

## Wettability and interception in relationship with the seasonal changes on the *Fagus sylvatica* leaf surface

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**Abstract.** Interception is the amount of water held on the canopy at the end of a rainfall event. Rainfall interception and contact angle of raindrops on the surface of plants has a significant meaning in ecohydrology. Leaves are the plant organs in which during development, changes in the composition of the epicuticular wax can be observed. These differences can be explained by phenological changes.

In the present study, there was a hypothesis that seasonal phenological changes of leaf surface can highly affect the amount of rainwater retained by plants (interception) and the angle of contact between the droplets and leaf's surface.

This above-mentioned hypothesis was assessed based on the designed measurement series, combining:

- 1) direct leaves spraying in various stages of growth with water at a constant temperature
- 2) images obtained by scanning electron microscope (SEM) to analyse changes in the structure of the epicuticular wax
- 3) photographic methods, images acquired in the light box
- 4) measurement and analysis of the angle of contact by using simulated raindrops.

The leaves of *Fagus sylvatica* L. were analysed. Samples were taken in the Niepołomice Forest District (southern Poland) from well-developed crown trees.

The result of the experiments conducted makes a database of changes in wettability of raindrops on beech leaves throughout the whole vegetative season. The internal slope of drops ranged from 110°–150° in April up to 20°–40° at the beginning of November.

Based on the obtained results, we can classify the degrees of leaf wettability and interception under the influence of morphological changes occurring during the vegetative season.

**Key words:** contact angle, cuticular waxes, ecohydrology, hydrophobicity, scanning electron microscopy (SEM)

## Introduction

Studies on wettability in the context of changes on the leaf surface provide a practical use of the knowledge on changeable surface properties of leaves during the growing season to quantify the amount of water which can be retained on the surface during rainfall events.

Retention process of the tree crown is understood as the retention of precipitation (Holder 2013), which reduces or delays the amount of water reaching the forest cover (Nanko 2011). This rainy interception cannot be ignored in equation describing the water balance of the atmosphere (Klamerus-Iwan 2014, Sadeghi et al., 2014). Researchers have established the interception at 10–50% of the total precipitation,

although this estimate corresponds to the range between 0.2 and 8.3 mm (Gash et al., 1995, Klaassen et al., 1998, Xiao et al., 2000, Chang 2006). We should focus on factors that can increase or decrease them (Holder 2012).

Factors affecting the magnitude of interception are related to: the characteristics of precipitation – intensity and size of rain drops (Nanko 2011), meteorological factors – ambient temperature, wind, general humidity (Keim et al., 2004, Holder 2013, Klamerus-Iwan 2014) and plant factors such as the size of leaves, surface characteristics, physico-chemical characteristics of chemical compounds covering leaves (Fernandez et al., 2013) and seasonal variations in terms of these characteristics as well. Therefore, interception is a function of rainfall intensity and factors related to the condition of leaves'

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surface. The literature review summarizing the issues on leaf water repellency from ecohydrological viewpoint, was conducted by Rosado and Holder (2013) and Holder (2013).

Water repellency is mainly caused by epicuticular wax crystalloids that cover the cuticular surface (Pandey and Nagar 2003; Roth-Nebelsick et al., 2012; Urrego-Pereira et al., 2013). Interactions between the surface of leaves and the amount of water retained play an important role in ecohydrological processes (Limm et al., 2009; Adamec 2013 and Berry et al., 2014); however, the mechanism for water capturing and water drops adhesion is not fully understood (Fernandez and Eichert 2009; Burkhardt and Hunsche 2013). Wettability and water absorption exemplified by the oak were presented by Fernandez et al. (2014), while Victor and Erb (2010) focused on the studies of seasonal changes in *Populus tremuloides*.

Chemical composition of plant epicuticular layers protecting leaves against water loss, air pollutants and pathogenic organisms changes during the vegetation period and constitutes adaptation to life under the existing conditions in changing seasons (Pallardy 2008). The structure of wax layer responsible for drop adhesion is associated with chemical changes occurring during their development, especially in senescent leaves (Barthlott et al., 2003). The wax content in the leaves differs within the range of trace quantities up to about 15% of dry weight.

