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Scientia Horticulturae





The effects of chestnut orchard microclimate on burr development

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A B S T R A C T			
Chestnut crop is regaining its fame worldwide with powerful investment perspectives. Unluckily the climate change effects are posing high threat to its cultivation with less available resources and increased production cost both in traditional and specialized orchards. Additionally, the chestnut physiological knowledge is still limited, especially as concern the burr development (<i>i.e.</i> , the economical production target) and its relationship with the environmental parameters. The aim of the present study was to evaluate the seasonal, daily, and hourly burr growth pattern associated to environmental parameters for improving physiological knowledge on this species. The study was carried out in a traditional rainfed sweet chestnut orchard located in the Tuscan-Emilian Apennines (Monterenzio, Italy). The chestnut burr growth was measured, along the entire season, both with a digital calliper and through the use of plant-based sensors (fruit-gauges) that permitted to measure, in real-time, the burr growth pattern. Environmental data were recorded by a weather station placed in the middle of the orchard. Results evidenced a higher burr growth rate, in the last part of the season (from middle-end of August to full fall) while the daily growing pattern was characterized by increased oscillation, along the season, of night-swelling and daily-shrinkage. The night-swelling was found to be influenced by high nocturnal air relative humidity while the daily-shrinkage was influenced by the higher wind speed, solar radiation and vapour pressure deficit. Thus, the burr daily net growth. These results could be promising for the adoption of sustainable (<i>e.g.</i> , late season grass mowing, sprinkler irrigation) and smart practices for improving chestnut management in both traditional and specialized orchards.			

1. Introduction

Chestnut is regaining its importance in the global market with an exponential increase of new hectares planted worldwide (*e.g.*, Portugal, Spain, Chile) every year (Beccaro et al., 2019). Unfortunately, at the same time, due to the climate change effects, areas where chestnut could be easily grown in the past, are nowadays reduced. Thus, it is necessary to start investigating chestnut physiological traits to increase orchards productivity and to cope with the climate change effects. To date, most of the studies relate to the plant physiological traits (*e.g.*, leaf gas exchanges, water relations) and few regard on burr growth physiology comparing to other fruit tree species (Rossi et al., 2022). The fruit (*i.e.*, nut) is the economical production target and its growth represents the integrated result of many physiological processes going on at whole tree level (Morandi et al., 2007). Therefore, if the burr is sufficiently

growing, all the physiological processes at plant level are being efficient, hence the orchard is being appropriately managed (Morandi et al., 2017). Perulli et al. (2020) studied the seasonal chestnut burr growing pattern in two different seasons (dry and mild) but with manual measurements carried out with a digital calliper on a weekly basis. To date, literature is lacking on real-time plant-based data evaluating the burr daily and hourly growth dynamics and trend during the whole season. Furthermore, studies highlighting the burr growth based on its phenological stages and on the environmental conditions have not been carried out yet. Usually fruit growth (*e.g.*, apple, peach) is indeed tightly controlled and influenced by exogenous (environmental), as well as endogenous, factors as light levels, soil water and temperature (Corelli-Grappelli and Lakso, 2004). Improving the knowledge of burr growth dynamics and its relationship with the environment would bring chestnut to be better managed, similarly to the other fruit tree crops and

https://doi.org/10.1016/j.scienta.2023.112183

Received 14 February 2023; Received in revised form 9 May 2023; Accepted 22 May 2023 Available online 26 May 2023

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allowing to optimize resources (*i.e.*, water). The objective of the present study is to determine the seasonal, daily, and hourly burr growth in relation to environmental conditions for improving chestnut physiological knowledge.

