Gas exchange and chlorophyll fluorescence in spearmint (*Mentha spicata* L.) leaves influenced by mineral nutrition

Intercambio gaseoso y fluorescencia de la clorofila en menta (*Mentha spicata* L.) influenciados por la nutrición mineral

LUCAS ESTEBAN CANO-GALLEGO[1,3]
NUBE MINCHALÁ-BUESTAN[2]
RUBY ALEJANDRA LOAIZA-RUÍZ[2]
JOSÉ RÉGULO CARTAGENA-VALENZUELA[2]
**OSCAR DE JESÚS CÓRDOBA-GAONA**[2]

Dark-adapted leaf.

Photo: L.E. Cano-Gallego

ABSTRACT

The production of export-quality spearmint is limited in Colombia because of low production volumes, poor compliance with good agricultural practices, nutrient availability, and fertilization management. This study aimed to identify how NPK fertilization influences photosynthesis and photochemistry in *Mentha* plants during vegetative growth in a mesh house. Increasing doses of chemical fertilization were evaluated with a 10-30-10 (N-P-K) formula at 0, 60, 90, 120, and 180 kg ha^{-1}. The evaluated variables were net photosynthesis (A), transpiration (E), stomatal conductance (g_s), leaf temperature (T_{leaf}), quantum yield (Qy), Non-photochemical quenching (NPQ), photochemical quenching (qP), and dry matter (Dm). The highest A, Qy, E, and g_s values were in the plants treated with high NPK doses; the NPQ and qP increased in the plants with low NPK doses. These findings elucidated the influence of NPK on photosynthesis and other physiological parameters in the growth and development of spearmint.

Additional key words: Lamiaceae, aromatic plants, plant nutrition, perennial herb.
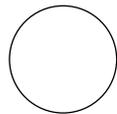
1. Corporación Colombiana de Investigación Agropecuaria – Agrosavia, Centro de investigación La Selva, Rionegro (Colombia). ORCID Cano-Gallego, L.E.: 0000-0002-2819-9694
2. Universidad Nacional de Colombia, Facultad de Ciencias Agrarias, Medellín (Colombia). ORCID Minchalá-Buestan, N.: 0000-0002-4572-7217; ORCID Loaiza-Ruíz, R.A.: 0000-0003-0074-1186; ORCID Cartagena-Valenzuela, J.R.: 0000-0001-6196-2439; ORCID Córdoba-Gaona, O.J.: 0000-0003-2642-4146
3. Corresponding author. lcanog@agrosavia.co

RESUMEN

La producción de menta con calidad de exportación es limitada en Colombia, debido a los bajos volúmenes de producción, el poco cumplimiento de buenas prácticas agrícolas, la disponibilidad de nutrientes y el manejo de la fertilización. Este estudio tuvo como objetivo identificar cómo la fertilización NPK influye en la fotosíntesis y la fotoquímica de la menta, durante el crecimiento vegetativo. En casa malla, se evaluaron dosis crecientes de una fertilización química a partir de la fórmula 10-30-10 (N-P-K) en las cantidades de 0, 60, 90, 120 y 180 kg ha^{-1}. Las variables estimadas fueron fotosíntesis neta (A), transpiración (E), conductancia estomática (g_s), temperatura de la hoja (T_{hoja}), rendimiento cuántico (Qy), desactivación no fotoquímica (NPQ), desactivación química (qP) y materia seca (Dm). Los mayores valores A, Qy, E y g_s se presentaron en plantas tratadas con altas dosis de NPK; mientras que el NPQ y el qP aumentaron en las plantas con bajas dosis de NPK. Nuestros hallazgos proveen bases a cerca de la influencia del NPK sobre la fotosíntesis y otros parámetros fisiológicos, con relación al crecimiento y desarrollo de la menta.

Palabras clave adicionales: Lamiaceae, plantas aromáticas, nutrición vegetal, hierbas perennes.

INTRODUCTION

Crops such as spearmint (*Mentha spicata* L.) are high added-value agricultural production alternatives; however, the global planted area is small (Karkanis *et al*., 2017). Spearmint is a perennial and aromatic herb in the Lamiaceae family, native to southern Europe and northern Africa (Pedraza and Henao, 2008). It is used fresh in the form of dried or powdered leaves as a condiment and herb or as herbal tea (Cirlini *et al*., 2016). Its oil is in demand in the pharmaceutical and cosmetic industries, ranking among the ten most commercialized extracts globally (Chrysargyris *et al*., 2017). In Colombia, the production of export quality spearmint is limited by low volumes that satisfy demand and comply with the norms of good agricultural practices (Pedraza and Henao, 2008). The availability of nutrients and fertilization management are two critical factors that affect crop productivity and some physiological processes, where photosynthesis stands out (Karkanis *et al*., 2017). The deficiency of nutrients, such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), and iron (Fe), alters functions in the photosynthetic apparatus by decreasing the photochemistry efficiency of Photosystem II (PSII) and modifying the values of chlorophyll parameters (Smethurst *et al*., 2005). Nitrogen, P, and K are essential nutrients for plants that are needed to complete the life cycle (Carstensen *et al*., 2019). Nitrogen is the main mineral nutrient needed for chlorophyll production, which is essential for the photosynthesis process and other components of plant cells. Crop yield is affected by the N content of plants; thus, optimizing nitrogen fertilization has become an object of intense research because of its environmental and economic impact (Muñoz-Huerta *et al*., 2013). Phosphorus is a crucial element in ATP and NADPH generation, nucleic acid synthesis, membrane stability, enzyme activation/inactivation, glycolysis, respiration, and photosynthesis (Hawkesford *et al*., 2012). Potassium influences enzymatic activity and controls stomatal movement, and its deficiency causes early leaf senescence, resulting in low crop yield (Zhao *et al*., 2016). The requirements of N, P, and K and management of these nutrients have become a focus of research in terms of their form and absorption rate (Pandey *et al*., 2015). Therefore, early detection of nutrient deficits is essential for the sustainable management of natural resources available in agricultural systems, providing a good indicator of plant stress. The fluorescence emission of chlorophyll a in PSII (ChlF) has been used as a reference to determine the response of plants to the availability of different mineral nutrients (Cendrero-Mateo *et al*., 2015). Recent studies have shown that a ChlF analysis can be used as a sensitive indicator of deficiencies of N (Muñoz-Huerta *et al*., 2013), P (Carstensen *et al*., 2019), and K (Wang *et al*., 2015; Zhao *et al*., 2016) in different plant species.

The ChlF analysis is one of the most widely used techniques to study the photochemical efficiency and thermal dissipation of absorbed energy during the photosynthetic process (Guidi *et al*., 2019). The ChlF measurement is a quick and non-invasive indicator of plant photochemistry. This procedure has

been used as a helpful indicator to monitor a wide range of biotic and abiotic stresses in higher plants because it provides information on the identity of various pigments, their organization, and various specific electron transfer reactions in PS II (Kalaji *et al*., 2016; Carstensen *et al*., 2019; Sánchez-Reinoso *et al*., 2019).

The ratio of variable fluorescence (Fv) and maximum fluorescence (Fm), expressed as Fv/Fm (Qy), has been used as an indicator of quantum yield for PSII (Rodríguez *et al*., 2015); therefore, it is a reference for photosynthetic activity in plants. A decrease in this ratio indicates a reduction in PSII efficiency (Guidi *et al*., 2019). On the other hand, the introduction of ChlF analyses to dissipation processes has meant an additional advance in detecting PSII photoinhibition. Few studies have been developed regarding the use of ChlF analysis in spearmint cultivation. The photosynthetic rate, stomatal conductance, internal concentration of CO_2, and ChlF (in terms of Qy) are affected by saline stress (Chrysargyris *et al*., 2019a) and applications of P (Chrysargyris *et al*., 2019b). Therefore, this method is a potential indicator of the balance of nutrients that define plant productivity and is of great importance to responding to the technological demands of horticulturists in Colombia. The objective of this study was to identify how NPK fertilization influences the efficiency of the photosynthetic apparatus and photochemistry during the different stages of vegetative growth in spearmint.

