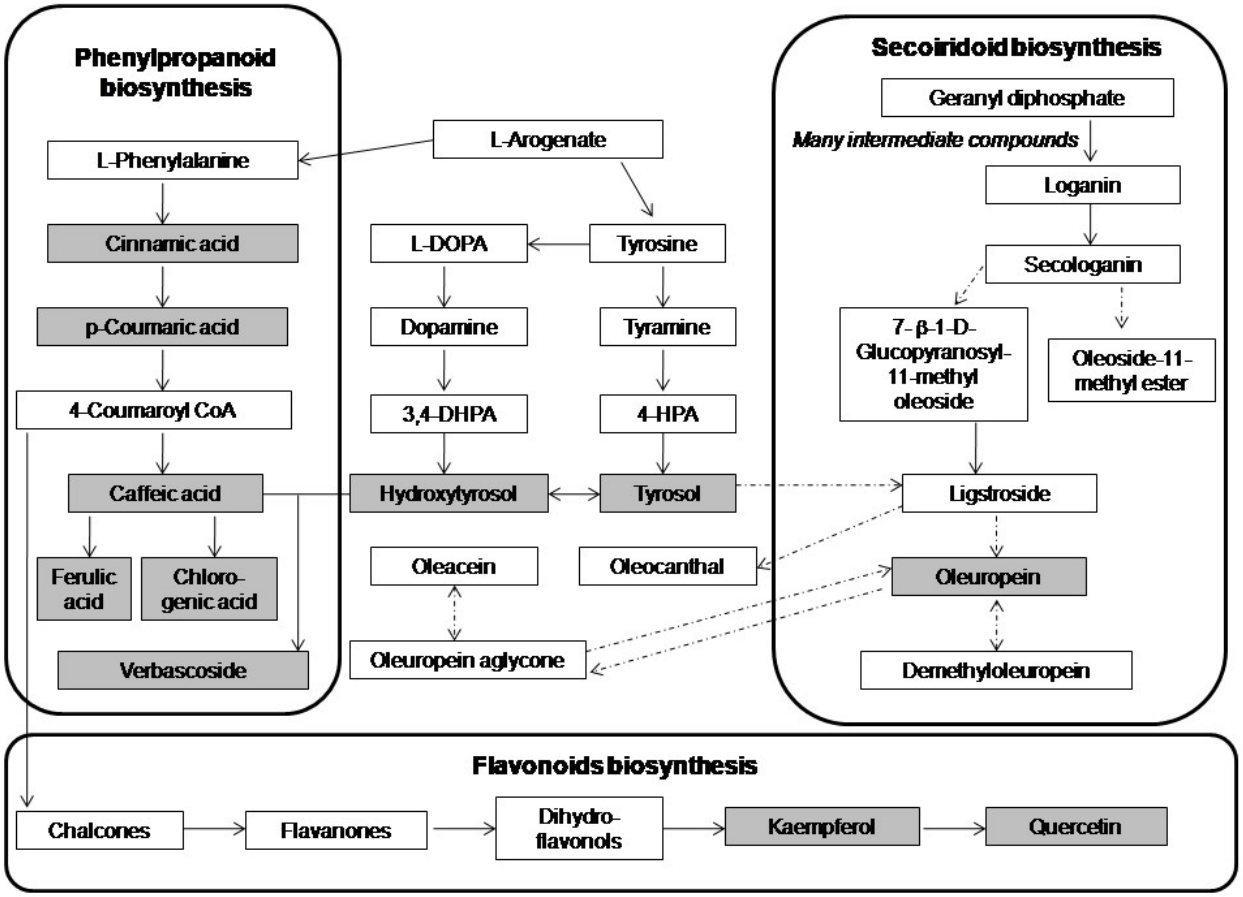

Figure 1. Metabolic pathway of phenolic compounds for olive trees. Dashed arrows indicate steps not yet elucidated. Gray phenolic compounds were evaluated in the study. Adapted from: Obied et al., 2008; Saimaru and Orihara, 2010; Agati et al., 2012; Alagna et al., 2012, Alagna et al., 2016; Mougiou et al., 2018.

2.3 Statistical analysis

Factorial analyses of variance (ANOVA) were used considering the treatments, time sampling and blocks. Significant differences were assessed by means of the Tukey's test ($p \leq 0.05$) performed using Sisvar version 5.8. Differences in the content of B and phenolic compounds between olive groves were evaluated by the Mann-Whitney test ($p \leq 0.05$) and represented by a box-plot using the program Sigma Plot version 11.0. Correlation between variables was determined with Pearson's correlation considering a confidence level of 95 % ($p \leq 0.05$). The data set was evaluated using Principal Component Analysis (PCA) in order to better explore the correlations of variables and highlight sample clusters. For the PCA, the B content and the phenolic compounds were used as variables, arranged in a correlation matrix using the Statistica version 7.0 program.

3. Results

3.1 Boron content

The leaves B concentration e in two olive groves was above the threshold level for all treatments, in the three evaluated periods, ranging from 18.6 to 73.2 $mg.kg^{-1}$.

The application of B fertilizers did not significantly affect the B content in leaf tissues (Table 2). However, B contents in leaves differed according to the sampling time. No interaction between treatments x time of sampling was observed.It was verified that B content decreased in the flowering period compared to pre-flowering. At the beginning of fruiting, B levels in leaves showed values twice as high as in flowering, regardless of fertilization (Table 2).

Boron content in leaves differed between olive groves, with CS exhibiting higher levels (49.72±3.05 $mg.kg^{-1}$ DW) than in SG (35.98±2.35 $mg.kg^{-1}$ DW).

Table 2. The effect of soil and foliar boron (B) application on B concentration in mature olive leaves (mg.kg^{-1} DW) in two olive groves in three periods: T0 = before boron application/pre-flowering; T1 = after soil boron application/flowering; T2 = after foliar boron application/initial fruit development.

	Caçapava do Sul	**São Gabriel**
Treatment	NS	NS
0 g Ulexite; NF	50.9 ± 5.8	34.3 ± 3.3
100 g Ulexite; NF	49.1 ± 5.3	38.7 ± 4.4
100 g Ulexite; WF	49.1 ± 5.3	34.9 ± 4.7
Period	p<0.001	p<0.001
T0	54.9 ± 1.9 b	35.6 ± 3.1 b
T1	31.0 ± 0.9 c	24.4 ± 1.4 c
T2	64.9 ± 2.9 a	48.0 ± 2.7 a
Block	NS	NS

Mean values ± SE are presented (n = 9). Different letters in the same column are significantly different at p ≤ 0.05 according Tukey's test. NF = no foliar boron; WF = with foliar boron.

3.2 Phenolic compounds

Significant differences were observed in the levels of phenolic compounds among treatments and among sampling time (Table 3; Figure 2).

Considering the response to B fertilization, only kaempferol in SG exhibited higher levels when B was added by both soil and foliar application (100 g Ulexite/WF treatment) than the B exclusively via soil and the unamended soil (Table 3). Regarding the period of sampling, it was found that all compounds, with the exception of cinnamic acid, differed statistically in at least one of the olive orchards (Figure 2).

Phenolic acids exhibited different behavior with respect to the sampling time and local. In the CS olive grove, caffeic, coumaric, cinnamic and ferulic acids showed no changes in their levels in the three periods, while chlorogenic acid exhibited higher concentration in T0 and T2. In the SG olive grove, the chlorogenic acid showed the same levels in the three evaluated periods, but the other acids showed a tendency of higher concentrations in T2 (Figures 2A-E).

Table 3. Concentrations (mg.100g^{-1} DW) in leaves of phenylpropanoids, flavonoids, phenolic alcohols and secoiridoid, considering boron application in two olive groves (Caçapava do Sul – CS; São Gabriel – SG).

Phenolic compound/ Treatment	**0 g Ulexite; No Foliar B**	**100 g Ulexite; No Foliar B**	**100 g Ulexite; With Foliar B**
Caffeic acid	CS 3.4 ± 0.3	3.5 ± 0.3	4.1 ± 0.5
	SG* 2.9 ± 0.3	3.0 ± 0.4	3.1 ± 0.4
Chlorogenic acid	CS 8.9 ± 0.2	9.0 ± 0.3	9.3 ± 0.5
	SG* 8.0 ± 0.1	8.0 ± 0.1	8.0 ± 0.1
p-Coumaric acid	CS 12.4 ± 0.4	12.8 ± 0.5	13.8 ± 0.6
	SG 10.7 ± 0.2	10.8 ± 0.2	10.7 ± 0.2
trans-Cinnamic acid	CS 6.8 ± 1.0	6.1 ± 0.2	6.1 ± 0.2
	SG 10.2 ± 0.8	10.9 ± 0.8	8.6 ± 0.4
trans-Ferulic acid	CS 25.5 ± 2.1	28.5 ± 2.4	29.0 ± 2.2
	SG* 13.9 ± 0.9	14.0 ± 0.9	14.6 ± 0.9
Kaempferol	CS* 30.8 ± 1.3	31.6 ± 1.4	31.0 ± 1.3
	SG 22.9 ± 0.2 b	22.8 ± 0.5 b	24.4 ± 1.1 a
Quercetin	CS 36.1 ± 1.9	36.0 ± 1.3	38.1 ± 2.3
	SG* 31.3 ± 1.6	29.3 ± 0.9	31.1 ± 1.8
Hydroxytyrosol	CS* 63.3 ± 4.3	66.0 ± 4.6	63.9 ± 5.8
	SG 60.7 ± 5.0	63.7 ± 5.6	68.5 ± 4.8
Tyrosol	CS 30.9 ± 3.6	31.3 ± 2.9	29.9 ± 3.6
	SG 14.6 ± 1.0	13.5 ± 1.1	13.8 ± 0.6
Oleuropein	CS* 454.4 ± 87.2	427.0 ± 60.4	518.9 ± 82.4
	SG 446.3 ± 110.7	582.6 ± 177.1	519.4 ± 106.3
Verbascoside	CS 227.3 ± 27.8	247.8 ± 23.7	307.0 ± 42.0
	SG 133.6 ± 19.7	158.6 ± 29.2	132.9 ± 13.1

*difference between blocks. Mean values ± SE are presented (n = 9). Different letters on the same line are significantly different at $p \leq 0.05$ according Tukey's test.

Flavonoids showed opposite trends considering the sampling time. The concentration of kaempferol increased from pre-flowering to the initial fruit development, while quercetin showed a decrease in its concentration considering the same period (Figures 2F-G).

Phenolic alcohols showed different behavior in the two olive groves. Hydroxytyrosol exhibited higher concentration in leaves in the reproductive period analyzed in CS, but in SG there was no statistically significant difference. Tyrosol levels did not differ in CS, however it showed higher values in T2 in the SG olive grove (Figures 2H-I).

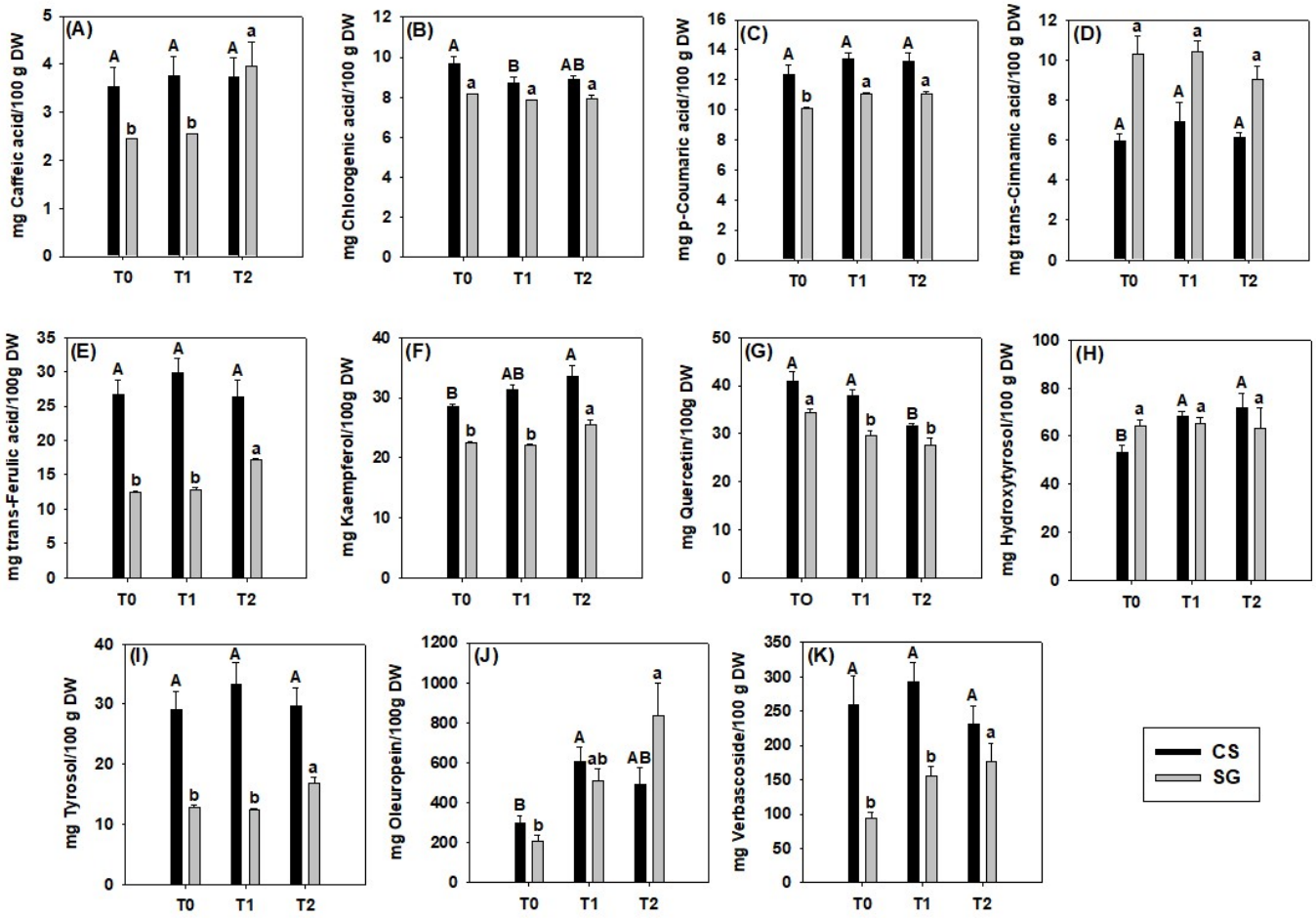

Figure 2. Concentration of phenolic compounds in two olive groves in three periods: T0 = before boron application/pre-flowering; T1 = after soil boron application/flowering; T2 = after foliar boron application/initial fruit development.

