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Morphological characteristics, trichomes, and phytochemistry of inflorescences of *Humulus lupulus* L: Comparison of cropping systems and varieties

Olivia Pak Campos[1,*], Flávia Maria Leme[2], Gabriel Cássia Fortuna[1], Jordany Aparecida de Oliveira Gomes[1], Caio Scardini Neves[1], Rosani do Carmo de Oliveira Arruda[2], Filipe Pereira Giardini Bonfim[1]

[1]São Paulo State University, School of Agriculture, (UNESP/FCA), Plant Breeding Department, Av. Universitária, nº 3780, 18610-034, Botucatu, SP, Brazil
[2]Universidade Federal de Mato Grosso do Sul (UFMS), Av. Costa e Silva, s/nº, 79070-900, Campo Grande, MS, Brazil

***Corresponding author: olivia.pakc@gmail.com**

Abstract

This study aimed to assess the influence of cropping systems (conventional and organic) and hops varieties ('Cascade', 'Chinook', 'Columbus', 'Hallertau Mittelfrüh', and 'Nugget') on morphological and anatomical aspects, as well as the contents of essential oil and phenolic compounds of hops inflorescences. Thus, morphological description and cone length; identification of trichomes and peltate glandular trichome density; anatomical and histochemical analysis of peltate glandular trichomes; essential oil content; and total phenolic compound content were evaluated. The results showed no influence of treatments on morphology; however, higher cone lengths were observed in organic cropping and in 'Cascade' and 'Chinook'. These results report the first record of hops trichomes grown in Brazil. Three types of trichomes were identified in inflorescences: nonglandular, peltate, and bulbous glandular. An intense presence of peltate trichomes was observed on flowers. The trichome density in bracts differed among varieties, and 'Cascade' had the highest mean (8.19). There was a significant interaction between cropping systems and varieties for trichome density in flowers: with the exception of 'Nugget' (59.50), the varieties had higher means under organic cropping, especially 'Hallertau Mittelfrüh' (92.12) and 'Chinook' (88.12). Histochemical analysis revealed the presence of polysaccharides, starch, lipids, phenolic compounds, and proteins in peltate trichomes. The cropping systems did not affect the oil content, although there were significant differences between varieties. 'Hallertau Mittelfrüh' showed the lowest oil content (0.25%). Phenolic compounds showed no effect of treatments. Therefore, the results indicate that organic cropping and the 'Cascade' and 'Chinook' varieties are more promising under the conditions studied.

Keywords*:* Essential oil; Glandular trichome micromorphology; Hops; Lupulin glands; Phenolic compounds; Scanning electron microscopy (SEM).

Introduction

Hops (*Humulus lupulus* L.) belongs to the Cannabaceae family and Rosales order. It is native to Europe and Western Asia and is a perennial, herbaceous, dioecious, anemophilous, and dextrogyrous vine species (Jeliazkova et al., 2018; Spósito et al., 2019).

Ethno-knowledge in relation to hops descends from antiquity; the first records date back to the 1st century, when the Roman writer Pliny described hops as a common plant found in gardens, being a very popular horticultural species (Jeliazkova et al., 2018). Hops have been used medicinally for over 2000 years; in the 11th century, Arabicmedicus Mesue described their anti-inflammatory effects.

Hops have multiple applications, including medicinal, food, and cosmetic purposes; furthermore, they are an alternative source of fiber for the textile industry. However, approximately 97% of world hops production is destined for the brewing industry (Raiser, 2011; Jeliazkova et al., 2018).

In 2016, Brazil produced 13.3 billion liters of beer, making it the third-largest producer in the world. This production resulted in revenue of R$77 billion, corresponding to 2% of its GDP (Sindcerv, 2019). The scarcity of commercial hop cultivation in the country, even in the face of high domestic demand, requires Brazil to be a large importer. A total of 3.24 thousand tons of hops were imported in 2020, at a cost of approximately US $28 million (Comexstat, 2020).

Hops are considered a commodity in many countries, and the commercial interest in this species is in its female inflorescences, also called cones. Plants are capable of producing a wide variety of secretions; in many cases, these exudates are bioactive and contribute to the evolutionary success and perpetuation of species since they are fundamental to the defense mechanism (Ascensão, 2007). These secretions can be synthesized and/or stored in specialized cells or in highly differentiated glandular structures (Fahn, 2000). Hop cones develop trichomes that secrete lupulin (Almaguer et al., 2014), composed mostly of the terpenes linalool, myrcene, and α-humulene; the phenolic compounds humulones (α-acids) and lupulones (β-acids); and the flavonoid xanthohumol (a potent cancer

preventive) (Farag et al., 2011; Stevens and Page, 2004), whose properties characterize the qualities of hop (Boulton, 2013; Rodrigues et al., 2015).
The description and classification of plant organ structures are objects of study of plant anatomy. This information helps in the identification of adaptive trends and in understanding plant functioning (Dickison, 2000). Concomitantly, histochemistry is used to characterize substances and their sites of synthesis and/or storage. The use of these techniques allows more efficient management strategies to be adopted to increase the production of metabolites of interest (Gobbo Neto and Lopes, 2007).
Hop plants are predominantly cultivated in temperate regions, but there is potential to cultivate this species in different climates due to the large number of available varieties adapted to different climatic conditions (Kavalier et al., 2011). The cultivation of hops in Brazil is recent and has undergone experimental phases; however, this scenario has been changing (Sarnighausen et al., 2017). Different varieties can present different agronomic performances and different proportions and compositions of specialized metabolites. Even within the same variety, these variables can show variability since they depend on interactions with external edaphoclimatic and biotic factors (Patzak et al., 2010; Savithramma et al., 2011).
Thus, management practices can directly influence productivity and quality, as the plant is dynamically interrelated with its environment. The fundamental differences between organic and conventional cropping systems concern fertilization methods, soil fertility management, and plant protection protocols, and these factors affect the composition of plants (Grzyb et al. 2012). In this sense, Keukeleire et al. (2007) and Solarska et al. (2015) reported that some hops varieties perform better under organic cropping systems than under conventional cropping systems, since these systems promoted an increase in the content of secondary metabolites and different growth patterns. Such responses may be linked to the stress that occurs in the plant due to the complex interactions among biotic and abiotic factors in this system.
In this context, the current study aimed to assess the influence of cropping systems (conventional and organic) and hops varieties ('Cascade', 'Chinook', 'Columbus', 'Hallertau Mittelfrüh', and 'Nugget') on morphological aspects of the plants, anatomical features of their trichomes, and the essential oil and phenolic compound contents of their inflorescences when grown under subtropical conditions.

