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Optimization the growth and quality of 'Picual' olive plants according to the dose of slow-release fertilizer

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ABSTRACT

Due to its importance for oil production in Brazil and worldwide, the 'Picual' olive trees deserves special attention of to its good climatic adaptation and high yield and stability in the oil produced. However, there is a need to generate more technical information to improve the propagation and production system for nursery plants. The use of slow-release fertilizer (SRF) affects the quality standard of the plants formed in the nursery. The purpose of this study was to evaluate the effect of doses (0, 1.5, 3.0, 6.0, and 12 g L^{-1}) of the SRF, Osmocote® (NPK 14-14-14), on morphological, biochemical and nutritional parameters of 'Picual' olive plants. It was found that the average value of maximum technical efficiency dose (MTED) for several morphological variables (plant height, stem diameter, total plant dry weight and root volume) was 7.18 g L^{-1}. Furthermore, an Osmocote® dose of 8.35 g L^{-1} resulted in optimal leaf number and area. For the carbohydrate concentration (sucrose, starch and Total Soluble Sugar) in leaves, the best average values were obtained at 4.51, 5.48 and 6.12 g L^{-1} of Osmocote®, respectively. Additionally, the best growth responses of plants may also be due to increased internal macronutrient concentration such as nitrogen (N), phosphorus (P) and potassium (K) after treatment with 7.87 g L^{-1} of Osmocote®. The use of SRF (Osmocote®) is a viable alternative in the production of 'Picual' plants, as it promotes good morphological characteristics, synthesis of photosynthetic products as well as satisfactory nutritional status and plant growth.

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Clonal propagation; *Olea europaea* L.; Osmocote®; seedling production; substrate fertilization

Introduction

The Olive (*Olea europaea* L.) tree is a crop predominantly grown in the Mediterranean region, representing 97% of the total area of olive trees worldwide (Solomou and Sfougaris 2021). However, the areas traditionally used for cultivating the olive tree are insufficient to supply the growing global demand for its derivatives. Among the cultivars widely utilized in the production of olive oils in Brazil and the globally, the 'Picual' is one of the most important. Only in Spain, the largest olive oil producer in the world, the Picual cultivar contributes to almost half of the oil production (Lama-Muñoz et al. 2020), but this country does not have the capacity to expand significantly its planting areas (Wrege et al. 2015). For this reason, over the past decades, the cultivation of the olive tree has been expanded in several regions worldwide, mainly in nontraditional areas like Brazil (Martins, Reis, and Pinheiro 2012; Tanasijevic et al. 2014; Wrege et al. 2015).

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Brazil stands out as the world's second-largest importer of olive oil, importing approximately 68.3 thousand tons of oil in 2018 (IOC, 2020). Based on this condition and in order to ensure the future consumption needs, significant investments in olive cultivation have been made in recent year (Coutinho, Jorge, and Costa 2015), particularly in the states of Rio Grande do Sul and Minas Gerais (Martins et al. 2014; Santos, Martins, and Torres 2017; Wrege et al. 2015).

Doe to the low availability of technical information on local conditions, many olive orchards in Brazil were stablished under the strong influence of international publications (regarding plant nutrition and orchard management), which contributed to the use of low-quality nursery stock, resulting in the formation of low-quality olive orchards. Therefore, the productivity of an orchard is directly linked to success in all stages of the orchard planting, starting with the quality of the seedlings used. According to Zaccheo et al. (2013), approximately 60% of the success of a commercial crop depends on the quality of the seedlings, making it necessary to produce them using indispensable techniques, such as fertilization, irrigation and adequate phytosanitary treatments.

To optimize the productivity of an olive orchard, it is crucial to ensure an adequate nutrient supply during the production of plantlets (Corcioli, Borges, and Jesus 2016) in order to obtain vigorous plant. Typically, soluble fertilizers are the most commonly choice for fertilization during the seedling production phase. However, these fertilizers are prone to losses through leaching (Almeida et al. 2012; Natale et al. 2018), which often result in elevated costs, resulting in higher environmental impact and low nutrient use efficiency. Adequate fertilization is essential during this initial phase, as it promotes the rapid growth of seedlings with optimal nutritional status (Dias et al. 2012; Souza et al. 2015), leading to enhanced physiological quality and improved survival rates, as well as rapid growth upon orchard transplantation. This fact is closely related to the development of robust root system and the exploration of larger substrate volumes, facilitating increased water and nutrient absorption (Natale et al. 2018), thus potentially acceleration time to fruit production.