The concentration of oleuropein exhibited lower values in the pre-flowering period for both olive groves. On the other hand, the levels of verbascoside did not differ in the CS olive orchard, however in SG it presented an increase in its contents over the analyzed time (Figures 2J-K).

The concentration of phenolic compounds differed between olive groves, with the exception of hydroxytyrosol and oleuropein (Figure 3). In general CS exhibited higher content of phenolic compounds than SG, with the exception of cinnamic acid. It was also observed that in SG the concentration of verbascoside, tyrosol, kaempferol, chlorogenic, coumaric, caffeic and ferulic acids exhibited a smaller range of variation in the data compared to CS.

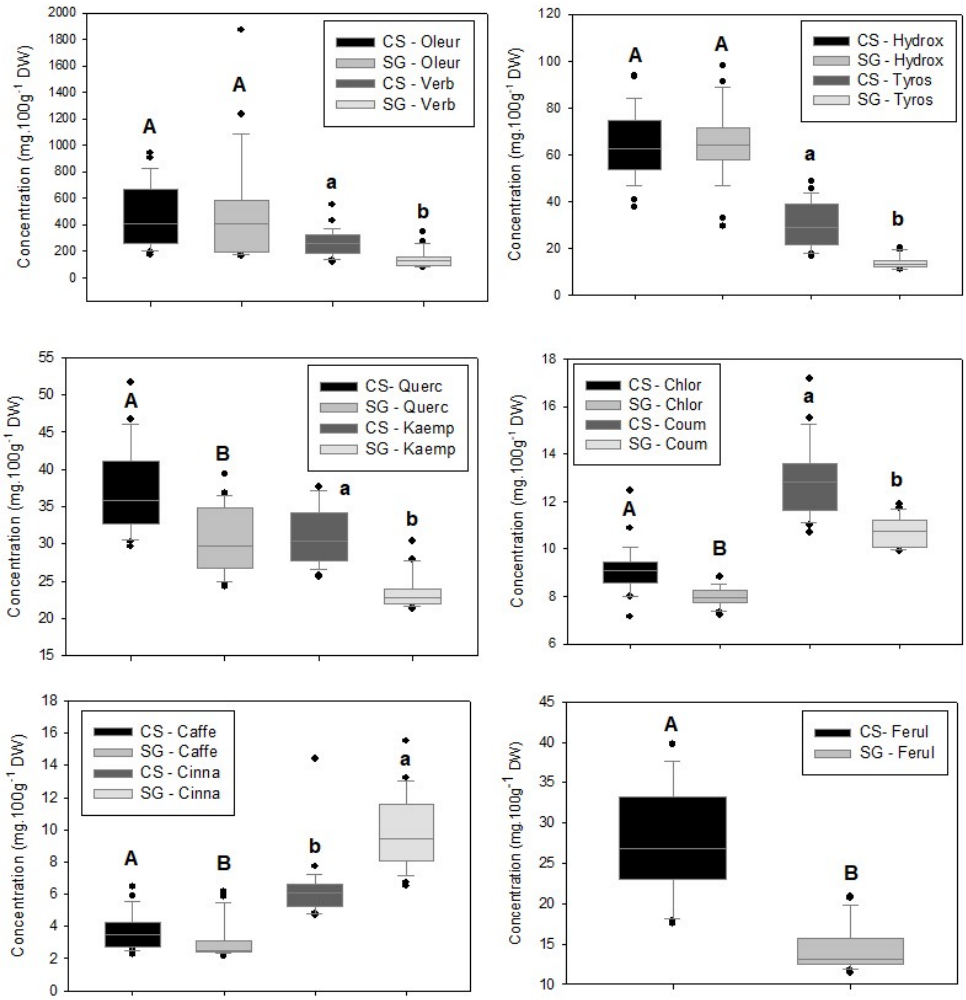

Figure 3. Box-plot of phenolic compounds in leaves of mature olive trees in two olive groves: Caçapava do Sul (CS) and São Gabriel (SG).

3.3 Correlations

Boron levels were significantly correlated with most of the phenolic compounds evaluated (Table 4). The positive correlation with kaempferol ($r = 0.51$; $p \leq 0.001$) stands out, being the same compound that showed a response in the treatment with B in soil and foliar (Table 2; Table 4). All significant correlations were positive, with the exception of cinnamic acid ($r = -0.46$; $p \leq 0.001$).

Cinnamic acid was inversely correlated with all other phenolic acids, in addition to kaempferol, tyrosol and verbascoside. It was observed that the proximity in the metabolic pathway in the formation of such polyphenols may not be the preponderant factor associated with the results found (Figure 1).

Table 4. Correlation between boron levels and phenolic compounds in leaves.

	2	3	4	5	6	7	8	9	10	11	12
Boron	0.28 *	0.33 *	0.30 *	-0.46 ***	0.35 **	0.51 ***	NS	NS	0.35 **	NS	NS
Caffeic acid (2)	-	0.34 **	0.62 ***	-0.41 **	0.73 ***	0.59 ***	NS	0.58 ***	0.64 ***	0.71 ***	0.70 ***
Chlorog. acid (3)	-	-	0.47 ***	-0.34 **	0.66 ***	0.52 ***	0.31 *	NS	0.70 ***	NS	0.50 ***
Coumaric acid (4)	-	-	-	-0.55 ***	0.82 ***	0.82 ***	0.34 **	0.32 *	0.72 ***	0.37 **	0.90 ***
Cinnamic acid (5)	-	-	-	-	-0.61 ***	-0.61 ***	-0.42 **	NS	-0.51 ***	NS	-0.49 ***
Ferulic acid (6)	-	-	-	-	-	0.86 ***	0.33 *	0.33 *	0.94 ***	0.29 *	0.78 ***
Kaempferol (7)	-	-	-	-	-	-	NS	0.37 **	0.81 ***	0.27 *	0.68 ***
Quercetin (8)	-	-	-	-	-	-	-	NS	NS	-0.33 **	0.32 *
Hydroxytyrosol (9)	-	-	-	-	-	-	-	-	0.28 *	0.62 ***	0.33 **
Tyrosol (10)	-	-	-	-	-	-	-	-	-	NS	0.71 ***
Oleurop. (11)	-	-	-	-	-	-	-	-	-	-	0.53 ***

NS = not significant. Significance levels: * (0.05); ** (0.01); *** (0.001). 12 = Verbascoside. n=54.

Significant positive correlation was observed between ferulic, coumaric, caffeic and chlorogenic acids, as well as verbascoside, kaempferol and tyrosol, in many cases with correlation values above 0.7. Quercetin, on the other hand, showed a contrary trend, so that most of the correlations were not significant, even with its precursor kaempferol (Table 4; Figure 1).

For oleuropein and hydroxytyrosol, it was found that most of the correlations with other compounds were not significant or were less than or equal to 0.5, with the exception of the correlation between them (0.62; $p \leq 0.001$). There was also a positive correlation between hydroxytyrosol and caffeic acid (0.58; $p \leq 0.001$) and oleuropein and caffeic acid (0.71; $p \leq 0.001$). It is noteworthy that caffeic acid and hydroxytyrosol are precursors of verbascoside, however oleuropein is found in another metabolic route far from such acid (Table 4; Figure 1).

3.4 Principal Component Analysis (PCA)

Multivariate exploratory analysis with PCA was performed using B content and concentration of 11 phenolic compounds as variables (Figure 4). The first two factors explained about 70% of the total variation in the data and the first five axes explained 90% of the variance (Figure 4A).

A

Value numb.	Eigen value	%Total variance	Cumul. Eing.	Cumul. %
1	6,20	51,6	6,2	52
2	2,15	17,9	8,3	70
3	1,04	8,6	9,4	78
4	0,88	7,3	10,3	86
5	0,53	4,5	10,8	90
6	0,39	3,2	11,2	93
7	0,32	2,7	11,5	96
8	0,22	1,9	11,7	98
9	0,13	1,1	11,9	99
10	0,08	0,7	11,9	100
11	0,03	0,3	12,0	100
12	0,02	0,2	12,0	100

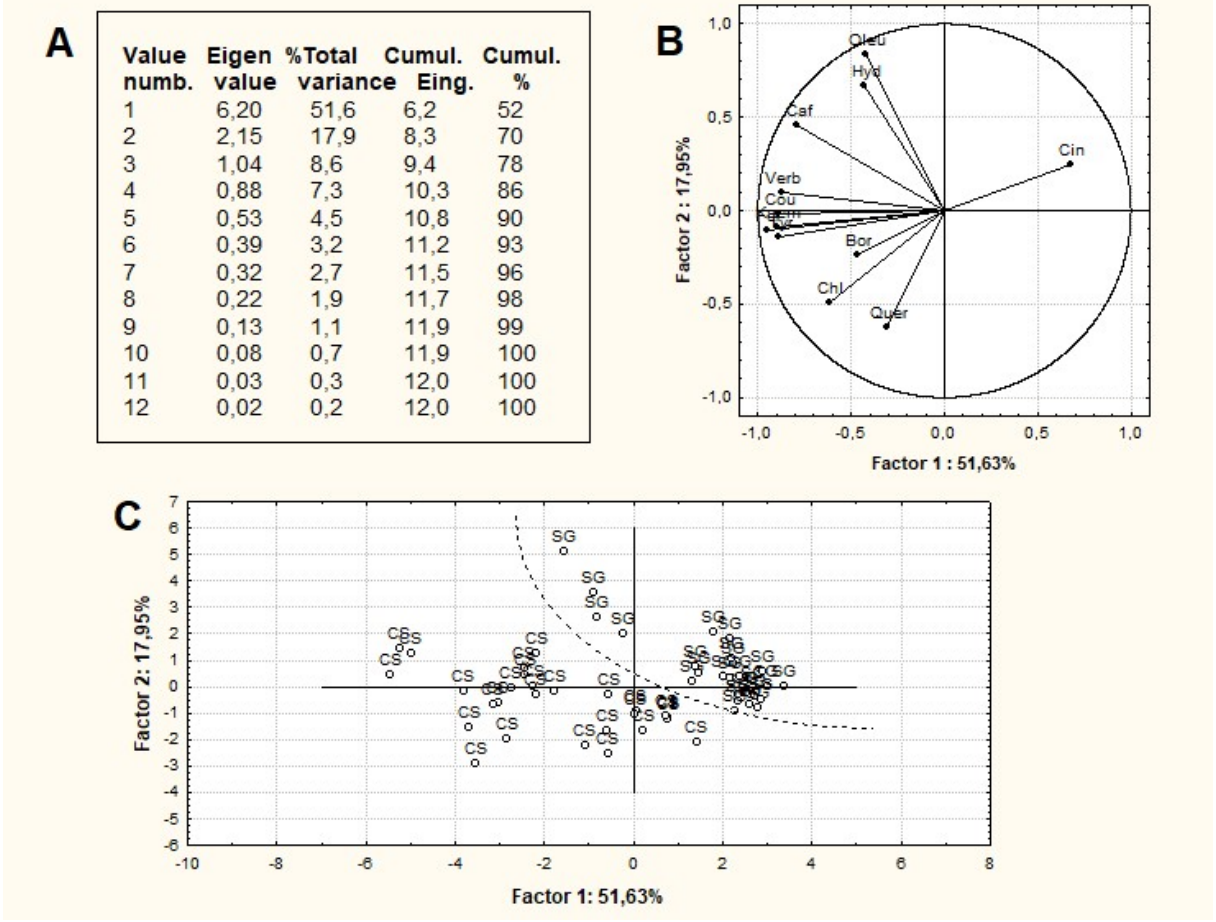

Figure 4. Results of the principal components analysis (PCA), represented by eigenvalues of correlation matrix and related statistics (A); loadings plot of boron and phenolic compounds in the leaves of olive trees (B); Scores plot of two olive groves, Caçapava do Sul (CS) and São Gabriel (SG). Boron (Bor); Oleuropein (Oleu); Verbascoside (Verb); Hydroxytyrosol (Hyd); Tyrosol (Tyr); Quercetin (Quer); Kaempferol (Kaem); Chlorogenic acid (Chl); p-Coumaric acid (Cou); Caffeic acid (Caf); trans-Cinnamic acid (Cin); trans-Ferulic acid (Fer).

Cinnamic acid had a great influence in the positive quadrant on PC1 and since was the only compound located in the positive quadrant, while the other phenolic acids, tyrosol and verbascoside contributed in the negative quadrant (Figure 4B). PC2 was influenced by oleuropein and hydroxytyrosol in the positive quadrant (Figure 4B). As shown in the Pearson's correlation, it was observed that cinnamic acid in PCA was negatively correlated with the other phenolic compounds and boron (Figure 4B). The positive correlation between oleuropein and hydroxytyrosol (0.62; $p \leq 0.01$) was also represented in the PCA, as well as the positive correlations between

verbascoside, tyrosol, kaempferol, coumaric and ferulic acids (Figure 4B). Cinnamic acid was the main variable that distinguished SG from CS, followed by oleuropein and hydroxytyrosol. The dataset in SG exhibited a more concentrated distribution than in CS, which exhibited a more dissimilar pattern (Figure 4C).

4. Discussion

4.1 Boron concentration: fertilization and time

The B content in the leaves was within the levels considered adequate for olive trees, between 19-150 $mg.kg^{-1}$ (Tiecher et al., 2020). In studies with olive trees, Tsadilas and Chartzoulakis (1999) observed more visible visual symptoms of foliar deficiency with B levels below 15 $mg.kg^{-1}$, while Fernández-Escobar et al. (2016) found deficiency symptoms with B concentrations of around 33 $mg.kg^{-1}$, after 20 weeks of micronutrient omission. In the present study, no deficiency symptoms were observed in any of the analyzed periods.