MATERIALS AND METHODS

Location: This study was developed at the La Selva Research Center of the *Corporación Colombiana de Investigación Agropecuaria* (AGROSAVIA), located in Rionegro, Antioquia, Colombia, at 6° 12' 96" N and 75° 41' 40" W, and 2,117 m a.s.l. During the evaluation period (2021/04/01 to 2021/07/31), the climatic conditions included a maximum temperature of 27.1°C, a minimum of 8.4°C, and an average of 17.3°C, with a cumulative precipitation of 396.4 mm (Fig. 1).

Plant material: Spearmint cuttings were used, previously rooted with a 10 cm long stem and four pairs of leaves, which were transplanted into 5 kg bags (40 cm high × 20 cm wide). The substrate was a mixture of soil (70%), sand (20%), and coffee husk (10%), with a pH of 5.5 and concentrations of OM (18 %), P (7 mg kg^{-1}), B (0.09 mg kg^{-1}), K (0.69 cmol kg^{-1}), Ca (3.4 cmol kg^{-1}), and Mg (1.3 cmol kg^{-1}). The substrate was disinfected with 50 g of Dazomet 98%®.

Experiment design: A randomized complete block design with five treatments and five replicates was used. The treatments consisted of five doses of chemical fertilization with the 10-30-10 (N-P-K) formula at 0, 60, 90, 120, and 180 kg ha^{-1}. Each experiment unit consisted of 20 plants. The treatments were applied at two times: half the dose at 15 days after transplant (DAT), and the other half at 30 d after the initial application. The fertilizer application was made to drench the substrate directly in each plant,
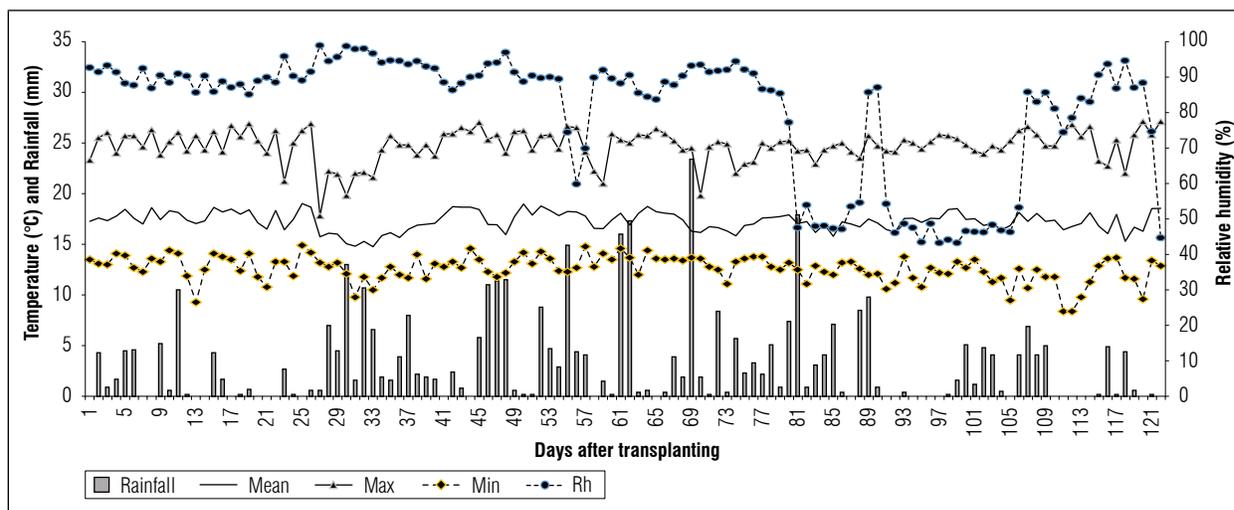

Figure 1. Daily rainfall, mean temperature (Mean), maximum (Max) and minimum (Min), and relative humidity (Rh) in a mesh house from 04/03/2021 to 07/31/2021. La Selva Research Center, Rionegro, Antioquia, Colombia.

and the equivalent dose per plant was 2.2 g (180 kg ha^{-1}), 1.5 (120 kg ha^{-1}), 1.1 (90 kg ha^{-1}), and 0.73 (60 kg ha^{-1}).

Variables evaluated: Starting 14 DAT and up to 112 DAT, 10 simultaneous measurements of gas exchange and ChlF were taken weekly in the fully expanded upper leaves between 9:00 a.m. and 11:00 a.m. With the same frequency, 11 measurements of the accumulation of dry matter in the roots, stems, and leaves were taken during the vegetative stage of the plant.

Net photosynthesis (*A*), transpiration (*E*), stomatal conductance (*g$_s$*) and leaf temperature (*Tleaf*) was recorded in the youngest fully expanded leaves, with a photosynthetic photon flux density of 1,100 μmol m^{-2} s^{-1} using a portable LCpro ADC® (BioScientific Ltd. Hoddesdon, UK) that was conditioned with an external halogen ADC® lamp (Bioscience Ltd, Hoddesdon, UK).

The variables associated with *ChlF* were determined with a Fluorpen FP100® fluorometer (Photon System Instrument, Drásov, Czech Republic). Measurements were taken on leaves kept in the dark for 30 min, which were evaluated for quantum yield (*Qy*), Non-photochemical quenching (*NPQ*), and photochemical quenching (*qP*).

The dry matter was quantified in the leaves (*LDM*), stems (*SDM*), and roots (*RDM*), where each organ was placed separately in a Memmert UL 80® oven (Memmert GmbH + Co. KG, Büchenbach, Germany) at 70°C until reaching constant weight. The final weight was the biomass of the structures and their sum, recorded as the total dry weight of the plant (*PDM*).

The leaf area reached by each plant (*LA*) was estimated with a Li-3000C® leaf area meter (Li-Cor Biosciences, Lincoln, NE).

Statistical analysis. Variations between the different treatments were analyzed using a one-way ANOVA at each evaluation time. Multiple comparisons were made with a Tukey test with a probability of 5% using the "Agricolae" package in R project version 4.1.0 (R Core Team, 2017).

RESULTS AND DISCUSSION

Gas exchange. The NPK doses showed significant differences (*P*<0.05) for the variables *A*, *E*, and *g$_s$*. In the first two evaluations (14 and 21 DAT), an irregular behavior was observed for *A*, *E*, and *g$_s$* between the treatments, without following a trend as a function of the increasing NPK doses (Fig. 2A, B, C). It was evident that, after 35 DAT, the 120 and 180 kg ha^{-1} treatments presented the highest *A*, surpassing the 60 and 90 kg ha^{-1} treatments by up to 71% (105 DAT) and the 0 kg ha^{-1} treatment by 148% (91 DAT). The highest *A* values were recorded in the treatment with 180 kg ha^{-1}, with the lowest in treatment 0 kg ha^{-1} (Fig. 2A). For *E*, starting at 35 DAT, the 180 kg ha^{-1} treatment had values that did not differ from 120 and 90 kg ha^{-1}. On the contrary, the plants subjected to a low dose (60 kg ha^{-1}) or no dose (0 kg ha^{-1}) of NPK presented the lowest transpiratory rates (Fig. 2B). For *g$_s$*, the treatment with 0 kg ha^{-1} did not differ significantly from the treatments with 180 and 120 kg ha^{-1} at 35, 56, and 98 DAT, presenting values similar to the treatments with 60 and 90 kg ha^{-1} (Fig. 2C).

Chlorophyll fluorescence. There were significant differences for the variables *Qy*, *NPQ*, and *qP*. It should be noted that, in the first two evaluations (14 and 21 DAT), a variable behavior was observed for *Qy*, *NPQ*, and *qP*, with no significant difference for the increasing NPK doses. Starting at 35 DAT, the plants without chemical fertilization (0 kg ha^{-1}) presented the lowest *Qy*, with values between 0.63 (DAT 42) and 0.78 (DAT 91) (Fig. 3A). The treatments with 60, 90, 120, and 180 kg ha^{-1} did not show differences for *Qy* in response to the NPK doses. The *NPQ* showed a trend contrary to the values of *Qy*. From 21 DAT, the 0 and 60 kg ha^{-1} treatments were characterized by the highest values for this variable, departing from the 60, 90, 120 and 180 kg ha^{-1} treatments, which did not present statistical differences (Fig. 3B). The *qP* varied over time, where, between 14 and 56 DAT, the treatments with 0 and 60 kg ha^{-1} presented higher values than the treatments with 120 and 180 kg ha^{-1}. However, after 63 DAT, the 0 kg ha^{-1} treatment had the lowest *qP*, differing from 180 kg ha^{-1} in several evaluations, but was similar to the treatments with 60, 90, and 120 kg ha^{-1} (Fig. 3C). For Tleaf after 56 DAT, there was a significant difference between the treatments, where the 0 kg ha^{-1} leaves reached the highest temperature, followed by the treatments with 60, 90, and 120 kg ha^{-1}. The 180 kg ha^{-1} treatment presented the lowest value, which fluctuated between 2 and 5°C below the temperatures recorded in the 0 kg ha^{-1} treatment, and between 1 and 4°C below those in the 60, 90, and 120 kg ha^{-1} treatments (Fig. 3D).

