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Physiological and productive performance of hop (*Humulus lupulus* L.) varieties grown under subtropical conditions in Brazil

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Abstract

The primary focus of hop cultivation is the brewing industry. Brazil is one of the largest producers and consumers of beer worldwide and therefore one of the largest importers of hops. Therefore, it is essential to develop research that promotes the national production of hops. This study aimed to analyze the photosynthetic, morphometric and productive capacities of five hop varieties outside of their natural cultivation conditions in a subtropical climate; thus, to analyze the performance of post-tropicalized hops. A randomized block experimental design was used, including five cultivar treatments (Comet, Chinook, Cascade, Nugget and Columbus varieties) with four blocks and two plants per plot, totaling 20 plots and 40 sampling units. Physiological, morphometric and productive data were collected during the reproductive phase (final phase of plant development). Photosynthetic data were collected using an infrared gas analyzer (IRGA) coupled to a portable fluorometer to obtain gas exchange and fluorescence data. The collected morphometric and productive data are related to the vegetative and productive capacity of the hops. All varieties analyzed showed remarkable physiological responses. However, the highest quantum yield and effective yield of PSII were obtained from Comet (0.94 and 0.50) and Nugget (0.94 and 0.52), respectively. Comet exhibited the best morphometric results related to vegetative development (561.12 cm for plant height) but did not exhibit similarly high productive performance, while Cascade was the variety with the best productivity. Moreover, this work shows that tropicalized hops have satisfactory performance.

Keywords: gas exchange, morphometric, fluorescence, *Humulus lupulus* L., productivity, Cannabaceae.
Abbreviations: A_Carbon assimilation; A/Ci_Carboxylation efficiency; B_Boron; Ci_Internal concentration of carbon dioxide; CL_Cones length; CO_2_Carbon dioxide; Cu_Copper; DM_Dry mass; E_Transpiration rate; ETR_Relative electron transfer; Fe_Iron; FM_Fresh mass; F_m_Maximum fluorescence; F_v_Variable fluorescence; F_0_Initial fluorescence; F_v/F_m_Potential quantum efficiency of photosystem II; F_v'/F_m'_Quantum efficiency of the antennas; g_s_Stomatal conductance; HIFC_Height of insertion of the first cone on the plant; ID_Internode distance; IRGA_Infrared Gas Analyzer; KCl_Potassium chloride; $kg.ha^{-1}$_Kilograms per hectare; LLB_Length of the lateral branches; Mg_Magnesium; Mn_Manganese; Mo_Molybdenum; N_Nitrogen; NI_Number of internodes; PH_Plant height; PPFD_Photosynthetic photon flux density; PSII_Photosystem 2; S_Sulfur; $t.ha^{-1}$_Tons per hectare; WUE_Water efficiency; Zn_Zinc.

Introduction

Hops (*Humulus lupulus* L.) belong to the Cannabaceae family and include three known species: *H. lupulus, H. japonicus* and *H. yunnanensis*. However, only the first two are commercially cultivated because they have lupulin glands, which are responsible for commercial interest in the plant (Almaguer et al., 2014).
Although hops are useful because of some medicinal components, such as the antimicrobial and anti-inflammatory components that are present in the lupulin glands, their main use is in the brewing industry (Zawadzki et al., 2018).
Approximately 97% of hop production is destined for this industry, and the medicinal properties of hops protect beer from oxidative processes and microbiological contamination (Durello et al., 2019). Brazil is the third largest beer producer in the world and the largest importer of hops in South America (Fortuna et al., 2023). There is a big demand for national hop production to supply the domestic market.
The scarcity of hops considering competition for what is available in the foreign market means that there is great demand for imports in Brazil (Campos et al., 2023), but this scenario has begun to change with new productive fields emerging throughout the country.
In 2019, there was large growth in the number of brewing establishments, with 320 new breweries and microbreweries registered (MAPA 2020). According to data from CERVBRASIL (2020), this sector is responsible for 1.6% of national GDP and generates 2.7 million jobs. It is clear that the cultivation

Table 1. Mean values of cone length (cm), internode distance (cm) and plant height (cm) for the five *H. lupulus* varieties.