It depends on such factors as: plant species, genotype, leaf age and environmental conditions. For instance, *Fraxinus americana* leaves are characterized by a thin layer of waxes, while *Acer saccharum* leaves not only have a much thicker wax layer, but also numerous stomata are occluded by wax crystals (Pallardy 2008). The amount of wax in the cuticle of leaves per unit leaves area is much smaller in young leaves and the amount of wax generally decreases during leaf senescence (Janick 1999). Gulz and Muller (1992) presented the seasonal changes in the composition of hydrocarbons, wax esters, aldehydes, alcohols and fatty acids in common oak leaves.

The objective of this study was to investigate whether interception and degree of wettability are variable characteristics during the vegetation period.

We also used electron microscopy to characterize the surface, subsurface areas, chemical composition and structure of plant materials. The analysis of epicuticular wax and cuticle using SEM technique in the context of hydrophobic properties, was performed by Koch and Barthlott (2009), Khayet and Fernandez (2012) and Fernandez et al. (2014). Differences between species in terms of wettability were studied by Nanko et al. (2013) and Dorr et al. (2015). Studies with similar methodology and terminology used to describe the parameters of a drop and contact angle with leaves' surface were presented by Nanko et al. (2013), Rosado and Holder (2013) and Owsiak et al. (2013).

## Methodology

### Study area and general methodological assumptions

Leaves of common beech (*Fagus sylvatica* L.) were analysed. Samples were collected in the area of Niepołomice For-

est District (20°19'37,26"E; 50°1'36"W). The average annual temperature was 8.2°C and the average annual amount of precipitation was 650 mm. This was a fresh mixed forest habitat at loamy sand. Three trees with properly developed crown were selected: diameter at breast height ( $d$ ) was  $d = 21.2$  cm,  $d = 25.1$  cm and  $d = 35.1$  cm. Twigs were collected with telescopic tree pruner. In each month, 6 twigs were collected in different locations of the crown to adjust for the effect of sunlight exposure and variability of leaves in terms of properties and thickness of layers of chemical compounds covering the leaves. The entire research methodology was repeated between 15<sup>th</sup> and 20<sup>th</sup> day of each month from April to November. Twigs that had more than one meter, were protected from drying by waxing the cutting area and were transported to the laboratory in plastic containers. All experiments were performed immediately after transportation to the laboratory.

### Measurement of wettability of leaves

Above individual leaves, condensation of water from the sprinkler nozzle of a diameter of 0.45 mm was proceeded. Simulated spraying was carried out at 22°C ( $\pm 1^\circ\text{C}$ ) under laboratory conditions. Water drops condensation was carried on until 50 images of drops were generated and further used to analyse the contact angle between the drop and the adaxial surface of the leaf. To complete the photographic documentation, Canon Eos 450D camera with EF 100 mm f/2.8 Macro USM lens was used. Internal contact angle ( $\alpha$ ) between a drop and a leaf was taken into account, which is schematically presented in Fig. 1. The measurements of contact angles ( $\alpha$ ) of drops was performed using Sigma Scan v5 program.

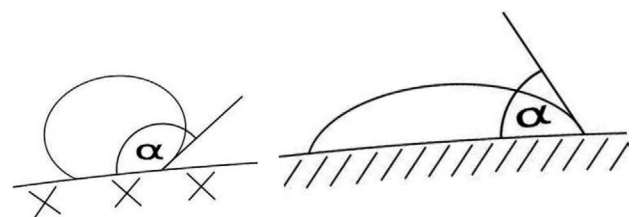


Figure 1. Scheme of contact angles ( $\alpha$ ) between a drop and a leaf

The contact angle of drops to the leaf surface indicates the degree of its wettability. When determining the range of results obtained, we used the classification proposed by Aryal and Neuner (2010).

### Measurements of interception

Under laboratory condition, the amount of water that can be retained on twigs of trees, after single and simulated precipitation, was determined. For the study of interception, 25–30 cm twigs were used. The collected twigs were weighed and further subjected to a simulation with a constant dose of precipitation followed by re-weighting under wet condition.

