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RESEARCH ARTICLE

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Agronomic and chemical evaluation of hop cultivars grown under Mediterranean conditions

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Abstract

Aim of study: Evaluation of the agronomic performance and chemical profile of four hop cultivars grown under Mediterranean conditions.

Area of study: The study was undertaken in Bragança, north-eastern Portugal.

Material and methods: The newly introduced cultivars ('Columbus', 'Cascade' and 'Comet') were compared with the well-stablished 'Nugget'. The field experiment was carried out between 2017 and 2019. Dry matter (DM) yield (plant and cones), tissue elemental composition and bitter acid and nitrate (NO3-) concentrations in the cones were assessed.

Main results: 'Comet' was the most productive cultivar with the highest total DM yield (1,624 to 1,634 g plant^{-1}), cone yield (572 to 633 g plant^{-1}), and dry weight of individual cones (0.28 to 0.79 g cone^{-1}). 'Cascade' showed the lowest average total DM yield (723 to 1,045 g plant^{-1}). The year affected the average values of DM yield and the concentration of bitter acids in the cones, with 'Cascade' showing the highest sensitivity between cultivars. The concentrations of α and β-acids in the cones were within or close to the normal ranges internationally accepted for all cultivars. 'Columbus' exhibited the highest levels of α-acids, ranging between 12.04 % and 12.23%, followed by 'Nugget' (10.17–11.90%), 'Comet' (9.32–10.69%) and 'Cascade' (4.46–8.72%). The nutrient accumulation criteria in cone and leaf tissues seem to be a differentiating factor between cultivars with influence on bitter acid biosynthesis and biomass production.

Research highlights: All cultivars showed notable performance in terms of DM yield and bitter acid concentration in the cones when compared to international standards.

Additional key words: *Humulus lupulus*; aroma; bitter acids; cone yield; cone quality.

Authors' contributions: Analysis and first draft of the manuscript: SA. All authors contributed to the conception and design of the study, material preparation and data collection, read and approved the final manuscript.

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Introduction

The cones of hop (*Humulus lupulus* L.) female plants are a primary ingredient in beer production and, although other substitutes can be used, the supremacy of hops has always prevailed. It is in the lupulin glands of the cones of female plants that a yellow resinous powder (lupulin) is synthetized. It contains the most valued compounds for brewing purposes such as the resins and aromatic compounds (Almaguer *et al.*, 2014). Hop soft resins include the so-called bitter acids, the α-acids (cohumulone, humulone and adhumulone), which are the most valued constituents of the hop resins, and the β-acids (colupulone, lupulone and adlupulone) (De Keukeleire, 2000; Almaguer *et al.*, 2014). The thermal isomerization of α-acids accounts for most of the bitterness of beer (Ting & Ryder, 2017), though β-acids can also make some contribution (Schönberger & Kostelecky, 2011).

Hop oils are considered to be the main source of beer aroma, consisting of three groups of compounds: hydrocarbons, oxygenated compounds, and sulphur-containing compounds (Schönberger & Kostelecky, 2011). Most of the hop aroma volatiles are lost when hops are added to the beer at the beginning of boiling, though derived compounds such as oxygenated terpenoids and oxygenated sesquiterpenes can impart aromas described as 'floral' or 'spicy’ (Lafontaine & Shellhammer, 2019). To produce beers with intense hop aromas, brewers add aroma varieties at the end of boiling (late hopping) and/or to cold beer (dry hopping) and, in this instance, the concentrations of hop oil volatiles and aroma precursors become of great relevance due to the impact on final beer aroma and flavour (Rettberg *et al.*, 2018; Lafontaine & Shellhammer, 2019). The volatile composition of hop essential oils can present great variability between different hop cultivars (Kishimoto *et al.*, 2006; Inui *et al.*, 2013). The concentrations in cones of essential oils and bitter acids are commonly used to differentiate between cultivars (Shellie *et al.*, 2009; Štěrba *et al.*, 2015; Ocvirk *et al.*, 2016).

Hop cultivars can be classified conventionally as ‘bittering’ or ‘aroma’ based on their chemical composition, though a more recent classification of ‘flavour’ hops is being used for cultivars that suit a dual purpose of imparting both aroma and bitterness (Almaguer *et al.*, 2014). The breeding efforts to improve hop cultivars have allowed the development of a great variety of cultivars with specific traits, such as higher yielding, more resistant to diseases, and with increased bitterness or specific aroma, to meet the demands of the world market (Seigner *et al.*, 2009; Hieronymus, 2012).

The increasing popularity of craft beer has changed the market demand from bittering cultivars, destined for a few macrobrewers, to aroma cultivars, sold in small quantities to a larger number of smaller brewers. Although the craft beer revolution is a global phenomenon (Garavaglia & Swinnen, 2017), it has experienced a huge increase in the USA of 273% in the last decade (Teghtmeyer, 2018). Craft brewers are more interested in cultivars with a strong aroma, different flavours and fewer α-acids (Hieronymus, 2012). The American ‘Cascade’ cultivar is one of the most popular aroma hop cultivars, while ‘Nugget’ is an important bittering cultivar (Almaguer *et al.*, 2014).

The hop crop has specific requirements for optimal growth, which include long day lengths to flowering and winter temperatures below 4.4 ºC. Thus, the cultivation areas considered to be adequate for hop growth should be at latitudes between 35 and 50 degrees (Sirrine *et al.*, 2010). In Europe, hops are mostly produced in the north, because of the favourable growing conditions. Germany is the main European country and a major world producer (32,527 t in 2018), while in the Mediterranean, Spain is the country with the largest production (915 t in 2018) (FAOSAT, 2020). Italy is beginning hop cultivation, and the ‘Cascade’ cultivar has been one of the most studied for its adaptability to Mediterranean growing conditions (Forteschi *et al.*, 2019; Rodolfi *et al.*, 2019; Mozzon *et al.*, 2020). However, there still remains scarce information available on hop growing under Mediterranean conditions.

In Portugal, the commercial production of hops began in the early sixties in the north of the country, with the Brewer´s Gold cultivar, and quickly spread to other areas with the production reaching sufficient levels to satisfy national demand and even for export. However, the downturn in production, which followed a promising start, was caused by years of reduced productivity and other constraints (Rodrigues *et al.*, 2015). At present, only a restricted number of producers, located in the Bragança region, maintain the crop, and all of them are growing the hop bitter cultivar ‘Nugget’. This seems to be well adapted to the drier climate of the north-eastern region, though some fields are currently showing a heterogeneous growth (Afonso *et al.*, 2020). Recently, the increasing popularity of craft beer in Portugal (Euromonitor, 2019) has awakened the interest in hop aroma cultivars, motivating current producers to invest in these cultivars and opening up new opportunities for growers.