The use of SRF can serve as an alternative fertilization method in plantlets establishment, yielding high-quality plants at a reduced cost and with minimal environmental impact. This source has macro and micronutrients within an organic resin, controlling the gradual release of nutrients. The increase in temperature favors the growth of seedlings, thus enhancing the release of nutrients. Consequently, the peak release of nutrients from SRF coincides with the highest demand from seedlings (Almeida et al. 2012; Rossa et al. 2015). Among the SRFs, Osmocote® is characterized by having soluble mineral nutrients (NPK) within granules protected by a semi-permeable membrane, which are slowly released to the plant root system, allowing a better performance in the production of seedlings (Brito et al. 2018). The potential of using SRF for nutrition purposes of seedlings in nursery has been evaluated in several cultures, such as *Persea americana* (Costa et al. 2011), *Eugenia uniflora* (Elli et al. 2013), citrus (Almeida et al. 2012), *Euterpe oleracea* (Almeida et al. 2018), peach (Souza et al. 2019; Menegatti, Souza, and Bianchi 2020), among others.

The amount of fertilizers used during fruit tree plantlet production is often not precisely defined or determined empirically in many nurseries in Brazil. Furthermore, fertilization during the nursery phase is frequently conducted without adequate technical support due to a lack of scientific information to assess of the nutritional status of the plants (Souza et al. 2015). The importance of adjusting the doses of nutrients to be applied is emphasized, considering the delicate balance between scarcity and toxicity (Lima Neto, Natale, and Modesto 2015), which allows for the optimization of production time and the physiological quality of the plantlets.

In Brazil, many nurseries utilize small containers for olive plant production. This coupled with the absence of parameters to define the nutritional status, results in plantlets with underdeveloped root systems in comparison to their aerial parts. Consequently, this negatively impacts initial growth in the field and leads to high mortality rates after transplanting in the orchard. Given the challenges faced in olive cultivation expansion in Brazil, we hypothesized that the use of SRF,

particularly Osmocote®, will enhance the productivity and quality of 'Picual' olive nursery plantlets. It is expected that the appropriate dosage of controlled-release nutrients provided by Osmocote® will optimize balanced plantlet growth, therby contributing to reduce production costs. Therefore, the present study aims to estimate the optimal dosage of a SRF (Osmocote®) that enhances the production and physiological quality of 'Picual' olive plantlets.

Materials and methods

Plant material and environmental parameters of the site

The experiment was carried out in a greenhouse at the Federal University of Pelotas (UFPel), (31°48'08.62″S 52°30'45.39″W, 8 m altitude) from October 2019 to May 2020 (Figure 1). Cuttings measuring 10 cm in length were taken from three-year-old 'Picual' olive trees. Only the mid-position of the shoots with a pair of leaves and a lesion at the cutting base were utilized (Figure 1a). The cutting bases were dipped in indole-3-butyric acid (IBA) solution (3000 ppm for 5s) and transferred to plastic trays (62 × 42 × 16 cm) containing a mixture of perlite:vermiculite (1:1) (Figure 1b), where they received two daily irrigations from a mist system (Figure 1c) and remained in this condition for 90 days (Figure 1d).

Fertilizer treatments and plant growth parameters

After rooting, the plants were transplanted into 1-liter plastic bags filled with commercial substrate Turfa Fértil® without fertilization (Control plants) and with four doses (1.5; 3.0; 6.0; 12.0 g L^{-1}) of SRF (Figure 1e). The SRF used was the Osmocote® (14% N, 14% P_2O_5, 14% K_2O), with total release of nutrients over a period of 90 to 120 days at a temperature of 21 °C. This fertilizer was incorporated into the substrate before transplanting the plants. Calcium (Ca), magnesium