Low response of olive trees to B fertilization was observed, both in terms of foliar B content and the possible effect on phenolic compounds. In general, studies of B fertilization in olive trees have been conducted with plants with boron levels in mature leaves below 23 $mg.kg^{-1}$ before flowering (Delgado et al., 1994; Perica et al., 2001a; Ateyyeh and Shatat, 2006; Hegazi et al., 2018; Pascović et al., 2019). In the present work, the average levels of boron in the leaves in this period (T0) were higher than those reported in the literature (CS – 55 $mg.kg^{-1}$; SG – 36 $mg.kg^{-1}$). The application of B also did not influence fruit yield in the 2019/2020 season (Figueiredo, unpublished results). Ateyyeh and Shatat (2006) did not observe any effect of B fertilization on production, suggesting that plants with adequate levels of B may not respond significantly to the application of this nutrient.

Despite trees showed an adequate levels of B, was observed temporal variation in the micronutrient content is emphasized, since the decrease in flowering evidences the mobilization of B in the leaves to supply the demand for B for reproductive structures, which is in line with the assumption that reproduction demands more B than vegetative growth, or even that the reproductive structures store more B (Dell and Huang, 1997; Blevins and Lukaszewski, 1998). For olive trees, Delgado et al. (1994) verified that the B content in young leaves decreased during the anthesis period and Perica et al. (2001b) found evidence of B

remobilization from leaves of different ages to inflorescences and fruits. Literature also found a higher concentration of B in reproductive organs compared to vegetative structures in the olive trees (Perica et al., 2001a; Hegazi et al., 2018).

At the beginning of fruiting, B levels in leaves were higher than pre-flowering, indicating that for fruit formation, this nutrient is not so crucial for their development, as for flower formation and fertilization. In addition, the work demonstrates the dynamism of B mobilization in olive trees, even in mature leaves, as in less than two months the levels drastically decreased, followed by an increase after about three months, regardless of B treatment.

4.2. Relationship between B and phenolic compounds

Considering the effect of B fertilization on the levels of phenolic compounds in olive trees, it was observed that only kaempferol presented higher concentration in the complete treatment. Pascović et al. (2019) evaluated fertilization with B in young olive trees and observed that the levels of the majority of the 11 phenolic compounds analyzed did not differ from non-fertilized plants. Only oleuropein, caffeic and ferulic acid exhibited higher levels in fertilized plants, while cinnamic acid showed higher concentration in non-fertilized olive trees. Liakopoulos and Karabourniotis (2005) in an experiment with B deficiency in young and adult olive trees observed higher levels of coumaric and ferulic acid in young plants with deficiency and of quercetin in nourished plants. As for adult olive trees, the authors found that of the 11 phenolic compounds analyzed, only oleuropein showed a higher concentration in nourished plants, while the other compounds did not differ between treatments.

Studies with other species evaluating the relationship between fertilization with B and kaempferol levels showed discrepant results. Fertilization with B for tobacco (*Nicotiana tabacum* var. One Sucker) increased kaempferol levels (Watanabe et al., 1961). As for Norwegian spruce (*Picea abiens* L.), the concentration of kaempferol unchanging with B fertilization (Rummukainen et al., 2007). For soybean (*Glycine max* (L.) Merr.) fertilization with B was associated with a decrease in the levels of that compound (Al-Molla et al., 1990).

The response to treatment with B for kaempferol, as well as the positive correlation between them, indicates that the compounds may be related to physiological processes. Both B and kaempferol have been associated with pollen germination. It is assumed that for the formation of the pollen tube it is necessary to

use molecules with material from both the female floral parts and proteins from the pollen grains, emphasizing the importance of complexes of borate with residues of sugars, which provide the rapid pollen tube growth (Blevins and Lukaszewski, 1998). Kaempferol has been identified as a stigma exudate that stimulates pollen development and pollen tube growth (Mo et al., 1992; Guyon et al., 2000). Further studies are needed to assess the relationship between the two compounds in vegetative tissues for olive trees.

4.3 Phenolics compounds

The main phenolic compounds present in olive tree leaves were oleuropein, verbascoside and hydroxytyrosol. Such compounds help reduce free radicals, due to the presence of hydroxyl groups, which donate hydrogen and prevent oxidation (Hassen et al., 2015; Talhaoui et al., 2015; Marković et al., 2019). In addition to the antioxidant action, oleuropein contributes to a chemical defense mechanism through the enzyme β-glucosidase, which degrades part of the molecule. Subsequently, the aglyconated oleuropein acts as an alkylating agent, which participates in cross-linking with amino acids, producing a high molecular weight complex and decreasing lysine levels, thus reducing the nutritional value of predated/infected tissues (Konno et al., 1999)

Verbascoside, oleuropein, tyrosol, kaempferol, caffeic and ferulic acids were present in higher concentrations at the beginning of fruiting (spring) in at least one of the olive groves, which may be associated with the increase in temperature and with defense mechanisms against herbivory and fruit predation, leading to a increase in the activity of enzymes involved in the metabolism of polyphenols, such as phenylalanine ammonia lyase (PAL), β-glucosidase (GLU), polyphenol oxidase (PPO), among others. Quercetin, however, showed a reduction in its levels in the period of initial fruit development compared to pre-flowering, besides presenting a low correlation with the other polyphenols. Liakopoulos et al. (2006) observed that quercetin is found exclusively in the non-glandular trichomes of the leaves, emphasizing that its composition is more intense in the initial stages of leaf development. It is suggested that small changes in the trichome layer during the evaluated period may be associated with the pattern found.

The formation of phenylpropanoids starts with cinnamic acid, which is produced by phenylalanine through the PAL enzyme (Dixon and Paiva, 1995, Figure

1). It has been reported that when there is greater production of cinnamic acid than its utilization there is an inhibition of transcription and enzymatic activity of PAL, which is associated with other enzymes of the phenylpropanoid metabolic pathway, such as C4H (cinnamic acid 4-hydroxylase) and Chalcone Synthase (CHS) (Mavandad et al., 1990; Loake et al, 1991; Blount et al., 2000). In the present study, a negative correlation was observed between cinnamic acid and most of the analyzed phenolic compounds, through the Pearson's correlation and PCA, evidencing a regulatory mechanism in the production of polyphenols, especially phenylpropanoids. It is noteworthy that cinnamic acid levels did not differ in the treatments and sampling time. Blount et al. (2000) suggest that the pool of free cinnamic acid is not constant, with a compartmentalization of cinnamate occurring in plants, in which only a subfraction of the total pool is associated with the aforementioned feedback mechanism.

Regarding the differences reported in the levels of phenolic compounds between olive groves, variations might be associated with edaphoclimatic conditions, age and spacing of trees, tree pruning, among other factors. The CS olive grove is older, with less spacing between trees and less incidence of pruning, showing greater shading of the crowns and less weathered soil. Thus, it is possible that exposure to environmental heterogeneity may be reflected in a greater amplitude and concentration of polyphenols in the olive trees. SG apparently exhibits environmental conditions more homogeneous, corroborated by the results of the PCA and the distribution in quartiles in the box-plots. Further studies on edaphoclimatic conditions and management of olive groves will be important to complement these results.

It is concluded that the evaluated adult olive trees exhibited adequate levels of B content in the leaves, even before B fertilization. The levels of B in leaf tissue did not vary significantly ($p<0.05$) after soil and foliar fertilization, while of the 11 compounds analyzed, only kaempferol showed an increase in its concentration with fertilization (100g of Ulexite/with foliar) in one of olive groves. Regarding the period of leaf collection, there was a decrease in the levels of B at flowering in both olive groves, indicating mobilization of the nutrient to the floral tissues. It was observed that most phenolic compounds exhibited an increase in their concentration at the beginning of fruiting, regardless of boron treatment. The study showed evidence of dynamism in the metabolism of olive trees, as in the evaluated period statistically significant changes were observed in the levels of the analyzed metabolites,

indicative of the integrated action between the vegetative and reproductive structures.

Acknowledgments

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Supplementary material

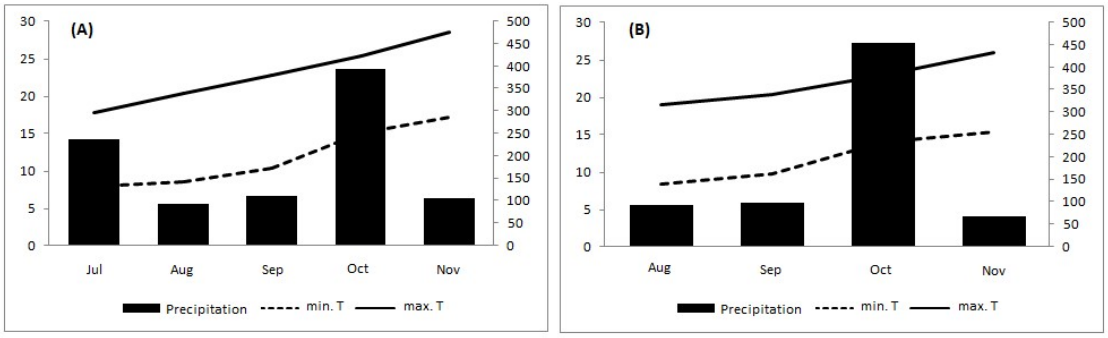

Figure 1S. Monthly rainfall and minimum and maximum temperatures during the study period in the municipalities of Caçapava do Sul (A) and São Gabriel (B), RS, Brazil.

Table 1S. Parameters of the calibration curves obtained for the phenolic compounds in the HPLC-DAD.

Phenolic compounds	**Concen-tration (µg/mL)**	**Min***	**Standard curve**	**R^2**
Hydroxytyrosol	2.5 - 50	4.4	$1.489 + 2.70x10^{-5} \times A$	0.999
Tyrosol	2.5 - 50	5.8	$1.805 + 5.36x10^{-5} \times A$	0.999
Chlorogenic acid	0.5 - 50	6.5	$0.949 + 1.79x10^{-5} \times A$	0.998
Caffeic acid	0.1 - 50	7.2	$0.384 + 6.97x10^{-6} \times A$	0.999
p-Coumaric acid	2.5 - 50	9.2	$1.742 + 7.86x10^{-6} \times A$	1
trans-Ferulic acid	2.5 - 50	10.0	$1.673 + 1.59x10^{-5} \times A$	1
Verbascoside	2.5 - 100	11.1	$2.205 + 1.17x10^{-4} \times A$	0.999
Oleuropein	5.0 - 500	14.0	$17.089 + 9.22 \times 10^{-5} \times A$	0.994
trans-Cinnamic acid	0.1 - 50	15.8	$0.227 + 3.12x10^{-6} \times A$	1
Quercetin	2.5 - 50	16.8	$4.276 + 4.00 \times 10^{-5} \times A$	0.994
Kaempferol	2.5 - 50	19.3	$3.213 + 3.67 \times 10^{-5} \times A$	0.990

*Time retention

Chemical composition of fruits and oils from olive trees with different fruit loads in southern Brazil

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ABSTRACT

With the aim of providing more data about the effect of location and fruit load on the chemical composition of fruits and oils, the objective of the study was to evaluate two olive groves (O1 and O2) in two sites in south of Brazil (2020 crop), and the same location considering trees (Arbequina cv.) with different fruit loads in the same orchard and year (2021 crop). Fruit analyzes included the quantification of moisture, oil content, phenolic compounds and nutrients, while oils were evaluated regarding quality indexes, fatty acid composition and phenolic compounds. The harvest of more mature olives promoted higher oil extraction in O2, and presented the quality indexes within the norms. Furthermore, O2 oil exhibited highest percentage of oleic acid and higher levels of oleacein, oleocanthal and hydroxytyrosol in oil than O1. Considering the effect of fruit load observed that the oil content did not differ, with subtle differences in the fatty acid profile. Greater differences were verified in the levels of phenolic compounds, as well as the levels of phosphorus and boron, indicating that such fruits have different metabolic demands, and such variations are reflected in the final product.

Key words: oil quality indexes, fatty acid profile, phenolic compounds, nutrients, Arbequina.

INTRODUCTION

Olive oil consists predominantly of fatty acids (98%), which compose the cellular structure of olive fruits, in the form of glycerides (mono-, di- and triacylglycerols) and phospholipids through ester bonds (Tena et al. 2015). The composition of fatty acids may vary according to the maturation of the fruits, cultivars, geographical location, edaphoclimatic conditions, alternate bearing, irrigation, fertilization, among other factors (Beltrán et al. 2004, Mailer et al. 2010, Dag et al. 2011, Bakhouche et al. 2013, Franco et al. 2015, Konuskan & Mungan 2016,Torres et al. 2017, Hernández et al. 2018, Vishekaii et al. 2019).The remaining 2% of olive oil represent minority compounds that mainly include aliphatics and triterpenes, sterols, hydrocarbons, volatile compounds, pigments and phenolic compounds (Servili et al. 2004).

The consumption of olive oil has increased in recent years associated to with health benefits, due to its composition with a predominance of monounsaturated fatty acids and compounds with antioxidant activity, especially phenolic compounds. Several papers, especially revisions and meta-analysis network, have evidenced that olive oil consumption may favor increased High Density Lipoprotein (HDL) cholesterol, cardiovascular protection, increased antioxidant activity, glucose metabolism regulation, and reduced risk of diabetes (Pedret et al. 2018, Schwingshackl et al. 2019, Tsartsou et al. 2019).

The Arbequina cultivar has Spanish origin in the region of Catalonia and is characterized by productive precocity, low vigor and high productivity (Criado et al. 2004). In recent years, Arbequina cultivation has extended to other regions of Spain, such as Andalusia and Aragón, as well as other countries around the world, including Brazil (Tous et al. 1997). Appreciated worldwide, its oil is fruity, sweet, slightly poignant, yellowish color (Aparicio & Luna 2002) and low bitterness (Franco et al. 2015).

Brazil is the second largest importer of olive oil and table olives in the world, so the country has attracted the attention of the international market (IOC 2020). In the 2020/2021 crop, 106,500 t of EVOO were imported by the country; considering the average price of approximately € 286.2/100 kg of a Spanish EVOO in 2021, for example, the cost would be around € 304,803.000 only with this type of oil (Data from IOC 2023). In this same crop, 202.000 l of olive oil were produced in the state of Rio Grande do Sul, the largest national producer of olive oil (João 2022). Considering the density of olive oil 0.9 $kg.L^{-1}$ (Mello & Pinheiro 2012), the amount of EVOO produced in Brazil in the 2020/2021crop was 181.8 t, which is equivalent to approximately 0.17% of the imported oil, thus emphasizing the high potential for expanding the olive cultivation area.