The present research was carried out on a hop farm in Pinela, in the district of Bragança, located in the north-east of the country, where hop aroma cultivars have been introduced in recent years. The trial includes the well-established bittering ‘Nugget’ cultivar and the recently introduced ‘Columbus’, ‘Cascade’ and ‘Comet’ aroma cultivars, all with North American germplasm provenance according to Patzak & Henychová (2018). It is recognized by the local hop farmers that American cultivars are more suited to the region because of its similar drier climate and these are therefore the farmers’ preferred option. The present research purpose is to study the agronomic performance and chemical profile of hop aroma cultivars grown under the Mediterranean conditions of the region, in order to produce useful data for both current and future growers. The newly introduced aroma cultivars (‘Columbus’, ‘Cascade’ and ‘Comet’) were evaluated and compared with the ‘Nugget’ cultivar for biomass production, tissue elemental composition and bitter acid and NO_3-concentration in the cones. Additionally, cone attributes (concentration of nutrients, bitter acids and NO_3-) and the concentration of nutrients in the leaves, were used for differentiation between cultivars, by performing a stepwise discriminant analysis.

Material and methods

Site characterization

A three-year field trial was conducted from 2017 to 2019 on a hop farm located in Pinela (41°40'33.6"N 6°44'32.7"W, 850 m a.s.l.), Bragança, NE Portugal. The region benefits from a Mediterranean-type climate, influenced by the Atlantic regime, with an average annual air temperature of 12.7 ºC and annual precipitation of 772.8 mm (IPMA, 2020). Meteorological data recorded during the experimental period at the weather station of Sta Apolónia farm in Bragança is presented in Fig. S1 [suppl].

The soil of the experimental field is a loamy textured, eutric Cambisol. Other physicochemical properties determined from composite soil samples (three samples composed by mixing the soil from 15 random points), collected just before the trial started, are presented in Table 1.

Field experiments

The field trial was arranged as a completely randomized design with four cultivars (three in 2017) and four replicates (four plants per treatment). In 2017, the experiment included the bitter cultivar 'Nugget' and the aroma cultivars 'Columbus' and 'Cascade'. In the next two years (2018 and 2019) the aroma cultivar 'Comet' was also included. All the cultivars were grown under similar agro-ecological conditions, on a standard 7 m high trellis system in a 'V' design. At planting, the rhizomes were placed at 3.0 m × 1.6 m between and within rows. Thereafter, a double tutor thread was placed in the position of each rhizome giving a planting density of 4,167 "plants" of three to four stems per hectare. The plots of 'Columbus', 'Cascade' and 'Nugget' were established in 2014 and the plots of 'Comet' in 2015. Sample collection began in 2017 for the first three cultivars and 2018 for 'Comet', when all were three years old, the age from which a hop plant can display its full productive potential.

Irrigation was performed through a surface irrigation system consisting of flooding the space between rows. The annual fertilization plan included the application of a compound NPK fertilizer (7:14:14) early in the spring and two side dress N applications performed during the growing season, the first with ~250 kg ha^{-1} of nitromagnesium (27% N as NH_4NO_3 + 3.5% MgO + 3.5% CaO) and the second with ~450 kg ha^{-1} of calcium nitrate (15.5% N as NO_3^- + 27% CaO). Additionally, the farmer applied 20 t ha^{-1} of farmyard manure late in the winter.

Hop tissue sampling

Hop tissue sampling for analysis was made at harvest (August 28th to 31st 2017; August 27th to 31st 2018; and August 29th to 31st 2019). The aboveground biomass was cut at ground level and separated into two samples of leaves (bottom and top half), stems and cones. The leaf samples included blade and petiole. Tissue samples were weighed fresh to obtain data on total dry matter (DM) yield. From each plant part, a subsample was taken and weighed again fresh. Thereafter, the subsamples were oven dried at 70 ºC and weighed dry for determination of DM yield of the different plant parts. Additionally, subsamples of 20 dried cones from each replication were randomly selected for determination of dry mass of the individual cones. Then, the samples were ground and analysed for elemental composition.

Chemical analyses

The soil samples were oven dried at 40 °C and sieved in a mesh of 2 mm. Thereafter, the soil samples were analysed for pH (H_2O) (soil: solution, 1:2.5), organic carbon (wet digestion, Walkley-Black method), exchangeable complex (ammonium acetate, pH 7.0), extractable P and K (Ammonium lactate) and extractable B (hot water and azomethine-H method) (Van Reeuwijk (2002). The availability of other micronutrients (Cu, Fe, Zn, and Mn) in the soil was determined by atomic absorption spectrometry after extraction with ammonium acetate and EDTA, according to Lakanen & Erviö (1971).

Elemental tissue analyses were performed by Kjeldahl (N), colorimetry (B and P), flame emission spectrometry (K) and atomic absorption spectrophotometry (Ca, Mg, Cu, Fe, Zn and Mn) methods after nitric digestion of the samples (Temminghoff & Houba, 2004). The nitrate concentration in hop cones was determined according with Clescerl *et al.* (1998), by UV-vis spectrophotometry in a

Table 1. Selected soil properties (average ± standard deviation) from soil samples collected between rows at 0-20 cm, just before the beginning of the experiment.

Soil properties		Soil properties	
pH (H_2O)[a]	5.64±0.06	Extract. P (mg P_2O_5 kg^{-1})[d]	284.51±43.81
Organic carbon (g kg^{-1})[b]	21.39±4.76	Extract. K (mg K_2O kg^{-1})[d]	238.67±1.15
Exchan. Ca (cmolc kg^{-1})[c]	4.35±1.32	Extract. B (mg kg^{-1})[e]	0.76±0.09
Exchan. Mg (cmolc kg^{-1})[c]	0.59±0.17	Extract. Fe (mg kg^{-1})[f]	100.31±1.01
Exchan. K (cmolc kg^{-1})[c]	0.42±0.07	Extract. Mn (mg kg^{-1})[f]	73.62±15.04
Exchan. Na (cmolc kg^{-1})[c]	0.69±0.31	Extract. Zn (mg kg^{-1})[f]	6.06±1.50
Exchan. acidity (cmolc kg^{-1})[a]	0.53±0.06	Extract. Cu (mg kg^{-1})[f]	5.77±1.37

[a]Potentiometry. [b]Wet digestion (Walkley-Black). [c]Ammonium acetate, pH 7. [d]Ammonium lactate. [e]Hot water, azomethine-H.; [f]Ammonium acetate and EDTA.

water extract (dry cone:solution, 2.5:50). Bitter acids (alpha and beta) in hop cones were extracted with methanol and diethyl ether by HPLC, according to the Analytica EBC 7.7. method (EBC Analysis Committee, 1998).