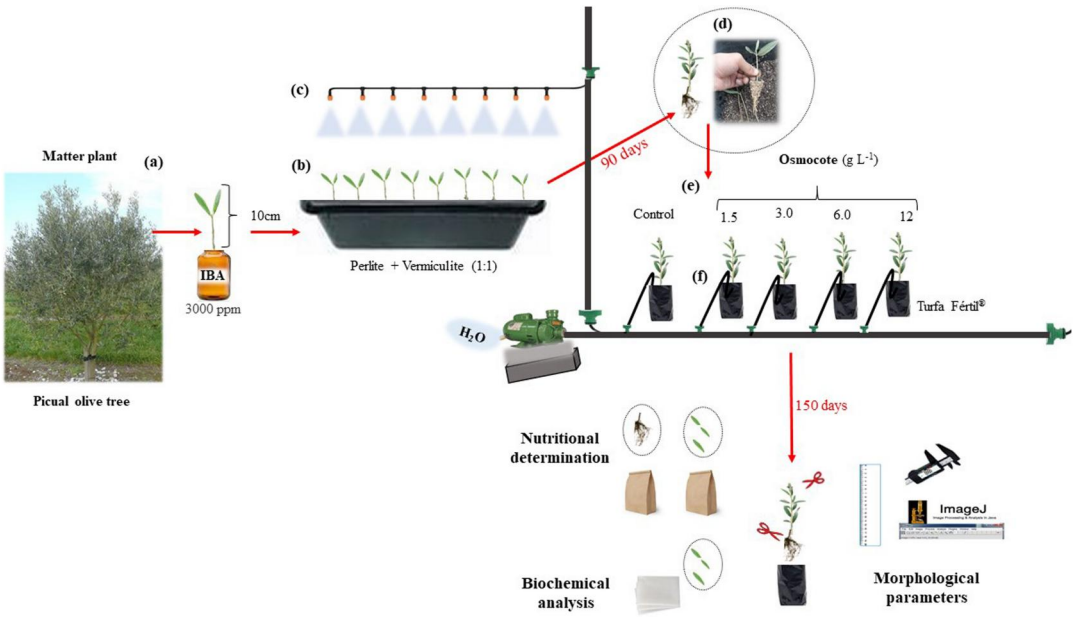

Figure 1. Schematic representation of experimental conditions adopted in experiment. (a) Mother plant ('Picual' olive tree) grown in agricultural center of palma (UFPel), (b) rooting of cuttings under (C) mist system. (d) Cuttings morphology after 90 days and (e) treatments applied under (f) drip system (detail see material and methods section).

(Mg), sulfur (S) and micronutrients were supplied through a nutrient solution (100 ml plant^{-1}) containing Ca (200.4 mg), Mg (48.6 mg), S (64.1 mg), boron (B) (500 μg), cupper (Cu) (39 μg), chlorine (Cl) (722 μg), iron (Fe) (5000 μg), manganese (Mn) (502 μg), molybdenum (Mo) (12 μg) and zinc (Zn) (98 μg) per liter H_2O (Sarruge 1975). This nutrient solution was applied every 15 days, starting 60 days after the establishment of the plants.

During the experiment, the plants were irrigated daily with 70 ml of water, divided two doses per day, using a drip system (Figure 1f). Phytosanitary management was carried out according to the observation of pests or diseases incidence. At 30 and 150 days after transplanting (DAT) of the plants, measurements were taken for plant height (PH) and leaves number (LN). Besides, the stem diameter (measured 1.0 cm above the substrate) was evaluated using a digital caliper. At 150 DAT, the experiment was concluded, and the following parameters were evaluated: main root length (RL), root volume (RV) and leaf area (LA) using ImageJ® software. Subsequently, the plants were dried in an oven at 65 °C until they reached a constant weight, and shoot dry weight (SDW), root dry weight (RDW) and total dry weight (TDW) were determined. Additionally, Dickson's quality index (DQI) was determined based on PH, stem diameter (SD), SDW and dry root weight (DRW), using the Dickson formula (Dickson, Leaf, and Hosner 1960): TDW/[(PH/SD) + (SDW/RDW)].

Extraction and pigments determination

To determine the chlorophylls '*a*' and '*b*' (Chl *a* and Chl *b*), olive leaf samples (150 mg) were extracted with acetone/water (80/20, v/v). The absorbance of the extracts was measured at 646 and 663 nm using a Ultrospec® 7000/7000PC UV–Visible spectrophotometer. The following equations were used to calculate the concentration of each pigment: Chl a = (12.21*Abs_{663} − 2.81*Abs_{646}) and Chl b = (20.13*Abs_{646} − 5.03*Abs_{663}) (Lichtenthaler and Wellburn 1983). The results were expressed in μg of the pigment per gram of fresh weight (μg g^{-1} FW).

