Variability of Leaf Wetting and Water Storage Capacity of Branches of 12 Deciduous Tree Species

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Abstract: Leaf surface wettability and factors which determine it are key in determining the water storage capacity of tree crowns and thus the interception of entire stands. Leaf wettability, expressed as the droplet inclination angle, and the surface free energy largely depend not only on the chemical composition of the leaves but also on their texture. The study concerns 12 species of trees common in Central Europe. The content of epicuticular waxes was determined in the leaves, and values ranging from 9.145 [μg/cm²] for horse chestnut (Aesculus hippocastanum L.) to 71.759 [μg/cm²] for birch (Betula pendula Roth.) were obtained. Each additional μg/cm² increases the canopy water storage capacity by 0.067 g g⁻¹. For all species, the inclination angles of water, diiodomethane and glycerin droplets to the leaf surface were measured and the surface free energy was calculated. It is shown that it is the wax content and the species that constitute independent predictors of water storage capacity. These factors explain the 95.56% effect on the value of canopy water storage capacity. The remaining 4.44% indicate non-species-related individual features or the ability to mitigate pollutants as well as possible environmental factors. Wax analyzed separately from other factors causes a slight increase (by 0.067 g/g) of S. Nevertheless, the influence of the surface condition as a result of species-related variability is decisive for the value of the canopy water storage capacity.

Keywords: ecohydrology; canopy water storage capacity; contact angle; wettability; surface free energy

1. Introduction

Determination of hydrological properties, i.e., water storage capacity and degree of leaf wettability, helps to determine the retention properties of trees and entire stands. The amount of water left in tree crowns is evaporated or reaches the soil with a delay, which is a significant problem [1]. Trees have their role in stormwater runoff mitigation via transpiration and stemflow [2]. The development and evolution of methods to determine water storage capacity and interception can be found in many practical studies [3–5]. Moreover, even the role of stable isotopes, related to interception, is pointed to in understanding rainfall interception processes [6].

Water storage capacity depends on numerous factors, which can be divided into “stand-related” and “meteorological”. Water storage capacity is not a constant value for a given species because it is
enough to change one element of the environment (add wind or reduce air humidity) and the water storage capacity, as a cause and effect mechanism, will change [7].

Water storage capacity largely depends on the surface condition of the plant material [8]. Researchers observed seasonal and species-related variability [9,10]. Changes in the chemical composition of the leaves themselves are an adaptation to life in the changing seasons of the year [11]. The structure of the wax layer responsible for the way droplets adhere to it is related to chemical changes taking place especially in ageing leaves [12]. The amount of wax in the cuticle can thus regulate surface free energy and, hence, wettability. Surface Free Energy (SFE) is a numerically equal value of work that will result in the formation of a new unit of area during the separation of two equilibrium phases, assuming a reversible isothermal process. The value of surface free energy is determined based on the measurement of the angle of contact of various liquids (water, glycerin, diiodomethane) with a surface [13–15]. A body is wetted by a liquid when the liquid spreads over its surface or penetrates into its pores. The phenomenon of wettability depends on many factors, including surface tension of the liquid (surface tension additionally depends on the temperature) or interaction of solid particles of the liquid [8,16]. A lower contact angle indicates that the liquid will spread over a larger surface area, i.e., the surface is hydrophilic [17]. A larger contact angle indicates that the liquid minimizes contact with the surface and forms a more spherical droplet of water [18].

There is a plant division system according to their classification by Aryal and Neuner [19] ranging from the most hydrophilic to the superhydrophobic plants. This classification is based on the size of the angle at which a drop of water touches a leaf. The contact angle as a physical feature of the plant material may influence hydrological properties, such as the water storage capacity of the canopy [20].

The leaf surface texture is also affected by the changing environment, mainly the amount of pollutants [21], and this has consequences in changes in the hydrophilicity of the assimilation apparatus of both deciduous and coniferous trees [22].

Additionally, superhydrophobicity offers protection against plant pathogens, such as fungi and bacteria, as infection is limited by lack of water and moisture [23]. Some oak diseases, such as powdery mildew (Microsphaera alpitoidea Griff. Et Maubl), need moisture and warm temperatures to develop conidia. The white coating of conidia clusters, which can be very numerous, is hydrophilic [24].

All these physical and hydrological properties are also very important for forest hydrology because they are related to interception, throughfall, and the amount of water reaching the forest floor, which is important for stand regeneration [25,26]. Even small changes in tree crown hydrophilicity can have large ecological consequences in natural and urbanized catchments [27,28]. Mutual relationships between the factors that may affect changes in water storage capacity of tree crowns cannot be ignored in ecohydrological research.

Since physical features enhance or weaken the mutual influence on hydrological properties, the aim of this paper is to investigate the interrelationships between species-related features, wax content, surface free energy and inclination angles. Our study takes into account differences in the structure of the upper and lower leaf sides. The influence of these characteristics of the leaves on water storage capacity has also been determined.