Twigs were weighed with a weight of up to 1000 g (Radwag PS1000/C/2) giving a result with accuracy up to 0.001 g. The amount of water retained, that is rainfall interception, was calculated taking into account the difference in weight of twigs before and after spraying. A continuous dose of distilled water (P) was established at 5.0 g. Based on the amount of water retained on twigs and treating the full dose of spraying as 100% precipitation, the proportion of water retained after a single precipitation was established. Weighed with a weight of up to 1000 g, giving a result with accuracy 0.001 g.

$$\text{Int [g]} = \text{mg}_2[\text{g}] - \text{mg}_1[\text{g}]$$

$$\text{Int [\%]} = (\text{Int [g]} * 100\%) / P [\text{g}]$$

P[g] = 5 g – constant amount of simulated rainfall

Int – rainfall interception

mg<sub>1</sub> – the mass of branches before spraying [g]

mg<sub>2</sub> – the mass of branches after spraying [g]

Twigs were sprayed from a fixed distance using water at 21°C to simulate real conditions for which the temperature of rainwater is about 1 degree lower than the air temperature in the environment (Woś 1996). Setting twigs during spraying was similar to their natural orientation on a tree.

### Recovery of images from electron microscopy

From a fraction of leaves, samples were collected and analysed using electron microscopy. The JEOL JSM5410 scanning electron microscope was used. Images were obtained by electron beam striking the sample.

Fragments of leaves were dried and fixed followed by spraying with gold. For such prepared samples, a series of images of leaves' surface were produced at different magnification ranges.

### Statistical analysis

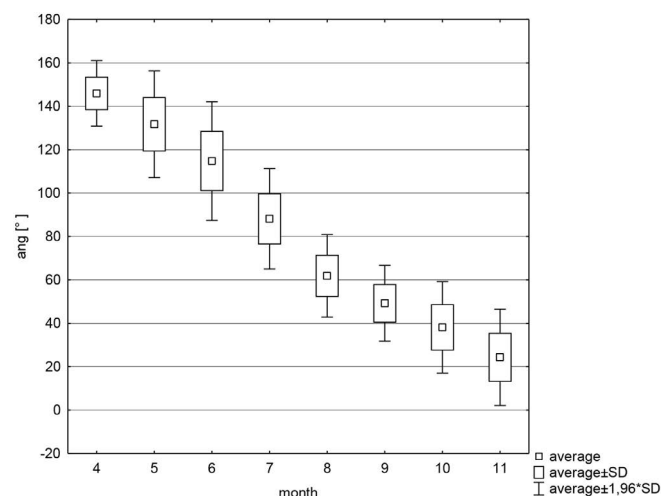
Dataset containing 50 measurements of interception and 50 measurements of contact angles between drops and the surface of leaves were obtained from April to November in each month. Differences in terms of mean values between the interception and contact angle in individual months, were evaluated using the nonparametric Kruskal-Wallis test. The statistical significance of the results was verified at the significance level of  $\alpha = 0.05$ . The analysis was performed using Statistica v10.

## Results

### Rain wettability

As a result of experiments, we generated a database containing changes in the rain adhesiveness of water drops on beech leaves throughout the whole growing season. Box-and-whisker plots presented the ranges of variation of contact angles between the drops and beech leaves in particular months (Fig. 2). The graph was plotted by measuring 50 contact

angles between the drops and leaves in each out of 8 months under study. All contact angles specifying the degree of wettability express the highest value in April decreasing in the course of subsequent months of the growing season.



**Figure 2.** Seasonal changes in contact angles of drops on the adaxial side of leaf

Comparing the average values with Aryal and Neuner classification, the following classes of wettability were obtained (Table 1).

**Table 1.** Wettability class achieved in individual months

Month	Mean ang [°]	Grades of hydrophobicity
IV	145.94	Highly non-wettable
V	131.69	Highly non-wettable
VI	114.76	Non-wettable
VII	88.14	Highly wettable
VIII	61.88	Highly wettable
IX	49.2	Highly wettable
X	38.12	Superhydrophilic
XI	24.33	Superhydrophilic

There are no ranges of contact angles within 90–100° identifying good wettability. Between June and July, we can observe a change within one class of wettability. Moreover, no superhydrophobic class denoting almost complete absence of adhesion of drops to leaves' surface, was reported. Kruskal-Wallis test was used to check whether there are significant differences between consecutive months and values of the contact angle (Table 2).