Extraction and carbohydrates determination

Total soluble sugar (TSS), sucrose (SUC) and starch were extracted from olive leaves (300 mg) using MCW (Methanol:Chloroform:Water) ratio of 12:5:3 (Bieleski and Turner 1966). The pellet obtained from the extraction of TSS and SUC was subsequently dried at room temperature (72 h), and used to determine the starch content (McCready et al. 1950). The determination of TSS and starch concentration was based on the anthrone method (Graham and Smydzuk 1965) and the SUC concenntration was determinated as previously described by Handel (1968). The results were expressed as mg g^{-1} of dry weight.

Nutritional determination

To determine leaf nutrient concentration, leaf samples (taken from the median portion of the plant) were collected at the end of the experiment and dried in an oven at 65 °C until they reached constant mass. Subsamples (0.5 g) were subjected to nitroperchloric acid digestion at 190 °C in block digester with $HClO_4$ (1.0 ml) + HNO_3 (6.0 ml). In the extract, the concentrations of P were determined by UV spectrophotometry (vanadate-molybdate method) and K by flame atomic absorption spectrometry (AAS, Perkin Elmer Analyst 200, EUA). The N was determined by the micro-Kjeldahl method, after digesting 0.2 g with H_2O_2 (1.0 ml) + H_2SO_4 (2.0 ml) and catalyst salts at 380 °C (Tedesco et al. 1995).

Statistical analysis

The treatments were arranged in a completely randomized design, with five replications, each represented by a plant. Data were assessed for normality using the Shapiro-Wilk test and then subjected to analysis of variance ($p \leq 0.05$). When they showed a significant difference, polynomial regressions were adjusted. By calculating the partial derivatives of the equations adjusted by the regression analysis, the MTED was determined for each variable, using the following formula: $X = -b_1/2b_2$ (X = point of maximum technical efficiency; b_1 e b_2 = coefficients of the equation) (Colwell, Suhet, and Raij 1988). The data obtained were evaluated using the statistical software SISVAR 5.6 (Ferreira 2019).

Results and discussion

The results obtained for RL, chlorophyll '*a*' and '*b*' and leaf P concentration did not differ depending on the doses of SRF. On the other hand, a quadratic response was observed for all other variables, with a MTED (Figures 2, 3 and 4). After 150 DAT, the MTED estimated for plant height was at a dose of 7.52 g L^{-1} of Osmocote®, reaching 95.77 cm (Figure 2a), while for the stem diameter was 7.94 g L^{-1} resulting in a diameter of 3.04 mm (Figure 2b). Similar results were found for plant height in other species. Evaluating the effect of tube sizes and Osmocote® doses on the production of seedlings of *Mimosa scabrella* (96 days after sowing), Stüpp et al. (2015) concluded that the most efficient dose was 7.70 g L^{-1}. In grapia seedlings (*Apuleia leiocarpa*), the DMET for plants height was 8.0 and 7.69 g L^{-1}, at 60 and 90 DAT, respectively (Pias et al. 2013).

According to Coutinho, Jorge, and Costa (2015), olive plants suitable for planting in the field must be between 90–100 cm in height and less than 2 years old. In the present study, an average height of 72.99 cm and a stem diameter of 2.58 mm were obtained. However, in the MTED, the height values rise to 95.77 cm and the stem diameter to 3.04 mm, remaining within the recommended standards for olive plants, approximately 150 days after transplanting. The height and the stem