2. Materials and Methods

2.1. Sampling Collection and Methodological Assumptions

The location of the city park where the samples were collected was 050°04′15.2″ N 019°59′32.8″ E (Krakow, Southern Poland). Branches of 12 deciduous tree species were collected for research on sunny days, between 19th and 28th of June 2019, in the morning (7:00–8:00 am.). The following species common in Central Europe were analysed: birch (Betula pendula Roth), willow (Salix caprea L.), privet (Ligustrum vulgare L.), ash (Fraxinus excelsior L.), oak (Quercus robur L.), sumac (Rhus typhina L.), robinia (Robinia pseudoacacia L.), small-leaved lime (Tilia cordata Mill.), lilac (Syringa vulgaris L.), olive (Elaeagnus angustifolia L.), maple (Acer platanoides L.), horse chestnut (Aesculus hippocastanum L.). Five trees were selected from each species, and two branches were sampled from each tree. In total, each analysis for one species was performed in ten replications.
The branches with a length of about 0.5 m were collected in the middle of crown height, on the north side. The samples selected had no visible signs of disease or dirt. From the entire branch sampled, a fragment of about 40 cm was used to simulate rainfall and determine water storage capacity (S). The leaves from the remainder of the branches were used to determine the water droplet inclination angles on the upper (Ang_ad) and lower (Ang_ab) sides of a leaf, the surface free energy, also on the upper side (SFP_ad) and lower sides (SFP_ab), and the wax content (Wax).

The above-mentioned components, measured in the study, were used in statistics but, additionally, measurements were performed for the inclination angles of diiodomethane and glycerin droplets (θ_c, θ_b), and calculations crucial to obtain the surface free energy were carried out. They are described in the methodology and the results are included in additional materials. The leaves had not been washed before the tests. Microscopic observations as well as analyses of wetting, SFP and S were carried out within 2 h of sampling. Until the tests, the samples had been stored in sealed polypropylene containers at 18 °C. The microscopic observations of the surface condition were performed with the use of the Zeiss Stereo Discovery.V8 microscope (Carl Zeiss Microscopy GmbH, Jena, Germany), equipped with an AxioCam ERC5S camera (Carl Zeiss Microscopy GmbH, Jena, Germany), and AxioVision v.4.8.2 software (Carl Zeiss Microscopy GmbH, Jena, Germany). Photos were taken of the top and bottom surfaces of the leaves using a 15× and 120× magnification. Figure 1 presents a sample image while all photos are in additional materials (Figure S1).

**Figure 1.** Examples of droplets of the liquids measured, obtained for *Elaeagnus angustifolia*, for the upper leaf surface: (a) water, (b) diiodomethane, (c) glycerine.

### 2.2. Canopy Water Storage Capacity Measurement

The water storage capacity of tree crowns (S) was measured by simulating rainfall on a 30–45 cm fragment of each sampled branch. The branches were weighed fresh to determine the biomass (BM). Precipitation was simulated by sprinkling the branch suspended on a scale using a fishing line. The weighing was repeated after sprinkling. The amount of water retained was calculated from the difference between the wetted and the dry branch (A). The arrangement of branches during sprinkling was similar to their natural arrangement on the tree [29]. A fixed dose of water (P) was established, amounting to 150 g. The amount of water was matched to the size of the branch and it had been checked beforehand what dose of water was necessary to saturate the branches with water, i.e., to obtain the maximum water storage capacity [20,24].

The water storage capacity of the branches was calculated according to the formula used by Garcia [30]: \( S = A/BM \) [g g\(^{-1}\)]. Water storage capacity can also be related to branch surface area, but in this case, it was decided to relate it to the biomass because it is a good predictor for calculating the water storage capacity of tree crowns [30].

Distilled water (Poch S. A., Gliwice, Poland) was used for the simulated wetting. The branches were sprinkled at a constant distance with water at a temperature of 21 °C, which was 1 degree lower than the temperature in the laboratory.
2.3. Methods to Measure Inclination Angles and Determine Surface Free Energy

The measuring liquids used in the analysis of the contact angle are: distilled water (Poch S. A., Gliwice, Poland), glycerin (Chempur, Piekary Śląskie, Poland) and diiodomethane (MERCK KGAA (Darmstadt, Germany)). Using a manual Vitrum micropipette with the 0.5 mm inner needle diameter and the volume range of 0.5–10.0 µL, ten drops of each liquid with the 0.5 µL volume each were deposited on each type of surface. This allowed for taking into account the surface layer heterogeneity and topography as well as for averaging errors. The temperature of the surroundings during the measurements was 22 ± 1 °C. After depositing on the surface, the drops were recorded at an equal time interval of 1 s. Using the SeeSystem 6.3 (Advex Instruments s.r.o., Brno, Czech Republic) software, the contact angles of water (θw), glycerin (θg) and diiodomethane (θd) were determined based on the Young–Laplace equation (Table 1).

| Measuring Liquid | γL | γL’ | γL’’ | γL’’’ | γL’’’’ |
|------------------|----|-----|------|-------|--------|
| Water            | 72.8| 21.8| 51.0 | 25.5  | 25.5   |
| Glycerin         | 64.0| 34.0| 30.0 | 3.9   | 57.4   |
| Diiodomethane    | 50.8| 50.8| 0    | 0     | 0      |

γL — surface free energy of the measuring liquid, γL’ — dispersion component of the surface free energy of the measuring liquid, γL’’ — polar component of the surface free energy of the measuring liquid, γL’’’ — acid and basic ingredients.