Results and discussion

Morphological description and length of the cones

The morphological behavior during the vegetative phase was similar under both cropping systems and in all five varieties. Hop is perennial plant with branched vines that are dextrogyrous (clockwise or right rotation), lack tendrils, and are fixed to the conduction system through adherent silica hairs (Fig. 1A, B, C). Of undetermined growth, the aerial part of the plant is formed by primary branches that grow vertically and lateral branches that may contain only leaves or host a combination of leaves and inflorescences (Spósito et al., 2019). Thus, a plant condution system is necessary and important because it is directly related to productivity.
Hops is a dioecious plant, and the commercial interest is in the female inflorescences (Fig. 1D, E, F). Thus, the cultivation areas have only female plants, which invariably come from vegetative propagation (Rossini et al., 2021) by herbaceous cuttings, rhizomes, or micropropagation. The female inflorescence of hops (Fig. 1E, F) consists of a rachis in which the flowers and bracts are inserted (Spósito et al., 2019). These trichomes are attached by a peduncle of only four cells, so handling during harvesting and processing should be done carefully to avoid losses (Campbell and Pearson, 2019).
The pistillate flowers of hop originate in the axillae of the stipular bracts (Fig. 2A), and the floral meristems occur in pairs at each floral node along the central axis of the cone (Shephard et al., 2000). The flowers are coated with many peltate trichomes (Fig. 2B, C, D).
Table 1 shows that there was a significant independent influence of treatment on the length of cones. In the organic cropping system, larger cones were observed (3.55 cm) than in the conventional system (2.97 cm). Among the varieties, the greatest lengths were detected in 'Cascade' and 'Chinook' and the shortest in 'Hallertau Mittelfrüh', at 3.56, 3.37, and 2.78 cm, respectively. According to the classification proposed by the American Society of Brewing Chemists (2010), 'Hallertau Mittelfrüh' presented small cones, while the other varieties had medium-length cones.
Each variety has its own morphological characteristics, including the size of the cone, which is also dependent on edaphoclimatic conditions (Roberts and Wilson, 2006). Small (1981) stated that European varieties have smaller cones than American varieties. Our results agree, as 'Hallertau Mittelfrüh' is of European origin and the others of American origin.
The cone length is a parameter used at the time of harvest. In this period, the cones reach their maximum size, which is associated with the point of physiological maturity (Čeh and Zmrzlak, 2012). The yield of cones per planted area depends on a number of factors that directly or indirectly influence production, and cone size directly affects yield.
As the cones elongate, due to the growth of the bracts, the glandular trichomes responsible for the synthesis and storage of lupulin develop. Kavalier et al. (2011) showed that there is a direct relationship between the development of cones and the accumulation of specialized metabolites. However, it should be noted that cones of excessive size (larger than what is considered large according to ASBC Hops-2 (2010), 7.6 cm) hinder the drying process because they require more time and may result in loss of quality when drying is not well planned (Raut, et al., 2020).

Types of trichomes

Trichomes of the nonglandular (Fig. 3A), bulbous glandular (Fig. 3C), and peltate glandular kinds (Fig. 3F) were identified in the hop inflorescence in all varieties studied in both cropping systems. The distribution of the different types of trichomes varied according to the analyzed region of the bracts and pistillate flowers.
Nonglandular trichomes (Fig. 3A) were more abundant on the abaxial surface of the bracts (Fig. 3B). These trichomes are mechanical protective structures against external factors, such as herbivores, pathogens, ultraviolet radiation, extreme heat, and excessive water loss (Valkama et al., 2003). The bracts have a protective function (Adams, 2021), and bracts with numerous nonglandular trichomes function as a protective barrier for the pistillate flowers of the hops.
The bulbous glandular trichome (Fig. 3C) is formed of two basal cells and two peduncular cells, and its glandular head consists of four to eight cells (Oliveira and Pais, 1988). This

trichome synthesizes and stores terpene phenols and essential oil (Oliveira and Pais, 1988; Kavalier et al., 2011); however, its contribution to the final amounts of these compounds is negligible compared to that of the peltate trichome, as its size is small, as is its density. This structure occurs mainly in the bracts (Fig. 3D, E).

The peltate glandular trichome (Fig. 3F) occurs predominantly in the basal region of the abaxial face of the bracts and in greater numbers in the abaxial face of the calyx of the pistillate flowers (Fig. 3G). This trichome synthesizes and stores specialized metabolites, which have been attributed to medicinal and organoleptic properties and bioactivities that have aroused commercial interest in the species (Srecec et al., 2011; Jeliazkova et al., 2018). Xanthohumol accumulates in these peltate glandular trichomes and has cytoprotective effects that function to detoxify carcinogens and metabolize oxidative radicals, in addition to being an antioxidant and free radical scavenger (Miranda et al., 2000a, 2000b). This phytochemical may be useful for the prevention and treatment of certain types of cancer. Bitter acids (alpha and beta acids) and essential oils are responsible for imparting bitterness and aroma to beer, as well as helping to preserve the beverage due to their antibacterial properties, and for stabilizing the foam (Keukeleire, 2000). Therefore, it is extremely important to study and understand this trichome, as it can provide a basis for the cultivation, management, handling, and processing of hops.