Studies on the production chain of oliviculture in Brazil have indicated that olive groves are financially viable, with significant competition with the international market and economically sustainable (Belarmino et al. 2022). Characterization studies of Brazilian olive oils are recent and have included the fatty acid profile and identification/quantification of minor compounds such as phenolic compounds, tocopherols, pigments, phytosterols, volatile compounds, as well as physical-chemical analyzes, such as quality indices, antioxidant activity, oxidative stability, in addition to sensory analysis (Mello & Pinheiro 2012; Silva et al. 2012, Ballus et al. 2014, Ballus et al. 2015, Borges et al 2017a, Borges et al 2017b, Bruscatto et al 2017; Rodrigues et al. 2019, Zago et al. 2019, Carvalho et al. 2020, Crizel et al 2020, Santos et al. 2021). However, there are still few scientific studies evaluating the extraction and quality of olive oils (Santos et al., 2021), with a gap on information regarding oil yield and extractability. In the same orchard/site, there is a marked oscillation in oil characteristics among distinct years and, in the same year, among olive trees with different fruit loads, but the driving factors and their interaction are also a gap for the edaphoclimatic conditions of south of Brazil. Therefore, the objective of this work was to evaluate the chemical composition of the major and some minor compounds of olive oils in two olive groves in the south of Brazil in the 2020 crop, and in the 2021 crop, to evaluate olive trees with different fruit loads in the same grove.

MATERIALS AND METHODS

Experimental design and plant material

The experiment was conducted in two commercial olive orchards in Southern Brazil (Caçapava do Sul municipality/Rio Grande do Sul state). The olive grove 1 (O1) (30°37'26.90”S, 53°20'44.33”W) had 10-year-old olive trees, planted at 7x6 m spacing in Luvisol Chromic Palicoabruptic soil, according to the Brazilian classification of soils (Santos et al. 2018). The olive grove 2 (O2) (30°24'50”S, 53°27'55”W) had 13-year-old olive and was planted with 5x3m spacing between trees in Regolithic Neosol soil (Santos et al. 2018). The clay content evaluated in 2020 was 10.2 and 7.4 $g.100g^{-1}$ for O1 and O2, respectively (Figueiredo, unpublished data). For the study, *Olea europaea* L. cultivar Arbequina was selected and all trees received recommended fertilizer in Brazil (CQFS-RS/SC, 2016) and no irrigation. The climatic data are reported in Figure 1.

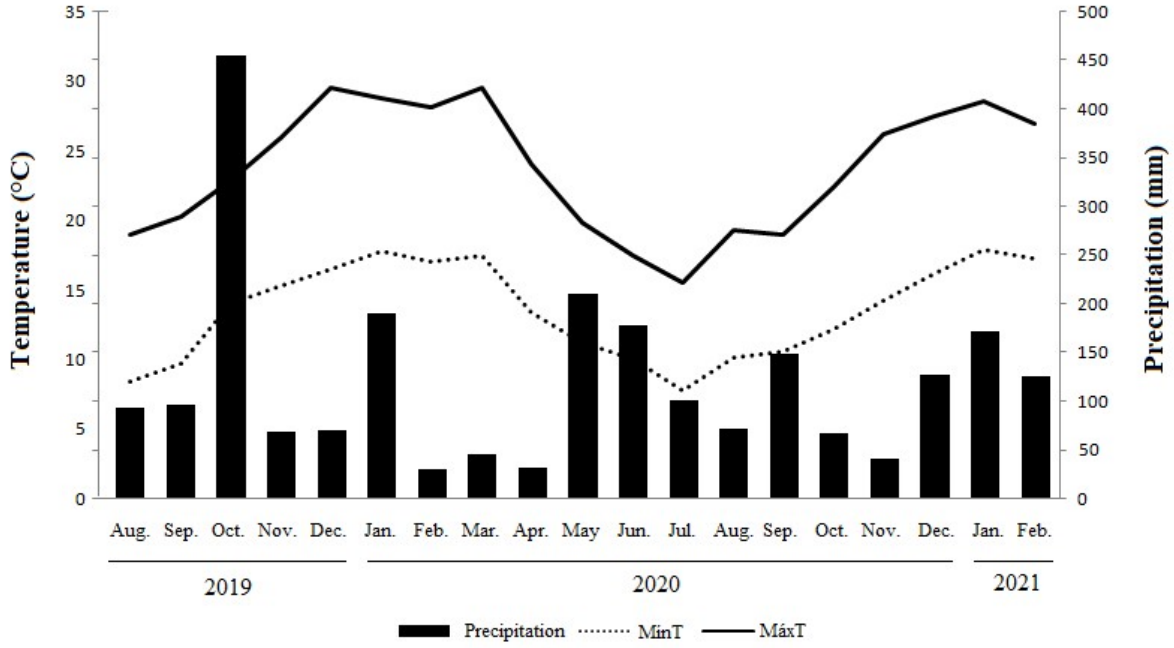


Figure 1. Monthly rainfall and minimum and maximum temperatures during the study period in the municipalities of Caçapava do Sul, RS, Brazil

Fruit sampling was carried out manually in two crop seasons (2020/2021), both in February (summer) at the beginning of the harvest. In the 2020 crop season, six olive trees were selected in each olive orchard. The fruits were harvested, the production was quantified and 0.5 kg of each olive tree was separated for analysis. The olive grove O2 was evaluated in the 2021 crop season, considering olive trees in two fruit load categories: high fruit load (19-40 kg of fruits/tree) and low fruit load (2-6 kg of fruits/tree), designated as O2-H and O2-L respectively. Six olive trees of each category were selected, quantified in terms of production, and 0.5 kg of fruit/tree was collected for analysis. After harvesting, the maturation index was determined, evaluating 100 fruits per olive grove/fruit load, which takes into account the visual analysis of the color stage of the epicarp and mesocarp of the fruits, with a scoring system ranging from 0 to 7 (least ripe to most ripe) (IOC 2011).

Olive oil extraction and lyophilized paste analysis

For the extraction of olive oil, initially the olives were crushed with a manual mill. Afterwards, malaxation was performed without adding water, using an anchor-type stirrer at 100 rpm, in a water bath at approximately 28°C, for 30 min, according to the method adapted from Miho et al. (2018). The paste was centrifuged at 3000 rpm for 10 min and the

supernatant liquid fraction was separated and centrifuged again at 4000 rpm for 10 min to separate the oil and aqueous phases. The moisture content of the ground paste and oil obtained was evaluated through moisture analysis in an oven at 105°C for 24 h. The oil extraction yield was obtained by the ratio of the oil mass/malaxed paste mass. The obtained oil samples were stored in dark glass bottles at 4°C until analyzes. A fraction of the ground paste was frozen at about -18°C for 24 h and subsequently subjected to lyophilization at -50°C for 48 h. The lyophilized paste was stored in dark bottles and kept in a dry place until use. The oil content of the lyophilized paste was determined using an oil extractor (Tecnal, TE-044, Brazil). Briefly, 2g of lyophilized paste packed in paper cartridges inside reboilers were inserted in triplicate. Then 100 ml of hexane was added as extracting solvent at 80°C for 1 h. Afterwards, the reboilers with the oil obtained and the residual solvent were placed in an oven at 105°C for 1 h. The extractability was determined by the ratio between the lipids extracted in the malaxation/lipids extracted by oil extractor (fresh matter basis).

Quality index

The quality index of the olive oil was evaluated according to the percentage of free acidity, peroxide index and specific extinction in the ultraviolet. Free acidity was determined according to the International Olive Council Method (IOC 2017a). The olive oil samples were dissolved in a mixture of solvents (diethyl ether and ethanol 1:1 V/V), followed by titration with sodium hydroxide and expressed as a percentage of oleic acid. Peroxide value was evaluated following the method prescribed to Olive Council Method (IOC 2017b). Chloroform and acetic acid were used as solvents, in addition to potassium iodide to release iodide in the reaction and subsequent titration with sodium thiosulphate, using starch solution as an indicator. The results were expressed in milliequivalents of active oxygen per kilogram of olive oil (mEq $O2.kg^{-1}$). The evaluation of secondary oxidative compounds was determined by specific extinction in the ultraviolet (IOC 2019a). The samples were dissolved in cyclohexane for the measurements at 232 nm and 270 nm using UV-Vis spectrophotometer. The ΔK was also determined, which corresponds to the extinction variation at the maximum wavelength of 270 nm. All quality index analyzes were performed in triplicate.

Fatty acid composition

Determination of the fatty acid composition of olive oil was carried out by GC-FID analysis after transesterification, according to the modified Hartman & Lago method (1973). In summary, 10 mL of olive oil were dissolved in 3.5 mL of potassium methoxide, kept in

reaction in a water bath at approximately 45°C, for 15 min. Afterwards, the material was placed in a decantation funnel, with the addition of 20 mL of hexane to facilitate the separation of glycerin from methyl esters. Subsequently, successive washes were performed with 0.5% HCl, after saturated NaCl and finally distilled water. Then the material was placed in a beaker and anhydrous magnesium sulfate was added to absorb the remaining moisture. After filtration, the material was rotaevaporated to remove the hexane and obtain the methyl esters. The separation and quantification of the methyl esters were performed using a Shimadzu GC-MS QP2010 Plus gas chromatograph, with a flame ionization detector (FID), split injector ratio 1:20, with a Rtx-WazRestec capillary column (30 m long, 0. 25 mm inner diameter, 0.25 µm film thickness). The chromatographic conditions used were: injector and detector temperatures of 240°C and 260°C, respectively; column with an initial temperature of 100°C, with a heating rate of 10°C/min up to 140°C (5 min), followed by heating of 4°C/min, holding (10 min); helium as carrier gas, with a linear velocity of 1.20 cm/s. The injection volume was 1 µL, obtained from HPLC grade methyl esters/hexane dilution (1:10 V/V). For each olive oil, two samples of methyl esters were produced and the injection was performed in triplicate, totaling six readings for condition. The results were expressed as a percentage of the methyl esters of the identified fatty acids.

Phenolic compounds

Phenolic compounds of olive oil and lyophilized pulp were extracted through maceration based on a modification of the International Olive Council Method (IOC 2017c). Briefly, 5.0 g of olive oil were mixed in 12.5 ml methanol/water (80:20 V/V) while for the lyophilized pulp were used 1g of pulp and 20 ml of methanol/water. The mixture was agitated in a horizontal shaker at 200 rpm for 1 h. After centrifugation (10 min at 4000 rpm) the supernatant was filtered through a 0.45 µm syringe filter. Identification and quantification of individual phenolic compounds from extracts were analyzed using a high performance liquid chromatography (HPLC). HPLC analysis were performed using an Agilent 64 (Agilent Technologies, Santa Clara, USA) equipped with a quaternary pump (1200 Series) and diode array detector (DAD) (Agilent 1260 Series Photo Diode Array Detector). The separation was conducted at 30°C using a reversed phase LC Column Eclipse Plus C18 (4.6 x 150 mm, 5 µm) (Supelco, Bellefonte, PA, USA). A ternary elution gradient was established at a flow rate of 1.0 mL min^{-1} with (A) water 0.2 % acetic acid (v/v), (B) methanol and (C) acetonitrile, as follows (A/B/C): 0 min (96:2:2 %); 5 min (80:10:10 %); 10 min (70:15:15 %); 20 min (50:25:25 %); 30 min (96:2:2 %). The injection volume was set at 20 µL and detection of

phenolic compounds was performed at 280 nm. Phenolic standards (Sigma-Aldrich®) of oleacein, oleocanthal, oleuropein, verbascoside, hydroxytyrosol, tyrosol, gallic, chlorogenic, caffeic, coumaric, ferulic and cinnamic acids were used to quantify the phenolic compounds. Analyzes were performed in quadruplicate and expressed in $mg.kg^{-1}$ (DW).

Nutrients

The contents of total nitrogen (N), phosphorus (P), potassium (K) and boron (B) in the lyophilized pulp were determined following the methods prescribed to Tedesco et al. (1995). In order to determine the levels of N, P and K, wet digestion of the samples was carried out for 3.5 h. Nitrogen levels were determined by distillation using a Kjeldahl steam distiller, followed by titration with sulfuric acid using boric acid as indicator. For the assay of the P contents, an aliquot of the digestion extract was used and evaluated by the colorimetric method using UV-Vis spectrophotometer (Murphy & Riley 1962). Potassium levels were determined by flame spectrometry, using an aliquot of the digestion product, diluted in distilled water (1:10). Boron contents were evaluated colorimetrically by the Azomethine-H method, modified by Tedesco et al. (1995). The trials were performed in triplicate and the results for N, P, K were expressed in $g.100\ g^{-1}$ and in $mg.kg^{-1}$ for B.

Statistical analysis

Statistical differences between locations and fruit loads were analyzed separately using the t test ($p<0.05$), performed in the Sigma Plot program version 11.0. For a better understanding of the interaction between the analyzed factors and the characteristics of each location/fruit load, a multivariate analysis of principal components (PCA) was carried out, including the variables of fruits and oil, arranged in a correlation matrix using the Statistica version 7.0 program.

RESULTS AND DISCUSSION

Maturity index, oil extraction, water and oil content

In the 2020 crop, the maturation index of the fruits analyzed in the two olive groves was between 3 and 4 (Table 1), which falls within the ideal range to harvest the olives, associating a better fruit yield to the olive oil without compromising the quality parameters (IOC 2011). In the 2021 crop, the harvest started with more immature fruits, expressed in maturation indices between 1 and less than 3 (Table 1).

Table 1. Fruit characteristics and oil extraction process for Arbequina cv., considering two olive groves (O1 and O2 in the 2020 crop) and one olive grove (2021 crop) for olive trees with two categories of fruit loads.

