



# Contact angle measurements and water drop behavior on leaf surface for several deciduous shrub and tree species from a temperate zone

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## Abstract

**Key message** Leaf CA measurement should take into account angle variation during measurement time. Leaf wettability of common deciduous forest plants is characterized by wetting contact angles ranging from 60° to 140° with a significant variation between species of the same family.

**Abstract** Leaf wettability is an important phenomenon that has an influence on several processes such as the hydrological cycle, plant pathogen growth, or pollutant and pesticide absorption/deposition. The main objective of this research was to investigate the leaf wettability differences of 19 species (16 trees and 3 shrubs) of deciduous plants commonly occurring in Polish forests (temperate climate). The measurements were gathered as follows: 20 undamaged leaves were selected for each species and the wettability was determined by contact angle measurements with an optical goniometer CAM 100 using the sessile drop method. The contact angle was measured with 1-s intervals during 2 min from droplet deposition on adaxial and abaxial leaf surface. Laboratory analyses were completed during the summer of 2016 during full vegetation growth. A general CA decrease with time was observed on both leaf sides. The contact angle values ranged from 60° to 140° depending on species and leaf side. Differences between contact angle values at the beginning and the end of measurement reached 23.6° and engendered changes of wetting classes for some species. In many cases, no wettability class change was observed despite a CA lowering of 20°. The abaxial side was found to be the more repellent for 14 out of 19 species. Altogether, the leaves were classified from highly wettable to highly non-wettable, probably depending on the plant-survival strategy.

**Keywords** Deciduous tree · Temperate climate · Leaf wettability · Contact angle · Water drop

## Abbreviations

CA	Contact angle (°)
CA <sub>in ad</sub>	Initial contact angle ( $t = 1$ s) for adaxial leaf side (°)
CA <sub>in ab</sub>	Initial contact angle ( $t = 1$ s) for abaxial leaf side (°)
CA <sub>fin ad</sub>	Final contact angle ( $t = 120$ s) for adaxial leaf side (°)
CA <sub>fin ab</sub>	Final contact angle ( $t = 120$ s) for abaxial leaf side (°)
CA <sub>in-fin ad</sub>	Differences between the initial and final CA for adaxial surface (°)
CA <sub>in-fin ab</sub>	Differences between the initial and final CA for abaxial surface (°)
CA <sub>R</sub>	Contact angle measured on the right side of the droplet (°)
CA <sub>L</sub>	Contact angle measured on the left side of the droplet (°)
DA	Drop asymmetry coefficient (–)

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$DA_{ad}$	Drop asymmetry coefficient for adaxial leaf side (–)
$DA_{ab}$	Drop asymmetry coefficient for abaxial leaf side (–)
LWR	Leaf water repellency (°)

## Introduction

Leaf wettability is understood as a plant's ability to retain water on its surface as a result of the presence of mist, rain, or dew. Most commonly, it is determined by the value of the contact angle (CA) (Bradley et al. 2003; Aryal and Neuner 2010; Goldsmith et al. 2016) which is the angle formed between the tangent to the liquid surface and the leaf surface at the interface between solid and liquid phases (Fig. 1).

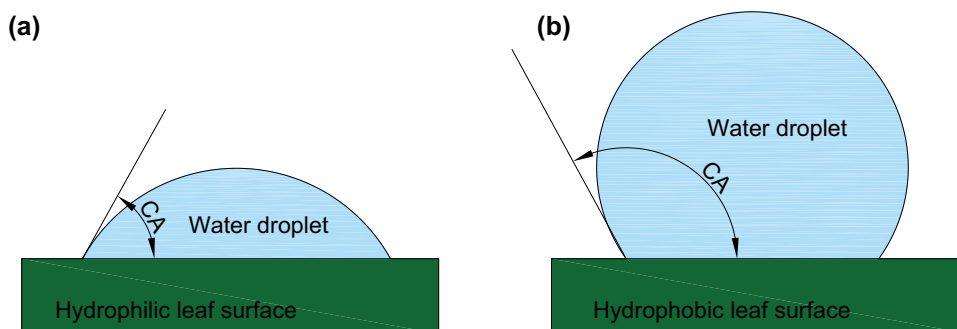
Depending on leaf wettability, the water drop placed on a leaf surface takes different shapes that determine the evaporation time (Pinon et al. 2006). In the case of an easily wettable surface, the droplet dissipates over the surface, covering it with a thin layer of water that dries faster. A poorly wettable surface on which the droplet does not dissipate but retains its shape dries more slowly. A longer water retention time along with a greater amount of water on the leaf surface creates favorable conditions for plant pathogens growth (Pinon et al. 2006) and increases plants' biochemical stress (Goldsmith et al. 2016). The amount of water retained by the plants depends on leaf wettability, leaf surface area, air pollution, plant condition, and plant species characteristics, as well as rainfall intensity (Klamerus-Iwan 2014; Klamerus-Iwan et al. 2017) but also on water temperature which affect the process of water drop formation (Owsiak et al. 2013). The presence of water drops on the leaf surface may also act as a photosynthetic inhibitor due to the fact that carbon dioxide penetrates stomata 10,000 times slower in the presence of water than in the presence of air (Brewer and Smith 1997). Therefore, high leaf water repellency (LWR) may help the plants to survive through periods of prolonged rainfalls (Brewer et al. 1991; Hanba et al. 2004; Rosado and Holder 2013). The leaf surface plays a significant role in protecting the plant against many biotic and abiotic threats.