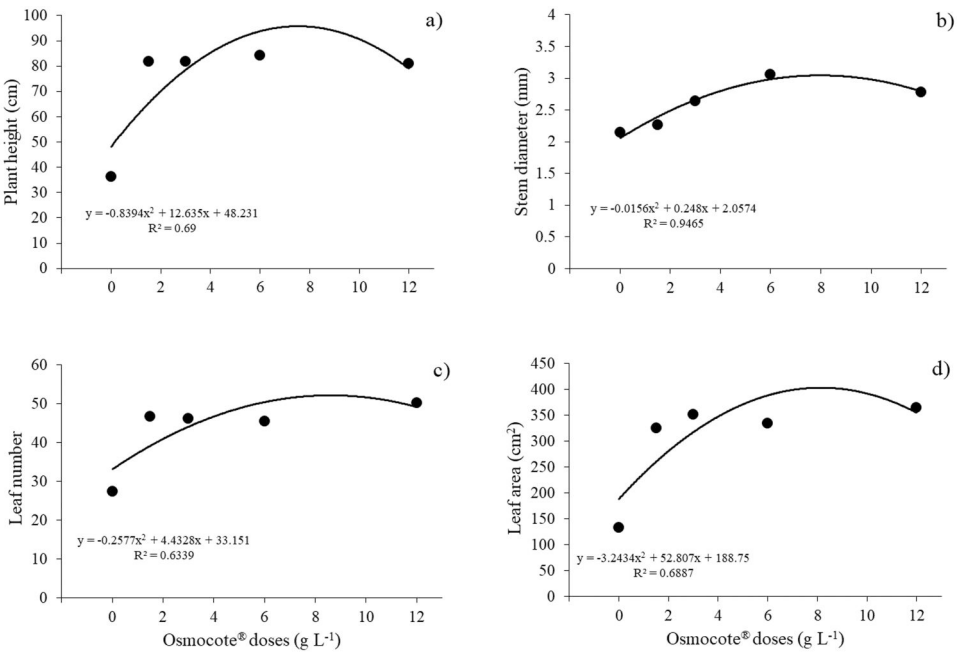

Figure 2. Morphological parameters of 'Picual' olive seedlings under different Osmocote® doses (g L^{-1}). Plant height (a), stem diameter (b), leaves number (c) and leaf area (d). Significant at $p < 0.05$.

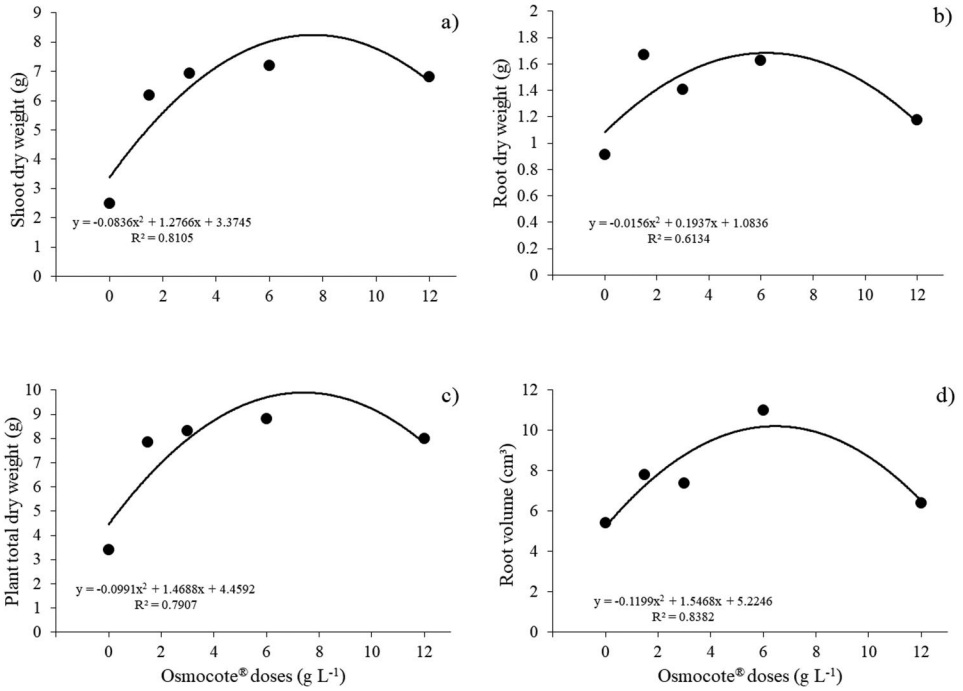

Figure 3. Biometric parameters of 'picual' olive seedlings under different osmocote® doses (g L^{-1}). Shoot dry weight (a), dry root weight (b), total dry weight (c) and root volume (d). Significant at $p < 0.05$.