Anatomy and histochemistry of the peltate glandular trichome

The peltate glandular trichome originates from a protodermal cell. At the end of its development, it consists of four basal cells, four peduncular cells, and a glandular head composed of a disc of 30 to 72 secretory cells along with a subcuticular cavity (Fig. 4) (Oliveira and Pais, 1988).

Before the secretory phase, the peltate glandular trichomes have a concave shape, similar to a cup (Fig. 5A, B). These trichomes develop their pointed shape as the subcuticular cavity fills, resulting from the biosynthesis and accumulation of lupulin (Fig. 5C, D, E, F), which in turn is rich in resins (alpha and beta acids), essential oils, and phenolic compounds. For this reason, these trichomes are also known as lupulin glands (Sugiyama et al., 2006; Nagel, 2008).

The histochemical tests indicated a positive response to all reagents used in the five varieties in both cropping systems (Table 2).

A strong reaction to Sudan black B was detected, indicating high accumulation of lipid substances both in the subcuticular space and in the secretory cells of the peltate trichome (Fig. 6C). In the other evaluations, it was not possible to identify the presence of the compounds tested in the subcuticular cavity of the trichome because the sections obtained did not permit such visualization. However, neutral polysaccharides, starch grains, phenolic compounds, and proteins were found in the cells that made up the head of the trichome (Fig. 6D, E, F and G).

Santagostini et al. (2019), when performing histochemical studies of the peltate trichomes of hops, also identified an intense response to the reagent that identifies lipids within the subcuticular cavity. Oliveira and Pais (1988) also detected the presence of phenols through tests. Some of the results are associated with the structure of the trichomes and not necessarily with the secreted exudate, as is the case for polysaccharides (Oliveira and Pais, 1988), which may also extend to starch and proteins.

Density of peltate glandular trichomes

Significant differences were detected in the density of peltate glandular trichomes in the bracts only between varieties (Table 3, Fig. 7), with 'Cascade' showing the highest density and 'Hallertau Mittelfrüh' showing the lowest density, at 8.19 and 3.44 trichomes per 1.26 mm^2, respectively.

There was a significant interaction between cropping systems and varieties regarding the density of peltate trichomes in the calyx of the flowers (Table 4, Fig. 8). Four of the varieties showed higher densities when grown under organic cropping, and only 'Nugget' showed a higher density in the conventional treatment (Fig. 8E). In the conventional system, 'Chinook' and 'Nugget' (Fig. 8B, E) showed approximately 33% more trichomes than 'Cascade' (Fig. 8A). In the organic cropping system, 'Chinook' and 'Hallertau Mittelfrüh' (Fig. 8G, I) showed 25% more trichomes than the others.

The different varieties vary widely in the number of peltate trichomes in their cones (Neve, 1991). These differences are of great relevance since these structures contain elements of commercial value. Patzak et al. (2015), when analyzing different varieties of hops, concluded that the density of peltate trichomes is closely correlated with the content of alpha and beta acids and polyphenols. Therefore, varieties that have a higher density of peltate trichomes and a cropping system that promotes an increase in this trait are highly desirable.

Essential oil and total phenolic compound contents

There was no significant difference between the cropping systems in essential oil content, with an average of 0.53%. On the other hand, there were significant differences between the varieties, in which the essential oil content in 'Hallertau Mittelfrüh' was 58.33% lower than the average of the other varieties (Table 5). For total phenolic compounds, there was no significant difference between the cropping systems and among the varieties (Table 5).

The essential oil content obtained corroborates the literature, as 'Hallertau Mittelfrüh' has a lower amount of essential oil than the other varieties studied (Hopslist, 2021). The contents found are within the overall average of 0.50 to 3.0% of the dry cone mass (Almaguer et al., 2014), ranging from 0.58 to 0.63%, with the exception of 'Hallertau Mittelfrüh', which had a content of 0.25%. It is important to note that this is the second year of cultivation, so the crop has not yet been fully established (Sirrine et al., 2014), *i.e.*, it has not expressed its maximum production potential.

The phenolic compounds of hops, unlike the essential oil, are not synthesized and stored exclusively in peltate trichomes (Durello, et al. 2019) but are also found in the bracts and in the central axis of the inflorescences, with the exception of prenylflavonoids (xanthohumol, isoxanthohumol, and related compounds), which are found mainly in the lupulin glands (Roberts and Wilson, 2006). Their synthesis routes also differ. For these reasons, these compounds do not necessarily follow the same statistical patterns as the essential oil.

Although the treatments did not influence the phenolic compound content, Goiris et al. (2014), when studying polyphenols and their relationship with the palatability of beer, found that these compounds are flavor activators. In

Table 1. Cone length of *Humulus lupulus* L. varieties grown in conventional and organic cropping systems.

Treatment	Cone length (cm)
Cropping systems	
Conventional	2.97 ± 0.4 [b]
Organic	3.55 ± 0.5 [a]
p value	< 0.05
Varieties	
'Cascade'	3.37 ± 0.7 [a]
'Chinook'	3.56 ± 0.5 [a]
'Columbus'	3.27 ± 0.4 [ab]
'Hallertau Mittelfrüh'	2.78 ± 0.3 [b]
'Nugget'	3.33 ± 0.4 [ab]
p value	< 0.05

Values are expressed as the mean ± standard deviation. Values followed by different letters differ significantly (Tukey test, $p < 0.05$).

Fig 1. General aspects of the morphology of *Humulus lupulus* L. (Cannabaceae). A: Branched vine without tendrils. B: Details of the dextrogyrous herbaceous branches, which are fixed through adherent hairs. C: Adherent hairs (arrows). D: Flowering stage. E: Cones in the final stage of development. F: Longitudinal section of the cone, highlighting the location of the peltate glandular trichomes in the bract (white arrow) and flower (black arrow).

Table 2. Histochemical data obtained from peltate glandular trichomes of bracts and flowers of varieties of *Humulus lupulus* L.