Data analysis

Data was firstly tested for normality and homogeneity of variance using the Shapiro-Wilk and Bartlett's test, respectively. Thereafter, data was subject to analysis of variance (one-way ANOVA). When significant differences were found ($p < 0.05$), the means were separated by Tukey HSD ($\alpha = 0.05$). A stepwise discriminant analysis was applied to understand if the cone attributes, namely the concentration of nutrients, bitter acids and NO_3^-, were different between hop cultivars. A similar analysis was also performed for nutrient concentrations in the bottom and top half leaves of the plants. The stepwise methods were used for this purpose and the de Wilks lambda method was chosen; the F value criteria were applied for the removal ($F < 2.71$) and inclusion ($F > 3.84$) of the variables in the discriminant functions. The cone attributes (concentration of nutrients, bitter acids and NO_3^-) and the concentrations of nutrients in the leaves were used as independent variables, with the cultivars ('Nugget', 'Columbus', 'Cascade', and 'Comet') as dependent variables. Self-validation and cross-validation methods were used to test the accuracy of the model. The statistical analyses were performed with the SPSS v. 25.0 programme.

Results

Plant dry matter yield

The comparison of total DM yield between cultivars provided a very consistent result over the years (Fig. S2 [suppl]). The aroma cultivar 'Comet' gave the highest average values, followed in descending order by 'Nugget', 'Columbus' and 'Cascade'. The order of the last three was the same in 2017, when 'Comet' was not included in the study. However, in 2018 significant differences between cultivars were not found. In the comparison of the three years, the lowest average values for total aboveground DM yield were recorded in 2017 in 'Cascade' (723 g plant^{-1}) and the highest in 2018 in 'Comet' (1,634 g plant^{-1}). DM yield of cones ranged between 326 ('Nugget') and 363 g plant^{-1} ('Columbus') in 2017, 445 ('Cascade') and 633 g plant^{-1} ('Comet') in 2018 and 323 ('Cascade') and 572 g plant^{-1} ('Comet') in 2019. Differences in cone DM yield were only significant in 2019, with 'Cascade' showing significantly lower values than the other cultivars. 'Cascade' and 'Columbus' also registered significant lower average values for leaf and stem total DM yield, in comparison to 'Nugget' in 2017 and 2019 and to 'Comet' in 2019.

In 2017, cone dry weight did not differ significantly between cultivars and the average values ranged from 0.26 ('Nugget') to 0.30 g cone^{-1} ('Columbus' and 'Cascade') (Fig. 1). However, in 2018 the differences between cultivars increased, with 'Comet' registering significantly higher values than 'Cascade' and 'Nugget' (Fig. S2 [suppl]). The average values in 2018 ranged between 0.29 ('Nugget') and 0.79 g cone^{-1} ('Comet'). In 2019, average values varied between 0.16 and 0.28 g cone^{-1}, with 'Cascade' and 'Comet' showing, respectively, significantly lower and higher values than the other cultivars.

Tissue nutrient concentration

N concentrations in the bottom half leaves varied significantly between cultivars (Table 2). 'Cascade' showed consistently higher leaf N concentration than the other cultivars. When present, 'Comet' showed the lowest

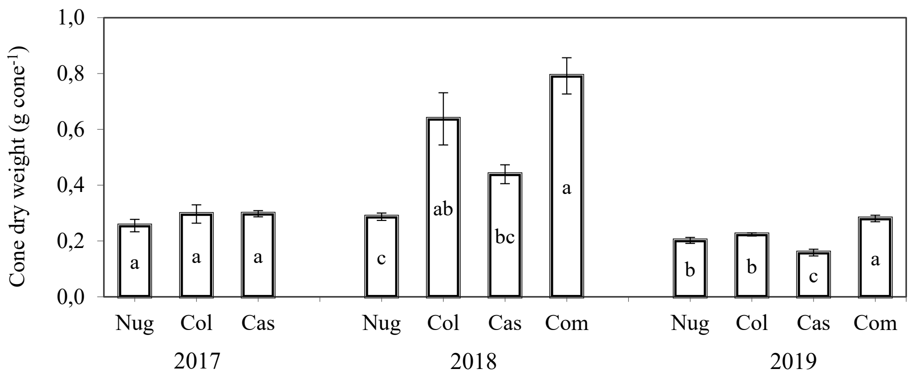

Figure 1. Dry weight of individual cones in 2017, 2018 and 2019, as a function of cultivar (Nug, Nugget; Col, Columbus; Cas, Cascade; and Com, Comet). Within each year, means followed by the same letter are not statistically different by Tukey HSD test ($\alpha = 0.05$). Error bars are the confidence intervals of the means ($\alpha = 0.05$).

Table 2. Leaf nutrient concentration (average ± SD) in August, at harvest, from the bottom and top half of the plants as a function of cultivar. For each year, means followed by the same letter are not statistically different by Tukey HSD test (α = 0.05).