Varieties	CL	ID	PH
Comet	2.94 bc	440.37 a	561.12 a
Chinook	3.26 ab	395.37 ab	512.04 ab
Cascade	2.68 c	366.12 b	442.12 bc
Nugget	2.85 bc	380.50 ab	498.50 ab
Columbus	3.40 a	285.87 c	375.87 c
CV (%)	6.47	8.17	7.21

Legend: CL: cone length; ID: internode distance; PH: plant height. Means followed by the same letters do not differ significantly according to the Tukey's test at a 5% probability.

of hops can positively impact the beer production chain concerning the generation of jobs and income.

The crop originates from the Northern Hemisphere, and blooms are induced by shortening of the day length. The lupulin glands are located in the flowers, and the ultimate interest in hop cultivation is related to their harvest. In the Northern Hemisphere, this induction occurs when the day length is shorter than 16 hours (Thomas and Schwabe, 1969; Iskra et al., 2019).

Day length varies in relation to latitude; ideal latitudes that induce good hop development are usually between 33° and 55° north or south of the equator (Dodds 2017). Better plant development and productivity occur when the day length ensures good vegetative growth before floral induction occurs with a decrease in the photoperiod (Agehara et al., 2020).

Botucatu is located at latitudes 22°30' to 23°05'S and longitues of 48°15' to 48°52'W and has an average altitude of 800 m. The photoperiod varies from approximately 10 hours on the shortest day of the year to 13 hours on the longest day of the year, never reaching the ideal photoperiod of 16 hours required for culture and early floral induction (Pearson et al., 2016; Kolenc et al., 2016).

To better understand crop development, it is important to correlate physiology with plant development; and thus to understand how photosynthetic variables change under different abiotic and biotic influences (Pokorný et al., 2011; De Lucena Costa et al., 2012). Neves et al. (2023) evaluated different varieties in two types of production systems, organic and conventional, and obtained different responses of gas exchange characteristics, which showed that cultivation practices have the potential to alter the physiological development of plants.

Thus, it is essential to study the ecophysiological and productive performance of hops in the central southern region of São Paulo, where the natural conditions are different from those of the place of origin of hops. Thus, the objective of this work was to analyze and describe the production and physiological performance of five hop varieties: Comet, Chinook, Cascade, Nugget and Columbus.

Results

Morphometric and productive characteristics

There were significant differences in the means of the following morphometric characteristics: cone length, internode length and plant height (Table 1).

The lengths of the cones ranged from the lowest value of 2.68 cm (Cascade) to the highest value of 3.40 cm (Columbus). According to the ASBC Hops – 2 (ASBC, 2021) methodology, no variety had large cones (> 5.1 cm), Columbus and Chinook had medium-sized cones (3.40 and 3.26 cm, respectively), and Comet, Nugget and Cascade had small cones (2.94, 2.85 and 2.68 cm).

Comet had the greatest total internode length of 440.37 cm; while the lowest value was 285.87 cm. ID represents the measurement of the main branches and is therefore related to plant height; PH was 561.12 cm for Comet, and the lowest value was 375.87 cm for Columbus.

Regarding the insertion height of the first cone and the length of the lateral branches, there were no significant differences (Table 2).

For the insertion height of the first cone, Cascade presented the lowest value of 76 cm. Comet presented the highest value of 120.75 cm, where the first cone formed. Chinook had the longest lateral branches, with an average length of 50.25 cm, and Comet had the shortest branches, with an average length of 40.25 cm.

The morphometric characteristics can be divided into groups related to the vegetative and productive development of the plants; for example, data such as the internode distance and the height of the plants are related to their growth capacity, and the length of the cones, the height of insertion of the first cone and the length of the lateral branches are related to the productive capacity.

For the examined productive characteristics, including the fresh and dry masses of the cones, there were no significant differences among the five varieties (Table 3).