Reagents	Target compound	CAS	CHI	COL	MH	NUG	Fig.
SBP	Neutral polysaccharides	+	+	+	+	+	6 E
Lugol	Starch	+	+	+	+	+	6 D
Sudan black B	Total lipids	+	+	+	+	+	6 C
Ferric chloride	Phenolic compounds	+	+	+	+	+	6 F
Bromophenol blue	Protein	+	+	+	+	+	6 G

CAS: 'Cascade'; CHI: 'Chinook'; COL: 'Columbus'; HM: 'Hallertau Mittelfrüh'; NUG: 'Nugget'; (+) presence.

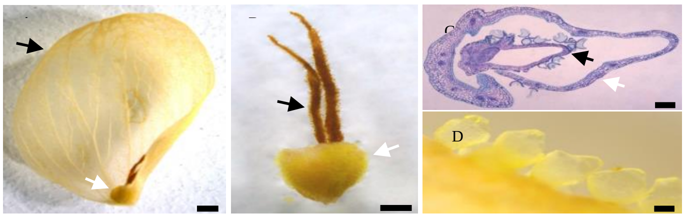

Fig. 2. Stereomicroscope images (A, B and D) and light microscopy (C), detailing the pistillate flower and peltate glandular trichomes of *Humulus lupulus* L. (Cannabaceae). A: Flower (white arrow) protected by the bract (black arrow). B: Pistillate flower with calyx covered with peltate trichomes (white arrow); note the long stigma (black arrow). C: Cross-section of the base of the pistillate flower next to the bract; note more peltate trichomes adhered to the calyx of the flower (black arrow) than to the bract (white arrow). D: Peltate trichomes on the abaxial surface of the flower calyx. Scale bars A: 1 mm; B: 0.5 mm; C: 62.5 µm; D: 0.2 mm.

Table 3. Density of glandular peltate trichomes (per 1.26 mm^2) on abaxial surface bracts of *Humulus lupulus* L. varieties grown in conventional and organic cropping systems.

Treatment	Density of trichomes in the bracts
Cropping systems	
Conventional	6.30 ± 3.1
Organic	7.00 ± 3.7
p value	0.37
Varieties	
'Cascade'	8.19 ± 2.9 ^{a}
'Chinook'	7.44 ± 4.1 ^{ab}
'Columbus'	7.94 ± 3.1 ^{ab}
'Hallertau Mittelfrüh'	3.44 ± 1.8 ^{b}
'Nugget'	6.25 ± 2.8 ^{ab}
p value	< 0.05

Values are expressed as the mean ± standard deviation. Values followed by different letters differ significantly (Tukey test, $p < 0.05$).

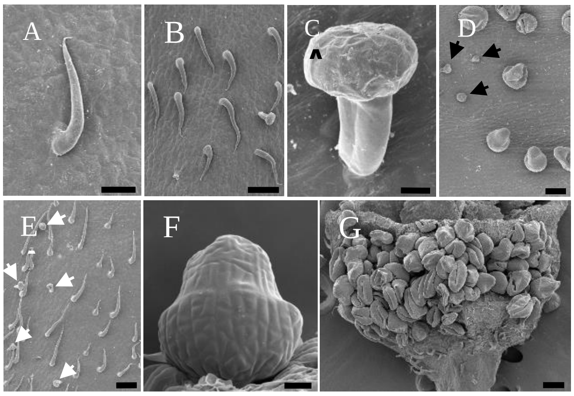

Fig 3. Abaxial surface of the epidermis of bracts and flowers of *Humulus lupulus* L. showing the different types of trichomes and their distributions (SEM). A: Non-glandular trichome on the abaxial surface of the bract. B: Middle region of the bract, abaxial surface with intense presence of non-glandular trichomes. C: Bulbous glandular trichome on the abaxial surface of the bract. D: Basal region of the bract, with the presence of bulbous (black arrows) and peltate trichomes. E: Middle region of the bract, with non-glandular and bulbous trichomes (arrows). F: Peltate glandular trichome in the last phase of morphogenesis - maturity, under the abaxial surface of the flower calyx. G: Pistillate flower; note the numerous trichomes peltate in different stages. Scale bars: A: 50 µm; B: 100 µm; C: 10 µm; D: 100 µm; E: 100 µm; F: 25 µm; G: 100 µm.

Table 4. Density of glandular peltate trichomes (per 1.26 mm^2) on calyx flowers of *Humulus lupulus* L. varieties grown in conventional and organic cropping systems.

Varieties	Cropping systems	
	Conventional	Organic
'Cascade'	50.12 ± 7.9 [cB]	70.75 ± 6.7 [bA]
'Chinook'	73.75 ± 2.4 [aB]	88.12 ± 9.5 [aA]
'Columbus'	56.75 ± 10 [bcB]	72.50 ± 15.2 [bA]
'Hallertau Mittelfrüh'	71.37 ± 4.7 [abB]	92.12 ± 5.8 [aA]
'Nugget'	76.37 ± 4.3 [aA]	59.50 ± 7.7 [bB]
p value	< 0.001	

Values are expressed as the mean ± standard deviation. Values followed by different letters, lowercase letters in columns and uppercase letters in rows, differ significantly (Tukey test, $p < 0.05$).