Year	Treatment	N	P	K	Ca	Mg	Fe	Mn	Cu	Zn	B
		($g\ kg^{-1}$)					($mg\ kg^{-1}$)				
		Bottom half									
2017	Nugget	24.8±0.58b	1.2±0.04ab	19.4±1.87a	26.9±6.05a	4.5±0.14b	319.9±59.5a	331.8±37.0a	11.8±1.85a	27.9±12.7a	87.4±5.84a
	Columbus	21.2±0.97b	1.3±0.15a	13.1±4.74ab	15.2±3.49b	4.6±1.09ab	312.0±105.8a	314.1±112.8a	12.3±2.56a	5.0±0.90b	79.4±8.32ab
	Cascade	40.9±6.93a	1.0±0.10b	10.7±2.32b	16.5±3.05b	6.8±1.59a	293.6±55.2a	198.7±37.1a	12.5±6.03a	6.4±1.16b	61.7±12.3b
2018	Nugget	31.2±0.65ab	1.2±0.04a	34.9±2.35a	14.9±1.45a	5.5±0.54b	262.7±10.1b	367.7±63.2ab	6.4±0.35b	16.4±0.58b	78.6±3.56a
	Columbus	23.3±1.69ab	1.2±0.06a	18.1±1.89b	17.0±3.68a	5.6±0.92b	238.3±47.4b	344.6±58.2ab	7.2±0.54ab	16.3±5.56b	58.0±5.33b
	Cascade	32.3±8.65a	0.7±0.07b	13.3±1.88c	16.4±0.52a	4.4±0.23b	384.2±66.0a	259.0±17.6b	7.0±1.03ab	7.1±1.48c	42.0±9.45c
	Comet	21.9±2.43b	1.1±0.07a	15.3±1.11bc	29.2±15.40a	9.8±1.45a	382.4±39.7a	410.1±65.3a	8.1±0.54a	29.1±4.10a	72.7±5.44a
2019	Nugget	27.9±0.84b	1.2±0.05a	17.0±3.21a	14.9±1.69a	6.2±0.99a	320.3±37.3a	387.2±20.4a	7.3±0.46a	22.5±4.33a	71.4±6.57a
	Columbus	28.0±1.22b	1.1±0.19a	12.3±1.72b	18.5±2.57a	5.2±1.18a	160.9±23.1c	396.5±72.6a	5.3±0.75b	16.4±4.83ab	42.3±1.40b
	Cascade	44.0±3.63a	1.0±0.08a	12.8±1.53ab	16.1±2.00a	5.5±0.21a	218.7±22.9b	380.1±97.2a	5.8±0.68b	9.7±1.68b	34.8±4.61b
	Comet	21.5±2.60c	1.1±0.09a	11.9±1.12b	10.0±1.26b	6.0±0.77a	168.1±4.33bc	342.6±37.3a	6.4±0.41ab	18.2±2.26a	41.6±2.89b
		Top half									
2017	Nugget	29.0±0.86b	1.3±0.06a	16.4±1.51a	19.1±8.68a	2.8±0.76b	162.2±26.9a	231.2±47.9a	8.9±0.76a	20.0±11.9a	58.7±5.55a
	Columbus	26.4±1.69b	1.4±0.14a	12.8±3.96a	11.1±2.81a	3.0±0.30b	197.1±31.9a	220.4±72.3a	9.4±2.89a	4.7±0.83b	58.3±7.87a
	Cascade	47.4±3.99a	0.9±0.10b	11.3±3.21a	15.5±3.35a	6.7±2.30a	181.0±57.7a	164.5±17.0a	10.8±3.36a	5.5±0.59b	56.6±7.18a
2018	Nugget	34.1±1.53a	1.5±0.03a	21.2±4.33a	12.5±1.81ab	3.1±0.61b	225.9±16.5b	289.1±94.3a	7.0±0.45ab	12.7±1.75a	63.7±8.82a
	Columbus	26.0±1.88a	1.3±0.11ab	16.3±2.33ab	11.6±1.23b	3.1±0.57b	257.8±11.2ab	232.3±40.8a	6.5±0.43bc	10.5±1.32a	39.2±5.74b
	Cascade	36.8±10.97a	0.7±0.05c	11.4±1.76b	13.1±2.50ab	3.4±0.59b	343.7±38.7a	198.9±39.3a	6.2±0.12c	6.2±0.52b	35.0±10.7b
	Comet	26.4±1.97a	1.2±0.15b	10.4±2.14b	15.5±1.21a	6.5±0.72a	324.3±72.7a	309.1±39.4a	7.7±0.34a	12.7±3.16a	49.0±3.44ab
2019	Nugget	32.9±1.69b	1.4±0.24a	16.1±2.05a	11.0±1.82a	3.6±0.36a	244.4±29.5a	328.3±85.4a	6.9±0.93a	20.2±3.99a	65.0±9.95a
	Columbus	31.4±0.92b	1.1±0.20ab	12.0±3.29a	14.1±5.60a	4.0±1.38a	151.8±9.8b	302.0±102.7a	4.3±0.98c	10.1±3.04b	36.5±6.81b
	Cascade	47.3±5.51a	0.9±0.12b	11.0±1.94a	12.4±2.41a	4.5±1.54a	179.8±21.5b	276.7±67.9a	5.0±0.80bc	8.5±1.00b	27.3±5.56b
	Comet	23.4±2.41c	1.1±0.07b	10.5±3.71a	8.2±1.40a	5.2±1.56a	167.1±11.8b	250.1±30.4a	6.4±0.47ab	10.6±1.79b	30.4±4.02b

values. Regarding leaf P concentrations, 'Cascade' showed consistently the lowest average values, in some years with significant differences to the other cultivars. The leaf K levels were consistently higher in 'Nugget', with significant differences for some of the other treatments. 'Cascade' and 'Comet' showed the lowest values. Regarding Ca and Mg, significant differences between cultivars were found for the majority of samplings, but a consistent pattern was not observed. Some cultivars displayed a high average value in a given year that was not maintained in the other years and vice-versa. The concentration of micronutrients in the leaves also did not show a consistent trend in spite of significant differences between cultivars being found for some samplings. However, 'Cascade' showed consistently the lowest average values of B.

'Cascade' showed a tendency to display the highest average values of leaf N concentration when the results of the top half leaves were analysed (Table 2). 'Cascade' also showed the lowest average P levels in the top half leaves. The top leaves also showed the highest K values in 'Nugget' and the lowest values in the 'Cascade' and 'Comet' cultivars. In spite of significant differences between cultivars often being found, no consistent trends were observed in the levels of Ca, Mg, Fe, Mn, Cu and Zn from the top half leaves. The lowest average levels of B, however, were found in 'Cascade' as observed in the bottom leaves. In general terms, N and P levels were found to be higher in the top leaves and many other nutrients such as Ca, Mg and B were found to be higher in the bottom leaves.

The concentration of the nutrients in the stems (Table 3) followed the most important trends observed in the leaves (Table 2). 'Cascade' showed high and low levels of N and P respectively, and 'Nugget' showed high levels of K. However, for the majority of the nutrients their levels in stems were lower than the values found in the leaves.

The patterns observed in the concentration of most of the nutrients in the leaves, when the different cultivars were compared, were not reflected in general terms in the cones (Table 3). However, 'Cascade' showed consistently the lowest levels of P in the cones in comparison to the other cultivars as observed in the leaves. Several nutrients, such as N, Ca, Mg, Mn and B appeared to be less concentrated in the cones than in the leaves, whereas the concentration of P was higher in the cones than in the leaves. Several nutrients, and in particular K, showed a

Table 3. Stem and cone nutrient concentration (average ± SD) in August, at harvest, as a function of cultivar. For each year, means followed by the same letter are not statistically different by Tukey HSD test (α = 0.05).