A plant's physiology and structure is the result of its adaptation to the prevailing conditions to make the best use of available resources and to be able to defend it effectively against unfavorable external conditions. For example, the presence of galls or trichomes on a leaf surface may have a significant impact on changes in the leaf surface roughness and therefore on its wettability (Koch et al. 2006; Bhushan and Jung 2010; Ensikat et al. 2011; Khayet and Fernández 2012). The pH, chemical composition of waxes, and presence of microflora can affect the wettability (Knoll and Schreiber 2000). The structure and chemical composition of epicuticular waxes depends to a considerable degree on atmospheric conditions such as climate (Aryal and Neuner 2010), rainfall intensity (Baker and Hunt 1986), air humidity (Koch et al. 2006), and air pollutants quantity and quality properties, such as the presence of atmospheric aerosols (Burkhardt 2010). Leaf wettability can also have a positive effect in reducing transpiration or absorbing water directly through the leaf surface, thus improving the plant condition and allowing its survival (Revilla et al. 2016). In dry habitats, the leaf surface is generally less susceptible to wetting and the probability of retaining water drops on its surface is smaller (Brewer and Nuñez 2007). This is due to an increase of hydrophobic wax concentration and density with low atmospheric moisture (Koch et al. 2006). The relationship between leaf water repellency and leaf water retention is unclear. In some studies, correlation between leaf wetness traits was not observed (Matos and Rosado 2016), while other studies have found positive (Holder 2012) or negative (Brewer and Nunez 2007) relationships.

According to Kardel et al. (2012), leaf wettability determined from the value of CA can also be used as bio-indicator of habitat quality. Moreover, on a larger scale, it may be meaningful in describing water cycle processes in nature because of its significant role in transpiration and interception processes. Water quantity and leaf surface retention time vary between species (Brewer and Nuñez 2007; Aryal and Neuner 2010).

Forest surface retention capacity is of great importance for regulating the hydrological cycle (Tyszka 2009). It was also suggested that leaf wettability can influence urban

**Fig. 1** Shape of water droplet and CA for **a** hydrophilic leaf surface and **b** hydrophobic leaf surface. Modified from Bradley et al. 2003



hydrology and that the choice in plant selection for landscaping should be taken with care (Holder and Gibbes 2016; Karczmarczyk et al. 2014). Forest canopy interception is an important process, retaining 10–50% of seasonal or annual precipitation, depending on forest characteristics and climate (Carlyle-Moses and Gash 2011). Moreover, water interception can affect the efficiency of insecticides, fungicides, or fertilizers in agriculture (Aston 1979). All these processes are related to LWR, thus showing the importance of improving the knowledge and understanding of hydrophobicity mechanisms.

When studying leaf wettability with the sessile drop method, the decrease of the contact angle with time is always ignored. As a rule, only one CA value is given, but it is not clear at what moment the measurement occurred. This value often depends on the operator accuracy and on his rapidity to turn on the camera. This is the reason why we suggest for future leaf wettability research to measure and to present the change of contact angle with time. This approach was sometimes applied to other fields of research: e.g., in wood studies (Rodríguez-Valverde et al. 2002), in pharmaceuticals (Muster and Prestidge 2002), or soil studies (Leelamanie and Karube 2009; Papierowska et al. 2018). The contact angle value decrease is caused by the change of the drop shape due to water spreading on the solid surface and to water absorption by the material but also to evaporation. The change of droplets shape with time on leaf surface was observed in literature. Xu et al. (2010) studied the evaporation rate and the wetted area in relation to the use of pesticides and other plant protection products. They observed the different spreading of water drops caused by the appearance of secondary vein, puckered ridges, or the presence of hydrophobic crystalline waxes. The change of the drop shape on the leaf surface can also be explained by the absorption of water droplets by the leaf surface (Fernández et al. 2014).

The laboratory approach to measure contact angle assumes a thermodynamic equilibrium during the measurement which is attained when a system does not change its properties with time. A relatively small droplet volume (microliter order) ensures that the gravity force does not affect the droplet. The laboratory approach also assumes that a drop of liquid does not react with the surface on which it is placed (Lages and Méndez 2007). Many factors (wind impact, radiation, etc.) are excluded from laboratory approach but were observed in environmental studies (e.g., Wittenberghe et al. 2012; Matos and Rosado 2016). However, such approach, in which the leaf surface is examined without the impact of external factors, allows the examination of the influence of the leaf surface on contact angle changes with time and a possible comparison with other studies.

The main objective of this research was to investigate differences in leaf wettability for various deciduous tree species

and shrubs from a temperate climate and to explore the dynamic of wettability changes during CA measurements.

## Materials and methods

The research was carried out during the summer of 2016 (July–August) at a time of full vegetation growth (in the middle of growing season) in the Lower Pilica River Valley in the vicinity of Inowłódz, Spała, and Tomaszów Mazowiecki (łódzkie Voivodship, Poland). The research area is located in a temperate climate zone. The average air temperatures in January and July are  $-2.9$  and  $18.0$  °C, respectively, with an average annual air temperature of  $7.8$  °C. The average annual precipitation is equal to 575 mm and the length of the growing season is about 220 days per year (Podstawczyńska 2010). For the study, 19 species of trees and shrubs commonly found in deciduous forests were selected for the study. These species were *Acer negundo* L., *Acer platanoides* L., *Acer pseudoplatanus* L., *Aesculus hippocastanum* L., *Alnus glutinosa* Gaertn., *Betula pendula* Roth, *Betula pubescens* Ehrh., *Carpinus betulus* L., *Corylus avellana* L. (shrub), *Fagus sylvatica* L., *Fraxinus excelsior* L., *Padus avium* Mill., *Populus tremula* L., *Prunus serotina* Ehrh., *Quercus robur* L., *Salix cinerea* L. (shrub), *Salix repens* L. (shrub), *Tilia cordata* Mill., and *Ulmus laevis* Pall.