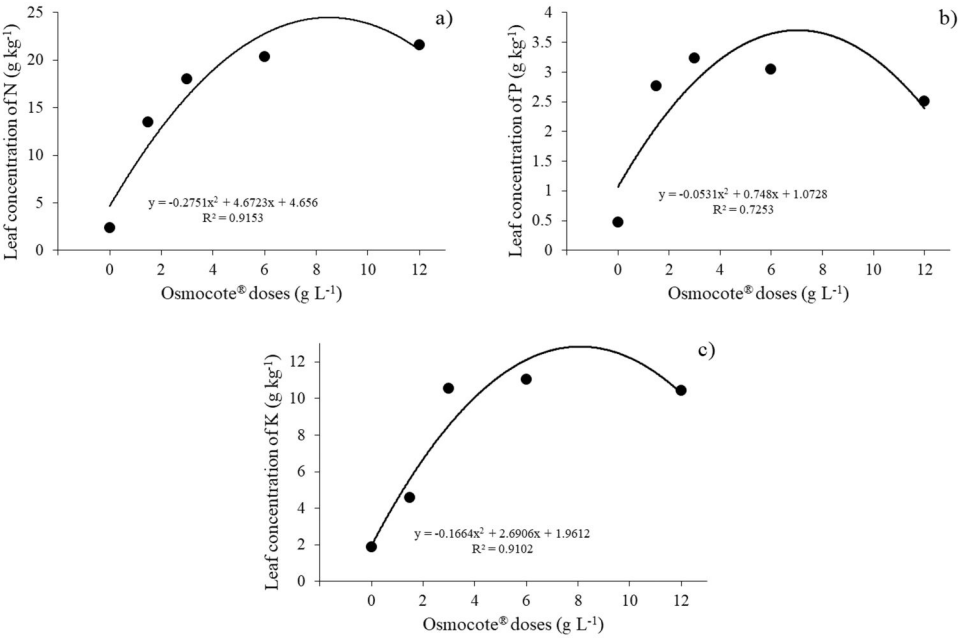

Figure 4. Nutritional parameters evaluated in leaves samples of 'Picual' olive seedlings under different Osmocote® doses (g L^{-1}). Leaf concentration of N (a), leaf concentration of P (b) and leaf concentration of K (c). Significant at $p < 0.05$.

diameter of the plants are relevant features for establishment in the field, where the species of interest needs to compete for light and other edaphoclimatic conditions (Grossnickle and MacDonald 2018).

The number of leaves and the leaf area are also worth highlighting, as they provide an indication of the photosynthetic capacity of the plant and therefore its ability to absorb carbon, thereby increasing the vigor and quality of the plants (Gomes et al. 2017). According to our results the MTED for the highest estimated value of the leaves number (52.2 per plant) was 8.60 g L^{-1} of Osmocote® (Figure 2c), while for the leaf area (403.7 cm^2) was 8.1 g L^{-1} (Figure 2d). Due to their ability to promote plant growth and development, some experiments were performed to evaluated effect of SRF, Osmocote® on morphological parameters, including leaves number, and the results reported in literature show different responses. Freitas et al. (2011) observed the MTED of 4.42 g L^{-1} for the leaves number of micropropagated pineapple seedling, reaching 34.8 per plant, while Menegatti et al. (2017) found a similar MTED (5.14 g L^{-1}) in *Aspidosperma parvifolium* seedlings, but observed only 10 leaves per plant.

Regarding SDW, RDW, TDW and RV, the DMET values were obtained at doses of 7.63, 6.20, 7.41 and 6.45 g L^{-1} of SRF, with 8.24, 1.68, and 9.9 g^{-1} dry weight (DW) and 10.21 cm^3, respectively (Figure 3a, b, c and d). Damian et al. (2016) when studying doses of Osmocote plus® in the production of *Sebastiania schottiana* seedlings, recorded the MTED corresponding to 7.88 g L^{-1} for SDW. Meanwhile Zamunér Filho et al. (2012) obtained maximum values of SDW and TDW at doses of 6.6 and 6.4 g L^{-1} in rubber rootstocks, respectively. The values of dry mass and RV indicate the robusteness of a plant; therefore, the higher the values, the greater the capacity of the plants to withstand adverse conditions in the field, as they are related to the accumulation of structural and nonstructural reserves (Dutra et al. 2017).

The leaf nutrients concentration also showed changes according to the different doses of SRF (Figure 4). The N leaf concentration was 24.4 g kg^{-1}, with 8.49 g L^{-1} Osmocote® (Figure 4a), showing a value higher than 15–20 g kg^{-1}, which is considered normal for the culture. The normal range of P concentration in olive leaves is typically between 1.0 to 3.0 g kg^{-1} DW. However, in our study, the P concentration was 3.70 g kg^{-1} dry weigh for the MTED of 7.04 g L^{-1} of SRF (Figure 4b). The average K concentration was 12.83 g kg^{-1} DW (Figure 4c) at the estimated dose 8.08 g SRF per kg^{-1} substrate, which is also considered a normal concentration for the crop, ranging from 8.0 to 12 g kg^{-1} DW.