Dry matter and foliar area. There were significant differences between the accumulated dry matter and
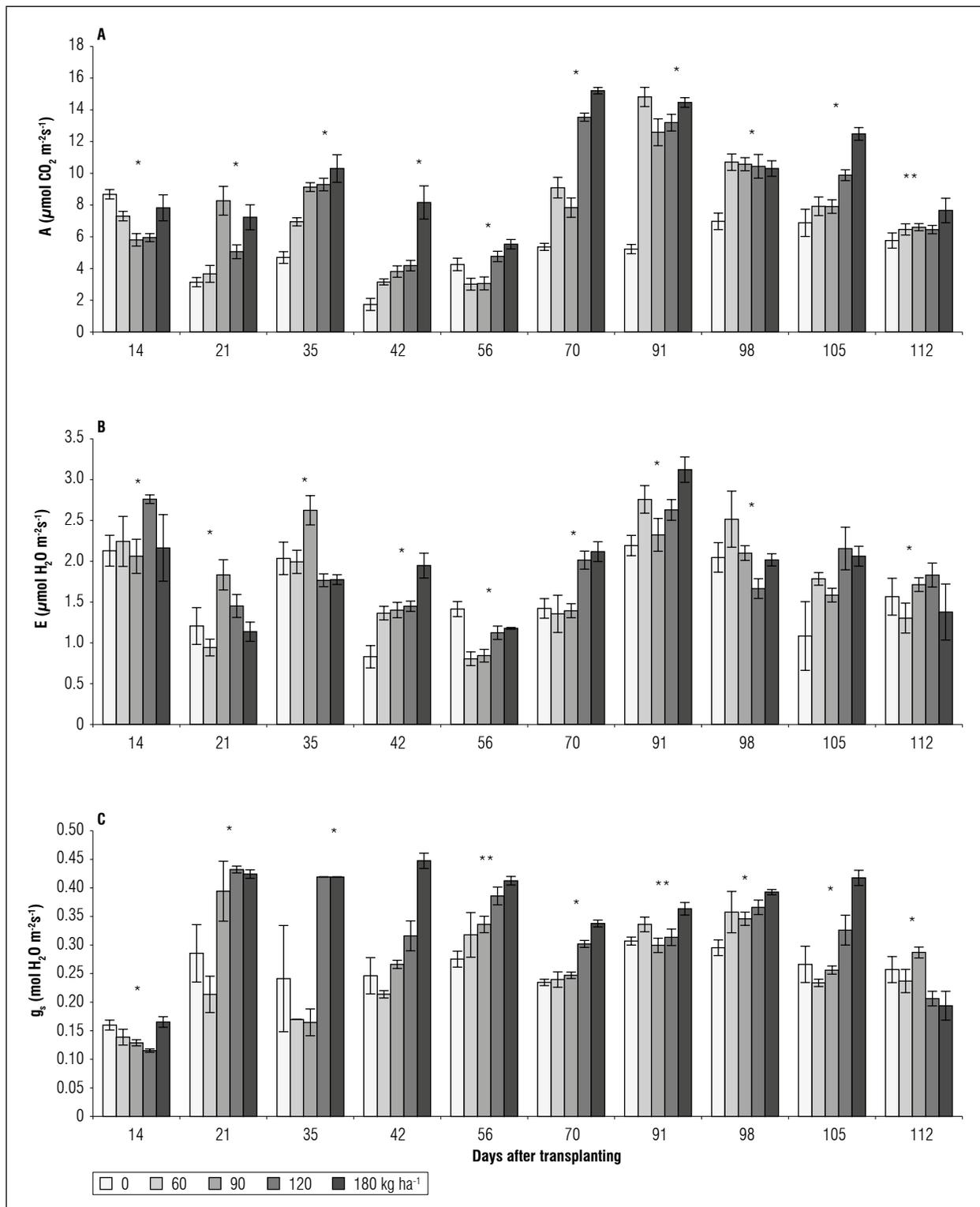
Figure 2. Effect of NPK doses on net photosynthetic rate - *A* (A), transpiration rate - *E* (B), and stomatal conductance – g_s (C) of recently fully-expanded spearmint leaves. Bars represent mean ± standard error.
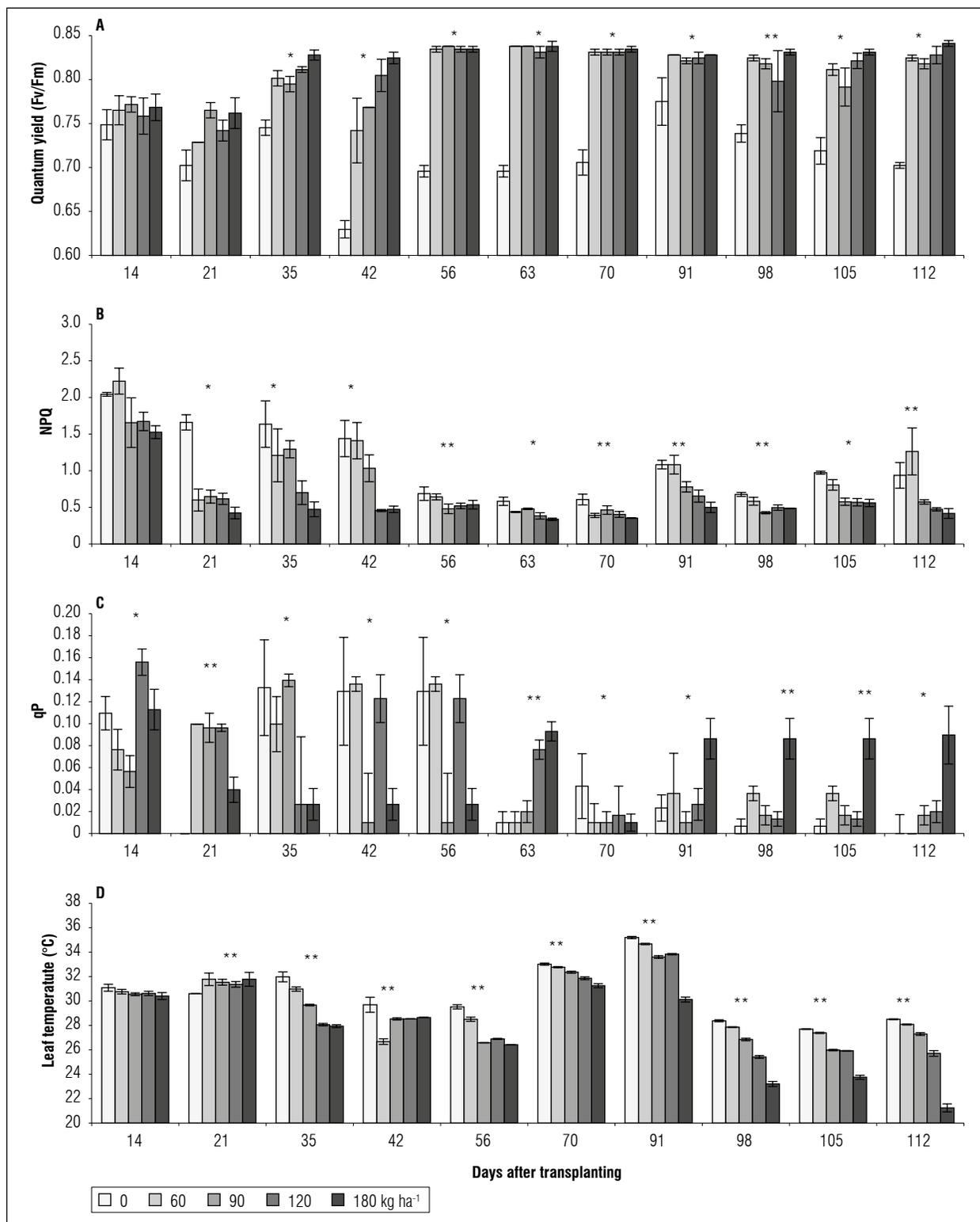
Figure 3. Effect of NPK doses on quantum yield – *Qy* (A), Non-photochemical quenching – *NPQ* (B), photochemical quenching coefficient – *qP* (C), and leaf temperature (D) of recently fully-expanded spearmint leaves. Bars denote mean ± standard error. Asterisks represent the difference between treatments according to Tukey's HSD test, at 1* and 5%** probability.
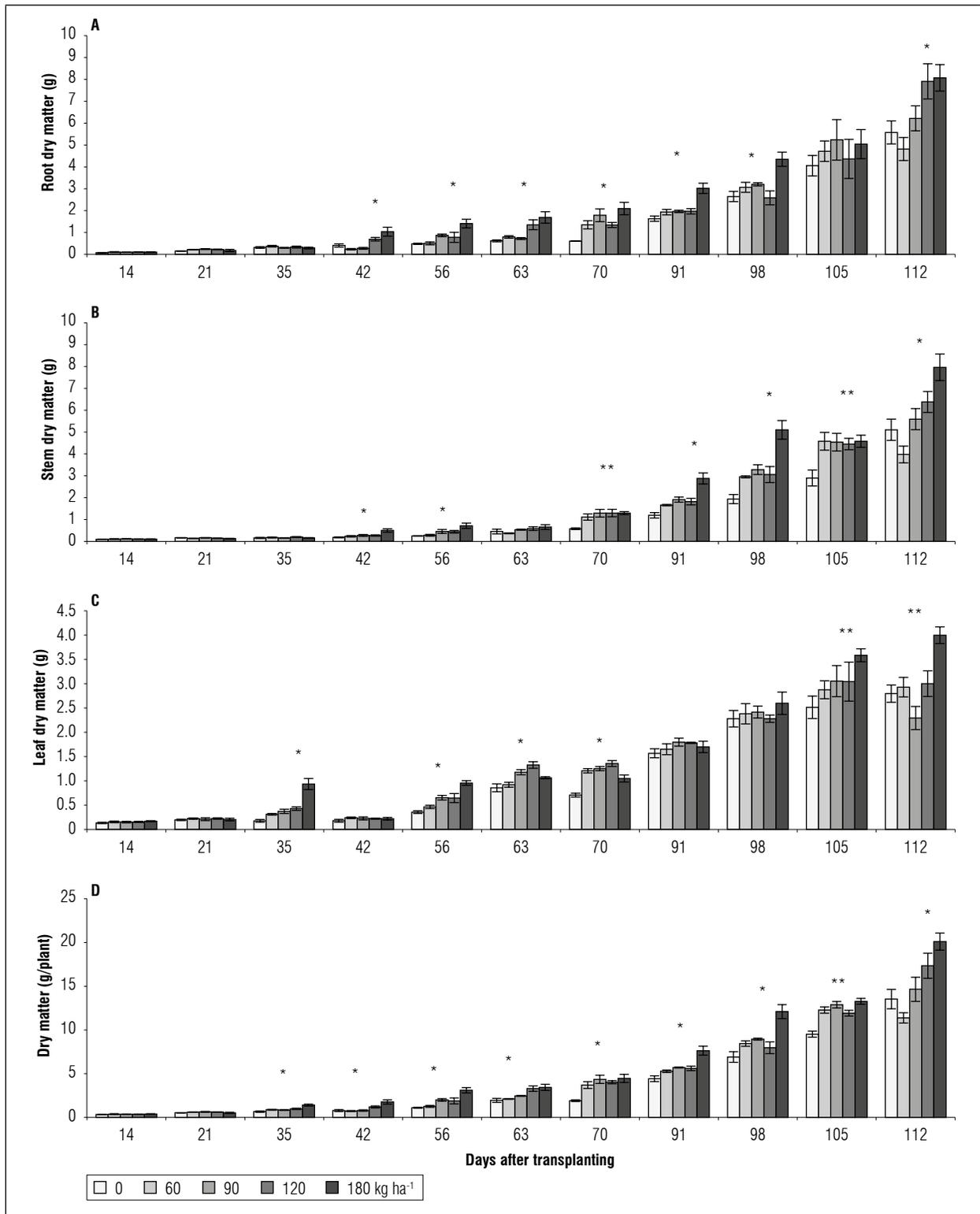
Figure 4. Effect of NPK doses on biomass partitioning in roots (A), stems (B), and leaves (C), and accumulated biomass per spearmint plant (D). Bars represent mean ± standard error. Asterisks denote the difference between treatments according to Tukey's HSD test, at 1* and 5%** probability.