Measurements of CA were conducted on freshly harvested leaves collected in situ and transported to the laboratory in humid and cool conditions (refrigerated). All leaves were taken from the same height of 1.5–2 m, since adaxial leaf wettability has been proved to varies along a canopy gradient (Van Wittenberghe et al. 2012). Measurements were conducted in the laboratory at a temperature of 20 °C within 1–2 days after sample collection. For sessile drop method measurements, the CAM 100 goniometer (manufactured by KSV Instruments, Finland) was used. Full description of the sessile drop method can be found in Papierowska et al. (2018). The instrument was connected to a computer equipped with image recording software, drop shape analysis, and CA measurement tools. For each plant species, ten healthy leaves were selected from different individuals. The selected leaves were carefully fixed with an adhesive tape on the glass slide. A distilled water drop was placed on a horizontal leaf surface using a syringe with a 0.13 mm internal diameter needle. The droplets were placed on both abaxial and adaxial sides of the leaf with a total of 20 water drops per species. To determine the dynamics of CA change with time, the shape of the liquid droplet was recorded by the camera starting from second zero (placing the drop on a leaf) to the 120th second with 1-s intervals. The following data were used for further analysis: initial CA ( $t = 1$  s) for adaxial ( $CA_{in\ ad}$ ) and abaxial ( $CA_{in\ ab}$ ) leaf surface; final CA ( $t = 120$  s) for

adaxial ( $CA_{fin\ ad}$ ) and abaxial ( $CA_{fin\ ab}$ ) leaf surface; and CA changes (differences between the initial and final CA) for adaxial surface ( $CA_{in-fin\ ad}$ ), and abaxial surface ( $CA_{in-fin\ ab}$ ). For each drop, CAs from the right ( $CA_R$ ) and left ( $CA_L$ ) sides of the droplet were obtained, as well as the mean value of both results for every second of measurement (for both adaxial and abaxial leaf sides). A short measurement time was selected to preserve changes of the drop shape due to evaporation. The heterogeneity of the leaf surface that can be the result of wax erosion and/or quality degradation was estimated by the drop asymmetry coefficient proposed by Kardel et al. (2012) and expressed as:

$$DA = 2 \times \left| \frac{CA_R - CA_L}{CA_R + CA_L} \right| \quad (1)$$

where  $CA_R - CA$  describes the right side of the droplet ( $^\circ$ ) and  $CA_L - CA$  the left side of the droplet ( $^\circ$ ).

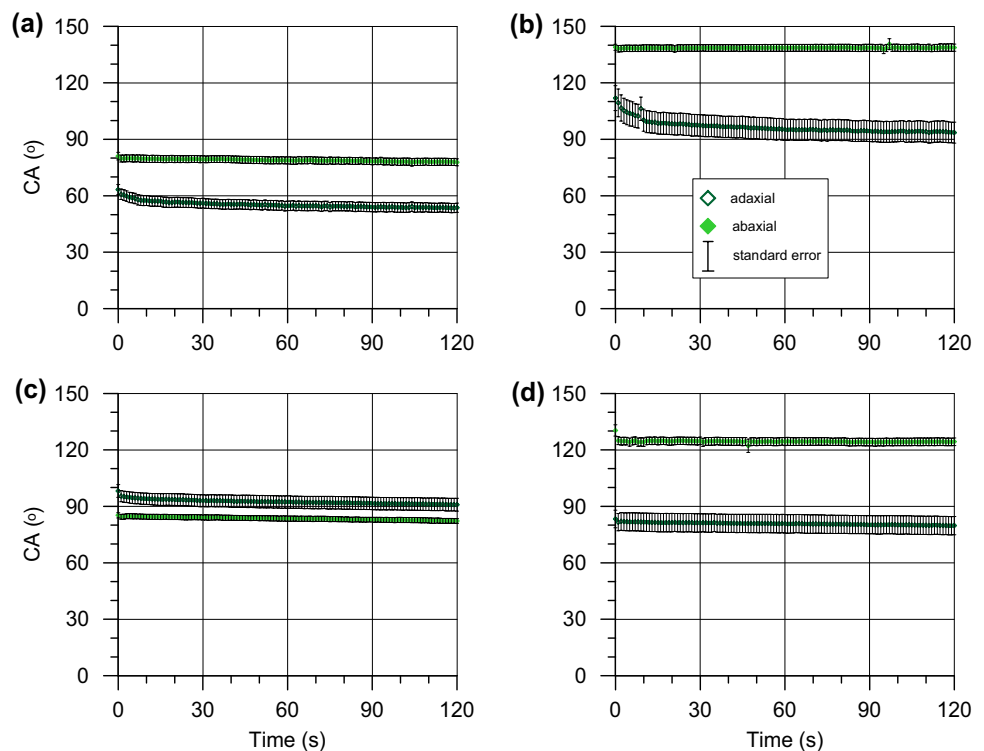
The statistical analysis was performed using Statgraphics plus (STSC Inc.–Statistical Graphics Corporation, 1996). Two-way analysis of variance (ANOVA) was used to compare the CA values. The mean comparison was done using Tukey's range test at  $p < 0.05$ . Principal component analysis (PCA) was performed on the  $CA_{in\ ad}$ ,  $CA_{fin\ ad}$ ,  $CA_{in\ ab}$ ,  $CA_{fin\ ab}$ ,  $CA_{in-fin\ ad}$ ,  $CA_{in-fin\ ab}$ ,  $DA_{ab}$ , and  $DA_{ad}$  results of the 19 leaves species to explore the spatial variability of the data and the relation between the investigated leaf properties. The PCA was conducted with Unscrambler

X 10.3 (Camo, Norway) using full cross-validation (19 segments). In addition, cluster analysis was performed to classify plant species according to their leaf CAs values (initial and final) on abaxial and adaxial sides. Ward's method was used with Euclidean distance to define the clusters. The Ward's method minimizes the total within-cluster variance.