Fruit charact. and oil extraction	Olive grove		Fruit load	
	O1	O2	O2 - High	O2 - Low
Maturation index	3.41	4.24	1.34	2.76
Extraction yield (%)	6.43	9.66	3.57	5.41
Extractability (%)	56.76	65.04	21.64	38.00
Water content (% FW)	64.03±0.70 A	59.30±0.25 B	63.29±1.10 a	64.37±0.50 a
Oil content (% FW)	11.33±0.51 B	14.85±0.60 A	16.52±0.26 a	14.24±0.64 b
Oil content (% DW)	31.51±1.42 A	36.49±1.47 A	45.00±0.71 a	39.96±1.81 a

Values are presented as mean and standard error (n=3). Different capital letters indicate statistical difference between olive groves and different small letters indicate difference between fruit loads, according to t test ($p \leq 0.05$).

The harvest of more mature olives influenced higher oil extraction yield and extractability, since in O2 the extractability was three times higher than in O2-H, which exhibited a lower maturation index (Table 1). This result has great relevance for the producer, since the yields in commercial olive orchards can be increased with the harvest in a shorter time in the adequate period of fruit maturation. Franco et al. (2015) obtained an oil extraction yield up to twice as high for mature olives compared to green olives for the Arbequina cultivar.

Regarding the moisture content of the olive oils obtained, all presented a value equal to 0 (data not shown), being below the limits established for EVOO of ≤0.2 % m.m (IOC 2019b). The average values of the moisture content of the paste evaluated in the study (Table 1) were within the range of values presented in other studies with the Arbequina cultivar in Uruguay (57.8-63.8%) and Italy (63.6-65.2%), however they were higher than in studies in Spain (42.3-55%) and Portugal (58.5%) (Franco et al 2015, Ellis & Gámbaro 2018, Famiani et al. 2020a, Ferro et al. 2023). Paste water content was higher for O1 compared to O2, however it did not differ between olive trees with different fruit load (Table 1). The lower

moisture content displayed in the paste of O2 may also have influenced the higher extractability of the olive oil, as higher water contents can form greater emulsions during crushing step, which makes malaxation difficult (Aguilera et al. 2010).

The oil content (% FW) differed between olive groves and for trees with different fruit load, however it did not differ between them when analyzed on a dry basis (Table 1). Tombesi et al. (1999) found lower levels of lipids (% DW) in olive trees with lower fruit load. Franco et al (2015) pointed out that the oil content on a dry basis is a more reliable parameter, making it possible to analyze materials with different moisture contents. The oil content (% DW) found in the study (31.5-45%) was similar to studies with Arbequina cv. in Uruguay (35.1-43.5%), United States (20-44.6%), China (42.96%), Italy (30.1-32.3%; 31.5-37.1%) and Spain (36.3-53.4%) (Farinelli & Tombesi 2015, Franco et al 2015, Ellis & Gámbaro 2018, Wang et al. 2018, Famiani et al. 2020a, Polari et al. 2021). It is noteworthy that O2-H exhibited high oil content even with a low degree of maturation, providing evidence that a large part of the fatty acids has already been produced in this period, however they cannot be released from the cells, so that the oil droplets cannot coalesce during malaxation.

Quality index

The quality indices of the oils produced are presented in Table 2. The quality of olive oil based on the percentage of free acidity, peroxide index and specific extinction in the ultraviolet were below the limits established in Brazilian and international regulations for EVOO, indicating good quality of the olive oils produced (Brasil 2012, EEC 2013, IOC 2019b).

Comparing the two olive groves, it was found that O1 exhibited better quality indices than O2. Between trees with different fruit loads, no statistical difference was observed in the quality standards of the olive oil, with the exception of K232 nm (Table 2). The values of the quality indices found in general were similar or lower, especially for the peroxide index, in the literature for the Arbequina cultivar in Brazil and other countries (Silva et al. 2012; Farinelli & Tombesi 2015, Franco et al. 2015, Borges et al. 2017a; Bruscatto et al. 2017, Ellis & Gámbaro 2018, Wang et al. 2018, Rodrigues et al 2019, Crizel et al. 2020, Famiani et al. 2020a; Polari et al. 2021, Santos et al. 2021, Yu et al. 2021, Ferro et al. 2023). Commercial olive oils were not considered for comparison, since shelf life can affect the quality of the olive oil (Gonçalves et al. 2022).

Table 2. Free acidity, peroxide value and specific extinction in the ultraviolet (K232 nm, K270 nm, ΔK) of olive oils of the Arbequina cv., considering two olive groves (2020 crop) and one olive grove (2021 crop) for olive trees with two categories of fruit loads.

Quality index	Olive grove		Fruit load		Limits established*
	O1	O2	O2 - High	O2 - Low	
Free acidity (%)	0.29±0.01 B	0.36±0.01 A	0.17±0.02 a	0.21±0.01 a	≤ 0.8
Peroxide value (mEq O2. kg^{-1})	1.31±0.00 B	3.90±0.04 A	3.30±0.42 a	4.36±0.21 a	≤ 20
K232 nm	1.63±0.01 B	1.84±0.02 A	1.31±0.03 a	1.21±0.01 b	≤ 2,50
K270 nm	0.11±0.00 A	0.12±0.00 A	0.09±0.00 a	0.09±0.00 a	≤ 0,22
ΔK	-0.004± 0.001 B	0.013± 0.001 A	-0.005± 0.001 a	-0.007± 0.002 a	≤ 0,01

* For extra virgin olive oil according to national and international standards (BRASIL, 2012; EEC, 2013; IOC, 2019b). Values are presented as mean and standard error (n=3). Different capital letters indicate statistical difference between olive groves and different small letters indicate difference between fruit loads, according to t test ($p \leq 0.05$).

The percentage of free acidity indicates the freshness, handling and correct processing of the olives (Tena et al. 2015). In general, oils from fruits with less maturation have lower free acidity (Salvador et al. 2001, Konuskan & Mungan 2016, Yu et al. 2021), as indicated by the difference between O1 and O2. Higher peroxide index observed in O2 may be associated with primary oxidation, associated with the presence of Lipoxygenase enzymes (LOX) in fruits, which oxidize pigments and unsaturated fatty acids (Amanpour et al. 2019). Likewise, higher levels of specific extinction in the ultraviolet in O2 indicated that secondary oxidative compounds were formed, such as conjugated dienes and trienes, which negatively affect the characteristics of the oil (Tena et al. 2015, IOC 2019a).

Fatty acid composition

The profile of major compounds for the olive oils produced was represented by 12 fatty acids (Table 3). Oleic acid was the main fatty acid, followed by palmitic, linoleic, palmitoleic and stearic acids. It is noteworthy that oleic acid presented a lower percentage than expected in the standards, between 55-83%. Meanwhile, palmitoleic and linolenic acid were present in greater amounts than predicted. Even palmitic and linoleic acids being within the norms, they showed a high percentage for all olive oils.

Table 3. Fatty acid composition (%) of olive oils of the Arbequina cv., considering two olive groves (2020 crop) and one olive grove (2021 crop) for olive trees with two categories of fruit loads.

Fatty acid	Olive grove		Fruit load		Limits established*
	O1	O2	O2 - High	O2 - Low	
C 16:0	19.78±0.03 A	19.81±0.26 A	19.59±0.02 a	19.65±0.04 a	7.5-20.0
C 16:1	5.14±0.01 A	4.80±0.04 B	4.12±0.04 a	4.06±0.02 a	0.3-3.5
C 17:0	0.24±0.00 A	0.23±0.01 A	0.29±0.01 a	0.23±0.00 b	≤ 0.3
C 17:1	0.63±0.01 A	0.61±0.02 A	0.63±0.01 a	0.53±0.01 b	≤ 0.3
C 18:0	3.50±0.09 B	3.99±0.15 A	4.03±0.11 a	4.13±0.06 a	0.5-5.0
C 18:1	51.35±0.01 B	53.68±0.14 A	52.13±0.14 a	52.01±0.10 a	55.0-83.0
C 18:2	15.18±0.04 A	13.16±0.08 B	14.80±0.07 b	15.13±0.07 a	3.5-21.0
C 18:3	1.99±0.02 A	1.60±0.05 B	1.87±0.03 a	1.86±0.03 a	≤ 1.0
C 20:0	0.93±0.01 A	0.95±0.05 A	1.07±0.01 a	1.05±0.01 a	≤ 0.6
C 20:1	0.80±0.01 A	0.75±0.04 A	0.81±0.02 a	0.80±0.01 a	≤ 0.4
C 22:0	0.29±0.00 A	0.28±0.02 A	0.36±0.01 a	0.35±0.01 a	≤ 0.2
C 24:0	0.18±0.00 A	0.16±0.01 A	0.23±0.00 a	0.21±0.01 a	≤ 0.2

* For extra virgin olive oil and virgin olive oil according to national standard (BRASIL, 2012). Values are presented as mean and standard error (n=6). Different capital letters indicate statistical difference between olive groves and different small letters indicate difference between fruit loads, according to t test (p ≤0.05).

The olive oils produced in the study had low levels of monounsaturated acids (MUFA) and high levels of polyunsaturated (PUFA) and saturated acids (SFA) (Figure 2). The MUFA:PUFA ratio was also low with average values between 3.4 and 4.1 for all olive oils.

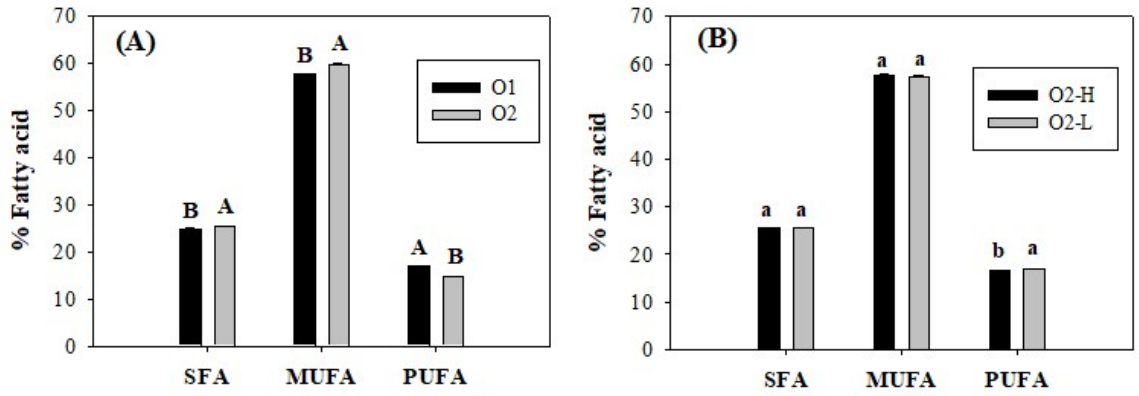


Figure 2. Proportion of fatty acids (%), considering two olive groves (O1 and O2) and one olive grove with high fruit load (O2-H) and low fruit load (O2-L) in olive trees. SFA = Saturated Fatty Acid; MUFA = Monounsaturated Fatty Acid; PUFA = Polyunsaturated Fatty Acid.

The Arbequina cultivar is characterized by a low amount of oleic acid and a high concentration of palmitic and linoleic acids (Aparicio &Luna 2002). The olive oils produced had low levels of unsaturated acids and high levels of saturated acids compared to others Brazilian olive oils (Borges et al. 2017a, Bruscatto et al. 2017, Zago et al. 2019, Crizel et al. 2020). Lower oxidative stability of olive oil has been associated with lower MUFA:PUFA and oleic acid:linoleic acid ratios (Beltrán et al. 2004). Some studies with olive oils produced in the state of Rio Grande do Sul with Arbequina cv. have exhibited oleic acid values below 60% and high levels of palmitic acid (above 17%) and linoleic acid (above 13%) (Mello & Pinheiro 2012, Bruscatto et al. 2017, Crizel et al. 2020). In the same state, Borges et al. (2017a) and Carvalho et al. (2020) found levels of palmitic acid (17-19%) and linoleic acid (9-14%).

Low levels of oleic acid for the Arbequina cultivar, even below international standards, have also been found in other countries such as Argentina, Australia and Egypt (Ceci & Carelli 2007, Mailer et al. 2010, Rondanini et al. 2011, Arafat et al. 2022). Mailer et al. (2010) found high levels of palmitic and linoleic acid and low levels of oleic acid for the Arbequina cv. in Australia in warmer regions, compared to colder climates. Bodoira et al. (2016) also found greater sensitivity in the metabolism of fatty acids for the Arbequina cv compared to Manzanilla cv and suggest that each cultivar responds differently to ambient temperature, which may be associated with variations in the enzymatic activity involved in the synthesis of fatty acids. Torres et al (2017) suggest that warmer regions have lower content of oleic acid compared to climate regions with more moderate temperatures and suggest the interaction between genotype x cultivar on fatty acids in relation to temperature, regardless of agronomic practices, harvest and fruit processing. Nissim et al. (2020) found differences in the expression level of some genes involved in fatty acid biosynthesis in response to higher ambient temperatures, identifying cultivar-dependent gene expression patterns.

Considering the differences between olive groves, it was observed that O1 had higher percentages of palmitoleic, linoleic and linolenic acids, while O2 had higher values of oleic and stearic acids (Table 3).Thus, the percentages SFA and MUFA were higher for O2, while the values of PUFA were higher for O1 (Figure 2). In the case of olive oils from trees with different fruit load, it was observed that the highest of fatty acids did not differ, with the exception of heptadenoic and heptadecenoic acids with higher values in trees with higher production and higher percentages of linoleic acid in trees with lower production (Table 3).

Consequently, only PUFA differed statistically, with O2-L exhibiting higher values (Figure 2).

The differences observed in this study regarding the fatty acid profile between olive groves within a same geographic area and even more within the same grove between olive trees with different fruit loads indicate that variations in fatty acid composition can occur on a small scale. For the analyzed olive groves, microclimate variations, such as soil type, in addition to agronomic practices may have influenced the composition of the fatty acids. In the case of olive trees from the same olive grove, with the same genotype, management and the same microclimatic conditions, it is suggested that plants with different fruit loads may have different strategies in the biosynthesis of fatty acids, especially in the route of palmitic acid and in the conversion of oleic acid into linoleic acid and linoleic acid into linoleic acid. Enzymes from the SAD family (stearoyl-ACP desaturase) are involved in the production of oleic acid and other fatty acids, while the conversion of oleic acid into linoleic acid involves the action of oleate desaturase enzymes from the FAD family (Hernández et al. 2018, Nissim et al., 2020). Future studies involving biochemical and molecular analyzes will be fundamental to better elucidate the presented results.