Cascade had the highest mean fresh and dry masses (90.97 g and 23.36 g), while Comet had the lowest mean values (27.65 g and 8.62 g).

Photosynthetic variables

For carbon assimilation (*A*), stomatal conductance (g_s), internal CO_2 concentration in the leaf (C_i), transpiration (*E*), water use efficiency (*WUE*), instantaneous carboxylation efficiency (A/C_i) and the relative electron transfer rate (*ETR*), there were no significant differences between the means of the varieties (Table 4).

All varieties showed similar values of *A*, g_s and A/C_i. For the other variables, there were small changes in the values, but no significant differences were found for any of the varieties.

The mean values of the photochemical quantum yield of photosystem II (F_v/F_m, PSII) and the effective photochemical yield of PSII (F_v'/F_m') showed statistically significant differences (Table 5).

Comet and Nugget presented the highest values of F_v/F_m, but only the F_v/F_m of Comet differed statistically from that of Cascade (0.89). For F_v'/F_m', Nugget exhibited the highest value (0.52), which differed significantly from that of Cascade.

Pearson correlation

A few perfect correlations were found, only one of which was negative. The other correlations were moderately or weakly positive or negative. It is worth noting the moderate positive correlations between the maximum quantum yield of PSII (F_v/F_m) and the length of the cones (LC) and between

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CV (%)	6.47	8.17	7.21

Legend: CL: cone length; ID: internode distance; PH: plant height. Means followed by the same letters do not differ significantly according to the Tukey's test at a 5% probability.

Table 2. Mean values of the insertion heights of the first cones on the plants (cm) and lengths of the lateral branches (cm) for the five *H. lupulus* varieties.

Varieties	HIFC	LLB
Comet	120.75	40.25
Chinook	116.66	50.25
Cascade	76	44.25
Nugget	118	48.25
Columbus	90	45.25
CV (%)	19.84	32.61

Legend: HIFC: Height of insertion of the first cone on the plant; LLB: Length of lateral branches.

Table 3. Mean values of fresh mass (g) and dry mass (g) for the cones of the five evaluated varieties of *H. lupulus***.**

Varieties	FM	DM
Comet	27.65	8.62
Chinook	73.57	22.21
Cascade	90.97	23.36
Nugget	80.97	13.50
Columbus	62.77	16.16
CV (%)	66.98	54.76

Legend: FM: fresh mass; DM: dry mass.

Table 4. Mean values of carbon assimilation (A, µmol CO_2 $m^{-2}s^{-1}$), stomatal conductance (g_s, mol $m^{-2}s^{-1}$), internal leaf CO_2 concentration (C_i, µmol CO_2 mol), transpiration (E, mmol water vapor $m^{-2}s^{-1}$), water use efficiency (WUE, µmol CO_2 $m^{-2}s^{-1}$/mmol H_2O $m^{-2}s^{-1}$), instantaneous efficiency of carboxylation (A/Ci) and the electron transfer rate (ETR) for the five evaluated *H. lupulus* varieties.

Varieties	A	gs	Ci	E	WUE	A/Ci	ETR
Comet	26.31	0.24	201.88	3.51	7.51	0.13	209.84
Chinook	27.59	0.23	267.81	4.57	6.29	0.10	168.69
Cascade	26.23	0.23	240.60	4.45	5.95	0.11	183.88
Nugget	26.52	0.27	217.95	4.06	6.85	0.12	216.50
Columbus	25.84	0.35	246.18	5.19	5.16	0.10	173.11
CV (%)	11.22	41.28	21.27	21.92	21.92	24.13	13.17

Table 5. Mean values of the photochemical quantum yield of photosystem II (F_v/F_m, PSII) and effective photochemical yield of PSII (F_v'/F_m') for the five evaluated *H. lupulus* varieties.