The station used to assess the value of the contact angle and determine the amount of surface free energy of the materials consists of a goniometer-type device from the Czech company Advex Instruments. It is a table with a possibility to control the level and movement in a plane parallel to the tested surface. The device is equipped with a digital camera that can move in the direction perpendicular to the tested surface. The position of the table and the camera is recorded and transferred to the programme integrated with the device. In this way, control over the test area is achieved. The recorded photo of the surface of the test material on which a droplet of the measuring liquid is deposited is transferred for further analysis. Using the software, it is possible to measure the value of the contact angle of various measuring liquids, and then apply the algorithms available in the programme to determine the SFP. This method had been tested for dead wood [31]. Measurements of the angle of the liquid droplets 3 to the leaf surface and the measurements needed to obtain free surface energy are presented in the additional material (Table S1 and Table S2).

The contact angle is defined by the Young law, based on the shape of a liquid droplet on the surface of a solid in the presence of the liquid vapours. Using the Young equation, i.e., the assumption of the equilibrium of forces on the body-S/liquid-L/gas-V interface, as shown in Figure 2, it follows that:

\[ γ_{SV} = γ_{SL} + γ_{LV} \cdot \cosθ \]  

(1)

The symbols γsv, γsl, γlv denote surface free energy [mJ/m²], respectively of solid S in contact with vapours V, solid S in contact with liquid L, and liquid L in contact with vapours V.

The work of adhesion Wad between the solid phase S and the liquid phase L is equal to the sum of the surface free energies of both components, minus the interphase free energy at the S/L interface:

\[ W_{ad} = γ_s + γ_L - γ_{SL} \]  

(2)

By introducing equation (2) in the form γsv = γsl + γlv · cosθ and assuming that γs = γlv, we obtain:

\[ W_{ad} = γ_s + γ_L - γ_{SL} = γ_s + γ_L - γ_{SL} + γ_{LV} \cdot \cosθ = γ_L \cdot (1 + \cosθ) \]  

(3)

Equation (3) became the basis for determination of the surface free energy of the solids, and for determination of the composition of the surface layers containing both hydrophilic and hydrophobic groups.
It is not possible to determine the total surface free energy of material $\gamma_s$ directly, therefore methods based on the measurement of the contact angle of two or three measuring liquids with known surface tension values are applied, respectively obtaining a system of two or three equations (Table 1). The Owens–Wendt, VanOss plus Good and Zisman methods were applied to determine $\gamma_s$ [32–34].

2.3.1. The Owens–Wendt Model

The Owens–Wendt model requires the use of two liquids (one polar and the other dispersive). Using this model, an approximation is obtained taking into consideration the geometric mean of polar components $\gamma^p_s$ and $\gamma^d_L$; The Owens–Wendt model is described by the following formulas:

$$\frac{1}{2}(1 + \cos\theta)\gamma_L = \sqrt{(\gamma^d_s\gamma^d_L) + (\gamma^p_s\gamma^p_L)}$$  \hspace{1cm} (4)

$$\gamma_S = \gamma^d_s + \gamma^p_s$$  \hspace{1cm} (5)

$$\gamma^d_s$$ - dispersion component (Lifshitz–Van der Walls).

$$\gamma^p_s$$ - polar component (Lewis acid-base).

2.3.2. The Van Oss–Chaudhury–Good Model

This model also assumes the division of energy into components. The $\gamma_s$ division includes long-range interactions called Lifshitz–Van der Waals interactions ($\gamma_{SW}^L$), as well as short-range interactions known as acid–base. The other component is considered as Equation (2), where $\gamma^d_s$ and $\gamma^p_s$ are the acidic and the basic components, respectively related to the acid–base interaction.

$$\frac{1}{2}(1 + \cos\theta)\gamma_L = \sqrt{\gamma^d_s\gamma^d_L + \gamma^p_s\gamma^p_L + \gamma^p_s\gamma^p_L}$$  \hspace{1cm} (6)

$$\gamma_S = \gamma^d_s + \gamma^p_s$$  \hspace{1cm} (7)

$$\gamma^d_s = 2\sqrt{\gamma^p_s\gamma^p_s}$$  \hspace{1cm} (8)

$\gamma_s$ - surface free energy of the tested material.

$\gamma^d_s = \gamma_{SW}^L$ - dispersion component of the surface free energy of the tested material, of Lifshitz–Van der Waals [LW].

$\gamma^p_s = \gamma_{AB}^L$ - polar component of the surface free energy of the tested material, Lewis acid–base [AB].

$\gamma_L$ - surface free energy of the measuring liquid

$\gamma^d_L$ - dispersion component of the surface free energy of the measuring liquid

$\gamma^p_L$ - polar component of the surface free energy of the measuring liquid

$\gamma^d_s, \gamma^p_s$ - acid and basic ingredients.