2. Material and methods

2.1. Chestnut orchard location and weather conditions

The study was conducted during 2019 season in the Tuscan-Emilian Apennines in Monterenzio (Bologna, Italy), at 500 m elevation (44° 16' N and 11° 24' E), in a mature (150-200 years old), commercial, rainfed sweet chestnut orchard (Castanea sativa Mill.). Trees were, most likely, of the 'Castel del Rio' ecotype ('Marrone type') grafted on seedling rootstocks. This ecotype is characterized by a medium tree vigour, an expanded canopy, medium productivity (Breviglieri, 1955; Bagnaresi et al., 1977; Mellano et al., 2012) and with burr typically containing 3 nuts for each burr (Fideghelli et al., 2016). Full bloom occurred in the middle of June (June 12th) while full burr falls on October 20th, corresponding to 120 days after full bloom (DAFB). Air temperature (T), air relative humidity (RH), solar radiation (PAR), wind speed (WS) and pluviometry (P) data were collected from a weather station (Wi-Net s.r. l., Cesena, Italy) placed in the middle of the orchard. Data were collected, at 15 min intervals, during the whole season. From these data, vapour pressure deficit (VPD) was also calculated.

2.2. Burr diameter-weight conversion equation

A total of 15 burrs, randomly picked every ten days along the whole season, was used to obtain the following conversion equation between burr diameter (D; mm) and burr fresh weight (W; g):

$$W = aD^2 - bD + c$$

where a, b and c were 0.019, 0.260 and 1.562, respectively. The coefficient of determination (R^2) of the relationship was > 0.99, as showed also in Fig. 1.

2.3. Burr seasonal measurement

The maximum equatorial diameter of 25 burrs (5 burrs tree⁻¹) was measured weekly throughout the growing season (n=14), using a digital calliper (Calibit, HK-Horticultural Knowledge s.r.l., Bologna, Italy). Measurements started 14 days after full bloom (DAFB) and ended some days before the beginning of burr fall, at burr valves still completely closed (108 DAFB). Burrs diameter data (mm) were then converted to fresh weight (g) using the equation in Fig. 1. The burr absolute growth rate, both in diameter (AGR_d, mm day⁻¹) and in weight (AGR_w, g day⁻¹), was then calculated as follows: AGR_{d,w} = (P_{t1} - P_{t0})/(t1 - t0); where P_{t1} and P_{t0} are the burr parameters (*i.e.*, diameter or weight) measured on a given day of year (t1) and on the previous sampling date (t0), respectively (Perulli et al., 2020).



2.4. Burr hourly and daily measurements

Burr diameter variations were measured constantly (excluding the days with technical problems *i.e.*, 21–27, 37, 38, 51, 62, 71, 72, 83–87 DAFB), along the season (15–112 DAFB), at 15 min intervals on four burrs (2 gauges tree⁻¹), using custom-built gauges interfaced to a wireless data-logger system provided with a general packet radio service (GPRS) modem for a real time cloud data monitoring (Wi-Net s.r.l., Cesena, Italy) (Fig. 2). The gauges consisted of a light, stainless steel frame supporting a variable linear resistance transducer (Megatron Elektronik AG & Co., Munchen, Germany). Temperature effects on the frame and the sensor were tested and showed negligible errors under normal field conditions (Morandi et al., 2007).

The monitored burrs were homogeneous in size and position in the canopy. At each recording time, diameter data (mm) from all monitored burrs were converted to fresh weight (g) using the equation in Fig. 1. For each recording time (15 min intervals during 24 h), data from the four burrs were hourly averaged and standard deviation calculated.

Burr net daily growth (BNG; g burr d^{-1}), mean hourly AGR (AGR_h. mean: mg burr h⁻¹), maximum hourly AGR (AGR_{h max}; mg burr h⁻¹), minimum hourly AGR (AGR_{h min}; mg burr h^{-1}), and burr daily shrinkage (SHR; mg burr h^{-1}) were also calculated. BNG was calculated as the sum of the hourly recorded instantaneous AGR during the 24 h. Maximum AGR and minimum AGR were the maximum and minimum instantaneous burr growth rates recorded during the 24 h, respectively. The absolute growth rate (AGR, mg day⁻¹) of the burrs was then calculated as follows: AGR = $(W_{t1} - W_{t0})/(t1 - t0)$; where W_{t1} and W_{t0} were the burr weights measured on a given hour of day (t1) and on the previous recorded hour (t0), respectively. Burr daily shrinkage (SHR) was calculated as the difference between the maximum and the minimum AGR (AGR $_{h,max}$ - AGR $_{h,min}$) recorded in the 24h. BNG daily pattern was represented throughout the whole season selecting the days characterized by homogeneous weather conditions (e.g., similar PAR) and distant at least four days by the last rain event.