the foliar area. A behavior similar to that of gas exchange and *ChlF* was observed. Between 14 and 21 DAT, no significant differences were found between the treatments for the variables *RDM, SDM, LDM,* PDM, and the total *LA* (Fig. 4). However, between 45 and 98 DAT, the *RDM* was higher in the 180 kg ha^{-1} treatment, significantly different from the treatments with 90, 60, and 0 kg ha^{-1}. However, at 105 and 112 DAT, these differences did not persist, especially between the extreme doses evaluated in the 0 and 180 kg ha^{-1} treatments (Fig. 4A). The *SDM* had a behavior similar to that of *RDM*. Up to 63 DAT, there were no differences between the treatments; however, between 70 and 112 DAT, the 0 kg ha^{-1} treatment was significantly lower than the 180 kg ha^{-1} treatment. The treatments with 60, 90, and 120 kg ha^{-1} did not differ for *SDM* (Fig. 4B). The highest *LDM* was in the 180 kg ha^{-1} treatment at 112 DAT, higher than that of the other treatments, which did not differ significantly from each other (Fig. 4C). For *LA*, starting at 63 DAT and until the end of the study cycle, the response to the 0 kg ha^{-1} treatment was lower than that obtained with the treatments with 90, 120, and 180 kg ha^{-1}, which did not differ significantly from the 60 kg ha^{-1} treatment (Fig. 5). Finally, for PDM, the trend was similar to that observed in the dry matter accumulated in the organs (*RDM, SDM, LDM*) independently. At 112 DAT, the 180 kg ha^{-1} treatment stood out over the other four treatments, presenting 45% more PDM (Fig. 4D).