2.3.3. The Zisman Method

By measuring the angles $\theta_i$ for the homologous series of compounds with a decreasing value of surface tension $\gamma_L$, Zisman observed linear dependence of $\gamma_L$ on $\cos\theta_i$ value:

$$\cos\theta_i = 1 - \beta \cdot (\gamma_L - \gamma_C)$$  \hspace{1cm} (9)
where $\beta$—constant, $\gamma_c$—critical surface tension, at which, when $\gamma_L = \gamma_c$, then $\cos\theta_i = 1$, i.e., the test surface is completely wetted by hypothetical solvent with surface tension value ($\gamma_c$). The $\gamma_c$ value is determined from $\gamma_L$ extrapolation, for which $\cos\theta_i = 1$.

2.4. Measurement of the Wax Content in the Leaves

The epicuticular wax content was determined by a modified colorimetric method [35], adapted to the needs of the microplate reader. The reagent was prepared by adding 20 g of potassium dichromate K$_2$Cr$_2$O$_7$ to 40 mL of distilled water, to which 1 L of concentrated sulfuric acid H$_2$SO$_4$ was then slowly added and, while stirring, it was heated until the sediment was completely dissolved.

The extraction of epicuticular waxes was performed in triplicate for each sample. A single sample consisted of the cut leaf discs with a total surface area of 30 cm$^2$. The sample was flooded with 1.5 mL of chloroform and shaken for 15 s, then poured into a new tube and centrifuged for 5 min at 8 °C (13,000 rpm). The supernatant was then transferred to a new tube and evaporated in a boiling water bath until the smell of chloroform disappeared. In the next step, 0.5 mL of reagent was added and the samples were heated in a boiling water bath for 30 min. After cooling for 5 min, each sample was diluted by adding 1.2 mL of deionized water and then, after waiting a further 5 min for colour development, it was transferred to a 96-well plate. Absorbance readings were taken at a wavelength of 590 nm with a microplate reader (Synergy 2, Bio-Tek, Winooski, VT, USA). The content of epicuticular waxes was read from a calibration curve prepared for carnauba wax and expressed in $\mu$g/cm$^2$ of leaf area.

2.5. Statistical Analyses

The multivariate analysis of the independent influence of many variables on S was performed using the linear regression method. The influence of inclination angles was analysed only for water because it reflects the response of trees to rainfall. The results of inclination angles for glycerin and diiodomethane were used to calculate the surface free energy and are included in additional materials. The results are presented in the form of regression model parameters with a 95% confidence interval. As the analysed quantitative variables did not have a normal distribution, the comparison of the species was performed using the Kruskal-Wallis test and, if statistically significant differences were found, post-hoc analysis with Dunn’s test was performed to identify statistically significantly different groups. The correlations between them were analysed using Spearman’s rank correlation coefficient. The strength of the dependencies was interpreted according to the following scheme: $|r| \geq 0.9$—very strong dependence, $0.7 \leq |r| < 0.9$—strong dependence, $0.5 \leq |r| < 0.7$—moderately strong dependence, $0.3 \leq |r| < 0.5$—weak correlation, $|r| < 0.3$—very weak (negligible) relationship [36]. The normality of distribution of the variables was tested using the Shapiro-Wilk test. The significance level of 0.05 was adopted in the analysis. Thus, all $p$ values below 0.05 were interpreted as showing significant relationships. The analysis was performed in the R programme, version 3.4.4 [37].

3. Results

The present study focused on the mutual relationships between the physical properties of leaves and water storage capacity as well as the mutual relationships between the analysed factors. The results of the analyses described in the methodology, and necessary to examine the dependencies in question, as well as examples of liquid droplet images, are included in the additional materials (Supplementary Materials).

3.1. The Influence of Factors: Species, Wax, SFP_ad, SFP_ab, Ang_ad, Ang_ab on Water Storage Capacity S

The linear regression model showed that the independent predictors of $S$ [g/g] ($p < 0.05$) were wax content and species. Each additional $\mu$g/cm$^2$ of wax content increases S by an average of 0.067 g/g, as evidenced by the regression parameter (B) (Table 2).
In the analysis of the influence of species, *Acer platanoides* was adopted as a reference species. *Aesculus hippocastani* lowers S by an average of 4.391 g/g; *Betula pendula* lowers S by an average of 4.905 g/g; *Fraxinus excelsior* lowers S by an average of 3.848 g/g; *Quercus robur* reduces S by an average of 11.104 g/g; *Rhus typhina* lowers S by an average of 12.04 g/g; *Robinia ps.* reduces S by an average of 13.941 g/g; *Syringa vul.* reduces S by an average of 7.336 g/g; *Tilia cordata* lowers S by an average of 2.164 g/g (Table 2).

| Variable          | Standardized Parameter (Beta) | Regression Parameter (B) | 95% CI | p    |
|-------------------|-------------------------------|--------------------------|--------|------|
| (absolute term)   |                               | 9.304                    | −0.494 | 19.101 | 0.062 |
| Wax [µg/cm²]      | 0.236                         | 0.067                    | 0.02   | 0.114 | 0.006 |
| SFP_ad            | −0.131                        | −0.074                   | −0.172 | 0.025 | 0.141 |
| SFP_ab            | −0.175                        | −0.101                   | −0.23  | 0.028 | 0.125 |
| Angle_ad          | 0.001                         | 0                       | −0.042 | 0.042 | 0.987 |
| Angle_ab          | 0.046                         | 0.019                    | −0.03  | 0.068 | 0.45  |