2.5. Statistical analysis

Principal Components Analysis (PCA) were performed to highlight which, among the considered factors, appeared to be more related to burr hourly and daily growth in different burr phenological stages from 15 to 112 DAFB (July, August, September, October). Both the PCA were performed with statistical R software (www.rproject), R version 4.1.2 (2021-11-01), utilizing "FactoMineR" and "factoextra" packages, respectively for data analysis and data visualization. Data were standardized and scaled before the analysis to make variables comparable and eigenvalues were calculated and ordered to determine the number of principal components to be considered in PCA. Vectors indicate the increase of the factors in a certain direction while points, based on their spatial position, indicate their relation to the factor. Hourly data of AGR,



Fig. 2. Gauge for the continuous monitoring of burr growth of sweet chestnut at 15 DAFB.

T, P, RH, VPD, WS and PAR and daily data of BNG, T, P, RH, VPD, WS, PAR and SHR, were analyzed along the entire burr development (from 15 to 112 DAFB).

3. Results

3.1. Weather conditions

During the burr development period (July-October), the total rainfalls was 140 mm. July and October were the month with the highest cumulated precipitations recorded (52.0 mm) while August the one with the lower amount (17.0 mm) (Fig. 3). Mean daily temperatures (T) were overall mild, with 23.5, 24.1, 18.5 and 14.8 $^{\circ}$ C registered in July, August, September and October, respectively. The mean air relative humidity and VPD were 64% and 1.8 kPa in July-August and 77% and 1.4 kPa in September-October, respectively.

3.2. Seasonal patterns of burr development

Chestnut burr diameter grew almost steady and linearly throughout the growing season till reaching 52.6 mm at 108 DAFB (Fig. 4a), while its fresh weight showed an almost constant growth till 56 DAFB (12.4 g) when burr growth rapidly increased till burr fall, with a final burr fresh weight of 40.7 g (Fig. 4a).

Between 23 and 37 DAFB, burr had the highest AGR_d of the season, reaching a peak of 0.74 mm burr day^{-1} at 37 DAFB, when its values started to sharply decrease to 0.38 mm burr day^{-1} at 51 DAFB (Fig. 4b). From this date, AGR_d slowly increased till 66 DAFB when its values progressively decreased till 94 DAFB when a peak of 0.44 mm burr day^{-1} was registered. At 101 DAFB, AGR_d registered its minimum value (0.17 mm burr day^{-1}) along the entire season, followed by a sharp AGR_d increase in the last burr measurement (108 DAFB).

The AGR_w increased sharply till 37 DAFB when a peak of 0.40 g burr day⁻¹ was registered (Fig. 4b). Except a rapid decrease between 37 and 42 DAFB, AGR_w progressively increased along the whole season till 94 DAFB, when it was reached the highest seasonal value of AGR_w (0.70 g burr day⁻¹). Afterwards the AGR_w sharply decreased to 0.30 g burr day⁻¹ (101 DAFB), reaching 0.50 g burr day⁻¹ at the end of the season (108 DAFB).

3.3. Hourly burr absolute growth rate and environmental parameters

The PCA evidenced that the Principal Component 1 (PC1) explained 47.8% of the variance of the results and Principal Component 2 (PC2) explained 17.7%. Night hours, especially around 22:00 (pink dots), where the main contributors to hourly AGR, while first afternoon hours (*i.e.*, 13:00; light-blue dots) contributed, with VPD, T, WS and PAR,

against the AGR (Fig. 4). Furthermore, the AGR was positively related to RH, showing the highest contribution especially in the early morning (7:00–9:00; green dots).

During the season the AGR_{h med} registered the lower values in July (8.42 mg burr h⁻¹) and August (9.67 mg burr h⁻¹) while rapidly increased, almost doubling the values, in September (17.3 mg burr h⁻¹) and October (15.7 mg burr h⁻¹) (Table 1). The AGR_{h max} instead progressively increased from July to October, reaching its maximum in September (106 mg burr h⁻¹). The AGR_{h min} registered the more negative values in October (-94.5 mg burr h⁻¹) while the less negative one in July (-22.1 mg burr h⁻¹). Burr had the highest shrinkages in September (199 mg burr h⁻¹) and October (185 mg burr h⁻¹) but still maintaining a high shrinkage, compared to July (67.1 mg burr h⁻¹), also in August (153 mg burr h⁻¹). The burr daily net gain was lower than 250 mg burr day⁻¹ both in July and August while almost doubled in September (411 mg burr day⁻¹) and October (377 mg burr day⁻¹) (Table 1).