DISCUSSION

Gas exchange. The spearmint leaves presented the highest *A* from 35 DAT in the treatments with 120 and 160 kg ha^{-1}, surpassing by up to 71% (105 DAT) the treatments with 60 and 90 kg ha^{-1} and by up to 148% (91 DAT) the treatment with 0 kg ha^{-1} (Fig. 2A). Hu *et al*. (2019) stated that N and K coordinate the diffusion and carboxylation of CO_2, improving photosynthetic activity. Photosynthesis in N- and K-deficient plants is mainly limited by mesophyll conductance and RuBP carboxylation. However, research on plants with an adequate supply of N and K has shown that photosynthesis is substantially improved through a balance of regeneration and carboxylation of RuBP, favored by higher availability of N and K (Xie *et al*., 2020). Likewise, when modeling photosynthesis, Walker *et al*. (2014) showed that foliar P is decisive since, with high N levels in the leaves (3 g m^{-2}), an increase in foliar P, from 0.05 to 0.22 g m^{-2}, doubled the carbon assimilation rate. Results obtained by Chen *et al*. (2018) when evaluating growth and photosynthetic parameters in *Eustoma grandiflorum* (Raf.) Shinn corroborated the synergistic effect between N, P, and K, reducing the net photosynthesis in leaves with a deficiency of these three nutrients. These authors indicated that the decrease was related to a low g_s; while leaves deficient in N are also accompanied by a higher intercellular concentration of CO_2 (*Ci*).
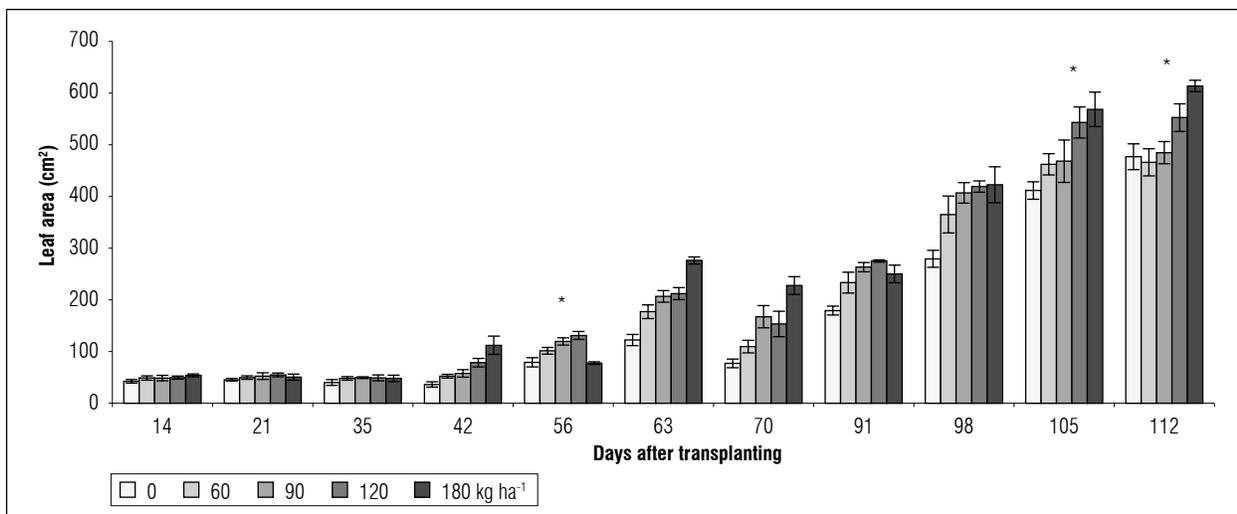

Figure 5. Effect of NPK doses on leaf area in spearmint plants. Bars represent mean ± standard error. Asterisks denote the difference between treatments according to Tukey's HSD test, at 1* and 5%** probability.

A reduction in stomatal opening and increased resistance are assumed to restrict g_s, resulting in decreased carbon fixation and photosynthesis rates (Wang *et al.*, 2016). The lower transpiration observed in the spearmint plants without chemical fertilization (0 kg ha^{-1}) was associated with a lower g_s (Fig. 2B) and greater resistance to water flow in the soil-plant-atmosphere continuum. Similar results were reported by Wang *et al.* (2016), who recorded a reduction in net photosynthesis with a low N supply in wheat (*Triticum* spp.) because of a decline in photosynthetic activity and low-efficiency carboxylation in the leaves. The g_s was affected by serial environmental factors (availability of water and nutrients, light, CO_2 levels, and temperature, among others), reducing the photosynthetic rate in C3 plants by up to 20% (Mu and Chen, 2021). Nitrogen is closely related to g_s or stomatal movement. Nitrate (NO_3^-), the main source of N for most plant species, regulates stomatal movements. Calcium chloride (ClCa), a NO_3^-/H^+ exchanger, is located in the vacuole membrane and can specifically accumulate NO_3^- in the main storage compartment of the cell (De Angeli *et al.*, 2006). On the other hand, a N deficiency promotes biosynthesis and increases the synthesis of abscisic acid (ABA), a hormone that has an essential role in the stomatal closure process (Mu *et al.*, 2018; Mu and Chen, 2021). In addition, N-deficient plants accumulate hydrogen peroxide (H_2O_2), which also promotes stomatal closure (Tewari *et al.*, 2007).

The causes of a reduced photosynthetic rate resulting from a K deficiency are attributed to a reduction in mesophyll conductance (Battie-Laclau *et al.*, 2014; Lu *et al.*, 2016) and g_s, as well as a decrease in the concentration of chlorophyll (Martineau *et al.*, 2017). However, Tighe-Neira *et al.* (2018) indicated that a K deficiency does not always limit the photosynthesis rate because a low demand in the K sink, associated with limitations in light supply and low transpiration, may not affect the fixing capacity. Pan *et al.* (2017) observed that the photosynthetic rate did not decrease consistently with the low availability of K in the species *Brassica napus* L.

Controversy exists for the main limiting effect that K has on photosynthetic activity. Oosterhuis *et al.* (2013) indicated that photosynthesis is limited by the opening and closing mechanisms of the stomata and, secondly, by biochemical processes. Jin *et al.* (2011) agreed that photosynthesis is limited by biochemical processes, such as the maximum rate of CO_2 fixation by RuBisCO and the maximum rate of electron transport but also attributed it to mesophilic conductance. However, in both situations, a K deficiency has adverse effects on the metabolic phases of photosynthesis (Jin *et al.*, 2011).

Chlorophyll fluorescence. Energy dissipation analysis is frequently used to monitor the response of the photosynthetic apparatus to environmental stress (Parkash and Singh, 2020). Stress in plants modifies the relative proportions of absorbed light energy used for quantum conversion in photosynthesis, chlorophyll fluorescence, and heat emission. Stress conditions also change the energy flow of photons through the leaf, altering its absorption, reflectance, and transmittance properties (Tremblay *et al.*, 2012). In this study, the absence of fertilization with N, P, and K was accompanied by a significant reduction in Qy, equivalent to 14%, a significant decrease in qP and an increase in NPQ (Fig. 3).

An N deficiency can affect PSII photochemistry by decreasing PSII electron transport and Qy (Lu and Zhang, 2000; DaMatta *et al.*, 2002). These findings are consistent with those of Du *et al.* (2019) for corn leaves (*Zea mays* L.), Huang *et al.* (2004) for rice (*Oryza sativa* L.), Wang *et al.* (2016) for winter wheat, and Lima *et al.* (1999) for beans (*Phaseolus vulgaris* L.), who concluded that Qy decreased significantly in the leaves of plants with a low N content.

A P deficiency generates PSII photoinhibition by reducing the efficiency of excitation energy in PSII reaction centers (Fv'/Fm') (Xu *et al.*, 2007). At the same time, the electron transport rate increases through PSII, and unused electrons are diverted to photorespiration, which reduces CO_2 fixation (Malhotra *et al.*, 2018). Despite the essential functions of P, Engels *et al.* (2012) indicated that a P deficiency had not been shown to have a direct impact on the photosynthetic electron transport chain. However, Carstensen *et al.* (2019) pointed out that a P deficiency reduces the rate of the enzyme ATP synthetase, which limits the output of protons to the chloroplast, acidifying the lumen of the thylakoid with consequent oxidation of hydroxy plastoquinone (PQH_2) in the Cytb6 f complex, a fact that further slows the linear flow of electrons towards PSI. P is associated with the loss of chlorophyll and decreases in Qy from photo-oxidative stress (Frydenvang *et al.*, 2015; Hernández and Munné-Bosch, 2015).

A K deficiency affects Qy by increasing thermal dissipation and reducing electron transfer efficiency, Fv'/Fm'

and qP. A reduction in the Fv'/Fm' decreases the power and affects the Calvin cycle, with a consequent decrease in the CO_2 assimilation rate. Likewise, Ye et al. (2017), who studied barley (*Hordeum vulgare*), indicated that plants with a K deficiency had a reduced ATPase activity, and Tighe-Neira et al. (2018) observed a decrease in the K concentration, associated with a 20% decrease in Qy, in broad beans (*Vicia faba* L.).

On the other hand, *NPQ* is an indicator of the process that dissipates the absorbed excesses of light energy as heat (Ruban, 2016) and corresponds to the dissipation mechanism of 96% of the radiation absorbed by leaves (Tremblay et al., 2012). Also, as a molecular adaptation, it represents a faster response of the thylakoid membrane to excess light (Demmig-Adams et al., 2014); this is critical since, under conditions without photoinhibition, a high and constant *NPQ* value is associated with a reduction in the CO_2 assimilation efficiency (Murchie and Ruban, 2020). As a mechanism for dissipating excess light energy, the spearmint plants without fertilization or low doses (60 kg ha^{-1}) presented the highest *NPQ* values, as compared to those that received the highest doses of compound fertilizer (> 120 kg ha^{-1}). This is a photoprotective mechanism of the photosynthetic apparatus in the leaves of plants subjected to low NPK doses. *NPQ* can improve productivity by limiting photoinhibition (Hubbart et al., 2018) but can also reduce the performance of plants through its excessively protective persistence, unnecessarily reducing quantum yield under low light conditions (Kromdijk et al., 2016). As such, the 60 kg ha^{-1} dose saw an *NPQ* increase, where the *Qy* was not affected in the leaves (Fig. 3A). However, the leaf temperature increased by up to 7°C in the plants that received the complete fertilization plan (180 kg ha^{-1}) (Fig. 3D).