Year	Treatment	N	P	K	Ca	Mg	Fe	Mn	Cu	Zn	B
		($g\ kg^{-1}$)					($mg\ kg^{-1}$)				
							Stem				
2017	Nugget	7.4±0.20b	1.0±0.07a	10.8±2.46a	8.4±1.27a	1.3±0.27a	26.9±6.11a	107.0±21.2a	3.9±0.87c	7.1±0.98a	12.8±0.64a
	Columbus	7.0±0.21b	1.0±0.18a	8.1±1.99a	6.6±0.76ab	1.1±0.29a	32.6±7.59a	86.9±45.9ab	5.3±2.25ab	6.3±2.02a	10.9±0.82b
	Cascade	13.1±2.62a	0.9±0.09a	8.1±1.38a	6.0±0.85b	1.0±0.16a	33.7±4.21a	44.3±3.93b	8.5±2.83a	7.2±1.02a	10.7±1.06b
2018	Nugget	8.0±0.14a	0.8±0.06a	15.3±1.24a	4.1±0.46ab	0.9±0.04b	45.3±5.43b	63.5±7.47b	6.4±0.42b	7.7±3.05a	12.2±0.56a
	Columbus	7.5±0.59a	0.8±0.06a	9.4±0.38b	5.3±0.90a	1.1±0.18a	40.1±2.62b	94.5±14.8a	9.6±0.49a	7.2±1.51ab	9.8±0.63a
	Cascade	8.7±1.50a	0.6±0.08b	5.7±1.20c	4.3±0.71ab	1.1±0.12a	55.7±5.97a	78.7±18.0ab	7.2±2.46ab	2.0±1.18c	10.6±1.75a
	Comet	7.2±0.72a	0.8±0.13a	8.0±1.82bc	3.4±0.18b	1.0±0.05ab	49.7±3.64ab	55.3±11.5b	7.0±0.73ab	3.3±1.14bc	10.0±2.17a
2019	Nugget	8.2±0.55c	0.8±0.06a	9.9±1.54a	6.2±0.49a	1.5±0.26a	70.8±8.14a	106.4±6.96b	8.6±0.36a	7.7±1.64a	15.0±0.81a
	Columbus	12.0±0.52b	0.6±0.03b	8.8±1.83a	3.6±0.80b	1.0±0.27bc	41.4±9.03b	155.8±17.6a	7.2±1.03b	5.4±2.81a	11.6±0.51bc
	Cascade	14.6±0.99a	0.6±0.09b	8.7±1.14a	3.1±0.32bc	1.2±0.07ab	56.9±5.24a	136.6±33.4ab	6.7±0.24bc	5.7±0.41a	13.0±1.85ab
	Comet	11.0±0.61b	0.6±0.04b	8.7±0.76a	2.3±0.17c	0.7±0.05c	39.7±5.62b	53.0±5.92c	5.6±0.13c	4.1±1.37a	9.6±0.26c
							Cone				
2017	Nugget	25.7±0.60b	3.0±0.13a	18.0±0.49b	5.6±0.26b	2.0±0.13b	138.6±3.51a	68.5±7.62a	6.5±0.36a	32.3±1.72a	27.2±1.36a
	Columbus	21.8±1.38b	2.8±0.63ab	20.5±0.80b	5.5±0.62b	2.1±0.27b	162.0±40.4a	63.3±19.02a	6.2±0.61a	28.5±3.86ab	27.6±0.41a
	Cascade	32.4±3.40a	2.1±0.06b	25.2±2.88a	9.0±1.15a	3.4±0.33a	177.1±30.5a	57.3±2.95a	6.2±0.28a	24.6±1.92b	27.3±2.83a
2018	Nugget	25.0±1.05a	2.6±0.07a	22.5±0.86a	2.3±0.08c	2.1±0.07c	143.6±15.9b	79.5±10.26a	6.4±0.12b	32.0±1.23a	25.2±1.02b
	Columbus	23.1±1.39ab	2.6±0.08a	17.5±1.32b	3.7±0.41b	2.2±0.18c	188.5±30.1b	79.4±7.02a	9.0±0.78a	26.2±3.29b	22.2±1.56b
	Cascade	20.2±2.96b	1.7±0.23b	18.7±1.14b	4.8±0.52a	2.5±0.07b	405.5±130.5a	81.3±5.48a	8.1±2.15ab	17.9±1.97c	21.8±3.56b
	Comet	21.7±1.41ab	2.4±0.20a	18.1±0.98b	3.7±0.30b	2.9±0.14a	276.8±42.8ab	88.0±8.23a	9.0±0.64a	20.9±1.86c	30.8±1.19a
2019	Nugget	25.9±1.63b	2.4±0.15b	13.8±1.87b	2.6±0.28ab	2.1±0.17c	174.0±18.8a	91.7±4.98a	6.8±0.52b	29.0±3.11a	26.9±2.18a
	Columbus	25.7±0.52bc	2.5±0.14ab	17.5±2.91ab	2.3±0.26b	2.0±0.09c	111.1±19.8b	95.5±12.57a	6.7±0.43b	21.5±1.43b	20.4±2.15b
	Cascade	30.5±1.48a	2.1±0.05c	18.1±0.60a	3.1±0.15a	2.7±0.06b	166.1±20.1a	105.3±9.91a	6.9±0.32b	23.0±2.13b	21.2±2.63b
	Comet	23.2±1.16c	2.8±0.18a	17.6±1.86ab	2.6±0.34ab	3.2±0.23a	160.6±15.7a	97.8±3.54a	8.1±0.43a	24.8±1.83ab	24.1±1.09ab

narrower range of variation between cultivars and years in the cones than in the leaves.

Bitter acid and nitrate concentration in hop cones

Significant differences between cultivars in α-acids were not found in 2018 and 2019 (Fig. 2). The highest average values were found in 'Columbus' and the lowest in 'Cascade'. The average values of α-acids ranged between 8.72% ('Cascade') and 11.9% ('Nugget') in 2018 and between 4.46% ('Cascade') and 12.2% ('Comet') in 2019. The concentrations of β-acids in cones only differed significantly between cultivars in 2018 with 'Cascade' recording the highest concentrations. The average values of β-acids varied from 3.89% ('Comet') to 8.00% ('Cascade') in 2018, and from 3.87% ('Comet') to 4.59% ('Cascade') in 2019.

The concentrations of NO_3^- in the cones ranged from 2.57 ('Comet') to 11.14 ('Cascade') g kg^{-1} in 2018 and from 9.19 ('Comet') to 14.56 ('Cascade') g kg^{-1} in 2019 (Fig. 3). 'Comet' and 'Cascade' consistently displayed, respectively, the lowest and highest average values, which differed significantly between both years.

Cone attributes in cultivar differentiation with stepwise discriminant analysis

According to the results produced by the stepwise discriminant analysis, using the cone attributes (concentration of nutrients, bitter acids and NO_3^-) as independent variables in the differentiation between cultivars ('Nugget', 'Columbus', 'Cascade', 'Comet'), three discriminant functions were constructed and four variables were selected with the stronger discriminant capacity (Mg, P, NO_3^- and Zn). The first function (F1) with an eigenvalue of 13.34 explained the greater differences between the cultivar groups, corresponding to 69.7% of variance explained, and the second (F2) and third (F3) functions explained 25.4% and 4.9% of the variance, respectively. The Wilks' lambda indicated a high significance of the three functions ($p < 0.001$). The functions of the cultivar group centroid (Table 4) indicated that function F1 differentiated the 'Comet' cultivar from the others and in particularly from 'Nugget', F2 separated 'Cascade' and F3 separated 'Nugget' from the other cultivars. The standardized canonical discriminant function coefficients (Table 4) reveal the more important variables in the construction of the functions, with Mg being more significant in