3.4. Daily burr growth dynamics

Burr showed variations in the cumulated net growth during the 24 h depending on the phenological stage (Fig. 6), with periods of rapid weight gain, usually recorded in the late evening/ night, followed by periods of reduced or even negative growth, between the midday and late afternoon (Fig. 6). At 16 DAFB, hourly variations in burr cumulated net growth were limited, with almost steady conditions during the whole day and with a limited net gain at the end of the day (0.06 g burr day⁻¹). As the burr developed, hourly variations in burr weight were wider, with more evident shrinkages in the early afternoon and swelling during the night (Fig. 6). Together with the increase of the burr shrinkage, the burr net daily growth increased, especially in the last period, with a daily net gain of about 0.5 g burr day⁻¹ (Fig. 6).

3.5. Daily burr net growth and environmental parameters

The PCA evidenced that the Principal Component 1 (PC1) explained 48.3% of the variance of the results and Principal Component 2 (PC2) explained 18.9% (Fig. 7). In July (red ellipse) and August (green ellipse) the main contributors (all grouped together) positively correlated to BNG, were VPD, PAR and T, while P and RH were negatively related to BNG (Fig. 7). In September (light-blue ellipse) and October (violet ellipse), the main variable positively contributing to BNG, were RH and P (Fig. 7). WS and SHR were the only two contributors explaining the PC2 (Fig. 7).

4. Discussion

From the burr diametral measurement, the recorded seasonal steady



Fig. 3. Weather conditions from 14 DAFB (4 July 2019) to 112 DAFB (10 October 2019).



Fig. 4. (a) Seasonal pattern of burr diameter (mm) and burr weight (g) (mean \pm standard deviation). (b) Seasonal pattern of burr absolute growth rate in diameter (mm day⁻¹) and weight (mm day⁻¹) (mean \pm standard deviation).

Table 1

Monthly mean (\pm SD) values of hourly mean burr absolute growth rate (AGR_{h_mean}), minimum burr absolute growth rate (AGR_{h_min}), maximum burr absolute growth rate (AGR_{h_max}), burr shrinkage (SHR) and daily burr net growth (BNG).

Parameter	AGR _{h med}	AGR _{h max}	AGR _{h min}	SHR	BNG
Month	(mg burr b ⁻¹ $)$	$(mg hurr h^{-1})$	$(mg hurr h^{-1})$	(mg hurr h ⁻¹ $)$	$(mq dav^{-1})$
July (15-41 DAFB)	$\frac{(119001111)}{842 + 529}$	$\frac{(110001111)}{450+232}$	-221 ± 165	$\frac{(110001111)}{671+373}$	$\frac{(110 \text{ day})}{210 + 124}$
August (42-70 DAFB)	9.67 ± 4.61	45.0 ± 25.2 88.5 ± 20.3	-65.0 ± 22.5	153 ± 37.0	246 ± 99.0
September (73-102 DAFB)	17.3 ± 8.27	106 ± 40.4	-93.3 ± 48.8	199 ± 82.9	411 ± 199
October (103-112 DAFB)	15.7 ± 7.36	90.7 ± 36.0	-94.5 ± 56.1	185 ± 82.1	377 ± 177

growth was confirmed also by Perulli et al. (2020) in the same sweet chestnut orchard in 2018 (Fig. 4a). A different behavior was instead showed by burr fresh weight where a curve increase was registered at 56

DAFB (15/8) (Fig. 4a). This was verified by the AGR_w that, contrary to AGR_d , started to rapidly increase at 56 DAFB till almost the burr fall, where the reduced AGR_w could be likely inferred to the nut pericarp and



Fig. 5. Principal component analysis (PCA) that describes the behavior of burr hourly absolute growth rate (AGR; mg burr h^{-1}) and hourly environmental parameters along the whole season (15-112 DAFB). Vectors represent the AGR and the environmental parameters while the coloured points represent the hour of the day in 24 hours. The contribution of a variable to the principal components is in gradient colour from -4 (black) to + 4 (light blue). The following abbreviations have been used: mean burr absolute growth rate (AGR), mean temperature (T), precipitation (P), air relative humidity (RH), vapour pressure deficit (VPD), wind speed (WS) and photosynthetic active radiation (PAR).