Dry matter and foliar area. The dry matter and leaf area in the plants subjected to different NPK levels (Fig. 4 and 5) presented a behavior similar to the A results (Fig. 2). With the low NPK rates, the initial growth rates were slow, and the increase in total dry matter was not remarkable, which only became evident after 42 DAT (Fig. 4). The dry matter distribution among the plant organs was strongly affected by the NPK treatments (Fig. 4). The highest dry matter accumulation occurred in the stems and roots, less so in the leaves in all treatments. The increase was greater with the higher NPK doses. In studies that compared three spearmint cultivars, Singh et al. (2001) recorded an accumulation of dry matter that varied between 7.07 and 9.18 g/plant, values lower than those of this study. The doses higher than 120 kg ha^{-1} saw an accumulation greater than 14.20 g/plant, distributed in the leaves (25%), stems (35%) and roots (40%).

The leaf area decreased as the NPK doses were reduced (Fig. 5). Zhao et al. (2016) highlighted the importance of N by confirming that a deficiency of the element in sorghum plants (*Sorghum* spp.) led to a reduction in leaf area, chlorophyll content, and photosynthesis, which resulted in a decrease in leaf area with a low accumulation of dry matter. Similar results were obtained by Roveda-Hoyos and Moreno-Fonseca (2019) when evaluating P deficiencies in cape gooseberry (*Physalis peruviana* L.) plants. Qiu and Israel (1994) pointed out that a reduction in leaf area expansion and a consequent decrease in biomass from a P deficiency is more important than the effect of P deficiencies on the photosynthetic rate per unit of the leaf area. On the other hand, Gerardeaux et al. (2009) showed that an early K deficiency in cotton plants (*Gossypium* ssp.) restricts the growth of the leaf area, where the number and individual size of the leaves are smaller.

According to Timlin et al. (2017), a smaller leaf area is associated with an accelerated decrease in the number of leaves because of faster senescence when the plants are subjected to a low supply of NPK, especially P. The growth and expansion of leaves involve two main processes: expansive growth and structural growth, which depend on water to increase cell volume and carbon and nutrients for structural growth (Pantin et al., 2011). Since leaves are essential for photosynthesis, NPK is indispensable to building initial infrastructure. Nitrogen, P, or K deficiencies suppress growth, accelerate senescence, and decrease chloroplastic pigment concentrations in leaves (Tewari et al., 2007).

As in the gas exchange variables, the synergistic effects between nutrients are recognized. Schlüter et al. (2013) indicated that P stress affects N dynamics, where the absorption of N is down-regulated under conditions with a P deficiency. At the same time, Hou et al. (2018) emphasized the importance of the favorable effect of the correlation between N and K on photosynthesis.

The nutritional management of spearmint is essential for the development of biomass; between 250 and 300 kg ha^{-1} of N, 55 and 110 kg ha^{-1} of P_2O_5, and about

375 kg ha^{-1} of K$_2$O are required to obtain better yields (Brown *et al*., 2003). These values are much higher than those used in Colombia for target fertilization, where 18 kg of N, 54 kg of P, and 18 kg of K are used in 1 ha, with a yield of 10-12 t ha^{-1} of fresh leaves (Agronet, 2021), less than the 15 to 20 t ha^{-1} of fresh leaves seen worldwide (Wikifarmer, 2021).

CONCLUSION

Increasing the NPK doses favored the photosynthetic performance and quantum yield in the spearmint plants. The highest *NPQ* values were recorded with the lowest NPK doses. High NPK doses increased the accumulated dry matter and the foliar area, contributing to a higher yield. For the gas exchange variables, in general, the doses of 120 and 180 produced the best rates of photosynthesis in the Mentha plants. A similar trend was observed for the accumulated dry matter and leaf area, parameters associated with yield.

Conflict of interests: The manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

BIBLIOGRAPHIC REFERENCES

Agronet. 2021. Estadísticas Agropecuarias - Agrícola. In: https://www.agronet.gov.co/estadistica/paginas/home.aspx?cod=1; Consulted: September, 2021.

Battie-Laclau, P., J.-P. Laclau, C. Beri, L. Mietton, M.R.A. Muniz, B.C. Arenque, M.C. Piccolo, L. Jordan-Meille, J.-P. Bouillet, and Y. Nouvellon. 2014. Photosynthetic and anatomical responses of *Eucalyptus grandis* leaves to potassium and sodium supply in a field experiment. Plant Cell Environ. 37, 70-81. Doi: 10.1111/pce.12131

Brown, B., J.M. Hart, M.P. Wescott, and N.W. Christensen. 2003. The critical role of nutrient management in mint production. Better Crops 87(4), 9-11.

Carstensen, A., A.E. Szameitat, J. Frydenvang, and S. Husted. 2019. Chlorophyll *a* fluorescence analysis can detect phosphorus deficiency under field conditions and is an effective tool to prevent grain yield reductions in spring barley (*Hordeum vulgare* L.). Plant Soil 434(1), 79-91. Doi: 10.1007/s11104-018-3783-6

Cendrero-Mateo, M.P., A.E. Carmo-Silva, A. Porcar-Castell, E.P. Hamerlynck, S.A. Papuga, and M.S. Moran. 2015. Dynamic response of plant chlorophyll fluorescence to light, water and nutrient availability. Funct. Plant Biol. 42(8), 746-757. Doi: 10.1071/FP15002

Cirlini, M., P. Mena, M. Tassotti, K.A. Herrlinger, K.M. Nieman, C. Dall'Asta, and D. Del Rio. 2016. Phenolic and volatile composition of a dry spearmint (*Mentha spicata* L.) extract. Molecules 21(8), 1007. Doi: 10.3390/molecules21081007

Chen, C.-T., C.-L. Lee, and D.-M. Yeh. 2018. Effects of nitrogen, phosphorus, potassium, calcium, or magnesium deficiency on growth and photosynthesis of *Eustoma*. HortScience 53(6), 795-798. Doi: 10.21273/HORTSCI12947-18

Chrysargyris, A., E. Nikolaidou, A. Stamatakis, and N. Tzortzakis. 2017. Vegetative, physiological, nutritional and antioxidant behavior of spearmint (*Mentha spicata* L.) in response to different nitrogen supply in hydroponics. J. Appl. Res. Med. Aromat. Plants 6, 52-61. Doi: 10.1016/j.jarmap.2017.01.006

Chrysargyris, A., S.A. Petropoulos, Â Fernandes, L. Barros, N. Tzortzakis, and I.C.F.R. Ferreira. 2019b. Effect of phosphorus application rate on *Mentha spicata* L. grown in deep flow technique (DFT). Food Chem. 276, 84-92. Doi: 10.1016/j.foodchem.2018.10.020

Chrysargyris, A., M. Solomou, S.A. Petropoulos, and N. Tzortzakis. 2019a. Physiological and biochemical attributes of *Mentha spicata* when subjected to saline conditions and cation foliar application. J. Plant Physiol. 232, 27-38. Doi: 10.1016/j.jplph.2018.10.024

DaMatta, F.M., R.A. Loos, E.A. Silva, and M.E. Loureiro. 2002. Limitations to photosynthesis in *Coffea canephora* as a result of nitrogen and water availability. J. Plant Physiol. 159, 975-981. Doi: 10.1078/0176-1617-00807

De Angeli, A., D. Monachello, G. Ephritikhine, J.M. Frachisse, S. Thomine, F. Gambale, and H. Barbier-Brygoo. 2006. The nitrate/proton antiporter AtCLCa mediates nitrate accumulation in plant vacuoles. Nature 442, 939-942. Doi: 10.1038/nature05013