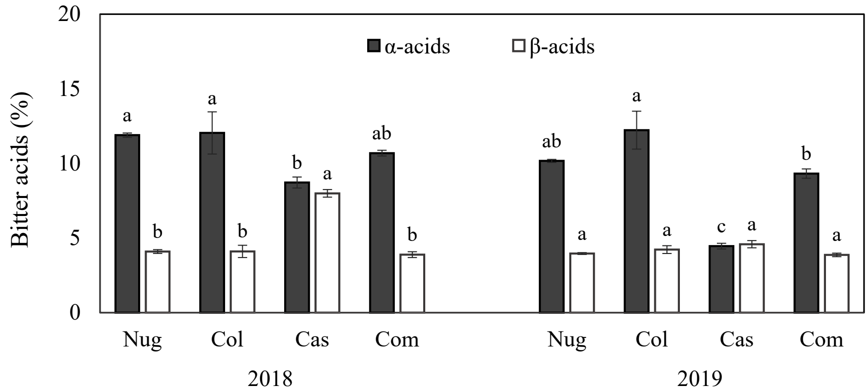

Figure 2. Cone α- and β-acid concentration in 2018 and 2019, as a function of cultivar (Nug, Nugget; Col, Columbus; Cas, Cascade; and Com, Comet). Within each year, same letters above the histogram bars indicate not significant differences by Tukey HSD test (α = 0.05). Error bars are the confidence intervals of the means (α = 0.05).

function F1, P and NO_3^- more significant in function F2 and Zn more significant in function F3. The interpretation of these results seems to indicate that the 'Comet' group cultivar was differentiated by the highest concentration of Mg in the cones, 'Cascade' was differentiated by the lowest concentration of P along with the highest concentration of NO_3^- in the cones, and 'Nugget' was differentiated by the highest concentration of Zn in the cones.

The self-validation classification results confirm that 96.9% of the original cases were classified correctly as 'Nugget', 'Columbus', 'Cascade' and 'Comet'. The cross-validation classification was of 90.6% and the misidentified cases belonged to 'Nugget' (12.5% classified as 'Columbus') and to 'Columbus' (25% classified as 'Nugget'). Therefore, the model based on the stepwise discriminant analysis was effective in the prediction of group membership. The classification of each cultivar group in the first two discriminant functions (F1 and F2), which explained most of the variance, showed that the centroids of 'Comet' and 'Cascade' were quite distant from each other and from 'Nugget' and 'Columbus', which were both close (Fig. 4).

Briefly, cone attributes such as the concentration of Mg, P, Zn and NO_3^-, helped to differentiate between the cultivars under analysis.

Leaf nutrient concentration in cultivar differentiation with stepwise discriminant analysis

The stepwise discriminant analysis was also performed for the nutrient concentrations in the leaves of the bottom or top halves of the plants as independent variables in the differentiation between cultivars. For both analyses (leaf bottom and top halves) three discriminant functions were constructed with the selected variables for each case (Fig. 5). Briefly, in relation to the results obtained for leaf nutrient concentration at the bottom half of the plant in the differentiation of cultivars, it can be noted that: i) Function 1 separated the 'Cascade' group cultivar by the highest N and lowest P concentrations in relation to the remaining groups and particularly to the 'Comet' group; ii) Function 2 separated the 'Nugget' group cultivar by

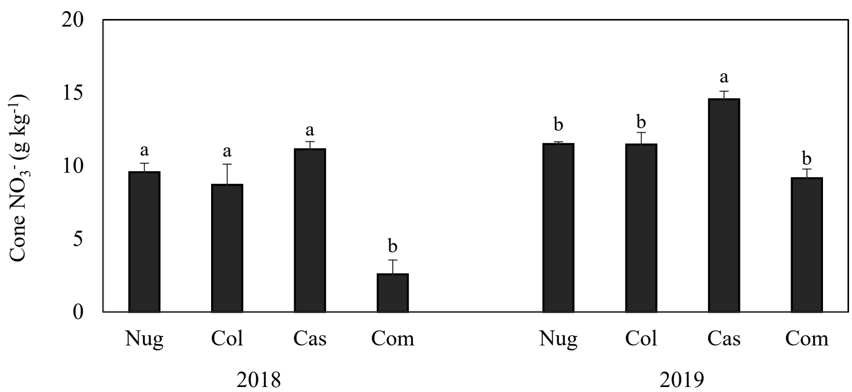

Figure 3. Cone nitrate (NO_3^-) concentration in 2018 and 2019 as a function of cultivar (Nug, Nugget; Col, Columbus; Cas, Cascade; and Com, Comet). Within each year, same letters above the histogram bars indicate not significant differences by Tukey HSD test (α = 0.05). Error bars are the confidence intervals of the means (α = 0.05).

Table 4. Discriminant functions generated with the stepwise method applied to hop cone attributes (concentration of nutrients, bitter acids and NO_3^-) as discriminant variables between hop cultivars.

Cultivar	Functions at cultivar group centroid			Select variables	Standardized canonical discriminant function coefficients		
	Functions				Functions		
	F1	F2	F3		F1	F2	F3
Nugget	-3.868	1.220	1.059	Magnesium	1.020	-0.324	0.339
Columbus	-1.387	1.291	-1.415	Phosphorus	0.803	1.024	-0.676
Cascade	-0.201	-3.572	-0.034	Nitrate	-0.452	-0.724	-0.369
Comet	5.456	1.061	0.390	Zinc	-1.047	0.054	1.155

the lowest concentration of Mg and highest concentration of B in relation to the remaining groups, and in particularly to the 'Comet' group; iii) Function 3 separated the 'Columbus' group cultivar by the lowest concentration of Zn and Mg, particularly from the 'Comet' group cultivar.

Regarding the results obtained for leaf nutrient concentration in the top half leaves, it can be noted that: i) Function 1 separated the 'Cascade' group cultivar by the lowest concentrations of P and Zn in relation to the remaining groups and particularly to the 'Nugget' group; ii) Function 2 separated the 'Comet' group cultivar by the highest Mg and lowest N concentrations in relation to the 'Nugget' and 'Cascade' groups; iii) Function 3 separated the 'Columbus' group cultivar by the lowest concentration of Zn and Mg particularly from the 'Comet' group cultivar.

The cumulative variance explained by the first two canonical discriminant functions (F1 and F2) was of 94.2% (F1 with 71.3%) for the bottom half leaf analysis and of 95.8% (F1 with 56.3%) for the top half leaf analysis. In both cases, in relation to the classification in functions F1 and F2, the centroid of the 'Cascade' group appeared as the most separated from the others, and the group separation was clearer from the top half leaf analysis (Fig. 5). The self-validation classification results confirmed that 88.6% of the original cases were classified correctly in the bottom half leaf analysis and 90.9% in the top half leaf analysis. The cross-validation classification was 81.8% for the bottom half leaf analysis and 90.9% for top half leaf analysis. With the exception of the 'Comet' group, all the others presented misidentified cases in the top half leaf analysis.