Phenolic compounds

The analysis of the phenolic compounds of the olives fruits in HPLC-DAD allowed the identification of ten compounds, distributed in secoiridoids (oleuropein), hydroxycinnamic acid derivatives (verbascoside), phenolic alcohols (hydroxytyrosol and tyrosol) and phenolic acids (gallic, chlorogenic, caffeic, coumaric, ferulic and cinnamic) (Table 4).Verbascoside, oleuropein, hydroxytyrosol and tyrosol were the most expressive phenolic compounds present in the olive fruits.

Oleuropein, verbascoside, hydroxytyrosol, tyrosol and coumaric acid were also reported in other studies with the Arbequina cultivar (Morelló et al. 2004, Gómez-Rico et al. 2008, Talhaoui et al. 2016). Gallic, chlorogenic, caffeic, ferulic and cinnamic acids were reported in papers with other cultivars (Arslan & Özcan 2011, Kanakis et al. 2013, Yorulmaz et al. 2013, Tekaya et al. 2014, Cirilli et al. 2016, Gucci et al. 2019, Emmanouilidou et al. 2020, Fernández-Poyatos et al. 2021) and, therefore, the present study is one of the first records of such phenolic acids in fruits of Arbequina cv. In this assay, verbascoside and coumaric acid exhibited high concentration compared to most studies for both Arbequina cv. as for the other cultivars.

Table 4.Quantification of phenolics compounds (mg.kg^{-1} DW) of olive fruits of the Arbequina cv., considering two olive groves (2020 crop) and one olive grove (2021 crop) for olive trees with two categories of fruit loads.

Phenolic compound	Olive grove		Fruit load	
	O1	O2	O2 - High	O2 - Low
Hydroxytyrosol	514.39±2.75 A	312.67±7.46 B	356.73±25.02 a	416.90±23.16 a
Tyrosol	214.68±0.51 A	151.37±4.33 B	165.83±1.74 a	128.94±3.97 b
Oleuropein	621.63±1.58 A	544.98±22.32 B	440.87±4.37 b	698.17±15.93 a
Verbascoside	2588.53±26.83 B	3517.23±127.71 A	1992.09±37.49 b	4791.52±290.70 a
Gallic acid	40.11±1.09 A	27.07±1.55 B	59.49±2.78 a	34.98±1.57 b
Chlorogenic acid	84.79±0.87 A	79.44±2.12 A	96.59±2.16 a	93.64±5.69 a
Caffeic acid	9.96±0.02 A	8.99±0.07 B	12.13±0.24 a	11.88±0.22 a
p-Coumaric acid	52.97±0.30 B	59.01±0.97 A	56.24±0.67 b	65.82±1.69 a
Ferulic acid	41.53±035 A	35.40±0.36 B	43.20±0.57 a	39.28±0.32 b
*trans*Cinnamic acid	5.98±0.05 B	8.78±0.21 A	6.39±0.07 b	7.36±0.13 a

Values are presented as mean and standard error (n=4). Different capital letters indicate statistical difference between olive groves and different small letters indicate difference between fruit loads, according to t test ($p \leq 0.05$).

The two olive orchards showed statistical difference in the levels of all phenolic compounds evaluated in the fruits, with the exception of chlorogenic acid (Table 4). O1 in general exhibited a higher concentration of phenolic compounds, including oleuropein, hydroxytyrosol and tyrosol. For O2, verbascoside stands out, whose content was about 36% higher than O1.The olive orchards have relatively similar climatic conditions on a regional scale, similar age (10-13 years), and the fertilization system follows the Brazilian recommendation manuals. The variations observed can be associated with different edaphic conditions, management practices and planting system, but it is difficult to identify, among the main factors, the contribution of each one and their interaction. Regarding the soil, although the orchards follow the same fertilization practices, there are differences in depth and clay content, which affect several attributes and processes, such as water retention capacity, drainage, nutrient adsorption and uptake by roots, root depth, among others. Plant density is also different, since O1 has spacing between trees typical of intensive planting (315 trees ha^{-1}) and O2 has a high-density planting system (714 trees ha^{-1}), similar to the "Pedestrian Olive Orchards" (Tous 2010, Bianco et al. 2021). The distribution and interception of luminosity varies between different planting systems, influencing the

distribution of fruits, as demonstrated by Pastor et al (2007) who found a more uniform distribution of fruits in olive trees planted in the intensive system, as opposed to olive trees in the super-high density, which showed fruits only in the upper layers and almost none in the lower layer. Melgar et al. (2009) observed that olive tree leaves in full sunlight had a significantly greater concentration of oleuropein (+138%) than shade leaves, corroborating the results of the present study for olive fruits. The high concentration of verbascoside in O2 may be associated with protection against pathogens. High-density olive groves have a higher incidence of pruning wounds and branches dieback, causing a series of fungal diseases (Agustí-Brisach et al. 2021). Markakis et al. (2010) reported the contribution of verbascoside in the defense mechanism in olive trees against fungal pathogens (*Verticillium dahliae*). However, further studies should be carried out to better elucidate the hypotheses raised.

Fruit load influenced almost all levels of phenolic compounds, with the exception of hydroxytyrosol, chlorogenic acid and caffeic acid (Table 4).O2-H exhibited about 29% above tyrosol compared to O2-L. Olive trees with lower fruit load had 58% more oleuropein and 2.4 times more verbascoside than O2-H. Studies on the effect of crop load on the levels of phenolic compounds for olive trees are still scarce, and evaluated the fruit load in different years, considering the years 'on' (high crop load) and 'off' (low crop load) (Ben-Gal et al. 2011, Dag et al. 2011), while contrary to this trial, which evaluated the effect of fruit load in the same harvest. For other fruit trees, studies have shown the relation of fruit load on the levels of phenolic compounds. Awad et al. (2001) did not observe the effect of fruit load on the levels of chlorogenic acid and flavonoids present in the epicarp of apples, as well as in this study in which the concentration of chlorogenic acid did not differ between olive trees with high and low fruit load. Buendía et al. (2008) reported lower levels of anthocyanins in peaches with low crop load, however phenolic acid levels did not differ compared to commercial crop load. Andreotti et al. (2010) found a higher concentration of phenolic compounds in the epicarp and mesocarp of nectarines with lower fruit load and inverse correlation between cinnamic acid and crop load. Higher cinnamic acid levels in olive trees with low fruit load were also found in this assay.

Six phenolic compounds were identified for olive oils, two secoiridoids derivatives (oleacein and oleocanthal), two phenolic alcohols (hydroxytyrosol and tyrosol) and two phenolic acids (coumaric acid and ferulic acid) (Table 5). Gallic, chlorogenic and cinnamic acids, as well as verbascoside present in the olive fruits, were not detected in the oil, while oleuropein and caffeic acid was detected, but it was below the limit of quantification. The

compounds present in the highest concentration for all oils were oleocanthal, oleacein and tyrosol (Table 5).

Table 5. Quantification of phenolics compounds (mg.kg^{-1} DW) of olive oils of the Arbequina cv., considering two olive groves (2020 crop) and one olive grove (2021 crop) for olive trees with two categories of fruit loads.

Phenolic compound	Olive grove		Fruitload	
	O1	O2	O2-High	O2-Low
Hydroxytyrosol	3.87±0.00 B	3.90±0.01 A	3.79±0.01 a	3.81±0.01 a
Tyrosol	6.70±0.01 A	6.47±0.04 B	6.19±0.02 b	7.92±0.03 a
Oleacein	14.15±0.05 B	21.94±0.39 A	10.78±0.13 b	39.68±1.37 a
Oleocanthal	64.88±0.35 B	129.51±0.87 A	52.94±0.80 b	98.80±2.42 a
p-Coumaric acid	4.49±0.01 B	4.65±0.01 A	4.65±0.01 b	4.96±0.01 a
Ferulic acid	NQ	NQ	4.48±0.00 b	4.52±0.01 a

Values are presented as mean and standard error (n=4). Different capital letters indicate statistical difference between olive groves and different small letters indicate difference between fruit loads, according to t test ($p \leq 0.05$). NQ= below the limit of quantification.

Secoiridoid derivatives predominate in olive oil, including oleacein, the dialdehyde form of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA) and oleocanthal, the dialdehyde form of elenolic acid linked to tyrosol (p-HPEA-EDA). These compounds are generally present in very low amounts in olive fruit (Servili et al. 1999, Morelló et al. 2004). The concentration of oleacein found in the olive oils produced showed lower values than most studies with oils from Arbequina cv., while the concentration of oleocanthal, hydroxytyrosol, tyrosol, coumaric and ferulics acids in general was similar or higher than reported for the cultivar Arbequina (Romero et al. 2002, Criado et al. 2004, Morelló et al. 2004, Gómez-Rico et al. 2008, Bakhouche et al. 2013, Ballus et al. 2014, Ballus et al. 2015, Talhaoui et al. 2016, Borges et al. 2017b, Crizel et al. 2020, Famiani et al. 2020a, Miho et al. 2020, Criado-Navarro et al. 2021, Criado-Navarro et al. 2022).

A large quantitative difference was observed in the levels of phenolic compounds present both in the olive fruits and in the oils. The contents of hydroxytyrosol, tyrosol, coumaric acid and ferulic acid in olive oil were respectively about 104, 25, 13 and 9 times lower compared to fruits. During the extraction of olive oil, a large part of the phenolic compounds are retained in the solid phase (wet pomace) due to the low lipophilic character exhibited by the phenolic compounds (Artajo et al. 2007).

Concerning the olive orchards, statistical differences were observed in the concentration of all phenolic compounds identified in the oil. In general, O2 showed a higher

amount of phenolic compounds than O1, with emphasis on oleocanthal and oleacein, with oleocanthal exhibiting twice the O2 content (Table 5). Although O1 exhibited higher levels of phenolic compounds in the fruits, an opposite trend was observed for olive oil, in which O2 showed a higher concentration for most of the analyzed compounds. It is known that during malaxation there is activation of a series of enzymes present in the fruit, such as LOX, peroxidase (POD), β-glucosidase (GLU) and polyphenoloxidase (PPO), which favor hydrolysis reactions and oxireduction in fatty acids and phenolic compounds (Clodoveo 2012, Taticchi et al. 2013). Thus, it is possible that during malaxation the O2 pulp exhibited greater enzymatic activity than O1, leading to the formation of a greater amount of the analyzed phenolic compounds, especially oleacein and oleocanthal.

For trees with different fruit load, it was observed that olive oil from O2-L olive trees exhibited higher amounts for almost all phenolic compounds, with the exception of hydroxytyrosol, which did not differ of O2-H. Enzymatic activity assays will be essential to quantify the levels of the main enzymes involved in the synthesis of phenolic compounds, considering the effect of location and fruit load.

Nutrients

Potassium was the macronutrient present in greater abundance in olive tree fruits (1.51-4.35 g.100 g^{-1}), followed by nitrogen (0.55-1.23 g.100 g^{-1}) and phosphorus (0.18-0.50 g.100 g^{-1}) (Table 6). The micronutrient boron exhibited mean values between 12.59- 55.25 $mg.kg^{-1}$.

Table 6. Content of macronutrients (N, P, K) and micronutrient (B) of olive fruits of the Arbequina cv., considering two olive groves (2020 crop) and one olive grove (2021 crop) for olive trees with two categories of fruit loads.

Nutrient	Olive grove		Fruit load	
	O1	O2	O2-High	O2-Low
N (g.100 g^{-1})	0.91±0.08 A	0.55±0.11 A	1.23±0.11 a	0.84±0.21 a
P (g.100 g^{-1})	0.18±0.03 A	0.27±0.05 A	0.21±0.04 b	0.50±0.06 a
K (g.100 g^{-1})	1.51±0.06 A	2.46±0.56 A	2.22±0.43 a	4.35±0.73 a
B($mg.kg^{-1}$)	12.59±0.47 B	16.56±0.32 A	55.25±1.16 a	21.81±1.30 b

Values are presented as mean and standard error (n=3). Different capital letters indicate statistical difference between olive groves and different small letters indicate difference between fruit loads, according to t test ($p \leq 0.05$).

The contents of N, P and K did not differ between sites, while B showed higher levels in O2 (Table 6). For olive trees with different fruit loads, it was observed that they did not

differ in N and K contents. The P content was 2.4 times higher in trees with a lower fruit load, compared to more productive trees, while the levels of B showed the opposite trend, being 2.5 times lower in trees with lower fruit load.

Fruit crops in general exhibit a high requirement for K, which acts on several physiological processes, being associated with fruit quality, by increasing the content of proteins, starch, vitamin C and soluble solids, promotes fruit size increase and improves color and flavor, reduces physiological disorders and incidence of pests and diseases, among other factors (Kumar et al. 2006). For olive trees, K has been identified as the main mineral present in the olive fruits (Fernández-Escobar et al. 2015), with documented values between 0.47-3.99 for different cultivars and regions (Nergiz & Engez 2000, Tanilgan et al. 2007, Fernandez-Hernandez et al. 2010, Bender et al. 2018, Fernández-Poyatos et al. 2021, Figueiredo et al. 2022).

Figueiredo et al. (2022) studied the nutritional state in the soil and plant tissues of olive trees in the 2020 harvest, in the same olive groves evaluated, reporting average K levels in fruits of 1.74 and 2.18 g $100g^{-1}$ in O1 and O2, respectively. The authors associated the variation found with the type of soil, since rocks rich in potassium feldspar favored higher levels of K in the fruits. In the case of the present study, such a trend also was observed, as O2 presents granitic soil, rich in feldspar, showing a higher average than O1, however they did not differ statistically. Potassium levels too did not differ statistically in trees with different fruit loads, indicating that similar K levels regulate metabolic processes in fruits, even with different crop loads.