Varieties	F_v/F_m	F_v'/F_m'
Comet	0.94 a	0.50 ab
Chinook	0.90 ab	0.49 ab
Cascade	0.89 b	0.47 b
Nugget	0.94 ab	0.52 a
Columbus	0.91 ab	0.48 ab
CV (%)	2.21	3.71

Legend: Means followed by the same letters do not differ significantly from each other based on Tukey's test at a 5% probability.

the effective photochemical yield of PSII (F_v'/F_m') and the fresh mass of the cones (FM), with r = 0.6 (Figure 1).

The first correlation cited above refers to the increase in the length of the cones with the maximum quantum yield achieved by the plants during the photosynthesis in photosystem II. The second correlation was an increase in the fresh mass of the cones with an increasing photochemical effective efficiency of photosystem II.

The plant height (PH) exhibited a perfect negative correlation (-1) with stomatal conductance (g_s) and a moderate negative correlation (-0.6) with transpiration (*E*). g_s and *E* were correlated with height; for example, Columbus and the varieties with the lowest g_s had the greatest height.

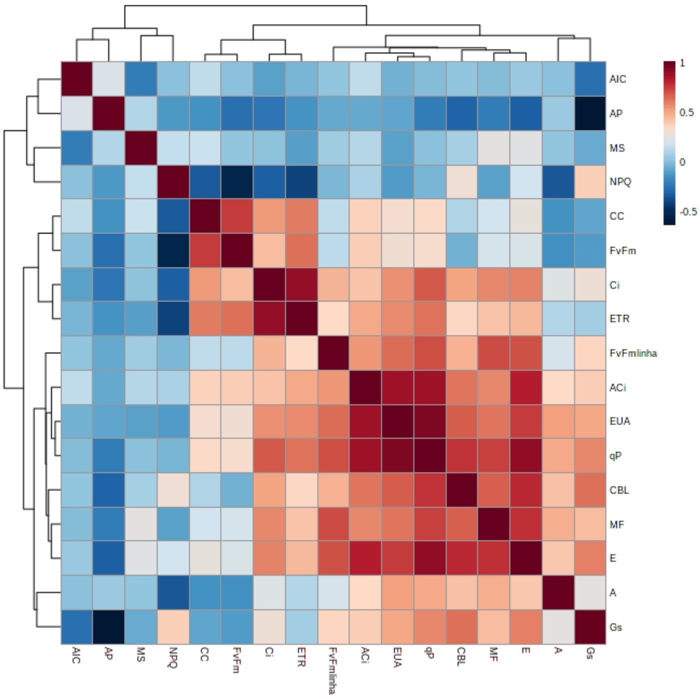

Figure 1. Graphical analysis to evaluate the strengths and weaknesses of Pearson's correlations of the morphometric, productive and photosynthetic parameters of the five varieties of *H. lupulus*.

Table 6. Chemical analysis of the soil at depths of 0 – 20 cm and 20 – 40 cm under hop cultivation.

Samples	pH	O.M.	P_{resin}	K	Ca	Mg	CEC	V%	B	Cu	Zn
	$CaCl_2$	g.dm3	mg.dm3	$mmol_c$.dm3					mg.dm3		
0 – 20	5.5	29	56	5.1	46	13	90	71	0.27	3.7	3.9
20 – 40	4.4	22	20	2.7	26	10	81	47	0.27	4.4	1.5

Legend: Department of Soils and Environmental Resources, Sector of Soil Sciences.

Discussion

The vegetative development of hops, analyzed on the basis of cone length, internode distance, plant height, the height of insertion of the first cone and the length of the lateral branches (CL, ID, PH, HIFC and LLB, respectively), has a strong impact on the yield and quality of hop cones produced (Howard and Tatchell, 1956).

The CL results obtained in this study were different from those found by Kondić et al. (2021), who reported an average length of 4.16 cm for the evaluated cones of Italian wild hops.

Cones have high contents of lupulin, including alpha- and beta-acids and essential oils (Spósito et al., 2019). Thus, the larger the cone is, the greater its capacity to produce lupulin. This is a characteristic intrinsic to varieties, but it can undergo changes due to factors such as temperature and drying time, which are essential processes to maintain quality in the postharvest stage (Raut et al., 2021).