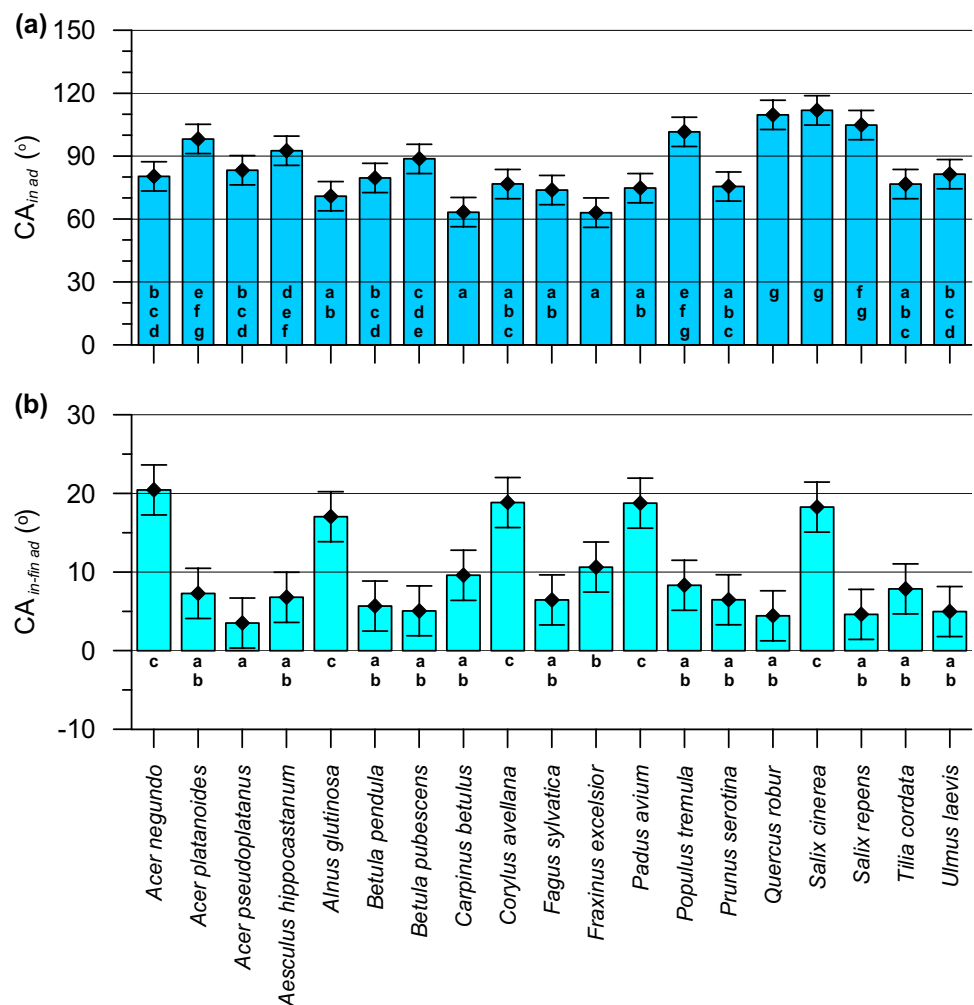
## Results

During measurements using the sessile drop method, a decrease in the CA value as a function of time was observed for the majority of the tested leaves. Overall, the CA behavior was similar for all species; therefore, selected results of CA measurements for the adaxial and abaxial leaf side vs. time are presented in Fig. 2. Figure 3 presents the initial CA ( $t = 1\ s$ ) values ( $CA_{in\ ad}$ ) along with adaxial value changes over the 120 s period of the experiment ( $CA_{in-fin\ ad}$ ), together with the results of the two-way ANOVA homogeneous groups. The highest initial  $CA_{in\ ad}$  values (Fig. 3a) were observed for *Salix cinerea*,  $111.9^\circ$ , and for *Quercus robur*,  $109.7^\circ$ . The lowest initial  $CA_{in\ ad}$  values were observed for *Fraxinus excelsior*,  $63.1^\circ$ , and for *Alnus glutinosa*,  $70.9^\circ$ . Species that were characterized by the highest leaf CA value changes ( $CA_{in-fin\ ad}$ ) over the duration of the experiment are presented in Fig. 3b. These species are as follows: *Acer negundo* ( $20.4^\circ$ ), *Corylus avellana* and *Padus avium* ( $18.8^\circ$ ), *Salix cinerea* ( $18.3^\circ$ ), and *Alnus glutinosa* ( $17^\circ$ ). The

**Fig. 2** Example of CA measurements for the adaxial and abaxial leaf side vs. time for **a** *Carpinus betulus*, **b** *Salix cinerea*, **c** *Acer platanoides*, and **d** *Acer pseudoplatanus*



**Fig. 3** Measurement CA results for the adaxial side for **a** initial leaf CA ( $CA_{in\ ad}$ ) and **b** leaf CA value changes in 120 s time ( $CA_{in-fin\ ad}$ ). Different letters indicate significant differences ( $p < 0.05$ ) among values after two-way ANOVA



lowest value changes ( $CA_{in-fin\ ad}$ ) were observed in the following species: *Acer pseudoplatanus* (3.5°), *Quercus robur* (4.5°), and *Ulmus laevis* (5°). The abaxial side of the leaf is characterized with greater leaf CA values compared to the adaxial side for a majority of species (Fig. 4).