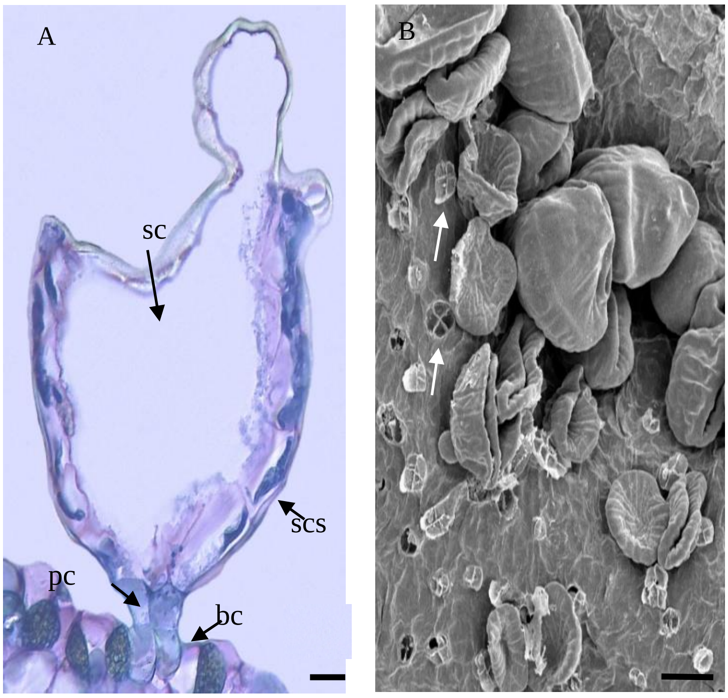

Fig 4. Details of the peltate glandular trichome. A: Longitudinal section of the trichome. Note the basal cells (bc), peduncular cells (pc), secretory cells (scs), and the space between the secretory cells and the cuticle that forms the subcuticular cavity (sc). B: Scanning electron micrograph. Note the scars left after detachment of the peltate trichome (arrows), evidencing the four basal and peduncular cells. Scale bars: A: 10 μm; B: 50 μm.

Table 5. Essential oil content and total phenolic content in inflorescences of *Humulus lupulus* L. varieties grown in conventional and organic cropping systems.

Treatment	Essential oil content (%)	Total phenolic content (mg GAE/100 g dry matter)
Cropping systems		
Conventional	0.53 ± 0.2	3327.17 ± 631.5
Organic	0.53 ± 0.2	2980.70 ± 500.8
p value	0.96	0.22
Varieties		
'Cascade'	0.60 ± 0.1 [a]	3158.54 ± 812.3
'Chinook'	0.58 ± 0.1 [a]	3244.44 ± 678.4
'Columbus'	0.63 ± 0.2 [a]	3138.54 ± 489.2
'Hallertau Mittelfrüh'	0.25 ± 0.1 [b]	2748.40 ± 325.0
'Nugget'	0.59 ± 0.1 [a]	3479.94 ± 383.2
p value	< 0.001	0.13

Values are expressed as the mean ± standard deviation. Values followed by different letters differ significantly (Tukey test, $p < 0.05$).

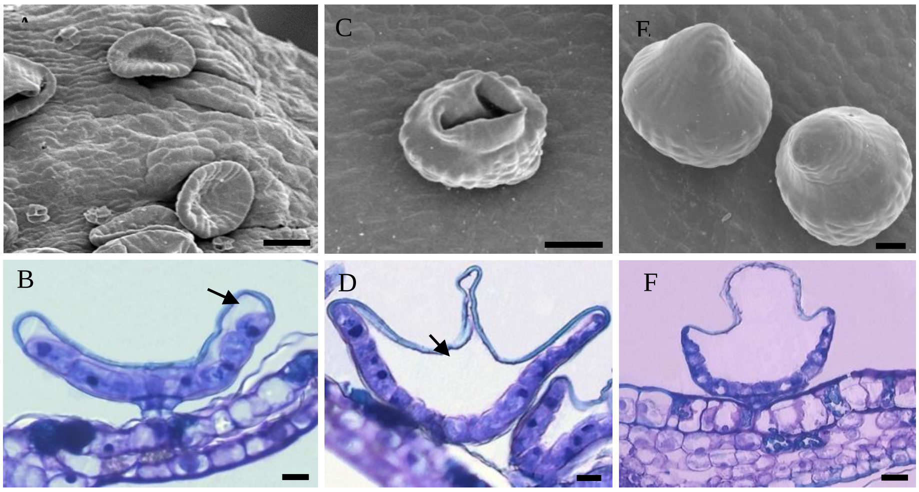

Fig 5. Details of the peltate glandular trichome under SEM (A, C, E) and light microscopy (B, D, F). A, B: Completely formed trichome disc, cup-shaped, with onset of lupulin accumulation (arrow). C, D: Stage of intense biosynthesis and accumulation of lupulin (arrow). E, F: Mature trichome, in bicone shape. Scale bars: A: 50 µm; B: 10 µm; C: 50 µm; D: 10 µm; E: 25 µm; F: 20 µm.

Table 6. Estimates of Pearson's correlations between trichome density in flowers (TDF), trichome density in bracts (TDB), cone length (CL), essential oil content (EO), and total phenolic compound content (PC) of five varieties of *Humulus lupulus* L. grown under conventional and organic cropping.

	TDF	TDB	CL	EO	PC
TDF	1	0.2388	0.1843	0.1029	0.9496*
TDB		1	0.0029	0.8132*	0.0783
CL			1	0.4835	0.6299*
EO				1	0.9226*
PC					1

*: Significant (t test, $p < 0.05$).

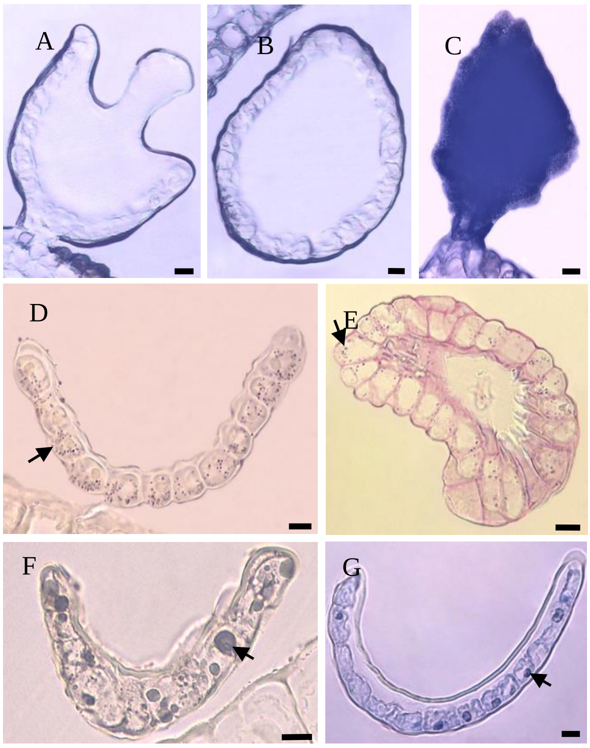

Fig 6. Photomicrographs of peltate glandular trichomes of *Humulus lupulus* L. histochemical analyses. A, B: No reagent. C: Presence of lipophilic substances after reaction with Sudan black B. D: Presence of starch grains (arrow) revealed by the reagent Lugol. E: Presence of polysaccharides after reaction with PAS. F: Presence of phenolic compounds (arrow) revealed by reaction with ferric chloride. G: Presence of proteins (arrow) in bromophenol blue reagent. Scale bars: 10 µm.