There is a difference in the productive capacity of plants that produce cones directly on the main stem and those that produce cones on lateral branches. However, with the production of lateral branches, there is a possibility of producing more cones. Therefore, it is important to increase the lengths of these branches.

Cone density is the most useful parameter for hop improvement, as it indicates the relationship between the number of knots and the length of the cone (Kondić et al., 2021).

The distance of the internodes is directly linked to the height of the plants and to the production capacity of new lateral branches, as described above. Thus, the greater the distance of the internodes, the greater the productive capacity of the plants. In relation to plant height, the values varied, similar to reports by Rossini et al. (2016).

Regarding the average mass of the cones, these values vary according to the growth vigor and the physiological and vegetative maturation of the plants (Rybácek 1991). The evaluated plants were analyzed in their first year of cultivation, and importantly, there was an increase in productivity when physiological maturity was reached, approximately in the third productive year (Dodds 2017).

No significant differences were found for most of the traits evaluated; for example, for the assimilation of CO_2 (*A*), all varieties showed similar values. These values were similar to those measured by Eriksen et al. (2020), who analyzed the *A* rates of the Cascade, Chinook, Centennial, Pride of Ringwood, Southern Brewer and Willamette varieties at different temperatures.

Carbon assimilation (*A*) and stomatal conductance (g_s) are related because CO_2 enters and exits through the stomatal openings of the leaves (Bonan et al., 2014); therefore, higher g_s rates may correspond to higher *A* values if the openings are sufficient.

Regarding C_i, Eriksen et al. (2020) also found higher values in Chinook than in the other varieties evaluated. In this experiment, Chinook presented the highest mean values of *A* and C_i. However, this assimilated carbon was only concentrated internally in the leaves, and for it to be efficiently used in photosynthesis, it must be reduced for organic synthesis.

An increase in the concentration of CO_2 in the leaf is linked to an increase in the concentration of atmospheric carbon (Da Rosa Dorneles et al., 2019). This is accompanied by a reduction in stomatal opening, which causes a decrease in transpiration and an increase in water use efficiency (Allen and Prasad, 2004).

Water use efficiency (*WUE*) was conceptualized by Briggs and Shantz (1913) as the relationship between plant productivity and water use as a measure of the amount of biomass produced per unit of transpired water (Hatfield and Dold, 2019). Water use efficiency is the ratio between CO_2 assimilation and stomatal conductance (Bogeat-Triboulot et al., 2019).

We measured the highest biomass response in Comet plants with the highest mean plant height and the highest water use efficiency, demonstrating the efficiency of this variety concerning the conversion of biomass.

When assessing the ETR, it is crucial to recognize that a higher rate indicates increased photosynthetic activity in the plants. This is because the electrons are utilized in compounds and processes essential for conducting photosynthesis (Martins 2011). The electrons are employed as energy sources and as boosters in the production of ATP, in photophosphorylation and in the production of NADPH that will be used in the carbon fixation reaction.

Considering the high *ETR* values obtained in the newest varieties and those described by Krause and Weis (1991) and Santabarbara et al. (2019) for the value considered normal for the maximum quantum yield of PSII (0.75), we concluded that the plants evaluated in our experiment did not suffer stress, regardless of the variety analyzed.

These data showed that the nutritional and water conditions of the plants under the adopted management regime allowed good plant development, even in the subtropical climate during cultural acclimation. Possible stress could cause damage to chloroplast structures and impose other biochemical limitations on photosynthesis (Peloso et al., 2017) that may compromise the vegetative and productive development of these varieties.

The only perfect correlation found was between PH and g_s; this correlation was negative, and we believe that the lower rate of stomatal activity resulted in taller plants. When stomata are closed, there is less water loss through transpiration (Hatfield and Dold, 2019) and, consequently, increased use of water for photosynthetic processes, thus increasing water use efficiency (*WUE*).