Nitrogen is a component of amino acids, nucleotides, chlorophyll, among other molecules (Raven et al. 2001), acting as a component of structural proteins and enzymes, especially in growth regions, regulating cell expansion and division, which is reflected in greater scale in increase of biomass and plant growth, as well as in crop production (Lawlor et al. 2001). In stone fruits (drupe) were reported the import and storage of nitrogen throughout the ontogeny of the fruits, between different tissues, such as from the endocarp to the seed (Famiani et al., 2020b). El-Fouly et al. (2014) found N levels in olives of 0.88-1.05 g.100 g^{-1} in the flesh and 1.13-1.34 g.100 g^{-1} in the pit. In southern Brazil, studies with Arbequina cv. showed N levels slightly higher than this test, with mean values of 1.53 and 1.28 g.100 g^{-1} (Bender et al., 2018, Figueiredo et al., 2022). Like potassium, nitrogen concentration did not differ statistically as a function of location and fruit load, suggesting that the stock of key nutrients in olive fruits is maintained at similar levels in order to provide the different metabolic reactions that occur in fruits.

Phosphorus is an essential element in photosynthesis, respiration and cell division, composing important molecules such as ATP and ADP, nucleic acids, coenzymes and phospholipids (Raven et al. 2001, Rausch & Buscher 2002). The levels of P presented in general were similar or higher than the values reported in the literature (0.001-0.24 g.100 g^{-1}) (Tanilgan et al. 2007, Bender et al. 2018, Fernández-Poyatos et al. 2021, Figueiredo et al. 2022).The olive fruit is a large sink of N, P and K, so the level of fruit load can be associated with the nutrient content in other tissues and organs in olive trees (Bustan et al. 2013). In studies with olive trees in 'on' and 'off' years, the mentioned authors found higher levels of P in different organs in the 'off' year, such as leaves, branches and main roots, suggesting a partition of P from these parts to the fruits. The high levels of P in trees with low fruit load, found in the present study, indicate the need for a greater P stock under these conditions, whose causes have not yet been elucidated.

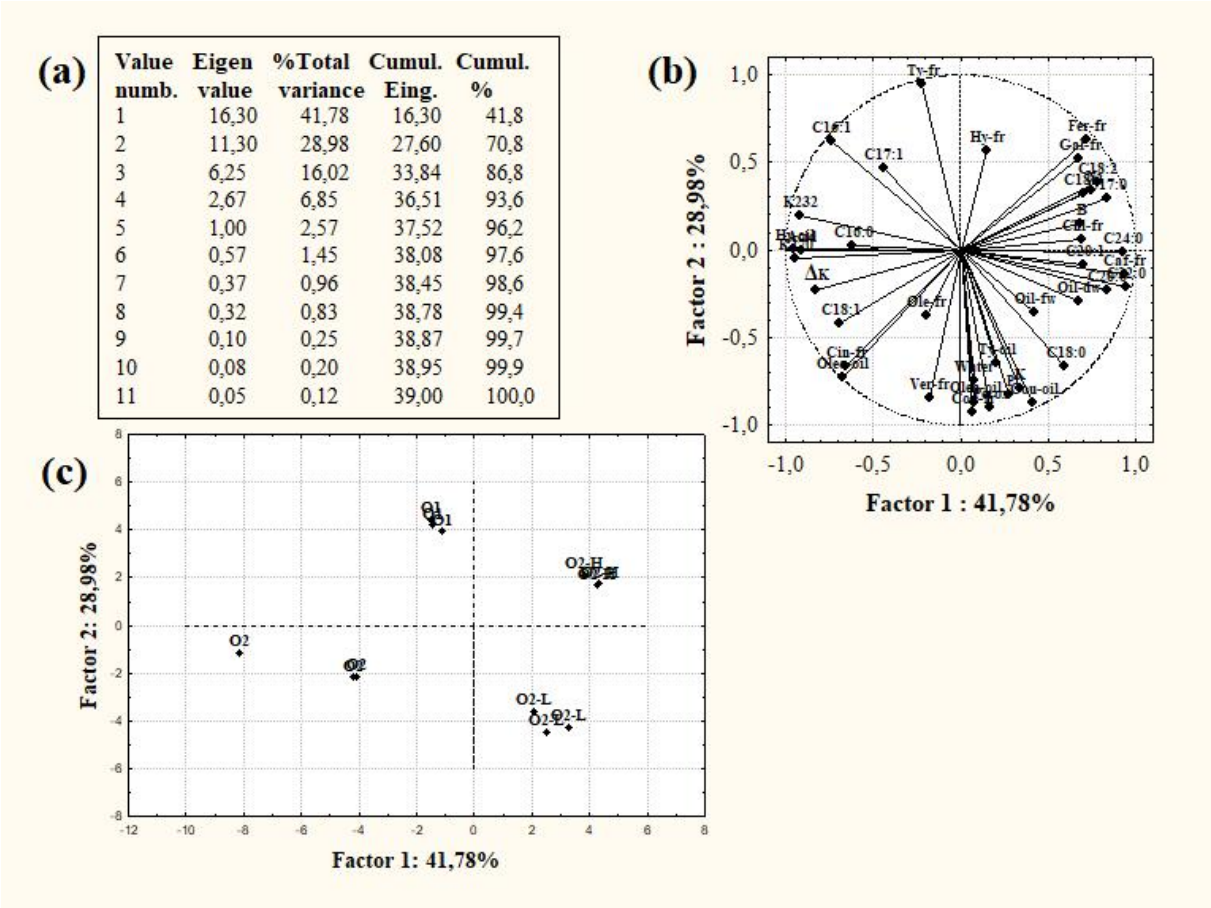
Boron acts in the primary metabolism in plants, participating in the structure of the cell wall, maintaining the integrity of the cell membrane, influencing the use of calcium ions and the synthesis of nucleic acids (Raven et al. 2001). In secondary metabolism, B plays a key role in pollen germination, pollen tube formation and fruiting (Blevins & Lukaszewski 1998). The highest concentration of B in fruits in O2 compared to O1 was also found by Figueiredo et al. (2022), in the 2020 crop season, and the authors associated the results found with the higher levels of B presented in the soil in O2, and the sandy soil in O1, which difficult the adsorption of B by plants. Boron levels in olive trees with a high fruit load were more than twice that of olive trees with a low fruit load, indicating the importance of this micronutrient for more productive trees. It is suggested that the concentration of B in olive trees with a high fruit load may be a mechanism to prevent fruit drop in the final phase of their ontogeny. Among the symptoms of B deficiency are fruit drop and the application of B in olive trees helped to reduce fruit drop, especially in the fruit set stage (Gul et al. 2017, Genaidy et al. 2020).

Principal Component Analysis (PCA)

For the PCA, 39 variables of fruits and oils were used (Figure 3). The first two factors explained about 71% of the variation and the first four axes explained 94% of the total variance (Figure 3a).

Factor 1 was influenced in the positive quadrant mainly by the fatty acid C17:0 and by the longer chain fatty acids, from C18:2 upwards, by the phenolic acids present in the fruits (caffeic, chlorogenic and ferulic) and by the N content in the fruits (Figure 3b). Besides,

factor 1 received the contribution in the negative quadrant of quality indices, with the exception of peroxide values, by fatty acids C16:1, C17:1 and C18:1 and by the content of hydroxytyrosol in olive oil.



Value numb.	Eigen value	%Total variance	Cumul. Eing.	Cumul. %
1	16,30	41,78	16,30	41,8
2	11,30	28,98	27,60	70,8
3	6,25	16,02	33,84	86,8
4	2,67	6,85	36,51	93,6
5	1,00	2,57	37,52	96,2
6	0,57	1,45	38,08	97,6
7	0,37	0,96	38,45	98,6
8	0,32	0,83	38,78	99,4
9	0,10	0,25	38,87	99,7
10	0,08	0,20	38,95	99,9
11	0,05	0,12	39,00	100,0

Figure 3. Results of the principal components analysis (PCA), represented by eigenvalues of correlation matrix and related statistics (A); loadings plot of olive fruit and olive oil parameters (B); scores plot of two olive groves (O1 and O2) and one olive grove with high fruit load (O2-H) and low fruit load (O2-L) in olive trees (C).

Factor 2 was influenced mainly in the positive quadrant by the concentration of tyrosol in the fruit and in the negative quadrant with the variables of the fruits as content of water, P and K, phenolic compounds (verbascoside and coumaric acid) and olive oil represented by phenolic compounds (coumaric acid, oleacein and oleocanthal) and peroxide index (Figure 3b). Factor 3 received the contribution in the positive quadrant of the oil FW and B content in the fruits and the phenolic compounds hydroxytyrosol and oleuropein in the fruits (Data not shown).

Figure 3c represents the distribution of variables for samples from different olives orchards and fruit loads. High tyrosol content in fruits was the main variable that distinguished O1 from other conditions (Figure 3c, Table 4). On the other hand, O2 showed a more dissimilar pattern between samples and the most important variables were the cinnamic acid content in the fruits and olive oil parameters, such as oleocanthal and oleic acid content and the quality parameter ΔK (Figure 3c, Tables 2-5). The variables that most represented O2-H were the concentration of phenolic acids (gallic, ferulic and chlorogenic) and B in the fruits (Figure 3c, Tables 4 and 6). In the case of O2-L, fruit parameters, such as the content of P and K nutrients and water, in addition to the phenolic compounds of olive oil (oleacein, tyrosol and coumaric acid) were more determinant to distinguish this set of data from the others (Figure 3c, Table 1, 5-6).

CONCLUSIONS

In the present study it was observed that O2, even with more mature fruits, showed a higher oil yield, but without losing quality, since the quality indices were slightly lower than O1, but were within the norms and, still, presented more oleic acid and high levels of oleacein, oleocanthal and hydroxytyrosol in olive oil. Considering olive trees with different fruit loads, no difference was observed in the oil content (% DW) in the fruits and the oil quality indices were similar, however the fatty acid profile was slightly different, with emphasis on linoleic acid, which was higher in O2-L. The profile of phenolic compounds was quite discrepant for olive trees with different fruit loads, especially in olive oil, with O2-L showing higher levels for almost all analyzed compounds. Variations in phosphorus and boron content were also observed in relation to fruit load, indicating different needs for these minerals at this stage of fruiting. Future studies on enzymatic activity and gene expression would help to increase the understanding of the variations found in olive groves on a small geographic scale, and even more, on olive trees in the same olive orchard with the same genotype, but with different fruit production.

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5 CONSIDERAÇÕES FINAIS

A privação de nutrientes produziu diferentes níveis de estresse nutricional para oliveiras jovens, sendo que a omissão por N gerou maiores danos ao crescimento e alterações nos teores dos metabólitos analisados. Já a omissão de K induziu os sintomas mais atenuados, exibindo inclusive maior comprimento e densidade de raízes, em relação ao tratamento completo. Deste modo, a hipótese do estudo 1 foi aceita, visto que todos os tratamentos com omissão apresentaram diferenças significativas em relação ao tratamento completo, em pelo menos um dos parâmetros analisados. Estudos posteriores utilizando o mesmo desenho experimental abordando outros nutrientes, como Mg, Ca e Zn, por exemplo, assim como a avaliação de parâmetros bioquímicos e moleculares auxiliarão a ter uma visão mais abrangente do efeito do estresse nutricional para oliveiras. Compostos fenólicos importantes para oliveiras como oleuropeína, verbascosídeo, hidroxitirosol e tirosol exibiram padrões diferentes com a privação de cada nutriente e com o tecido/órgão, indicando um dinamismo no metabolismo secundário, a fim de tolerar as condições adversas. A privação de N em oliveiras adultas e o efeito de tal estresse nutricional nos níveis dos compostos fenólicos individuais se constitui em estudo futuro importante, no qual ainda existem lacunas na literatura sobre o tema.

A adubação com B no solo e foliar não alterou o conteúdo de B nas folhas e dos 11 compostos fenólicos analisados, apenas o kaempferol apresentou maiores níveis em um dos olivais, no tratamento com 100 g de Ulexita/sem adubo foliar. Observou-se que as oliveiras exibiram teores adequados de B, mesmo antes da adubação, sugerindo que plantas bem nutridas podem não responder a adubação com tal micronutriente. Com isso, a hipótese do estudo 2 foi parcialmente aceita. Entretanto, observou-se uma diminuição significativa nos teores de B na floração, sendo um indicativo da mobilização de nutrientes das folhas maduras para os tecidos florais. Estudos posteriores que avaliassem oliveiras adultas com deficiência foliar de B e que utilizassem um maior número de aplicações de fertilizante foliar, além de um maior período de amostragem, poderiam auxiliar no entendimento sobre o efeito da adubação com B em diferentes períodos, avaliando também a produção de frutos e azeite.

Oliveiras com diferentes cargas de frutos apresentaram similaridade no conteúdo de lipídios em massa seca nos frutos, assim como nos parâmetros de

qualidade do azeite. As maiores variações se referiram aos níveis de compostos fenólicos nos frutos e azeite e nos teores de P e B nos frutos. As diferenças em alguns parâmetros analisados entre oliveiras com o mesmo genótipo e manejo, com condições microclimáticas similares, sugerem que oliveiras com diferentes cargas de frutos podem ter diferentes estratégias na biossíntese de ácidos graxos e compostos fenólicos, assim como na demanda por nutrientes nos frutos. Deste modo, estudos futuros envolvendo análises de enzimas que atuem na formação de tais compostos, assim como de expressão gênica serão fundamentais para elucidar os resultados apresentados.

A extração e qualidade do azeite diferiram entre os olivais, com maior rendimento e eficiência de azeite no olival que exibiu frutos mais maduros. O azeite de tal olival apresentou os parâmetros de qualidade dentro das normas, além de maior conteúdo de ácido oleico, oleaceina e hidroxitirosol no azeite. Com isso, observou-se que frutos mais maduros, com IM próximo a 4 podem gerar mais rentabilidade sem perder em qualidade, resultado de grande relevância para o produtor, sugerindo-se que a colheita seja realizada em menos tempo, mas próxima aos IM sugeridos pelo IOC. Além do IM, variações nos níveis dos parâmetros analisados entre olivais podem estar associadas com diferenças edáficas e de manejo, tais como o espaçamento entre as árvores e a frequência de realização de podas, entre outros fatores. Sendo assim, a hipótese do estudo 3 foi aceita.