**Table 2.** The p-value for multiple comparisons for different values of contact angle [ $^{\circ}$ ] in different months

ang [°]	4	5	6	7	8	9	10	11
4								
5	1.000							
6	0.007	1.000						
7	<0.001	0.001	0.319					
8	<0.001	<0.001	<0.001	0.359				
9	<0.001	<0.001	<0.001	<0.001	1.000			
10	<0.001	<0.001	<0.001	<0.001	<0.01	1.000		
11	<0.001	<0.001	<0.001	<0.001	<0.001	<0.01	1.000	

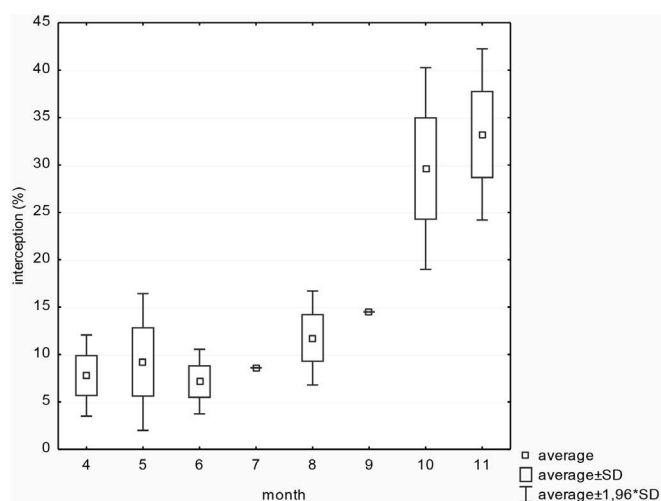
## Interception

The obtained proportion values of water retained on the surface of twigs after single and simulated spraying were presented as box-and-whisker plots (Fig. 3).

The values of interception increase from April (starting from 7.5%) to November (up to 33%). We can distinguish three stages of interception change on twigs. For the first four months, (April, May, June and July), we can observe a stable phase at the level of 7.5–8.7% of the amount of water retained from the total precipitation. We report an increase in May up to the value of 9.2%. August and September are characterized by the increase in the value of interception to an average value of 14.5% of the total simulated precipitation. In July and September, the amounts of retained water are characterized by extremely low range of variability.

In October and November, we observe a rapid increase in the capability to retain water through leaves. The level of interception is estimated at 33.2% on average in the last month analysed.

The Kruskal-Wallis test was used to check whether there are significant differences between consecutive months and the interception values (Table 3).



**Figure 3.** Seasonal changes of interception [%]

Correlations between the obtained values are shown in Table 4. Senescence of leaves expressed by consecutive months of the vegetation period is strongly positively correlated with the values of interception and inversely correlat-

**Table 3.** *P*-value for multiple comparisons for different values of interception [%] in different months

int [%]	4	5	6	7	8	9	10	11
4								
5	1.000							
6	1.000	0.320						
7	1.000	1.000	0.561					
8	<0.001	0.090	<0.001	<0.05				
9	<0.001	<0.001	<0.001	<0.001	0.247			
10	<0.001	<0.001	<0.001	<0.001	<0.001	<0.01		
11	<0.001	<0.001	<0.001	<0.001	<0.001	<0.01	1.000	

ed with the contact angle between drops and the surface. This confirms the hypothesis on the impact of seasonal changes on both parameters analysed.

**Table 4.** Correlations between the contact angle, interception and months on leaves sample collection ( $p < 0.05^*$ ;  $p < 0.01^{**}$ ;  $p < 0.001^{***}$ )

	month	ang	int
month	1.00	-0.96*	0.83*
ang		1.00	-0.75*
int			1.00

### Images of leaves surface

A set of images from the scanning electron microscope was generated. In Fig. 4 we can observe typical hairs on beech leaves collected in May.