Bogeat-Triboulot et al. (2019) stated that water use efficiency is the ratio of the biomass produced and the water used by the plant. Thus, plants with lower g_s rates show greater biomass gains. The lack of strong or perfect correlations can be attributed to the lack of physiological maturity of the plants according to Dodds (2017) because the data were collected at the end of the first harvest in the first year of cultivation. Thus, possible correlations may arise and facilitate the understanding of the productive development of the varieties.

Materials and methods

Experimental conditions

The experiment was conducted in the orchard of the Lageado Farm of the Department of Horticulture, belonging to the Faculty of Agronomic Sciences of São Paulo State University – FCA/UNESP, in Botucatu, São Paulo, between November 2020 and March 2021.

The region has a Cfa climate according to the Köppen classification (1948), which corresponds to a hot humid (mesothermal) temperate climate, with an average annual rainfall of 1428.4 mm and an average annual temperature of 20.3 °C (Cunha and Martins, 2009). The experimental area is located between 22°30' and 23°05'S and 48°15' and 48°52'W and has an average altitude of 800 m.

During the experimental crop period, the minimum mean temperature was 18.76 °C, the maximum mean temperature was 29.21 °C, and the maximum rainfall was 497.6 mm. The soil is a clayey dystroferric Red Latosol (Santos et al., 2018).

Treatments and experimental design

The experiment had a randomized block design involving five treatments corresponding to the varieties of hops (*H. lupulus*), including Comet, Chinook, Cascade, Columbus and Nugget, with four blocks and two plants per plot, totaling 20 experimental plots and 40 units.

Soil preparation

For soil preparation, scarification was performed, followed by plowing and harrowing, furrowing and opening of the pits. Soil correction and fertilization were performed by means of complete chemical analyses of the soil (Table 6), adapting the recommendations established for the crop in the international literature "Fertilizer Guide: Hops" (Gingrich et al., 2000).

For soil correction, limestone (3.15 $kg.ha^{-1}$) and gypsum (800 $kg.ha^{-1}$) were used, and for plant fertilization, plant compost (40 $kg.ha^{-1}$), Yoorin Master® (P_2O_5 - 17%; Ca - 18%; Mg - 7%; B - 0.10%; Cu - 0.05%; Mn - 0.30%; Si - 10%; Zn - 0.55%) (3.25 $kg.ha^{-1}$), calcitic limestone (1 $t.ha^{-1}$) and potassium sulfate (150 $kg.ha^{-1}$) were applied.

In the interrows of hops, different species of green manure were sown, such as sunn hemp (*Crotalaria breviflora*), jack bean (*Canavalia ensiformis*), millet (*Pennisetum glaucum*) and buckwheat (*Fagopyrum esculentum*), and these crops were sown parallel to the hop lines.

Topdressing fertilization was performed when necessary by applying bokashi (1.5 $t.ha^{-1}$), Nitrabor® (200 $kg.ha^{-1}$), calcium nitrate (1 $t.ha^{-1}$) and urea (700 $kg.ha^{-1}$) to meet the need for nitrogen. To meet the need for boron, boric acid was sprayed (4 $kg.ha^{-1}$), and potassium sulfate was applied (1.15 $t.ha^{-1}$) for potassium fertilization. Spraying was performed with the biofertilizers SuperMagro and micronutrient mix Oligogreen® (B - 0.5%; Cu - 1%; Fe - 2%; Mn - 4%; Mo - 0.05%; Zn - 3%) (20 g for 20 liters of water) and the application of Effective Microorganisms (EM) (2 liters of EM for 18 liters of water) for biological activation of the soil.

Crop treatment and harvesting

The irrigation system was set up using drip lines with two strips per hop line, emitter spacing of 0.5 m and a flow of 1.1 Lh^{-1}, and the plants were placed in a "V" shape. The most vigorous branches with a hexagonal shape were selected (Peragine 2011) for guidance along sisal.

The green manure was mowed at the time of floral initiation, and the biomass was deposited as a cover in the rows. Control of spontaneous plants was performed mechanically through mowing, weeding and uprooting when necessary.