It is worth noting that, according to CQFS-RS/SC (2016) and Tiecher et al. (2020), the SRF doses defined as appropriate for the nutrient composition of olive leaves would be in the range of 2.61 to 4.49 g L^{-1} for N, 1.37 to 3.39 g L^{-1} for P, and 2.69 up to 5.85 g L^{-1} for K. Although the N, P and K values were higher than those considered normal for the culture, there were no significant differences in the concentration of leaf pigments depending on the dosage used, showing that this physiological behavior did not change due to the amount of Osmocote®. Therefore, the absorption of higher N levels was sufficient to ensure an adequate nutritional status of the leaves, especially with regard to the synthesis of photosynthetic pigments, in addition to providing N and P for the synthesis of other nitrogenous compounds, amino acids and carbohydrates necessary for supporting plant growth within a maximum physiological efficiency limit

The photosynthetic pigments in the leaves are responsible for fixing carbon in the chloroplasts in the form of phosphate trioses, which are later converted into monosaccharides in the cytosol, which are then transformed into di-trisaccharides and sugar alcohols (Bhattacharya and Kundu 2020). Nonstructural sugars are the main soluble components of plant tissues, whose primary use is in energy production and as precursors in the biosynthesis of lipids, proteins, pigments and structural and reserve polysaccharides (Du, Lu, and Xia 2020). Thus, sugars play a key role in the composition of the cell wall structure, in the production of energy, and in the accumulation of reserves, in addition to acting as signaling molecules. Kaushal et al. (2013) also highlight the role of sugar alcohols, such as mannitol and sorbitol, as osmoprotective molecules under certain stress conditions.

The major TSS transported *via* phloem to sink tissues (young leaves and roots) in olive plants are mannitol, glucose, fructose and galactose, which are responsible for 60–90% of its composition (Ghoreishi and Shahrestani 2009; Gómez-González et al. 2010). According to Gómez-González et al. (2010), mannitol and glucose were the predominant sugars (about 55 and 75 mg g^{-1} FW) in leaves of three olive cultivars (Picual, Manzanilla and Hojiblanca). In this study, the effect of the Osmocote® dose on TSS, SUC, and starch concentration were evaluated (Figure 5), and the highest averages of starch and TSS concentration were 23.04 mg g^{-1} FW and 48.62 mg g^{-1} FW, with MTED of 5.48 e 6.12 g L^{-1} of SRF, respectively (Figure 5a, b). Concerning SUC, the highest estimated values was 0.99 mg g^{-1} of FW with MTED of 4.51 g L^{-1} of Osmocote® (Figure 5c). Thus, based on the data available in the literature, the detected values of TSS and starch are adequate levels to olive leaf tissues, since mannitol and glucose represent the main nonstructural sugars synthesized in olive tissues. Additionally, these values serve as an indicator that sufficient nutrients are being provide within the SRF Osmocote® dosage range to optimize plant growth.

Regarding the quality of plants, indices of individual variables, in most cases, do not allow to obtain a good rating of morphophysiological quality of the plant in formation. In this context, the DQI has been shown to be a good indicator for improving plant quality rating, as it considers the robustness and balance in the distribution of biomass in the plant (Dickson, Leaf, and Hosner 1960). In the present study, the estimated MTED was 8.46 g L^{-1}, with the highest estimated value for DQI being 0.26 (Figure 6). Therefore, it is above the value recommended by Gomes et al. (2017), where seedlings with DQI values $\geq$ 0.20 are considered of good quality.

Assessing the effect of different nutrient solutions and substrates on the production of peach rootstocks from the 'Okinawa Roxo' selection, Souza et al. (2019) registered DQI values ranging from 1.79 to 4.89, reinforcing the need for periodic supplementation of mineral nutrients during the plant formation phase, even using commercial substrates. Menegatti, Souza, and Bianchi (2020), studying the effect of the environment and doses of SRF on peach plant production, obtained the highest DQI value (2.2) in the MTED of 6.18 g L^{-1} for plants grown in a