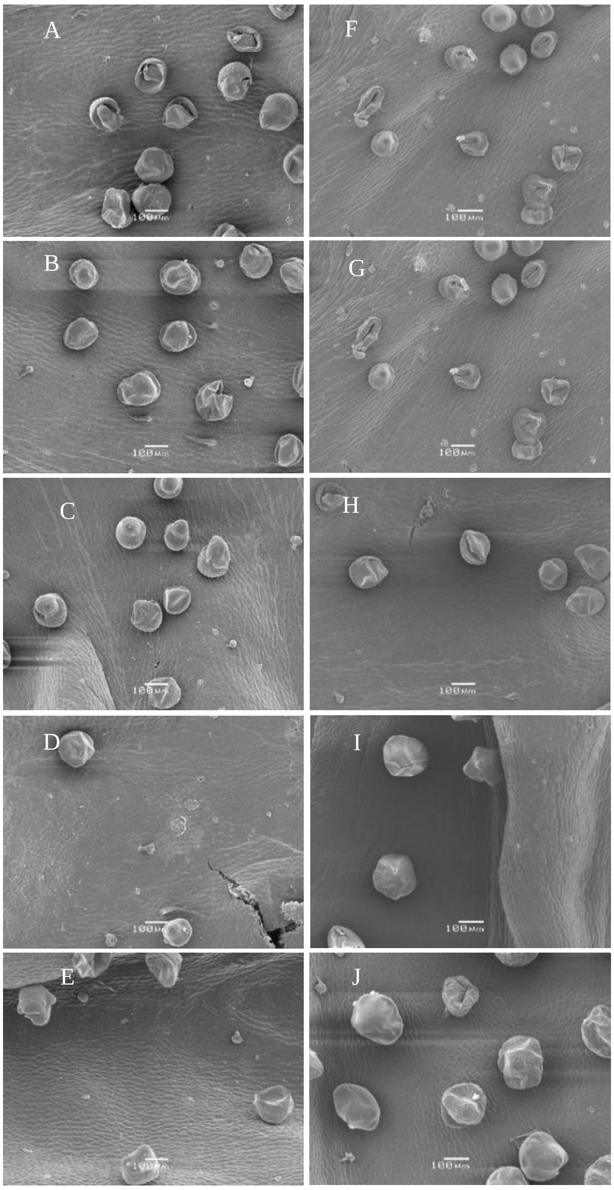

Fig 7. Density of trichomes in bracts of *Humulus lupulus* L. varieties under conventional and organic cropping. A: Conventional 'Cascade'. B: Conventional 'Chinook'. C: Conventional 'Columbus'. D: Conventional 'Hallertau Mittelfrüh'. E: Conventional 'Nugget'. F: Organic 'Cascade'. G: Organic 'Chinook'. H: Organic 'Columbus'. I: Organic 'Hallertau Mittelfrüh'. J: Organic 'Nugget'.

addition, antioxidant activity is attributed to these compounds; due to their ability to act as free radical scavengers, this mechanism plays an important role in human health, nutrition, and medicine (Tang et al., 2020). Thus, hop varieties with high levels of phenolic compounds are of great productive interest.

Although the cropping systems did not have an effect on essential oil or phenolic compound contents, organic cropping can promote an increase in specialized metabolites, as described by Keukeleire et al. (2007) in a study conducted with hop varieties grown in organic and conventional cropping systems and by Solarska et al. (2015), who found significant increases in xanthohumol, flavan-3-ols, and proanthocyanidins in hops grown in organic cropping compared to conventional cropping.