The values of interception and wettability of leaves' surface referred to changes on the surface of leaves in the following months; therefore, it is important to present

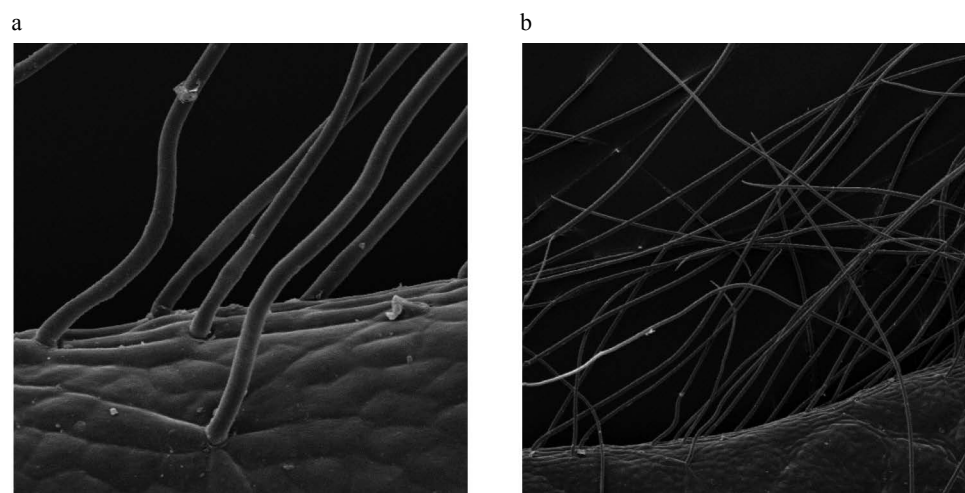
the image generated by the scanning electron microscope (SEM) (Fig. 5).

We can observe the erosion of wax crystals along with the length of exposure to external factors described above. In April, the leaf surface was most evenly covered with waxes; in July, these were rather clusters of larger crystals; and in September, we observed much smaller and more ragged waxes.

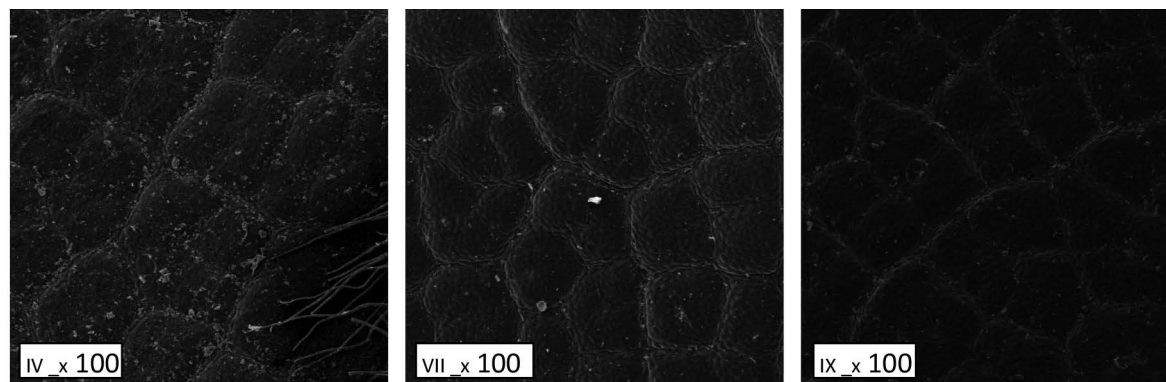
### Discussion

The combination of physiology and forest hydrology seems to be necessary and justified. Interception as a hydrological phenomenon has widely been described in terms of seasonal changes for individual trees and whole forest stands (Fathizadeh et al., 2013). Seasonal changes in the amount of water that reaches the forest cover has its consequences in the properties of the soil (Gruba et al., 2015; Brožek et al., 2015). It is necessary to explain the genesis of this phenomenon in accurate laboratory studies.

Such basic research can be translated into appropriate transposition models to the whole ecosystems (Seidl et al., 2013). The results obtained and change in the interception losses from 7.5 to 33.2% show that the issue of seasonality in wettability studies of leaves and interception of woody plants



**Figure 4.** Hairs on the leaf edge surface, registered in May and – zoom x500; b – Zoom x100



**Figure 5.** Seasonal changes in the surface with epicuticular waxes at beech in April, July and September, at zoom x100 (upper side of the leaf).



cannot be ignored. Interception losses are more and more pronounced every month in the course of duration of the growing season; therefore, we observe an increased amount of water that can be retained in trees' crowns.

The observed relationships between the magnitude of interception and the contact angles of drops as well as a high correlation between these parameters lead to the conclusion on the processes occurring in the tree crowns (Tables 2, 3, 4). The amount of water that can be retained in the crown, that is interception, was found to be closely correlated with the seasonal changes of leaves' surface expressed by consecutive months.