Por fim, sugere-se um estudo em escala regional sobre o perfil de ácidos graxos em oliveiras do cultivar Arbequina, pois identificou-se no presente estudo, assim como em outros estudos no estado do Rio Grande do Sul, baixos níveis de ácido oleico e elevados níveis de ácido palmítico e linoleico. Tal estudo deve abordar o efeito das mudanças climáticas sobre o referido parâmetro, uma vez que o cultivar Arbequina é sensível a maior temperatura ambiente, de modo que com o aumento da temperatura global pode ocorrer um severo decréscimo nos níveis do principal ácido graxo em oliveiras.

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APÊNDICE 1

Análise dos compostos fenólicos em HPLC (High Performance Liquid Chromatography)

Inicialmente foram realizados testes para ajustar as condições cromatográficas referentes ao tipo de eluente, volume dos eluentes no gradiente, tempo de corrida e volume de injeção. Usou-se como base o método sugerido por IOC, com adaptações (IOC, 2017b). Após os testes, definiu-se por utilizar como eluentes o ácido acético 0,2% (Solvente A), metanol (Solvente B) e acetonitrila (Solvente C). Foram avaliados vários tempos de corrida, de 15 a 82 min. O tempo de 82 min, proposto por IOC, separou bem os picos cromatográficos (Figura 1).

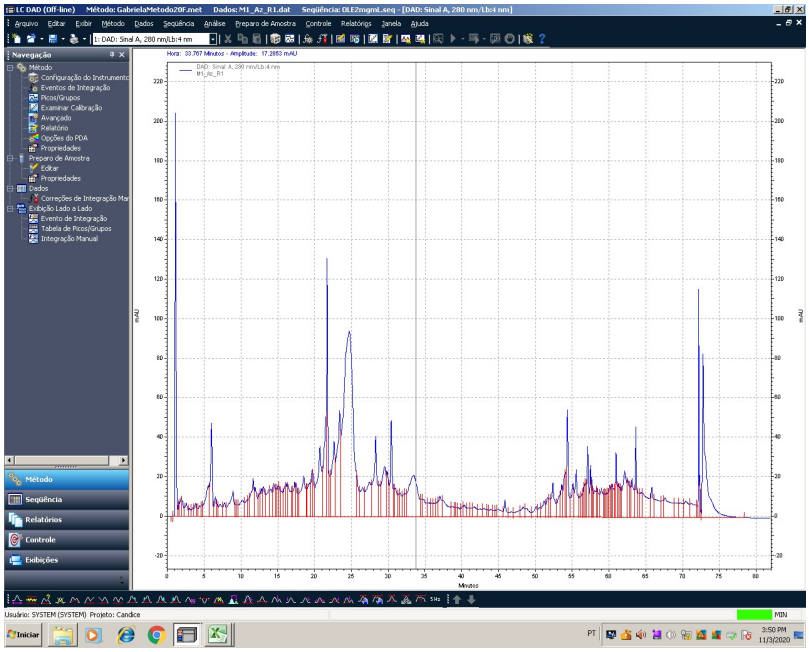


Figura 1. Cromatograma de frutos de oliveiras, com tempo de corrida de 82 min.

Contudo, em função do elevado número de amostras, o tempo de análise total seria muito elevado, de modo que foram avaliados outros tempos de corrida. Após os testes definiu-se o tempo de corrida de 30 minutos como ideal para a separação dos picos dos compostos fenólicos analisadas.

Para a obtenção das curvas de calibração foram preparadas soluções padrões de 1 mg. mL^{-1}, utilizando reagentes Sigma-Aldrich® e água ultra purificada (Mili Q). A partir da solução mãe foram feitas diluições e obtidas diferentes

concentrações, que variaram de acordo com cada composto (PETRIDIS *et al.*, 2012). Os parâmetros da curvas de calibração, assim como as condições cromatográficas foram apresentados nos artigos.

A identificação de cada composto foi realizada com base no tempo de retenção de cada padrão, obtido pelas curvas de calibração. Nos casos em que picos muito próximos estavam quase no mesmo tempo de retenção, as amostras eram “batizadas”, com a injeção do padrão em pequena concentração, auxiliando na identificação mais nítida de qual era o pico do composto de interesse.

Na figura 2 são apresentados cromatogramas típicos, a fim de elucidar os resultados. A integração dos picos foi realizada de forma manual, por meio do ajuste da linha base, em vermelho, nos cromatogramas.

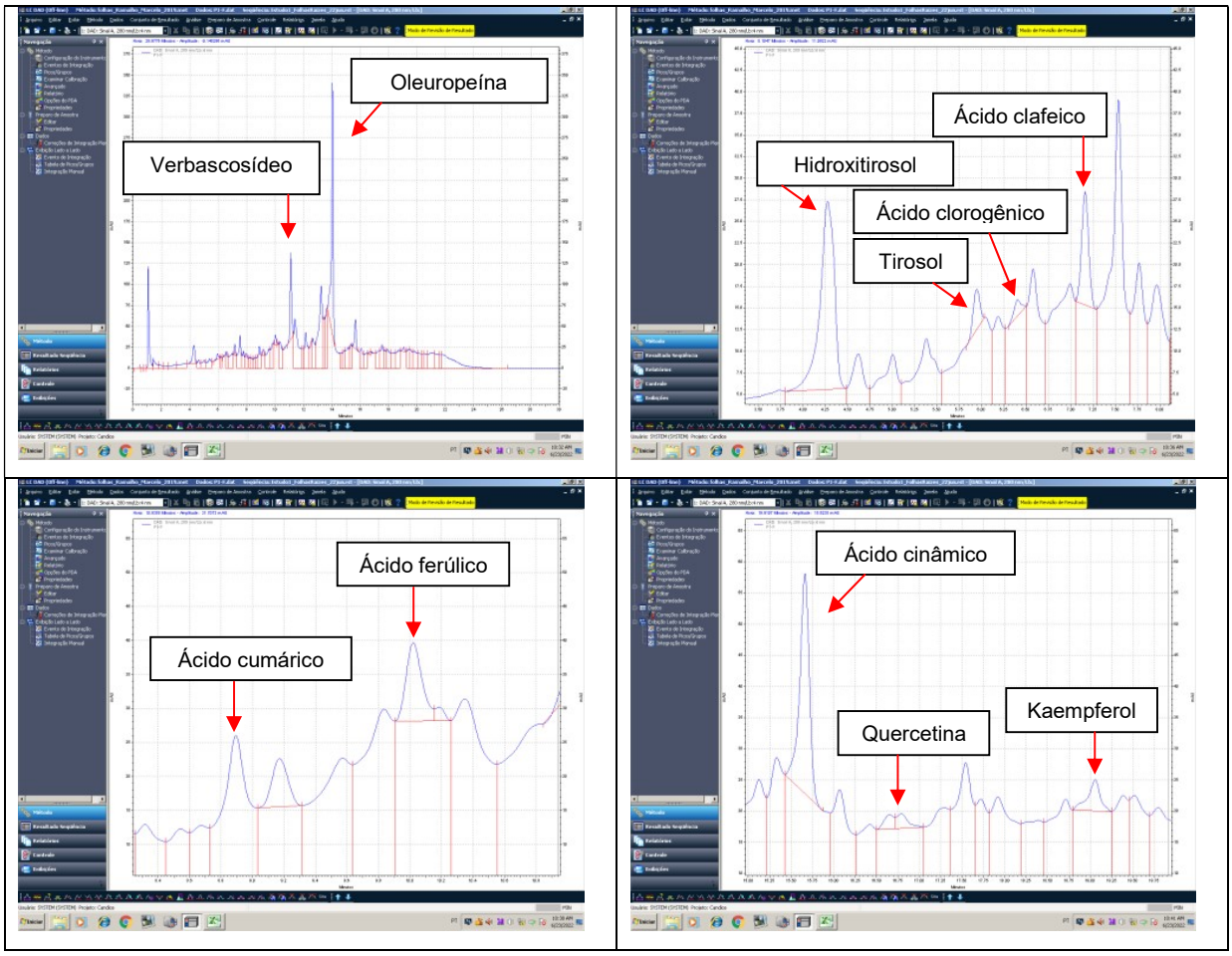


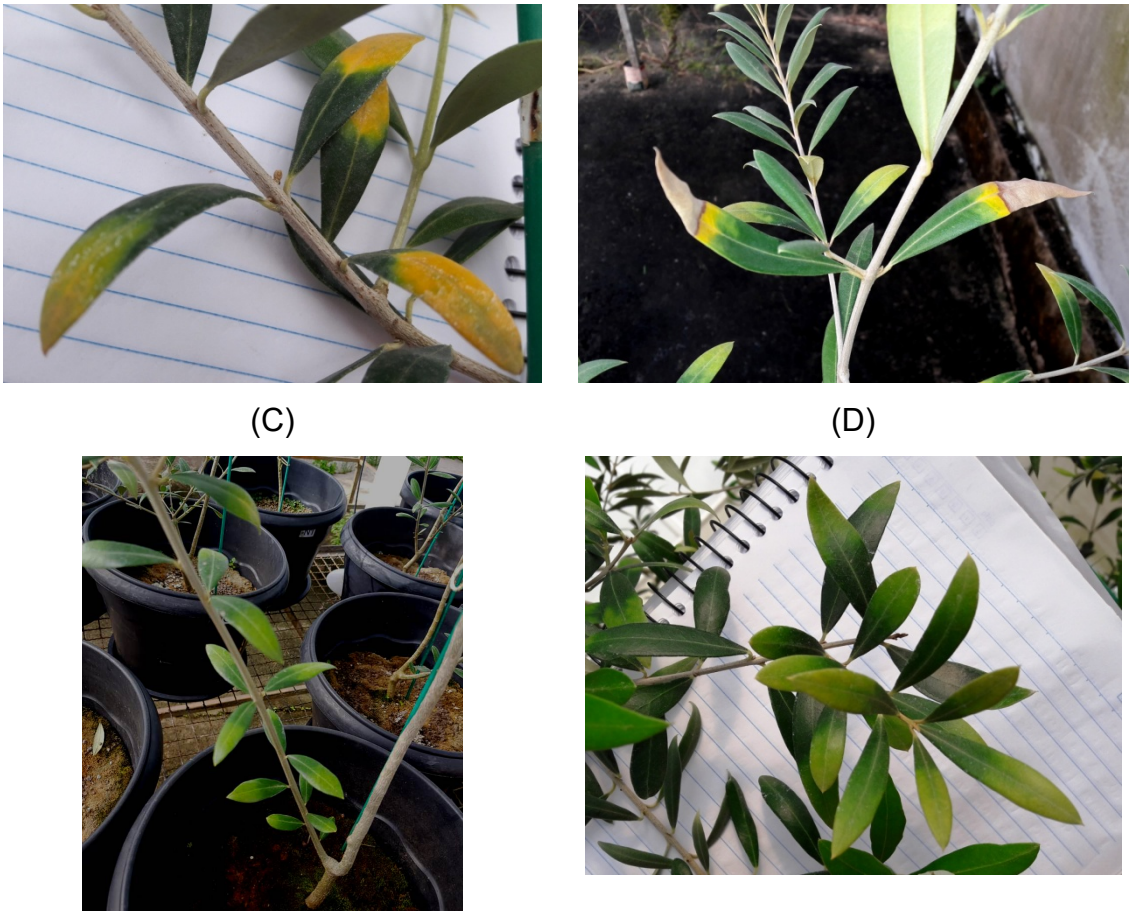
Figura 2. Cromatograma de folhas de oliveiras, com tempo de corrida de 30 min, com identificação de cada composto fenólico.

APÊNDICE 2

Sintomas visuais de deficiência nutricional

Com relação à morfologia dos ramos e folhas, os sintomas visuais não foram tão perceptíveis e característicos para cada deficiência nutricional, como descrito na literatura. Sintomas como clorose, com amarelecimento das folhas do ápice até metade das folhas, têm sido observados abundantemente em plantas -N, mas também em plantas -B e ocasionalmente em -K (Figura 4A). Tem sido documentado que na ausência de nitrogênio, oliveiras exibiram folhas maduras com tom pálido, seguido de clorose (amarelamento) por toda a folha.

(A) (B)



(C) (D)

Figura 1. Sintomas de deficiência nutricional em estudo com oliveiras jovens, utilizando o método do nutriente faltante em casa de vegetação em São Gabriel, RS. (A) clorose com amarelamento intenso em planta sem nitrogênio; (B) necrose no

ápice das folhas em planta sem potássio; (C) clorose no ápice até a metade das folhas em planta sem fósforo; (D) folhas jovens com clorose em planta sem boro.

Oliveiras privadas de potássio podem apresentar folhas mais velhas, com sintomas de clorose no ápice e margem das folhas, podendo chegar à necrose (FERNÁNDEZ-ESCOBAR *et al*., 2016; EREL, *et al*., 2017; SOUZA *et al*., 2019). No presente estudo folhas com clorose e necrose foram observadas em plantas -K, mas também em outros tratamentos (Figura 4B).

A deficiência de fósforo produziu poucas alterações visuais em 2019/2020, entretanto em 2021 observou-se várias folhas com sintomas de deficiência, com clorose no ápice até a metade das folhas (Figura 4C). Na literatura comenta-se que oliveiras privadas de fósforo apresentam redução do tamanho foliar, enrugamento das folhas mais velhas juntamente com leve clorose, que se torna gradualmente avermelhada até a queda das folhas, assim como crescimento em altura reduzido (HARTMANN e BROWN, 1953; JIMÉNEZ-MORENO e FERNÁNDEZ-ESCOBAR, 2016; SOUZA *et al*., 2019).

No caso do boro, dentre os sintomas de deficiência mais nítidos incluíram folhas novas em formato de rosetas, algumas folhas retorcidas, além de algumas folhas jovens com clorose apical (Figura 4D). Na literatura comenta-se que a deficiência de boro pode ocasionar diversas alterações morfológicas no sistema aéreo, como formação de folhas pequenas, distorcidas, com clorose apical nas folhas jovens; queda das folhas e morte nos pontos de crescimento; folhas novas em formato de rosetas; crescimento lateral e ramificações múltiplas; frutos mal formados e partenocárpicos (ARROBAS *et al.,* 2010; FERNÁNDEZ-ESCOBAR *et al.,* 2016; SOUZA *et al.,* 2019).