**Table 2. The influence of the analysed factors on canopy water storage capacity.**

Wax—leaf wax content; S—canopy water storage capacity; SFP_ad—surface free energy of the upper leaf surface; SFP_ab—surface free energy of the lower leaf surface; Angle_ad—angle of contact with the upper leaf surface (adaxial side); Angle_ab—angle of droplet contact with the lower leaf surface (abaxial side); A—*Acer platan.; B—*Aesculus hippocastani*; C—*Betula pendula*; D—*Elaeagnus ang.*; E—*Fraxinus excelsior*; F—*Ligustrum vul.*; G—*Quercus robur*; H—*Rhus typhina*; I—*Robinia ps.*; J—*Salix caprea*; K—*Syringa vulgaris*; L—*Tilia cordata*; 95% CI—95% Confidence Interval

Standardized (beta) parameters show the strength of the influence of particular variables on S [g/g]. Therefore, it becomes evident that among the species in question the strongest impact on S is exerted by *Quercus robur*, *Rhus typhina* and *Robinia ps.* The influence of the wax in the leaves is not dominant.

The *R²* coefficient for this model was 95.56%, which means that 95.56% of the variability of S [g/g] was explained by the variables chosen for the model. The remaining 4.44% depends on the variables not included in the model and on random factors.

In order to be able to fully answer the question of which of the analysed factors influence the hydrological properties, the interrelationships between species, Wax, S [g/g], SFP_ad, SFP_ab, Ang_ad, Ang_ab were also examined.

### 3.2. The Influence of Species on the Other Parameters

The species is a qualitative variable while the remaining parameters are quantitative. The measurements did not have a normal distribution (p from the Shapiro-Wilk test below 0.05) in the analysed groups, therefore the analysis was carried out using the Kruskal-Wallis test (Table 3), and the diagram shows the medians, quartiles and ranges of values for individual variables (Figure 3).

*p* values are lower than 0.05 for all parameters, so the analysed species differed significantly in all parameters. To answer the question of what exactly this relationship looks like, the post-hoc analysis was performed. The table has letter symbols for the following species: A—*Acer platan.; B—
The results of this analysis are presented in the last column of Table 3; e.g., for the leaf wax content (Wax), we can see that: for species C, this parameter was higher than for species A, B, D, H, I, K, L; for species J this parameter was higher than for species A, B, D, I, K, L; for species E, F, G this parameter was higher than for species A, B; for species H this parameter was higher than for species B.

Table 3. The Kruskal-Wallis test and post-hoc analysis (Dunn’s test) to determine the interrelationships between the analysed factors.