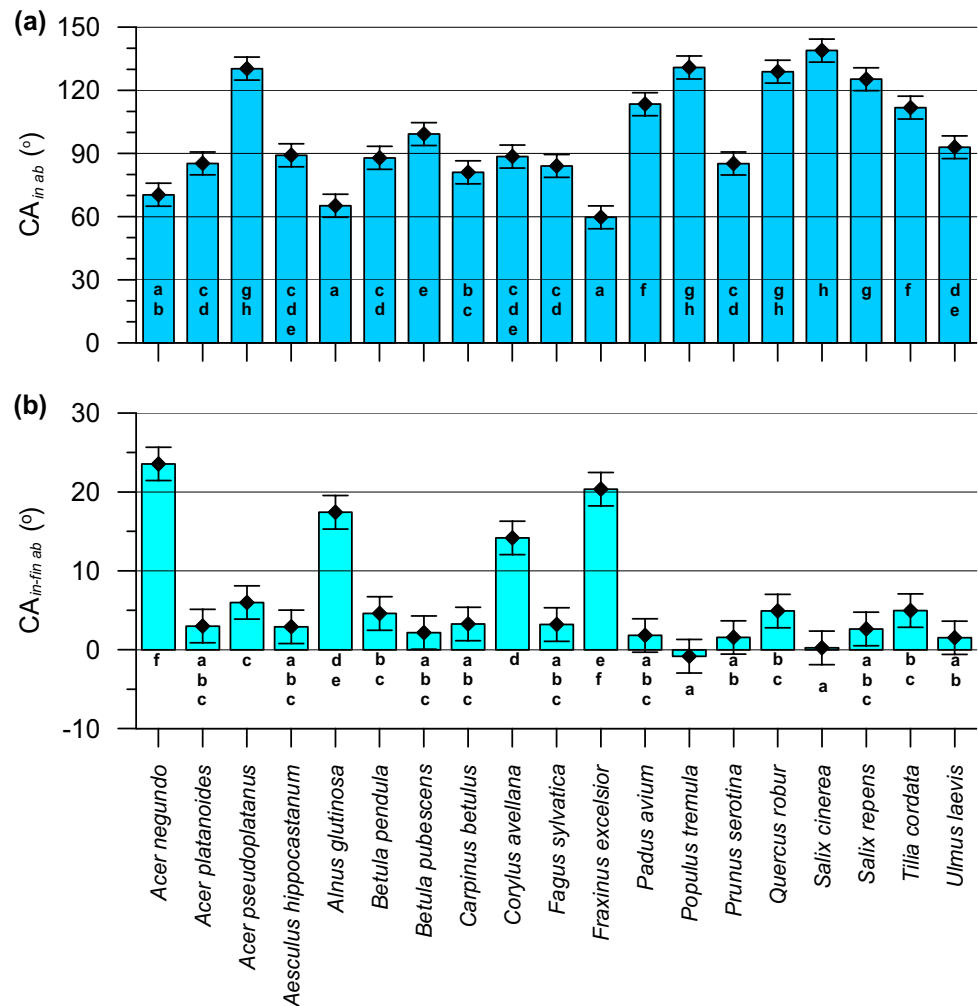
In the case of the abaxial side, the highest initial CA values  $CA_{in\ ab}$  (Fig. 4a) were observed in *Salix cinerea* (138.9°), *Populus tremula* (130.9°) and *Acer pseudoplatanus* (130.4°), and the lowest in *Fraxinus excelsior* (59.7°), *Alnus glutinosa* (65.2°) and *Acer negundo* (70.4°). The highest decrease of leaf CA values with time  $CA_{in-fin\ ab}$  was observed in *Acer negundo* (23.6°), *Fraxinus excelsior* (20.4°), *Alnus glutinosa* (17.4°), and *Corylus avellana* (14.2°), and the lowest in *Populus tremula* (−0.8°) and *Salix cinerea* (0.2°).

Figure 5 presents CA differences between the adaxial and abaxial sides of leaves. Analysis of the initial leaf CA ( $CA_{in\ ad} - CA_{in\ ab}$ ) shows (Fig. 5a) that the highest differences were observed in *Acer pseudoplatanus* (47.1°), *Padus avium* (38.7°), and *Tilia cordata* (35.1°), and the lowest differences in *Fraxinus excelsior*, *Aesculus hippocastanum* (3.4°), and *Alnus glutinosa* (5.7°). In the case of the final leaf

CA (measured after 120 s from the placement of the water drop), the highest differences between adaxial and abaxial side ( $CA_{fin\ ad} - CA_{fin\ ab}$ ) of the leaf were observed in *Padus avium* (55.6°), *Salix cinerea* (45.1°), and *Acer pseudoplatanus* (44.6°), and the lowest in *Aesculus hippocastanum* (0.5°) and *Alnus glutinosa* (6.1°).