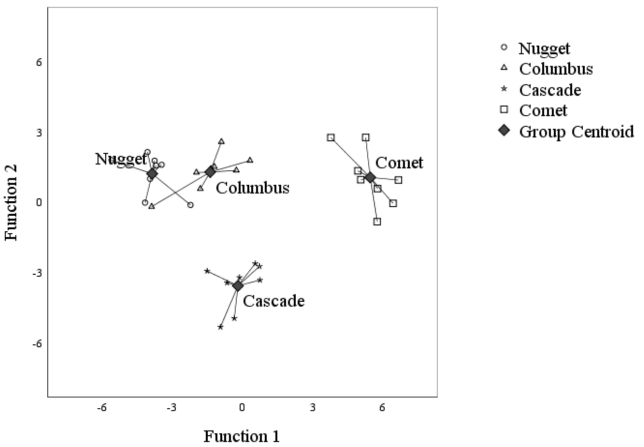

Figure 4. Distribution and centroid of each cultivar group in relation to the first two canonical discriminant functions generated with the cone attributes as independent variables (concentration of nutrients, bitter acids and NO_3^-).

Discussion

The productivity of the tested cultivars stressed the good adaptation of 'Comet', which consistently recorded the highest average values of total DM yield. The average DM yield of 'Columbus' was slightly above that of 'Nugget', but without significant differences. 'Cascade' exhibited the lowest values of total DM yield. Cone yield only differed significantly between cultivars in the last year (2019). 'Comet' registered the highest values in 2018 (633.5 g plant^{-1}, 2,640 kg ha^{-1}) and in 2019 (572.4 g plant^{-1}, 2,385 kg ha^{-1}), while the lowest average values were registered in the same period by 'Columbus' in 2018 (479.9 g plant^{-1}, 2,000 kg ha^{-1}) and 'Cascade' in 2019 (323.3 g plant^{-1}, 1,347 kg ha^{-1}). The reference values reported from Hopslist (2020) for cone yield indicate 'Comet' (1,900–2,240 kg ha^{-1}) and 'Nugget' (1,700–2,200 kg ha^{-1}) as having similar yield potential and 'Columbus' (2,000–2,500 kg ha^{-1}) and 'Cascade' (2,017–2,465 kg ha^{-1}) with slightly higher values. This is not in agreement with the present results, though the ranges of variation are similar.

Under Mediterranean conditions, Rossini *et al.* (2016) tested several hop cultivars including 'Cascade' and 'Columbus' and found that 'Cascade' was one of the highest yielding cultivars while 'Columbus' displayed a lower performance. Ruggeri *et al.* (2018) also found 'Cascade' to be the highest yielding of the cultivars tested in a two-year experiment and reported an average yield of 470 g of cones per plant (248.87 and 691.20 g plant $^{-1}$ in the first and second years, respectively). Mongelli *et al.* (2016), in an

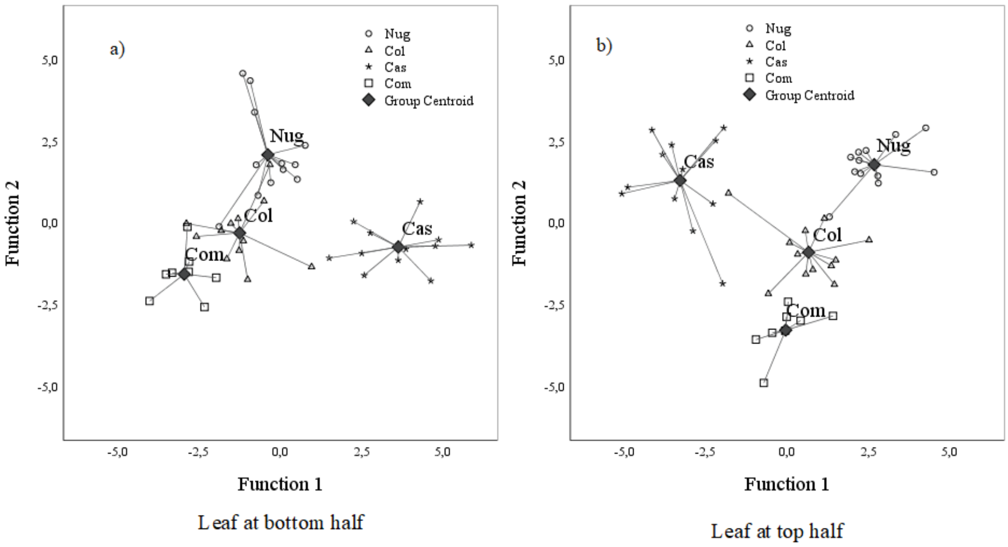

Figure 5. Distribution and centroid of each cultivar group (Nug, Nugget; Col, Columbus; Cas, Cascade; and Com, Comet) in relation to: a) the first two canonical discriminant functions generated in leaf bottom half analysis; b) the first two canonical discriminant functions generated in leaf top half analysis.

experiment to test the adaptation of several hop cultivars in northern Italy, also observed higher yields for 'Cascade' (952 kg ha^{-1}, dry weight) in comparison to 'Nugget' (292 kg ha^{-1}, dry weight). These results seem to disagree with those presented in this work, in spite of 'Cascade' showing good vegetative development during the growing seasons. Cone yield, in turn, was similar to that of the other cultivars and only in the last year was it significantly lower than that of 'Columbus' and 'Comet', but similar to that of 'Nugget'.

The year influenced the productivity of all cultivars. The highest average values of total biomass were found in 2018. Highest average cone yields were also achieved in the year 2018 for 'Nugget', 'Cascade' and 'Comet' and in 2019 for 'Columbus'. The meteorological data for the year 2018 showed higher precipitation levels between March and July. 2019 showed lower average monthly temperatures in June and July and higher precipitation in August and September. The higher precipitation levels in the middle of the growing season in 2018 may have contributed to increasing biomass production. Rossini *et al.* (2016, 2020) also stated that in central Italy the growth and yield of hop cultivars were affected significantly by the weather conditions, particularly by reduced precipitation and high temperatures. Marceddu *et al.* (2020) reported a high variability of hop yield according to crop management in the region of Palermo in Italy.

The dry weight of individual cones differed significantly among cultivars in 2018 and 2019 and 'Comet' exhibited once again the highest average values in both years (0.79 g cone^{-1} in 2018 and 0.28 g cone^{-1} in 2019). 'Nugget' displayed the lowest average value in 2018 (0.29 g cone^{-1}) and 'Cascade' in 2019 (0.16 g cone^{-1}). Between the years, the average weight of individual cones was higher in 2018 for 'Columbus', 'Cascade' and 'Comet' cultivars. 'Nugget' displayed higher average cone DM yield in 2018, though the values were lower than those of the other cultivars. Čeh *et al.* (2012) analysed the relationship between cone mass and length of the Slovenian cultivar Savinjski Golding and the weather conditions. They observed a significant effect of weather on cone traits which seems to be in accordance with the results presented in this study. The DM of 100 cones of the Slovenian cultivar Savinjski Golding varied between 10 and 16 g (Čeh *et al.*, 2012), which is less than the values found in this study (0.16 to 0.79 g cone^{-1}). These results seem to be a positive indication of the good hop growing conditions in the north of Portugal.