| Parameter | Type | N  | Mean   | SD    | Median  | min  | max  | Q1   | Q3   | p   |
|-----------|------|----|--------|-------|---------|------|------|------|------|-----|
| Wax [µg/cm²] |  |  |  |  |  |  |  |  |  |  |
| A         |  | 10 | 12.33  | 2.23  | 12.37   | 7.09 | 15.39| 11.75| 13.46| <0.001|
| B         |  | 10 | 9.14   | 0.73  | 9.43    | 7.87 | 10.14| 8.8  | 9.53 |
| C         |  | 10 | 71.76  | 9.06  | 68.33   | 61.93| 86.67| 64.48| 79.21|      |
| D         |  | 10 | 18.6   | 2.66  | 18.43   | 13.81| 22.58| 17.7 | 20.38|      |
| E         |  | 8  | 27.32  | 1.76  | 26.7    | 25.84| 30.1 | 26.29| 27.67|      |
| F         |  | 9  | 30.46  | 5.62  | 30.38   | 22.38| 39.68| 25.84| 34.98|      |
| G         |  | 8  | 26.25  | 1.39  | 26.35   | 24.31| 28.54| 25.16| 27.02|      |
| H         |  | 10 | 23.48  | 4.36  | 23.85   | 16.03| 28.8 | 20.16| 27.3 |
| I         |  | 10 | 21.13  | 2.88  | 21.92   | 16.9 | 24.73| 18.5 | 23.39|      |
| J         |  | 10 | 35.81  | 5.81  | 34.12   | 27.98| 45.85| 31.64| 40.65|      |
| K         |  | 8  | 18.86  | 3.97  | 17.94   | 13.88| 26.69| 16.54| 20.2 |
| L         |  | 10 | 19.47  | 5.76  | 20.68   | 9.01 | 26.1 | 18.77| 23.11|      |
| S [g/g]   |  | 10 | 19     | 0.71  | 19.34   | 17.84| 20.02| 18.47| 19.4 |
| A         |  | 10 | 12.14  | 0.81  | 12.07   | 10.89| 13.62| 11.6 | 12.39| <0.001|
| B         |  | 10 | 16.92  | 0.95  | 16.94   | 14.8 | 18.12| 16.68| 17.57|      |
| C         |  | 10 | 18.48  | 1.01  | 18.62   | 16.34| 19.92| 18.11| 18.91|      |
| D         |  | 8  | 13.59  | 0.39  | 13.66   | 12.9 | 14.03 | 13.47| 13.88|      |
| E         |  | 9  | 15.79  | 0.92  | 15.87   | 14.38| 17.34| 15.09| 16.32|      |
| F         |  | 8  | 8.07   | 2.51  | 9.07    | 3.67 | 10.29| 6.92 | 9.94 |
| G         |  | 10 | 7.81   | 0.62  | 7.93    | 6.97 | 8.85 | 7.27 | 8.24 |
| H         |  | 10 | 7.43   | 0.68  | 7.58    | 6.08 | 8.34 | 7.21 | 7.87 |
| I         |  | 10 | 16.1   | 1.18  | 16.37   | 13.89| 17.77| 15.92| 16.78|      |
| J         |  | 8  | 10.1   | 0.94  | 10.23   | 8.97 | 11.78| 9.3  | 10.58|      |
| K         |  | 10 | 21.02  | 1.47  | 21.19   | 18.23| 23.48| 20.19| 21.38|      |
| SEP_ad    |  | 10 | 26.95  | 2.28  | 27.04   | 23.62| 29.27| 25.13| 29.23| <0.001|
| A         |  | 10 | 26.05  | 1.88  | 26.22   | 23.12| 28.81| 24.77| 27    |
| B         |  | 10 | 25.37  | 1.23  | 25.68   | 23.43| 27.03| 24.38| 26.32|      |
| C         |  | 10 | 28.1   | 3.66  | 26.91   | 24.24| 35.56| 25.5 | 30.33|      |
| D         |  | 8  | 28.56  | 2.21  | 28.98   | 24.28| 31.23| 27.41| 30.1 |
| E         |  | 9  | 25.03  | 0.87  | 25.35   | 22.88| 25.86| 24.92| 25.44|      |
| F         |  | 8  | 23.61  | 1.94  | 23.83   | 19.74| 25.83| 22.75| 24.95|      |
| G         |  | 10 | 24.09  | 2.47  | 24.13   | 19.73| 27.56| 22.82| 25.84|      |
| H         |  | 10 | 5.31   | 0.73  | 5.39    | 3.9  | 6.25 | 4.85 | 5.81 |      |
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| SEP_ab | A   | B   | C   | D   | E   | F   | G   | H   | I   | J   | K   | L   |
|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| A      | 10  | 7.4 | 1.42| 7.64| 5.5 | 9.49| 6.21| 8.43|     |     |     |     |
| B      | 10  | 26.14| 2.68| 26.01| 22.45| 29.87| 23.9 | 28.29|     |     |     |     |
| C      | 10  | 16.84| 1.46| 17.3 | 14.27| 18.74| 15.71| 17.76|     |     |     |     |
| D      | 10  | 10.74| 1.14| 10.96| 9.09 | 12.73| 9.76 | 11.18|     |     |     |     |
| E      | 8   | 26.46| 1.54| 26.21| 24.22| 28.73| 25.74| 27.02|     |     |     |     |
| F      | 9   | 25.08| 1.94| 25.07| 22.57| 27.87| 24.11| 26.17|     |     |     |     |
| G      | 8   | 13.63| 2.61| 12.62| 10.8 | 17.45| 11.68| 16.19|     |     |     |     |
| H      | 10  | 7.53 | 1.66| 7.26 | 5.38 | 10.63| 6.54 | 8.53 |     |     |     |     |
| I      | 10  | 4.72 | 1.06| 4.62 | 3.45 | 7.1  | 3.98 | 4.92 |     |     |     |     |
| J      | 10  | 10.45| 0.64| 10.43| 9.11 | 11.49| 10.24| 10.68|     |     |     |     |
| K      | 8   | 26.06| 0.75| 26.05| 25.1 | 27.48| 25.74| 26.33|     |     |     |     |
| L      | 10  | 8.93 | 1.72| 8.59 | 7.02 | 12.78| 7.89 | 9.12 |     |     |     |     |

Wax—leaf wax content; S—canopy water storage capacity; SFP_ad—surface free energy of the upper leaf surface; SFP_ab—surface free energy of the lower leaf surface; Angle_ad—angle of contact with the upper leaf surface (adaxial side); Angle_ab—angle of droplet contact with the lower leaf surface (abaxial side); A—Acer platan; B—Aesculus hippocastanum; C—Betula pendula; D—Elaeagnus angustifolia; E—Fraxinus excelsior;
The diagrams present the medians, quartiles and ranges of values for individual variables (Figure 3). The diagrams (Figure 3) show that the angle of contact with the upper leaf surface is similar for most species. *Robinia pseudoacacia* can be distinguished for the highest contact angle, i.e., the lowest wettability, and *Salix caprea* for the smallest contact angles, i.e., high wettability. Low contact angles mean that a droplet adheres well to the surface.

The water storage capacity and the contact angle of the lower leaf surface display inversely proportional values. Where the angle decreases, the water storage capacity increases. This is due to the fact that the smaller the angle, the better the droplet adheres to the leaf surface, which translates into a higher water storage capacity of the plant material.

Pedunculate oak (*Quercus robur*), staghorn sumac (*Rhus typhina*) and black locust (*Robinia pseudoacacia*) have the lowest water storage capacity. Small-leaved lime (*Tilia cordata*) has one of the higher capacities.
Figure 3. Medians, quartiles and value ranges of individual variables. Wax—the leaf wax content; S—canopy water storage capacity; SFP_ad—surface free energy of the upper leaf surface; SFP_ab—surface free energy of the lower leaf surface; Angle_ad—angle of contact with the upper leaf surface (adaxial side); Angle_ab—droplet angle of contact with the lower leaf surface (abaxial side).
3.3. The Remaining Relationships

The analysed features did not have the normal distribution ($p$ from the Shapiro-Wilk test below 0.05), therefore the analysis was based on the Spearman correlation coefficient.