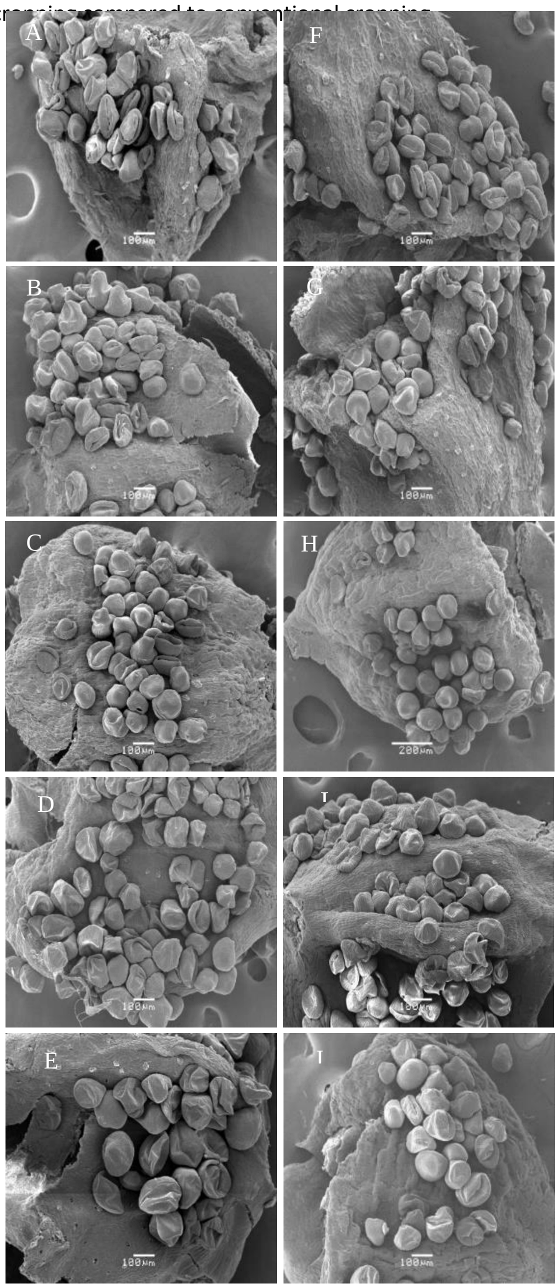

Fig. 8. Density of trichomes in the calyx of flowers of *Humulus lupulus* L. varieties under conventional and organic cropping. A: Conventional 'Cascade'. B: Conventional 'Chinook'. C: Conventional 'Columbus'. D: Conventional 'Hallertau Mittelfrüh'. E: Conventional 'Nugget'. F: Organic 'Cascade', G: Organic 'Chinook, H: Organic 'Columbus', I: Organic 'Hallertau Mittelfrüh', J: Organic 'Nugget'.

Pearson's correlations between trichome density in flowers, trichome density in bracts, cone length, essential oil content, and total phenolic compound content

Based on the tests applied (ANOVA, t test, and Pearson's correlation), the density of trichomes in bracts had a strong positive correlation with essential oil content (r = 0.81), as did the density of trichomes in flowers with the content of phenolic compounds (r = 0.95) (Table 6). This trend is explained by the composition of lupulin secreted by peltate trichomes, which are rich in essential oil and phenolic compounds (Liberatore, et al., 2019).

The essential oil content correlated strongly positively with the content of phenolic compounds (r = 0.92) (Table 6). Essential oils and phenolics share the same metabolic pathway as malonic acid (Taiz and Zeiger, 2016). The experimental conditions may have benefited this pathway, resulting in a positive correlation between these variables.

The cone length showed a strong positive correlation with the content of phenolic compounds (r = 0.63) (Table 6). Since phenolic compounds are found in bracts and in the central axis of the hop inflorescence (Roberts and Wilson, 2006), in addition to the peltate trichomes, the size of the cone will directly influence the amounts of these compounds.

Therefore, the morphological and anatomical characteristics evaluated were associated with production parameters and hop quality.

Materials and methods

Experimental area

The experiment was conducted in the "Didactic Orchard" of the Department of Horticulture belonging to the School of Agronomic Sciences of UNESP in the municipality of Botucatu, São Paulo (22°50′ S, 48°26′ W, and altitude 791 m). According to the Köppen classification, the climate is type *Cfa*, *i.e.*, a humid subtropical climate. During the harvest period (September 2019 - March 2020), the minimum average temperature was 17.94°C, and the maximum average temperature was 28.45°C, with a rainfall of 1257.61 mm. The soil is of the Red Nitosol type, and its chemical characteristics were defined through soil analysis.

Treatments and experimental design

A randomized block experimental design was adopted with a 2 × 5 split-plot, the main factor being the cropping systems, namely, conventional and organic, and the secondary factor being hops varieties: 'Cascade', 'Chinook', 'Columbus', 'Hallertau Mittelfrüh', and 'Nugget'. There were four blocks and four plants used per plot.

Characterization of the crop

For soil preparation, scarification was performed, followed by plowing and harrowing, furrowing, and opening of the pits. The correction and fertilization of the planting soil were performed by means of complete chemical analysis of the soil, following the recommendations established for hops in the international literature (Gingrich et al., 2018). In November 2018, hop seedlings were planted.

The differentiating factors between cropping systems relate to fertilization and phytosanitary control. The organic cropping was established according to the regulations established by Law No. 10.831, 12/23/2003, and the Technical Regulation of Normative Instruction 46, 06/10/2011, complemented by IN 17 of 06/18/2014.

Conventional cultivation

Fertilization was performed according to the needs observed from the soil analysis. In the first harvest, nitrogen topdressing was performed with calcium nitrate and urea, potassium in the form of potassium chloride, and micronutrients in the form of MIB®. For phytosanitary control, abamectin (Abamex®) was applied for mites, fipronil (Regent®) for ants, and tebuconazole (Folicur®) for powdery mildew (*Podosphaera macularis*). In the second harvest, nitrogen fertilization followed by calcium nitrate, urea, conventional poultry litter, potassium fertilization with potassium chloride, and micronutrients with MIB® were also added. Borate fertilization was performed with boric acid and leaf fertilization with zinc sulfate. The phytosanitary control of mites and ants was the same as above, and *Bacillus thuringiensis* (Dipel®) was applied when larvae were seen.

Organic cultivation

Fertilizations were performed according to the needs observed from the soil analysis. In the first harvest for nitrogen fertilization, cattle manure, sunn hemp (*Crotalaria breviflora*) biomass, and castor bean cake were used, and potassium was used with potassium sulfate. For phytosanitary control, sulfocalcic spray was applied for mites, organic formicide (Bioisca®) was applied for ants, and powdery mildew (*P. macularis*) was sprayed with raw milk and Bordeaux mixture. In the second harvest, nitrogen fertilization was performed with Bokashi, plant compost, castor bean cake, organic poultry litter, and biomass of radish (*Raphanus sativus*), sunn hemp (*C. breviflora*), and pigeon pea (*Cajanus cajan*). For potassium fertilization, potassium sulfate was used; for phosphate, Yoorin Master®; for borate acid, borate; and fertilization was also performed with bone meal. SuperMagro biofertilizer was sprayed, and biological activation of the soil was performed with effective microorganisms. *Metarhizium* + *Beauveria bassiana* was applied as a preventive pest control, and sulfocalcic spray was applied for curative mite control, Bioisca® for ants, and Dipel® for caterpillars.