It should be assumed that changes in the parameters investigated found within successive phases of leaves development are associated with diverse, growth phase-dependent structure and content of the cuticular layer of leaves, particularly in epicuticular and intracuticular waxes. While the production of waxes occurs mainly at the beginning of leaves' development, a fully-developed leaf loses the ability to produce large amounts of these compounds. During leaf senescence, wax crystals undergo degradation due to weathering by wind, solar radiation and mechanical abrasion (Schreuder et al., 2001).

Crockford and Richardson (1990) as well as Sase et al., (2008) demonstrated that older leaves were more hydrophilic and were wetted more evenly in comparison to young leaves. These studies were performed on both, deciduous and coniferous species. Tomaszewski and Zieliński (2014) also indicate the formation of cuticle form under the influence of external environment. In relation to cessation of their production, it leads to a reduction in the thickness of their layer, and in consequence, to an increase in the rate of transpiration, loss of minerals by leaching and smaller resistance to pathogens (Romberger et al., 1993, Pallardy 2008). The structure and thickness of wax layer affects the intensity of leaf transpiration and evaporation of water (including water retained through interception) from their surface, also by reflective characteristics of leaf surface, affecting their temperature. While the leaves characterized by physiologically developed wax layer reflect 15–25% of solar radiation on average, wax removal from their surface results in a decrease of light reflection by about 50%. This results in an increase of leaf temperature and more intense evaporation of water from their surface (either from the inner surface of the leaf through transpiration as well as evaporation of water from the outer surface of leaves – water retained as a result of interception). In spring, the greatest physiological changes are observed in deciduous trees. An interesting observation for the common beech is the occurrence of thick hairs especially at the edges and along the nerves. This phenomenon is explained by an increase in the amount of water retained in May (Figs. 4 and 5). The more severe the changes on the leaf surface, the more acute is the contact angle, which denotes increasing wettability. Based on the results obtained, it is possible to classify the degrees of wettability of leaves under the influence of changes in the properties, structure and contribution of individual compounds building the cuticle and occurring during the vegetation

period. The observed change within one class of wettability may be explained by the fact that in 2015, between June and July, very high temperatures and lack of rainfalls were reported, which resulted in the acceleration of seasonal changes on leaves (meteorological data IMGW). Weather conditions are less reflected by the changes in interception magnitude. The values of the amount of water retained on the twigs react more gently in comparison to the degree of wettability.

Studies on wettability and water absorption in beech leaves are limited. More studies on this subject refer to oak tree varieties (Fernandez et al., 2014) and wettability in the context of lotus effect of the leaves (Nienhuis and Barthlott 1997, Stosch et al., 2007, Tranquada and Erb 2014). For this reason, the analyses performed for the beech were justified. Canopy storage capacity is particularly important in regions with negative water balance (Martin and Von Willert 2000, Sadeghi et al., 2014), where the amount of water retained by the tree crowns are more important from a hydrological viewpoint. SEM images are increasingly used to assess the state of the leaves' surface (Tomaszewski 2004, Koch and Barthlott 2009; Ensikat et al., 2010). Methodology of sampling has been positively verified with the latest research on interception under laboratory condition (Xiao and McPherson 2016).

The impact of seasonal changes in terms of wettability of leaves on ecohydrological processes is important and the studies should be extended on other species examining the impact of biotic and abiotic factors on the contact angle between drops and leaves.

## Conclusions

From April to November, the changes occurring in *Fagus sylvatica* leaves lead to changes in the amount of water that can be retained in tree crowns.

Over time and under the influence of external factors, the increase in wettability of leaves can be observed with simultaneous increase in the magnitude of rainfall interception losses.

Classification of the degrees of wettability and the images of leaves' surface generated with the use of scanning electron microscope were found to be the most appropriate for a better interpretation of hydrological results obtained.

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The images using Scanning Electron Microscope (SEM) were taken at the Institute of Cell Imaging at the Jagiellonian University

## Authors' contribution

K.-I.A. – concept, article author, laboratory research, statistical analysis, hydrological issues, discussion of results; K.W. – co-author, physiological issues, discussion of results.

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