Using the wettability class proposed by Aryal and Neuner (2010), and based on the initial CA values for the adaxial leaf sides ( $CA_{in\ ad}$ ), the 19 analysed species can be grouped into the three following categories: highly wettable ( $40^\circ < CA < 90^\circ$ ), which described 13 species, wettable ( $90^\circ < CA < 110^\circ$ ), which described 5 species, and non-wettable ( $110^\circ < CA < 130^\circ$ ), which described 1 species (Table 1). In analysing the abaxial leaf sides ( $CA_{in\ ab}$ ), four groups were assigned: highly wettable (ten species), wettable (two species), non-wettable (four species), and highly non-wettable ( $130^\circ < CA < 150^\circ$ ), which described three species. Among the examined leaves, no species were characterized as super-hydrophilic ( $CA < 40^\circ$ ) or super-hydrophobic ( $CA > 150^\circ$ ). In Table 1, average values of the water drop asymmetry coefficient for the adaxial ( $DA_{ad}$ ) and abaxial

**Fig. 4** Measurement results for the abaxial side for **a** initial leaf CA ( $CA_{in\ ab}$ ) and **b** leaf CA value changes in 120 s time ( $CA_{in-fin\ ab}$ ). Different letters indicate significant differences ( $p < 0.05$ ) among values after two-way ANOVA



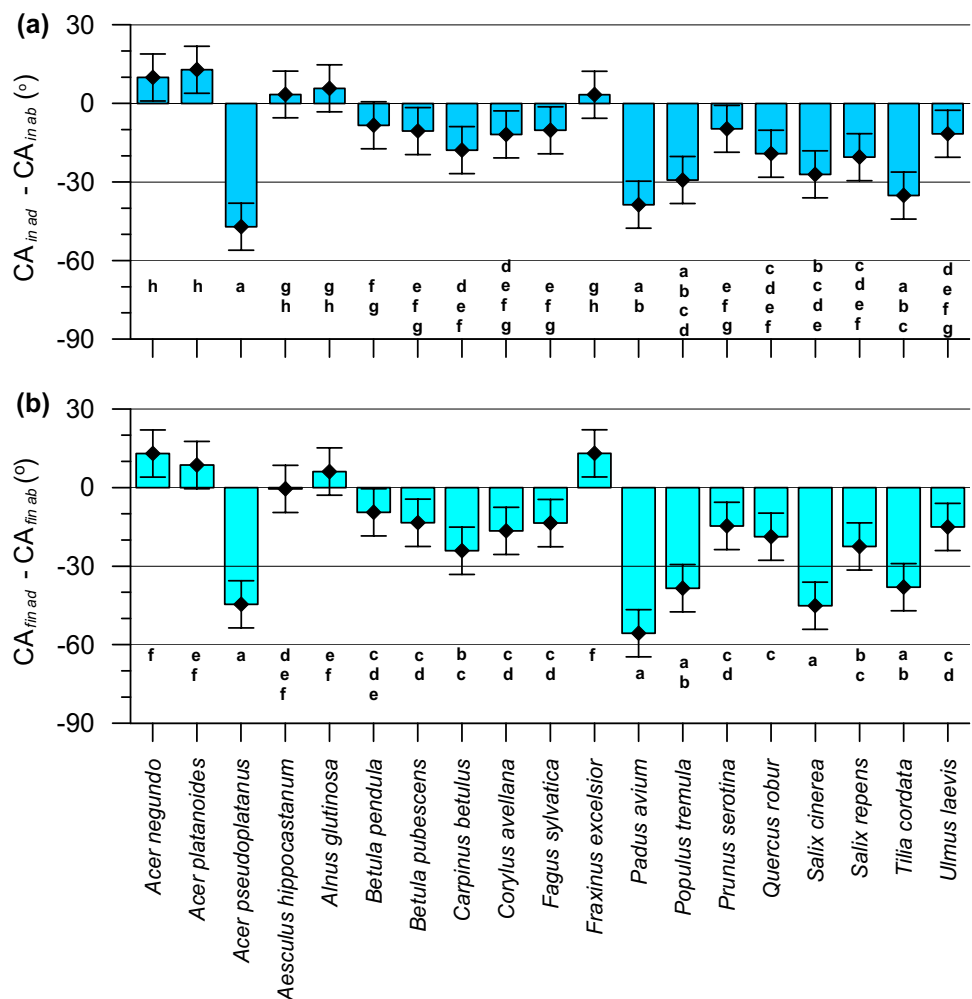
( $DA_{ab}$ ) leaf sides calculated using Eq. 1 are also presented. The highest values of the water drop asymmetry coefficient for the adaxial side of the leaves ( $DA_{ad}$ ) were observed in *Prunus serotina* (0.06) and the lowest in *Acer platanoides* (0.012). For the abaxial side of the leaves ( $DA_{ab}$ ), the highest coefficient values were observed in *Fraxinus excelsior* (0.116) and the lowest in *Acer pseudoplatanus* (0.005).

The scores of the PCA analysis on  $CA_{in\ ad}$ ,  $CA_{fin\ ad}$ ,  $CA_{in\ ab}$ ,  $CA_{fin\ ab}$ ,  $CA_{in-fin\ ad}$ ,  $CA_{in-fin\ ab}$ ,  $DA_{ab}$ , and  $DA_{ad}$  are presented in Fig. 6. The samples are distributed along the PC-1 axis and to the least extent along the PC-2 axis. There is no grouping according to family or genus rank. Correlation loadings of the PCA (Fig. 7) explain the correlation between the scores and the observed data. From this plot, it is visible that  $CA_{in\ ad}$  and  $CA_{fin\ ad}$  have a high positive correlation as for  $CA_{in\ ab}$  and  $CA_{fin\ ab}$  (the points are connected on the plot).  $DA_{ab}$  and  $CA_{fin\ ab}$  are also positively correlated (points close to each other). Those two properties are negatively correlated to  $CA_{in\ ad}$ ,  $CA_{fin\ ad}$ ,  $CA_{in\ ab}$  and  $CA_{fin\ ab}$  since located on the other side of the plot. The sum of the explained variance for PC-1 and PC-2 is 96% with

PC-1 covering most of the variation (87%). Six variables are explained by more than 50% (outside the inner circle).  $CA_{in-fin\ ad}$  and  $DA_{ad}$  are located in the inner circle (less than 50% of variance explained) and  $DA_{ab}$  and  $CA_{in-fin\ ad}$  are outside but near the border. These variables, therefore, do not contain enough structured variation to be discriminating for the leaf samples and were discarded from the following cluster analysis.  $CA_{in\ ad}$ ,  $CA_{in\ ab}$ ,  $CA_{fin\ ad}$ , and  $CA_{fin\ ab}$  were considered enough to group the data (leaves).