Fig. 6. Cumulative pattern of burr growth (g burr day⁻¹), over 24 hours in different phenological phases of burr development. Days (16, 34, 47, 59, 68, 77, 89, 103 DAFB) were chosen for the similar weather conditions. Lines were coloured in green, yellow, orange and red palettes, based on the related month: July, August, September and October, respectively. Each line represents the mean of four burrs.

integument hardening and browning, that normally takes place in that period (personal communication) (Fig. 4b). The AGR_d instead showed the highest values at the beginning of burr development while progressively reducing its diametral growth rate along the season. A similar AGR_d decreasing pattern was showed, in two consecutive seasons (2017 and 2018), also by Perulli et al. 2020. The different pattern of AGR_d and AGR_w, highlight that in the first part of burr development, burr is enlarging its size, likely being in cell division stage, while from 56 DAFB, burr start to expand and to accumulate starch in the nuts, thus explaining its rapid increase in fresh weight (Chen et al., 2017). A similar behavior was also documented for other fruits (*e.g.*, peaches) that appear to slow their diametral growth towards harvest although still growing at their maximum rate when fresh weight is considered (Corelli-Grappadelli et al., 2004).

These data were confirmed also by the real-time data acquired with fruit gauges. Indeed, the AGR_{h,mean} almost double its values in the month of September (17.3 mg burr h⁻¹) and October (15.7 mg burr h⁻¹) compared to July (8.42 mg burr h⁻¹) and August (9.67 mg burr h⁻¹), indicating an enhancement of the burr development in these last growing stages (Table 1).



Fig. 7. Principal component analysis (PCA) that describes the behavior of daily burr net growth (BNG; mg burr day⁻¹) and daily environmental parameters for each month (July, August, September and October). Vectors represent the BNG and the environmental parameters while the coloured points indicate the belonging month. The contribution of a variable to the principal components is in gradient colour from -5 (black) to + 5 (light blue). The following abbreviations have been used: burr net growth (BNG), mean temperature (T), precipitation (P), air relative humidity (RH), vapour pressure deficit (VPD), wind speed (WS), burr shrinkage (SHR) and photosynthetic active radiation (PAR).

At daily scale, hourly variations in burr growth, showed how burr typically swells during the late afternoon and night and shrinks around midday-early afternoon, almost independently of the burr developmental stage (Fig. 6). The night swelling responded to environmental changes (Fig. 5): nocturnal atmosphere humidity was able to positively influence burr rehydration (Fig. 5). Although that, burr swelling was probably also related to the cease of burr transpiration rate and likely favoured by the daily accumulation of osmotic active carbohydrates enhancing, in the night-time, burr turgor pressure, as occurs in peach fruit (Morandi et al., 2010).

During the day, burr was characterized by a strong shrinkage in the early afternoon when the highest values of WS, VPD, T and PAR were recorded. These environmental conditions likely enhanced the burr transpiration, by the skin surface and by the large amounts of burr live spines, with likely xylem inflows not anymore able to match the instantaneous rates of burr water losses (Fig. 5). A similar process was displayed by peach fruit, in which fruit daily diameter reduction was related to skin transpiration losses, enhanced by high VPD (Morandi et al., 2010).