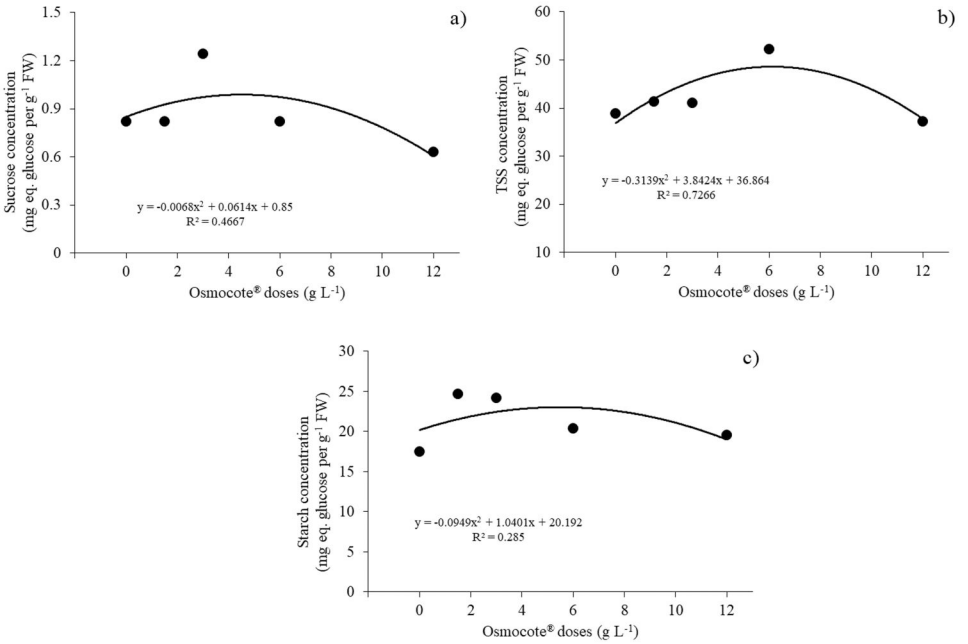

Figure 5. Carbohydrate's concentration evaluated in leaves samples in 'Picual' olive seedlings under different Osmocote® doses (g L^{-1}). SUC (a), TSS concentration (b), starch concentration (c). Significant at $p < 0.05$.

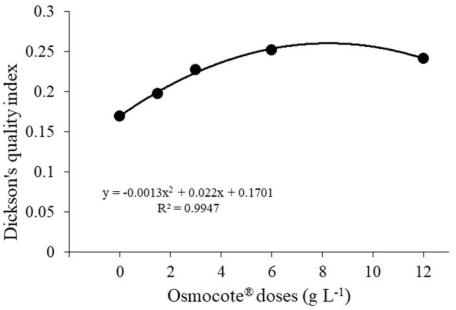

Figure 6. Dickson quality index in 'Picual' olive tree under different Osmocote® doses (g L^{-1}). Significant at $p < 0.05$.

greenhouse, while for plants grown outside the greenhouse, the highest DQI value was 1.83 in MTED of 5.72 g L^{-1}, a value considered below the ideal for the species. According to Gomes and Paiva (2011) the higher the DQI, the higher the quality standard of the plants, and DQI values close to 2 are considered ideal. Based on data from the present study and from other fruit trees and forest species, it must be considered that DQI values can vary greatly from species to species, therefore, the identification of adequate quality standards, based on the DQI, must be determined experimentally.

Expanding the cultivated area is currently the biggest challenge in Brazilian olive cultivation. In this context, the production and use of high-quality nursery plants are is very important, as they form the base of modern orchards. The use of SRF during the production of olive plants in the nursery proposes the optimization of production by reducing production time and labor, as well as mitigating nutrient losses due to leaching, resulting in less environmental impact and enabling the reduction of production costs. Based on this statement, our goals was to identify SRF dose that allows obtaining olive plants from the Picual cultivar quickly, efficiently, and with high-quality standards. According to our results, Osmocote® positively influenced the growth and development of olive plants, with MTED values of 7.18 g L^{-1} for growth parameters, 7.76 g L^{-1} for nutritional analysis and 5.37 g L^{-1} for the content of carbohydrates (products photosynthetic). In addition, the MTED for DQI was 8.46 g L^{-1} of Osmocote®.

Conclusion

Morphological, biochemical and nutritional analyzes showed that the use of slow-release fertilizer (Osmocote®) has positive effect on the production of 'Picual' olive plants and is an alternative to improving the efficiency of substrate fertilization.

For the production of 'Picual' olive plantlets, the use of 8.46 g L^{-1} of slow-release fertilizer permits to obtain the best rating for the most important variables analyzed.

Disclosure statement

The authors declare that they have no conflicts of interest.

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