Demmig-Adams, B., S.-C. Koh, C.M. Cohu, O. Muller, J.J. Stewart, and W.W. Adams III. 2014. Non-photochemical fluorescence quenching in contrasting plant species and environments. pp. 531-552. In: Demmig-Adams, B., G. Garab, W. Adams III, and Govindjee (eds.). Non-Photochemical quenching and energy dissipation in plants, algae and cyanobacteria. Advances in Photosynthesis and Respiration. Vol. 40. Springer, Dordrecht, The Netherlands. Doi: 10.1007/978-94-017-9032-1_24

Du, Q., X.-H. Zhao, L. Xia, C.J. Jiang, X.G. Wang, Y. Han, J. Wang, and H.-Q. Yu. 2019. Effects of potassium deficiency on photosynthesis, chloroplast ultrastructure, ROS, and antioxidant activities in maize (*Zea mays* L.). J. Integr. Agric. 18(2), 395-406. Doi: 10.1016/S2095-3119(18)61953-7

Engels, C., E. Kirkby, and P. White. 2012. Mineral nutrition, yield and source: sink relationships. pp. 85-133. In: Marschner, P. (ed.). Marschner's mineral nutrition

of higher plants. Elsevier, London. Doi: 10.1016/B978-0-12-384905-2.00005-4

Frydenvang, J., M. van Maarschalkerweerd, A. Carstensen, S. Mundus, S.B. Schmidt, P.R. Pedas, K.H. Laursen, J.K. Schjoerring, and S. Husted. 2015. Sensitive detection of phosphorus deficiency in plants using chlorophyll *a* fluorescence. Plant Physiol. 169(1), 353-361. Doi: 10.1104/pp.15.00823

Gerardeaux, E., L. Jordan-Meille, and S. Pellerin. 2009. Radiation interception and conversion to biomass in two potassium-deficient cotton crops in South Benin. J. Agric. Sci. 147(2), 155-168. Doi: 10.1017/S0021859608008381

Guidi, L., E. Lo Piccolo, and M. Landi. 2019. Chlorophyll fluorescence, photoinhibition and abiotic stress: does it make any difference the fact to be a C3 or C4 species? Front. Plant Sci. 10, 174. Doi: 10.3389/fpls.2019.00174

Hawkesford, M., W. Horst, T. Kichey, H. Lambers, J. Schjoerring, I.S. Møller, and P. White. 2012. Functions of macronutrients. pp. 135-189. In: Marschner, P. (ed.). Marschner's mineral nutrition of higher plants. 3rd ed. Elsevier, London. Doi: 10.1016/B978-0-12-384905-2.00006-6

Hernández, I. and S. Munné-Bosch. 2015. Linking phosphorus availability with photo-oxidative stress in plants. J. Exp. Bot. 66, 2889-2900. Doi: 10.1093/jxb/erv056

Hou, W., J. Yan, B. Jákli, J. Lu, T. Ren, R. Cong, and X. Li. 2018. Synergistic effects of nitrogen and potassium on quantitative limitations to photosynthesis in rice (*Oryza sativa* L.). J. Agric. Food Chem. 66(20), 5125-5132. Doi: 10.1021/acs.jafc.8b01135

Hu, W., T. Ren, F. Meng, R. Cong, X. Li, P.J. White, and J. Lu. 2019. Leaf photosynthetic capacity is regulated by the interaction of nitrogen and potassium through coordination of CO_2 diffusion and carboxylation. Physiol. Plant. 167(3), 418-432. Doi: 10.1111/ppl.12919

Huang, Z.A., D.A. Jiang, Y. Yang, J.W. Sun, and S.H. Jin. 2004. Effects of nitrogen deficiency on gas exchange, chlorophyll fluorescence, and antioxidant enzymes in leaves of rice plants. Photosynthetica 42(3), 357-364. Doi: 10.1023/B:PHOT.0000046153.08935.4c

Hubbart, S., I.R.A. Smillie, M. Heatle, R. Swarup, C.C. Foo, L. Zhao, and E.H. Murchie. 2018. Enhanced thylakoid photoprotection can increase yield and canopy radiation use efficiency in rice. Commun. Biol. 1, 22. Doi: 10.1038/s42003-018-0026-6

Jin, S.H., J.Q. Huang, X.Q. Li, B.S. Zheng, J.S. Wu, Z.J. Wang, G.H. Liu, and M. Chen. 2011. Effects of potassium supply on limitations of photosynthesis by mesophyll diffusion conductance in *Carya cathayensis*. Tree Physiol. 31, 1142-1151. Doi: 10.1093/treephys/tpr095

Kalaji, H.M., A. Jajoo, A. Oukarroum, M. Brestic, M. Zivcak, I.A. Samborska, M.D. Cetner, I. Łukasik, V. Goltsev, and R.J. Ladle. 2016. Chlorophyll *a* fluorescence as a tool to monitor physiological status of plants under abiotic stress conditions. Acta Physiol. Plant 38(4), 102. Doi: 10.1007/s11738-016-2113-y

Karkanis, A., C. Lykas, V. Liava, A. Bezou, S. Petropoulos, and N. Tsiropoulos. 2017. Weed interference with peppermint (*Mentha x piperita* L.) and spearmint (*Mentha spicata* L.) crops under different herbicide treatments: effects on biomass and essential oil yield. J. Sci. Food Agric 98(1), 43-50. Doi: 10.1002/jsfa.8435

Kromdijk, J., K. Głowacka, L. Leonelli, S.T. Gabilly, M. Iwai, K.K. Niyogi, and S.P. Long. 2016. Improving photosynthesis and crop productivity by accelerating recovery from photoprotection. Science 354(6314), 857-861. Doi: 10.1126/science.aai8878

Lima, J.D., P.R. Mosquim, and F.M. Da Matta. 1999. Leaf gas exchange and chlorophyll fluorescence parameters in *Phaseolus vulgaris* as affected by nitrogen and phosphorus deficiency. Photosynthetica 36(1), 113-121. Doi: 10.1023/A:1007079215683

Lu, Z., J. Lu, Y. Pan, P. Lu, X. Li, R. Cong, and T. Ren. 2016. Anatomical variation of mesophyll conductance under potassium deficiency has a vital role in determining leaf photosynthesis. Plant Cell Environ. 39(11), 2428-2439. Doi: 10.1111/pce.12795

Lu, C. and J. Zhang. 2000. Photosynthetic CO_2 assimilation, chlorophyll fluorescence and photoinhibition as affected by nitrogen deficiency in maize plants. Plant Sci. 151(2), 135-143. Doi: 10.1016/S0168-9452(99)00207-1

Malhotra, H., Vandana, S. Sharma, and R. Pandey. 2018. Phosphorus nutrition: plant growth in response to deficiency and excess. pp. 171-190. In: Hasanuzzaman, M., M. Fujita, H. Oku, K. Nahar, and B. Hawrylak-Nowak (eds.). Plant nutrients and abiotic stress tolerance. Springer, Singapore. Doi: 10.1007/978-981-10-9044-8_7

Martineau, E., J.-C. Domec, A. Bosc, M. Dannoura, Y. Gibon, C. Bénard, and L. Jordan-Meille. 2017. The role of potassium on maize leaf carbon exportation under drought condition. Acta Physiol. Plant. 39, 219. Doi: 10.1007/s11738-017-2515-5

Mu, X. and Y. Chen. 2021. The physiological response of photosynthesis to nitrogen deficiency. Plant Physiol. Biochem. 158, 76-82. Doi: 10.1016/j.plaphy.2020.11.019

Mu, X., Q. Chen, X. Wu, F. Chen, L. Yuan, and G. Mi. 2018. Gibberellins synthesis is involved in the reduction of cell flux and elemental growth rate in maize leaf under low nitrogen supply. Environ. Exp. Bot. 150, 198-208. Doi: 10.1016/j.envexpbot.2018.03.012

Muñoz-Huerta, R.F., R.G. Guevara-Gonzalez, L.M. Contreras-Medina, I. Torres-Pacheco, J. Prado-Olivarez, and R.V. Ocampo-Velazquez. 2013. A review of methods

for sensing the nitrogen status in plants: advantages, disadvantages and recent advances. Sensors 13(8), 10823-10843. Doi: 10.3390/s130810823

Murchie, E.H. and A.V. Ruban. 2020. Dynamic non-photochemical quenching in plants: from molecular mechanism to productivity. Plant J. 101(4), 885-896. Doi: 10.1111/tpj.14601