Based on the cluster analysis (Fig. 8), five groups of species can be distinguished. These five groups are clearly visible also on the PCA score plot (Fig. 6) with the exception of one sample (*Carpinus betulus*). These groups are characterized with similar leaf CA values and, therefore, similar leaf wettability. The first group (*Carpinus betulus*, *Alnus glutinosa*, *Fraxinus excelsior*, and *Acer negundo*) includes species presenting low leaf CA (initial and final) similar in both adaxial and abaxial sides. The second group (*Acer platanoides*, *Aesculus hippocastanum*, and *Betula pubescens*) includes species with higher leaf CA values compared to the previous group but which also have similar CA values

**Fig. 5** Differences in leaf CA values between adaxial and abaxial side of the leaf for **a** initial ( $CA_{in\ ad} - CA_{in\ ab}$ ) and **b** final ( $CA_{fin\ ad} - CA_{fin\ ab}$ ) values of the angle



for both adaxial and abaxial sides. The third group (*Betula pendula*, *Ulmus laevis*, *Fagus sylvatica*, *Prunus serotina*, and *Corylus avellana*) includes species characterized by the highest values of leaf CAs for the abaxial side. The fourth group, with species like *Acer pseudoplatanus*, *Tilia cordata*, and *Padus avium*, is characterized by higher abaxial values of the leaf CA. The last group of species was characterized by the highest values of leaf CAs, and furthermore by higher CA values for the abaxial side of the leaves than for the adaxial side. The species belonging to this group are *Quercus robur*, *Salix repens*, *Salix cinerea*, and *Populus tremula*.

## Discussion

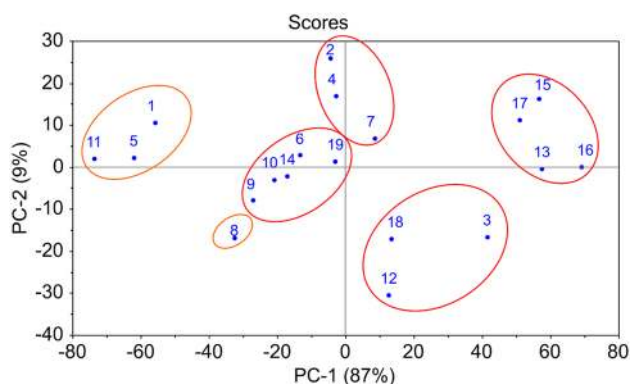
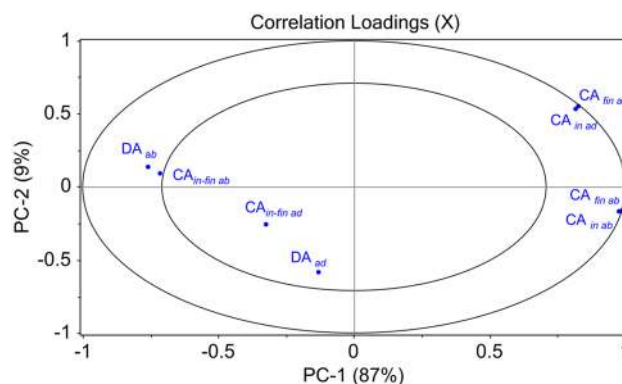
Research on plant leaf wettability has been carried out by many authors (Hall and Burke 1974; Barthlott and Neinhuis 1997; Brewer and Smith 1997; Brewer and Nunez 2007; Holder 2007; Fernández et al. 2014). Comparing wettability results is not easy, since the leaf wettability (adaxial side)

has been proved to change throughout the growing season (Van Wittenberghe et al. 2012). In our study, CA values varied from about 60° to about 140°. These data are comparable to the CA values found in the literature, which range from 20° to 180°. CA values depended on species and leaf sides, as confirmed by other studies (e.g., Brewer and Smith 1997; Brewer and Nuñez 2007; Holder 2007; Sikorska et al. 2017). The majority of the investigated leaf species presented CA values lower than 90° and during the 2 min of measurement, the water drop spread on the surface increasing the wet surface on the leaf which might cause an increase of evaporation of the intercepted water and reduce the amount of water reaching the root zone. This seems particularly important in the context of climate changes where temperate regions like Poland are believed to experience in the future a hotter and dryer climate (Anders et al. 2014).