Cultural treatment

An ASI automated drip irrigation system was installed (Medici et al., 2010). The plants were trained in a "V" shape, and the most vigorous branches with a hexagonal shape were selected for guiding on sisal (Peragine, 2011). Unguided branches were pruned. In the interrows of both cropping systems, green manure species were cultivated twice a year and then mowed at the time of floral initiation, and the biomass was deposited as mulching in the rows of the organic cropping. The control of spontaneous plants was performed mechanically, when necessary, by mowing, weeding, and uprooting in both cropping systems.

In the first crop, the cleaning of the leaves of the shoots (up to 1 m in height) of the hop plants was performed as a preventive phytosanitary control due to the appearance of powdery mildew (*P. macularis*). For the 2nd harvest, pruning was performed close to the soil of all the first emergent shoots after dormancy to standardize sprouting, eliminate thieving branches, and favor the emergence of more vigorous branches.

Evaluations

Morphological description and length of cones

Field observations and photographic records of the plant shoots were made to perform the morphological description along with the respective management necessary for the cultivation of the species. Such characteristics refer to the growth habit and morphological aspects of stems, inflorescences, and flowers.

Cone length was taken as the average of 30 samples. For this, 30 cones were lined up end to end next to a tape measure, and the total length was divided by 30.

Identification of trichomes and density of peltate glandular trichome

The trichomes were identified by scanning electron microscope (SEM) analysis. To determine the density of the peltate glandular trichome, the frequency of the structures still attached and the scars left after detachment were computed by scanning electron microscopy. The abaxial surface of the bracts was analyzed at two points, and the abaxial surface of the calyx of the pistillate flowers had an area of 1.26 mm^2. For this purpose, four samples of cones from the second harvest (February – March 2020) were collected from each plot at the point of physiological maturity. The material was fixed in neutral buffered formalin for 48 hours, dehydrated in an increasing ethanol series, and preserved in 70% ethanol. The samples were taken to the Laboratory of Plant Anatomy of the Biosciences Institute of the Federal University of Mato Grosso do Sul (UFMS), Campo Grande Campus, Mato Grosso do Sul, Brazil, for further analysis. The samples were dehydrated in absolute ethanol and then to the critical CO_2 point in a Polaron CPD 7501 device. They were fixed in carbon tape on a metallic support and coated with a thin layer of gold in a Denton Vacuum Table III device. Electron micrographs were taken under a JEOL-JSM 6380 LV scanning electron microscope (SEM) at the Physics Institute of UFMS.

Anatomical and histochemical analysis of peltate glandular trichomes

For anatomical and histochemical analysis of the peltate trichome, the samples underwent the above fixation and dehydration until preservation in 70% ethanol. To continue the analysis, segments of the bracts with the pistillate flowers of the median region of the cones were subjected to serial ethanol dehydration up to absolute alcohol. Then, they were infiltrated in histological resin (Historesin, Leica) and sectioned in a transverse plane (7 µm thick) in a Leica RM 2145 rotary microtome, and the slides were mounted.

For anatomical analysis, the material was stained with 0.05% toluidine blue at pH 6.5. For the identification and localization of compounds, the samples were exposed to the following reagents: periodic acid of Schiff (PAS) for the recognition of neutral polysaccharides (Jensen, 1962), Lugol for starch (Johansen, 1940), Sudan black B for total lipids (Pearse, 1972), 10% ferric chloride solution for phenolic compounds (Johansen, 1940), and bromophenol blue for proteins (Mazia et al., 1953). The results were documented using a MoticamPro 252B digital camera coupled to a Nikon Eclipse Ci light microscope.

Essential oil content

Cones were collected in February – March 2020 and dried in an oven at 35°C until reaching approximately 10% moisture. This material was stored in plastic vacuum bags and kept in a freezer at a temperature below 0°C until extraction. For this purpose, 50 g of cones from each plot was ground in a mill and then subjected to hydrodistillation in a Clevenger apparatus. The plant material was placed in a 2-L flask with 1 L of deionized water, and each cycle lasted 90 minutes after boiling. The essential oil content was determined based on the dry matter according to the following equation:

$$\frac{grams\ of\ oil}{grams\ of\ dry\ matter} \times 100$$

Total phenolic compound content

The total content of phenolic compounds in the ethanolic extract of the cones was determined using the Folin-Ciocalteu spectrophotometric method (Singleton et al., 1999). The dry cones were ground, approximately 0.1 g of the material was measured into a Falcon tube, and 10 mL of 80% ethanol was added. Next, the samples were placed in an ultrasonic bath for 15 minutes, and centrifugation was performed at 3500 rpm for 30 minutes. Aliquots of 0.2 mL of the supernatant were transferred to test tubes, and 0.3 mL of 80% ethanol, 2.5 mL of Folin-Ciocalteu reagent diluted in water 1:10, and 2 mL of 4% sodium carbonate were added. Then, the tubes were left to rest for 2 hours in the dark. Absorbance was measured in a BEL 1105 spectrophotometer at 760 nm. A blank sample was prepared under the same conditions. The results of the total phenolic compounds are expressed in gallic acid equivalents based on their calibration curve.

Statistical analysis

The data obtained were subjected to analysis of variance (ANOVA) to determine the effects of cropping systems, hop varieties, and their interaction. The comparison of the means of both factors was performed by Tukey's test at 5% probability using SISVAR software. To understand the relationships between the five characteristics evaluated, Pearson's correlation was performed using Excel.

Conclusion

The anatomical, micromorphological, and histochemical results refer to the first record of hops trichomes grown in Brazil. Peltate trichomes occur intensely on the abaxial surface of the calyx of hop flowers, *i.e.*, the floral organ makes a marked contribution to the total content of chemical compounds of interest of the inflorescences. The organic cropping was superior to conventional cropping in the promotion of the length of cones as well as the density of trichomes in the flowers in all varieties except 'Nugget'. Among the varieties, 'Cascade' and 'Chinook' had the longest cones. 'Cascade', 'Chinook', and 'Hallertau Mittelfrüh' stood out in the density of peltate trichomes. Therefore, the results indicate that organic cropping and the 'Cascade' and 'Chinook' varieties are more promising under the conditions studied.

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