Oosterhuis, D.M., D.A. Loka and T.B. Raper. 2013. Potassium and stress alleviation: Physiological functions and management of cotton. J. Plant Nutr. Soil Sci. 176(3), 331-343. Doi: 10.1002/jpln.201200414

Pan, Y., Z. Lu, J. Lu, X. Li, R. Cong, and T. Ren. 2017. Effects of low sink demand on leaf photosynthesis under potassium deficiency. Plant Physiol Biochem. 113, 110-121. Doi: 10.1016/j.plaphy.2017.01.027

Pandey, R., G. Zinta, H. AbdElgawad, A. Ahmad, V. Jain, and I.A. Janssens. 2015. Physiological and molecular alterations in plants exposed to high [CO_2] under phosphorus stress. Biotechnol. Adv. 33(3-4), 303-316. Doi: 10.1016/j.biotechadv.2015.03.011

Pantin, F., T. Simonneau, G. Rolland, M. Dauzat, and B. Muller. 2011. Control of leaf expansion: A developmental switch from metabolics to hydraulics. Plant Physiol. 156(2), 803-815. Doi: 10.1104/pp.111.176289

Parkash, V. and S. Singh. 2020. A review on potential plant-based water stress indicators for vegetable crops. Sustainability 12(10), 3945. Doi: 10.3390/su12103945

Pedraza, R. and M.C. Henao. 2008. Composición del tejido vegetal y su relación con variables de crecimiento y niveles de nutrientes en el suelo en cultivos comerciales de menta (*Mentha spicata* L.). Agron. Colomb. 26(2), 186-196.

Qiu, J. and D.W. Israel. 1994. Carbohydrate accumulation and utilization in soybean plants in response to altered phosphorus nutrition. Physiol. Plant. 90(4), 722-728. Doi: 10.1111/j.1399-3054.1994.tb02529.x

R Core Team. 2017. R: a language and environment for statistical computing. Vienna.

Rodríguez Torressi, A.O., M. Yonni, M. Nazareno, C.R. Galmarini, and C.A. Bouzo. 2015. Eficiencia fotoquímica máxima e índice de potencial fotosintético en plantas de melón (*Cucumis melo*) tratadas con bajas temperaturas. FAVE, Secc. Cienc. Agrar. 13, 1-2. Doi: 10.14409/fa.v13i1/2.4966

Roveda-Hoyos, G. and L. Moreno-Fonseca. 2019. Physiological and antioxidant responses of cape gooseberry (*Physalis peruviana* L.) seedlings to phosphorus deficiency. Agron. Colomb. 37(1), 3-11. Doi: 10.15446/agron.colomb.v37n1.65610

Ruban, A.V. 2016. Nonphotochemical chlorophyll fluorescence quenching: mechanism and effectiveness in protecting plants from photodamage. Plant Physiol. 170(4), 1903-1916. Doi: 10.1104/pp.15.01935

Sánchez-Reinoso, A.D., Y. Jiménez-Pulido, J.P. Martínez-Pérez, C.S. Pinilla, and G. Fischer. 2019. Chlorophyll fluorescence and other physiological parameters as indicators of waterlogging and shadow stress in lulo (*Solanum quitoense* var. *septentrionale*) seedlings. Rev. Colomb. Cienc. Hortic. 13(3), 325-335. Doi: 10.17584/rcch.2019v13i3.100171

Schlüter, U., C. Colmsee, U. Scholz, A. Bräutigam, A.P.M. Weber, N. Zellerhoff, M. Bucher, H. Fahnenstich, and U. Sonnewald. 2013. Adaptation of maize source leaf metabolism to stress related disturbances in carbon, nitrogen and phosphorus balance. BMC Genomics 14(1), 442. Doi: 10.1186/1471-2164-14-442

Singh, P., A. Misra, and N.K. Srivastava. 2001. Influence of Mn deficiency on growth, chlorophyll content, physiology, and essential monoterpene oil (s) in genotypes of spearmint (*Mentha spicata* L.). Photosynthetica 39(3), 473-476. Doi: 10.1023/A:1015107116205

Smethurst, C.F., T. Garnett, and S. Shabala. 2005. Nutritional and chlorophyll fluorescence responses of lucerne (*Medicago sativa*) to waterlogging and subsequent recovery. Plant Soil 270(1), 31-45. Doi: 10.1007/s11104-004-1082-x

Tewari, R.K., P. Kumar, and P.N. Sharma. 2007. Oxidative stress and antioxidant responses in young leaves of mulberry plants grown under nitrogen, phosphorus or potassium deficiency. J. Integr. Plant Biol. 49(3), 313-322. Doi: 10.1111/j.1744-7909.2007.00358.x

Tighe-Neira, R., M. Alberdi, P. Arce-Johnson, J. Romero, M. Reyes-Díaz, Z. Rengel, and C. Inostroza-Blancheteau. 2018. Role of potassium in governing photosynthetic processes and plant yield. pp. 191-203. In: Hasanuzzaman, M., M. Fujita, H. Oku, K. Nahar, and B. Hawrylak-Nowak (eds.). Plant nutrients and abiotic stress tolerance. Springer, Singapore. Doi: 10.1007/978-981-10-9044-8_8

Timlin, D.J., T.C.M. Naidu, D.H. Fleisher, and V.R. Reddy. 2017. Quantitative effects of phosphorus on maize canopy photosynthesis and biomass. Crop Sci. 57(6), 3156-3169. Doi: 10.2135/cropsci2016.11.0970

Tremblay, N., Z. Wang, and Z.G. Cerovic. 2012. Sensing crop nitrogen status with fluorescence indicators. A review. Agron. Sustain. Dev. 32, 451-464. Doi: 10.1007/s13593-011-0041-1

Walker, A.P., A.P. Beckerman, L. Gu, J. Kattge, L.A. Cernusak, T.F. Domingues, J.C. Scales, G. Wohlfahrt, S.D. Wullschleger, and F.I. Woodward. 2014. The relationship of leaf photosynthetic traits–V_{cmax} and J_{max}–to leaf nitrogen, leaf phosphorus, and specific leaf area: a meta-analysis and modeling study. Ecol. Evol. 4(16), 3218-3235. Doi: 10.1002/ece3.1173

Wang, X., L. Wang, and Z. Shangguan. 2016. Leaf gas exchange and fluorescence of two winter wheat varieties in response to drought stress and nitrogen supply. PLoS One 11(11), e0165733. Doi: 10.1371/journal.pone.0165733

Wang, X.-G., X.-H. Zhao, C.-J. Jiang, C.-H. Li, S. Cong, D. Wu, Y.-Q. Chen, H.-Q. Yu, and C.-Y. Wang. 2015. Effects of potassium deficiency on photosynthesis and photoprotection mechanisms in soybean (*Glycine max* (L.) Merr.). J. Integr. Agric. 14(5), 856-863. Doi: 10.1016/S2095-3119(14)60848-0

Wikifarmer. 2021. Información sobre la planta de menta. In: https://wikifarmer.com/es/; consulted: September, 2021.

Xie, K., Z. Lu, Y. Pan, L. Gao, P. Hu, M. Wang, and S. Guo. 2020. Leaf photosynthesis is mediated by the coordination of nitrogen and potassium: the importance of anatomical-determined mesophyll conductance to CO_2 and carboxylation capacity. Plant Sci. 290, 110267. Doi: 10.1016/j.plantsci.2019.110267

Xu, H.X., X.Y. Weng, and Y. Yan. 2007. Effect of phosphorus deficiency on the photosynthetic characteristics of rice plants. Russ. J. Plant Physiol. 54, 741-748. Doi: 10.1134/S1021443707060040

Ye, Z., J. Zeng, X. Li, F. Zeng, and G. Zhang. 2017. Physiological characterizations of three barley genotypes in response to low potassium stress. Acta Physiol. Plant. 39, 232. Doi: 10.1007/s11738-017-2516-4

Zhao, X., Q. Du, Y. Zhao, H. Wang, Y. Li, X. Wang, and H. Yu. 2016. Effects of different potassium stress on leaf photosynthesis and chlorophyll fluorescence in maize (*Zea mays* L.) at seedling stage. Agric. Sci. 7(1), 44-53. Doi: 10.4236/as.2016.71005