For phytosanitary control, the applied agents included Abamectin (Abamex®) (20 ml for 20 liters of water) for spotted spider mites (*Tetranychus urticae)* and fipronil (Regent®) for ants, and Dipel® (*Bacillus thuringiensis*) (500 ×

g for 20 liters of water) was applied in the presence of caterpillars.
Seedlings were purchased from a suitable, legal nursery, transplanted to the field on November 13, 2020, and harvested during the months of February and March 2021, with pruning close to the soil of the harvested plants. At this time, the plants were in their last stage of reproductive development, and they had mature cones ready for harvest, which are mainly characterized by the formation of bracts on the pistils that appear during floral initiation.
When the cones are formed, they are still green and have a large amount of moisture. The maturation of the cones is part of the final phenological stage of the plant, preceding natural senescence and characterized by the loss of water from the formed organs, the leaves and the cones. Thus, they reach the ideal dry matter point for harvesting from the field and can benefit from the postharvest process.

Morphometric and productive analyses
At the time of cone collection, the plants were cut and transported to the laboratory, where analyses related to vegetative and productive development were carried out. The harvest started 101 days after planting. As explained above, the harvesting of cones occurs when they reach the ideal maturation point after losing water and increasing the intensity of flavor and aroma.
To identify the ideal maturation point for harvesting, some sensory analyses were carried out in the field; thus, it was possible to determine the maturation of the cones sensorial. After this step, approximately five cones were collected and taken to the laboratory to determine the dry matter content of the cones and thereby confirm the maturation point of the cones and the beginning of the harvest.
The sensory analyses carried out in the field were mainly based on touch and smell. When the cones are new, they contain a large amount of water. Therefore, when they are squeezed with the fingers, they are soft and do not make any sound. With the maturation of the cones, there is a loss of water, and when they are squeezed, they feel dry and make a sound similar to the movement of butter paper. In addition to the change in color, when cones are new, they have an intense green color, and with the maturation process, the color becomes opaque.
At the beginning of the maturation of the cones, the aroma is weak and lacks intensity, whereas it changes with maturation. Thus, the aromatic intensity indicates that the approach of the harvest time, as the aroma becomes pungent, citric and less herbal. These general aromatic characteristics vary according to the chemical composition of each variety. Another factor analyzed was the color of lupulin, which changes with maturation. When the cones are new, lupulin has a dull light yellow color, and when the cones are mature, the color becomes intense and bright.
After carrying out these analyses in the field, the plants with the cones identified as appropriate for harvesting were marked, and approximately five cones were taken to the laboratory. Then, their fresh weight was obtained, after which they were microwaved for 1 minute and weighed again. This process was repeated until their weight stabilized in three repetitions. After stabilization, the dry matter test was performed using the following formula: final weight/initial weight * 100. The criterion for determining the beginning of the harvest was the point at which a 23% cone dry matter content was observed.
After harvesting the cones, the following analyses were carried out: fresh mass of the cones (g), dry mass of the cones (g), length of the cones (cm), height of insertion of the first cone on the plant (cm), length lateral branches (cm) and plant height (cm).
The fresh weight of the cones was obtained as soon as the cones were harvested from the plants. They were then stored in a forced ventilation oven at 35 °C until a 10% moisture content was reached, at which point they were removed from the oven and weighed. Thus, the dry mass of the cones was obtained.
The other analyses were performed using a 150 cm tape measure. To measure the lengths of the cones, 30 cones were randomly selected and measured, and the average length was calculated. The cone size classification methodology proposed by the American Society of Brewing Chemists (ASBC Hops – 2) (ASBC, 2021) was used, where a large cone has a value equal to or greater than 5.1 cm, an average cone has a value between 3.2 and 5.1 cm, and a small cone has a value less than 3.2 cm.
The height of cone insertion on the plant was measured in the field before the plants were cut. For this characteristic, the cones released on the lower part of the plant were observed, and their distance to the soil was measured where the stem began. The length of the lateral branches was determined by randomly selecting six lateral branches of each plant that were one meter above the ground. These were measured, and the mean was calculated. The height of the plant was defined as the sum of the distances between the internodes of the plants.