At higher AGR_{h mean} corresponded also higher burr net gain in weight (BNG) at the end of the day (Table 1). This is especially true in the month of September (0.41 g burr d^{-1}) and October (0.38 g burr d^{-1}) as the nut growing inside the burr is developing (Chen et al., 2017). Together with an increment of daily burr net gain, also the burr shrinkage-swelling pattern get intensified during the season, despite the increasingly lower VPD recorded in September and October (Fig. 6). This could be likely related to both the increased burr surface conductance (data not shown) and burr surface area. Furthermore, the high levels of starch, as an osmotically inactive carbohydrate, in the last phases of nut development, could intensify the burr daily water losses (Chen et al., 2017). Even if the phloem and xylem contributions were not assessed in this study, burr exhibited the typical passive fruit growth mechanism, as in peach (Morandi et al., 2007), with a likely still fully functional xylem until full fall. In this mechanism, the large quantities of water losses through the fruit epidermis are the base for achieving the accumulation of soluble solids in the fruit and thus for enhancing its net growth (Morandi et al., 2010).

The BNG, like the hourly AGR, was highly influenced by the external environmental parameters in the different burr phenological stages, with T, VPD and PAR playing a positive role for BNG in July and August (Fig. 7). The direct PAR stimulation on BNG, could be hypothesized to be related to a photosynthetic active process occurring also at the burr level (*i.e.*, burr is feeding itself), in a stage when the burr carbohydrate demanding is still low. These environmental-related positive effects on BNG were probably achieved as the registered mean air temperatures in July (23.5 °C) and August (24 °C) and related VPD values were not limiting plant physiological performances (Almeida et al., 2007).

As the season progressed, in September and October the main parameters positively influencing the burr net growth were instead precipitations and especially the air relative humidity (Fig. 7). Concerning the former, literature reported that rains occurring at the end of the season were influencing positively the burr growth (Perulli et al., 2020) and the nut yield (Gomes-Laranjo et al., 2007; Mota et al., 2018). As for the latter (RH), instead, literature data are still missing. The RH positive effect on BNG could be explained by an improvement of plant physiological performances (e.g., higher carbon fixation) when chestnut trees are subjected to high RH values, although a direct RH effect on the burr growth mechanism should not be neglected (Figs. 5 and 7). It is known that a suitable atmospheric humidity is among the major factor influencing chestnut physiological performances (Araujo-Alves et al., 1993; Perulli et al., 2022). Indeed, September and October are the most sensitive month for burr growth, as coincide with the nut endosperm filling, with a rapid increase in nut fresh weight (Mota et al. 2018b). In this phenological stage, burr represents a strong carbohydrate sink for the plant, that if not properly directed to the fruit could negatively penalize chestnut yield and quality.

5. Conclusion

These results helped to characterize the burr seasonal, daily and hourly pattern in a sweet chestnut cultivar. Burr showed to have and maintain higher burr growing rate in the last part of the season (from middle-end of August to full fall). The daily burr growing rate was characterized by progressively increased oscillation, along the season, of night-swelling and daily-shrinkage. The night-swelling was found to be highly correlated to the nocturnal air relative humidity while the dailyshrinkage was correlated to wind speed, PAR, temperature and VPD. The burr daily net growth was highly associated, depending on the phenological stages, to environmental parameters. Precipitation but especially the atmosphere humidity, in September and October, were the main microclimate drivers of burr daily growth. These data show that burr growing strategies change based on the phenological stages and on the environmental conditions. Based on these results, we conclude that, atmosphere humidity plays a fundamental role on burr growth (*i.e.*, nut) especially in its last development stages (September and October), suggesting rational orchard management practices as a late season grass mowing for preserving, as long as possible, a higher atmosphere relative humidity in the chestnut microclimate. For those orchards that possess enough water availability to irrigate, an under-canopy sprinkler irrigation could be likely the best irrigation management solution to rationally increase orchard air relative humidity and thus to enhance burr net growth and thus orchard productivity. These practices, together with the adoption of plant-based sensor (e.g., fruit gouges), could help to precisely manage, in real time, irrigation application based on the burr daily growth rate responses. These strategies would better improve chestnut orchard management and counteract the climate change effects.

CRediT authorship contribution statement

Giulio Demetrio Perulli: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. Alexandra Boini: Investigation, Writing – review & editing. Brunella Morandi: Investigation, Supervision, Validation, Writing – review & editing. Luca Corelli Grappadelli: Investigation, Supervision, Writing – review & editing. Luigi Manfrini: Conceptualization, Formal analysis, Investigation, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2023.112183.

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