Concerning tissue nutrient concentration, the most consistent trends between cultivars were observed in the leaves and to a lesser extent in the stems. 'Cascade' showed high levels of N but low levels of P, K and B in plant tissues. 'Comet', which was the highest yielding cultivar, displayed lower values of N and K in leaf tissues, probably due a dilution effect (Jarrel & Beverly, 1981). 'Nugget' consistently presented the highest levels of K in the leaves. In general, the nutrient concentration in the cones did not follow the same trend reported for leaves. At this point, the results do not seem to suggest that the differences in the productivity between cultivars can be explained by the differences in tissue nutrient concentration.

The concentration of bitter acids in the cones differed significantly between cultivars. 'Columbus' exhibited the highest levels of α-acids, ranging between 12.04 % and 12.23%, which are slightly below the general reference range of 14-18% (Hopslist, 2020), but higher than those reported by Mozzon *et al.* (2020) for 'Columbus' (7.41%)

grown in central Italy, also on a high trellis system. The average values of α-acids of 'Nugget' (10.17–11.90%) and 'Comet' (9.32–10.69%) were similar to those found in Hopslist (2020), as well as those of 'Cascade' (4.46–8.72%), in spite of being lower than those of 'Nugget' and 'Comet'. Mozzon *et al.* (2020) reported similar α-acid levels for 'Nugget' (10.61%) and 'Cascade' (4.47%). Forteschi *et al.* (2019) also obtained α-acid levels between 5.00 and 9.05% for 'Cascade' grown in Sardinia (Italy) on a low trellis system. Pearson & Smith (2018) reported α-acid levels slightly higher for 'Comet' (11.2%) in the first-year of growth in Florida (USA), on a high trellis system.

Regarding β-acids, the values obtained for 'Nugget', 'Columbus' and 'Cascade' were similar to those reported by Mozzon *et al.* (2020) and Forteschi *et al.* (2019) for 'Cascade'. The ratios obtained for β-acids were also generally in agreement with the reference values of the Hopslist (2020). The average values for α- and β-acids varied with the year, with the values of 'Cascade' being the most affected. The values of 2019 were particularly low. 'Cascade' seems to have good adaptation to high temperatures (Eriksen *et al.*, 2020). Probably, the lower temperatures and the higher precipitation than usual, at the middle and end of the growing season of 2019, may have contributed to these negative results. Despite the less favourable effect that the year may have had, all the cultivars displayed bitter acid contents close to the reference values.

The results of stepwise discriminant analysis performed with the cone attributes as discriminant variables presented a solution which differentiated mostly between 'Comet' and 'Cascade' and both these from the other cultivars ('Nugget' and 'Columbus'). 'Comet' seemed to display higher Mg concentration in the cones while 'Cascade' presented lower P concentration and higher NO_3^- concentration than the other cultivars. The accumulation pattern of NO_3^- in the cones was markedly different between 'Comet' and 'Cascade', which consistently exhibited the lower and higher average levels, respectively. Mg is involved in N metabolism and seems to be able to reverse ammonium toxicity (Guo *et al.*, 2016). 'Comet' presented higher Mg concentrations which may be related to the lower levels of NO_3^-. 'Cascade' and 'Columbus' displayed respectively the lowest and highest values of α-acids, which is in accordance with the reference levels.

The variables that seem to differentiate better between these cultivars were the concentration in cones of P (higher in 'Columbus') and NO_3^- (higher in 'Cascade'). The higher concentration of NO_3^- in the cones of 'Cascade', may mean that fewer amino acids were being synthetized via NO_3^- reduction (Lal, 2018). Consequently, the bitter acid biosynthesis was affected since branched-chain amino acids derived compounds are the essential building blocks for the biosynthesis of hop bitter acids (Xu *et al.*, 2013). On the other hand, the reduction of NO_3^- to amino acids is an energy consuming process. Thus, it involves P as the main nutrient in energy metabolism and also in the phosphorylation and dephosphorylation of the nitrate reductase enzyme (Kathpalia & Bhatla, 2018; Lal, 2018). Moreover, P compounds are required in bitter acid biosynthesis (Champagne & Boutry, 2017). Hence, the lowest levels of P in 'Cascade' may be related to a lower bitter acid biosynthesis, in contrast to 'Columbus' which has higher levels of P and bitter acids. Cultivars may differ in nutrient uptake, which is probably related to the rate of production of important compounds such as bitter acids, which are very stable in each cultivar.

The results of stepwise discriminant analysis performed with the leaf nutrient concentration as discriminant variables presented a solution which differentiated mainly 'Cascade' from the other cultivars. It also highlighted the lowest concentration of P in 'Cascade' leaves and the highest concentration of Mg in 'Comet' leaves. The higher uptake and accumulation of Mg by 'Comet' may be related to higher biomass production. 'Comet' stood out for its high biomass production compared with the other cultivars and interestingly presented the lowest concentrations of N and K in the leaves. These are macronutrients used in high amounts in plant growth (Hawkesford *et al.*, 2012). Perhaps the higher uptake levels of Mg improved the efficient use of N and K in biomass production. Mg has a relevant role in photosynthesis and N and C metabolism, and it is probably more important in hop growth than is usually considered (Guo *et al.*, 2016). The nutrient accumulation criteria in cone and leaf tissues seem to be a differentiating factor between cultivars with influence on bitter acid biosynthesis and biomass production.

In summary, 'Comet' was the most productive cultivar, displaying the highest total DM yield and cone production. 'Comet' was followed by 'Nugget' and 'Columbus', with similar values, with 'Cascade' giving the poorest performance. The concentration of α- and β-acids in the cones, which is a very important quality parameter, was within or close to the range established as normal in Hopslist (2020) for all cultivars. However, 'Cascade' showed high sensitivity to the year effect, which greatly influenced the average bitter acid yield. Cultivars greatly differed in leaf N, P, K and B concentrations. Cone attributes (concentration of nutrients, bitter acids and NO_3^-) and leaf nutrient concentrations were differentiating factors between cultivars. The results showed that the differences in the concentration of nutrients in the leaves and cones may be related to biomass and bitter acid production. 'Cascade' was the least similar of the cultivars and was differentiated by the lowest concentrations of P in the leaves and cones and the highest NO_3^- concentrations in the cones. 'Columbus', in turn, was differentiated by the highest leaf and cone P concentrations, while 'Comet' by the highest Mg concentrations in the leaves and cones.

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