The correlation coefficients between the analysed variables are shown in the so-called heat map below (Figure 4). The blue areas represent strong positive correlations whereas the red areas represent strong negative correlations. The white areas denote a lack of correlation. Altogether, six relationships are statistically significant ($p < 0.05$). Positive relationships are those where both variables “head in the same direction” (i.e., the greater the value of one of them, the greater the value of the other). We can observe that the greater the SFP_ad, the greater the S (and vice versa). Negative relationships are those where the variables are inversely proportional. There we can observe the following relationship: the lower the wax content in the leaves (Wax), the greater the angle of droplet adherence to the upper (Angle_ad) and lower (Angle_ab) leaf surfaces. Larger contact angles indicate that the leaves are hydrophobic, meaning that the droplet adheres less to the surface.

![Heatmap of correlation coefficients between analysed variables](image)

**Figure 4.** Correlation coefficients between the analysed variables, the so-called heat map. Wax—the leaf wax content; S—canopy water storage capacity; SFP_ad—surface free energy of the upper leaf surface; SFP_ab—surface free energy of the lower leaf surface; Angle_ad—angle of contact with the upper leaf surface (adaxial side); Angle_ab—droplet angle of contact with the lower leaf surface (abaxial side).

It can be observed that as droplet contact angles increase, the canopy water storage capacity decreases and the surface free energy (SFP) decreases. The details of the relationships are presented in the table in the additional materials (Table S3).

4. Discussion

The obtained results show that the factors significant for S are the wax content and the species-related features, i.e., morphology differentiating individual leaves. An increase in the wax content did not decrease the water storage capacity and wettability of the plant material.
Rather, the main role of leaf waxes is to retain water inside an organism. In the case of young leaves, the wax content is high and the adhesion of water to the leaf surface is greatly influenced by structures such as thorns and hairs, which are often characteristic only of young leaves (Appendix A).

Species-related differences have been described in many studies on ecohydrology [15,18,29]. The species itself is not an easy parameter because it has many immeasurable features. It is the texture that can significantly increase water storage capacity and wettability [24].

Among the analysed species, *Acer platanoides* retains the most water, while *Robinia pseudoacacia*, *Rhus typhina* and *Quercus robur* retain the least (Figure 3). Similar studies on species variability were conducted by Koch and Barthlott [38], Rosado and Holder [8] and Fernández et al. [13].

Moreover, what is significant for the whole process of water retention are the interrelations of the measured parameters, such as contact angles of the lower and upper leaf surface and the surface energy as related to species [39,40].

*Betula pendula* clearly has the highest percentage of waxes among the studied species and, at the same time, has a fairly high water storage capacity (Table 3, Figure 3).

The surface free energy of the upper and lower leaf sides differs significantly within a single species. For most species, the SFP.ad is similar; values lower than average were found for *Robinia ps.* and *Syringa vul.* and even lower for *Salix caprea* and *Tilia cordata*.

The SFE of the lower leaf side is less responsible for water retention, and it is here that the species-related features associated with the presence of stomata become apparent.

The contact angle of water droplets with the upper leaf surface is inversely proportional to the surface energy. The same is true of the lower side of the leaf. Thus, one can speak of a clear relationship between leaf wettability and surface free energy.

A comparison of the obtained results to the classification of hydrophobicity by Aryal and Neuner [19], taking into account the hydrophobicity of the upper leaf surface, leads to including *Salix caprea* among plants with high wettability. The species with good wettability include: *Fraxinus ex.*, *Tilia cordata*, *Acer platan.* and *Betula pendula*. The remaining species, namely: *Syringa vul.*, *Rhus typhina*, *Robinia ps.*, *Elaeagnus ang.*, *Aesculus hipp.*, *Quercus robur* are plants with low wettability.

An analysis of the lower leaf surface reveals that none of the studied species can be classified as highly wettable plants. On the other hand, only *Syringa vul.* can be classified as a plant with good wettability. However, *Fraxinus ex.*, *Ligustrum vul.*, *Salix caprea*, *Elaeagnus ang.*, *Aesculus hipp.*, *Quercus robur*, *Betula pendula* are species that are difficult to wet. The remaining species, namely: *Rhus typhina*, *Robinia ps.*, *Acer platan.* are very difficult to wet.

These considerations can be summed up in terms of a heat map (Figure 4), where we can see that water storage capacity is strongly positively correlated with SFP_ad and negatively correlated with the angle of inclination to the upper side of the leaf. That is, by changing the surface free energy, we can control the wettability of leaves, which is used in substances applied in horticulture in the form of spraying [41]. The heterogeneity of the influence of the amount of surface free energy on the adhesion of water was stated by Wang et al. [16]. A change of surface free energy through chemical treatments in fertilization and application of fertilizers makes us realize how important this process is [13].

The chemical composition and surface structure of the leaves are internal causes, but the environment can also influence wettability and water storage capacity [42].