Photosynthetic analyses
Data were collected during the final period of the productive development of the plants in the fourth month of the harvest (February 2021), when the plants had already released lateral branches with cones during the maturation process.
Photosynthetic data were collected, and fully expanded leaves in the middle third of the plants were selected for the analysis. The leaves were covered with aluminum foil for twenty minutes (so that all reaction centers were open, with the ability to receive electrons). Readings were performed between 8:00 am and 11:00 am (Ramos et al. 2015) using an open photosynthesis system with a CO_2 analyzer and infrared water vapor (IRGA), model LI-6400XT, LI-COR) coupled to a portable fluorometer (Portable Chlorophyll Fluorometer-PAM-2500).
After the dark period of 12 hours, the following characteristics were obtained with a fluorometer: basal fluorescence (F_0) was determined under sufficiently low irradiance (< 1 µmol $m^{-2}s^{-1}$), and maximum fluorescence (*Fm*) was determined after a 0.8 s saturation pulse at 4,200 µmol $m^{-2}s^{-1}$ on the dark-adapted leaves (30 min), from which variable fluorescence (*Fv*) was obtained. In the light-adapted leaves, the radiation of the saturation pulses to determine the maximal fluorescence (*Fm*) was 6,000 µmol $m^{-2}s^{-1}$ for 0.8 s, whereas the actinic light was 200 µmol $m^{-2}s^{-1}$. Measurements of the quantum yield of photosystem (PS) II photochemistry (PSII) were obtained by the application of a saturation light pulse (6,000 µmol $m^{-2}s^{-1}$ for 0.8 s) under ambient irradiance. The photochemical quantum yield of photosystem II (*Fv/Fm*) and the effective yield of PSII (*Fv'/Fm'*) as well as the relative electron transfer rate (*ETR*) were calculated.
Fluorescence parameters, such as the variable maximum quantum yield of PSII (*Fv/Fm*), effective quantum yield of

photosystem II (PSII) (*Fv′/Fm′*) and relative rate of electron transport (*ETR*), were calculated according to Schreiber et al. (1986).

The CO_2 assimilation rate (*A*, µmol CO_2 $m^{-2}s^{-1}$), transpiration rate (*E*, mmol water vapor $m^{-2}s^{-1}$), stomatal conductance (g_s, mol H_2O $m^{-2}s^{-1}$) and internal CO_2 concentration in the leaf (C_i, µmol $m^{-2}s^{-1}$) were determined with the IRGA using the data analysis program of the photosynthetic equipment (Von Caemmerer and Farquhar, 1981). At the same time, water use efficiency (*WUE*, µmol CO_2 $m^{-2}s^{-1}$/mmol-H_2O $m^{-2}s^{-1}$) was calculated according to the relationship between CO_2 assimilation and the transpiration rate, and the carboxylation efficiency of the enzyme ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) (*A/Ci*, µmol CO_2 $m^{-2}s^{-1}$/µmol $m^{-2}s^{-1}$) was determined according to the ratio between the assimilation of CO_2 and the internal concentration of CO_2 in the leaves.

Statistical analyses

The morphometric and photosynthetic data were subjected to homogeneity and normality analyses. After being accepted, they were subjected to analysis of variance ($p < 0.05$), and the means of the factors were compared by Tukey's test at a 5% probability using MiniTab® and AgroEstat® software.

Pearson's correlation (r) was performed between the morphometric and photosynthetic data using MetaboAnalyst 5.0® software.

Conclusions

In general, all varieties showed satisfactory efficient physiological performance and developed even during cultural acclimation in a subtropical climate. This showed that the evaluated varieties of *H. lupulus* did not suffer physiological limitations, and we believe that in future harvests, there will be an increase in vegetative and productive development.

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