It is known from the literature that contact angles increase with growing wax content. However, research has shown that contact angles are more dependent on wax structure complexity and surface microrelief itself than solely on the absolute amount of wax. The wax content, even within a single species, may vary depending on seasonal changes. For example, for *Quercus robur* it varies from 17 to 50 µg/cm² during the growing season [12]. The amount of wax may have a more complex effect on water storage capacity. The presented research is in the category of basic research. Only after these physical and hydrological relationships are identified, a wide window opens for population studies and taking into account genetic variation within a species [43,44].

Species with a high wax content accumulate more pollutants inside [45], which results in the acceleration of ageing and changes of texture [46]. Rougher leaves have higher water storage capacity [47]. What is important for water adhesion is also the form of wax as a film or crystals [42].
However, each species has specific characteristics that may individually affect the degree of droplet adhesion and the amount of water retained in the crowns. Species-related differences in terms of the amount of water storage capacity were also visible for small-leaved lime and poplar, regardless of whether the samples were collected in a forest area or in the city centre [48]. In the present study, it is the species-related features that turn out to have a significant impact on water storage capacity. These species-related features include, for example, the presence of large or tiny hairs, or the leathery character of the leaf surface. A study of leaf texture and roughness, based on an analysis of SEM photos, demonstrated the influence of changes in the surface condition of oak leaves infected with oak powdery mildew [24].

The adhesion of water droplets to the leaves also differed among species, between the upper and lower leaf sides [16]. As shown in Wang’s study [16], generally more water adheres to the adhesive surface than to the abaxial surface. In the present study, the mean for all angles of inclination to the adaxial surface for all species considered jointly is $107.97^\circ$ while for the abaxial surface it is $125.05^\circ$. Similar relationships between the upper and the lower side of the leaves were found for SFE, with values of 25.42 and 14.89 [mJ/m²], respectively.

The obtained results can be combined with the retention capacities of forests and mixed tree-covered areas. The amounts of water that are retained in tree crowns, and depending, among other things, on the amount of wax and surface roughness, do not reach the forest floor and reduce the amount of water available to the roots. In the face of increasingly lower rainfall and higher temperatures [49], these issues are worth further research. Assessment of water adhesion constitutes an easy and valuable tool that can be used to determine the retention properties of a given plant. The amount of retained water may have a positive effect on the microclimate of urban forests [28] as well as it may reduce the amount of water reaching the roots [47,50].

5. Conclusions

The conducted research is within the scope of basic research, which adds a lot to the knowledge about water retention processes in tree crowns. These issues are extremely important in the context of climate change and the diminishing soil water resources for forests. In Europe, even pine forests become sheltered and susceptible to pests and parasites during long periods without precipitation. The presence of trees protects the soil and increases soil retention, but with low rainfall, tree crowns may block water from reaching the forest floor.

The linear regression model showed that the significant predictors of S are waxes and factors related to the species-typical characteristics of the leaf structure. Each additional $\mu$g/cm² of wax in the leaf increases the water storage capacity. An important observation is the proportional relationship between the water capacity of the leaves and the wettability.

With the increase in wettability, which was described by the drop angle to the leaf, the water capacity also increased.

Research shows that the hydrological properties of leaves of various species resulting from the physical features of those leaves.

**Supplementary Materials:** The following are available online at www.mdpi.com/1999-4907/11/11/1158/s1, Figure S1: Droplets of the liquids measured, Table S1: Measurements of the angle of the droplet to the leaf surface, Table S2: Components necessary to calculate the surface free energy, Table S3: Mutual relations between the studied features.

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Appendix A: Analysis of Leaf Surface Photographs

In order to fully analyse the relationship between the wax content and the angles of contact of a liquid to the leaf surface, as well as the hydrological properties of the plant material, it is also necessary to compile photos showing the surface type. The texture of the leaves clearly differs between the species analysed (Figure A1).

*Syringa vulgaris* L. upper surface: (a) 15×, (b) 120×, lower surface (c) 15×, (d) 120×.

*Betula pendula* upper surface: (a) 15×, (b) 120×, lower surface (c) 15×, (d) 120×.

*Quercus robur* upper surface: (a) 15×, (b) 120×, lower surface (c) 15×, (d) 120×.
Fraxinus excelsior upper surface: (a) 15×, (b) 120×, lower surface (c) 15×, (d) 120×.

Aesculus hippocastanum upper surface: (a) 15×, (b) 120×, lower surface (c) 15×, (d) 120×.

Acer platanoides upper surface: (a) 15×, (b) 120×, lower surface (c) 15×, (d) 120×.
Ligustrum vulgare upper surface: (a) 15×, (b) 120×, lower surface (c) 15×, (d) 120×.

Tilia cordata upper surface: (a) 15×, (b) 120×, lower surface (c) 15×, (d) 120×.

Elaeagnus angustifolia upper surface: (a) 15×, (b) 120×, lower surface (c) 15×, (d) 120×.
As presented in Table 1, the influence of the leaf wax content is much weaker than such species characteristics as roughness or the presence of hairs. All of the analyses were performed within one microclimate and at the same time during the growing season, so we can take into consideration the individual characteristics.
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