The amount of water retained on the plant depends on its morphological characteristics, including the angle of placement, leaves, and stalks' size, but also leaf roughness and plant height. The vegetation affects the process of rain-water retention that is for the most part directly returned

**Table 1** Wettability classes and water drop asymmetry coefficient DA values for examined species

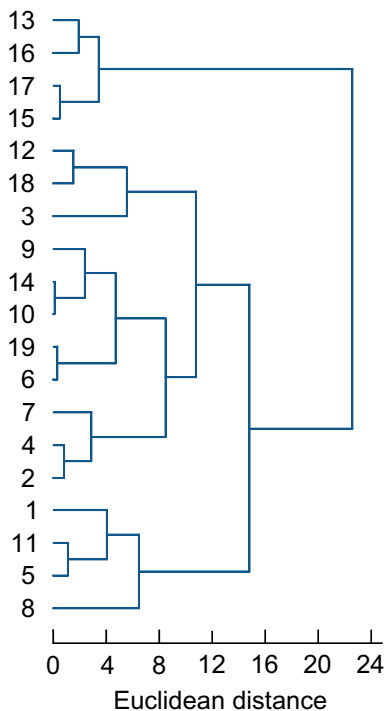
No.	Species	Wettability class		DA <sub>ad</sub>	DA <sub>ab</sub>
		Adaxial	Abaxial		
1	<i>Acer negundo</i>	Highly wettable	Highly wettable	0.028	0.089
2	<i>Acer platanoides</i>	Wettable	Highly wettable	0.012	0.016
3	<i>Acer pseudoplatanus</i>	Highly wettable	Highly non-wettable	0.020	0.005
4	<i>Aesculus hippocastanum</i>	Wettable	Highly wettable	0.014	0.016
5	<i>Alnus glutinosa</i>	Highly wettable	Highly wettable	0.027	0.063
6	<i>Betula pendula</i>	Highly wettable	Highly wettable	0.026	0.010
7	<i>Betula pubescens</i>	Highly wettable	Wettable	0.031	0.021
8	<i>Carpinus betulus</i>	Highly wettable	Highly wettable	0.050	0.028
9	<i>Corylus avellana</i>	Highly wettable	Highly wettable	0.048	0.022
10	<i>Fagus sylvatica</i>	Highly wettable	Highly wettable	0.034	0.015
11	<i>Fraxinus excelsior</i>	Highly wettable	Highly wettable	0.032	0.116
12	<i>Padus avium</i>	Highly wettable	Non-wettable	0.058	0.011
13	<i>Populus tremula</i>	Wettable	Highly non-wettable	0.024	0.008
14	<i>Prunus serotina</i>	Highly wettable	Highly wettable	0.060	0.020
15	<i>Quercus robur</i>	Wettable	Non-wettable	0.023	0.013
16	<i>Salix cinerea</i>	Non-wettable	Highly non-wettable	0.029	0.006
17	<i>Salix repens</i>	Wettable	Non-wettable	0.032	0.010
18	<i>Tilia cordata</i>	Highly wettable	Non-wettable	0.021	0.020
19	<i>Ulmus laevis</i>	Highly wettable	Wettable	0.020	0.027

**Fig. 6** PCA scores of the two first components based on 19 plant species for the following properties: CA<sub>in ad</sub>, CA<sub>fin ad</sub>, CA<sub>in ab</sub>, CA<sub>fin ab</sub>, CA<sub>in-fin ad</sub>, CA<sub>in-fin ab</sub>, DA<sub>ad</sub>, and DA<sub>ab</sub>, where 1-*A. negundo*, 2-*A. platanoides*, 3-*A. pseudoplatanus*, 4-*A. hippocastanum*, 5-*A. glutinosa*, 6-*B. pendula*, 7-*B. pubescens*, 8-*C. betulus*, 9-*C. avellana*, 10-*F. sylvatica*, 11-*F. excelsior*, 12-*P. avium*, 13-*P. tremula*, 14-*P. serotina*, 15-*Q. robur*, 16-*S. cinerea*, 17-*S. repens*, 18-*T. cordata*, and 19-*U. laevis*. The red ellipses are groups similar to those from the cluster analysis. (Color figure online)**Fig. 7** Correlation loadings for the two first components based on the 19 plant species and the following properties: CA<sub>in ad</sub>, CA<sub>fin ad</sub>, CA<sub>in ab</sub>, CA<sub>fin ab</sub>, CA<sub>in-fin ad</sub>, CA<sub>in-fin ab</sub>, DA<sub>ad</sub>, and DA<sub>ab</sub>. The outer ellipse explains 100% of variance and the inner ellipse indicates 50% of explained variance

to the atmosphere through evaporation, partly absorbed by plants, or flowing along plant stems down to the ground (Kołodziej 2011). Rainfall interception is the process of rainwater retention by the plant cover and plays an important role in the water balance. Interception losses in forest environment account for 10–50% of season-long or annual rainfall (Carlyle-Moses and Gash 2011) and for 30–40% in

cultivated field (Kołodziej 2011). Interception is affected by leaf water repellency. In our knowledge, LWR is not taken into account when building ecohydrological models; nevertheless, accurate data are needed. Moreover, to estimate the plant available water, it is important to use proper LWR data. Therefore, the correct estimation of LWR is crucial and the proposed methodology should help to minimize estimation errors and allows for result reproducibility. Further investigation are needed as underlined by Holder (2007), since the significance of LWR





**Fig. 8** Clustering results of deciduous shrub and tree species based on CA values (initial and final) of adaxial and abaxial side of the leaves, where 1-*A. negundo*, 2-*A. platanoides*, 3-*A. pseudoplatanus*, 4-*A. hippocastanum*, 5-*A. glutinosa*, 6-*B. pendula*, 7-*B. pubescens*, 8-*C. betulus*, 9-*C. avellana*, 10-*F. sylvatica*, 11-*F. excelsior*, 12-*P. avium*, 13-*P. tremula*, 14-*P. serotina*, 15-*Q. robur*, 16-*S. cinerea*, 17-*S. repens*, 18-*T. cordata*, and 19-*U. laevis*

as a mechanism that influences hydrological processes is underexplored.

In the view of our results, it appears that more attention should be paid to time scale during CA measurements. Through the first 2 min of measurements, a significant decrease in CA were observed with every leaves investigated (from 3.5° to 20.4° for adaxial side and from 0.2° to 23.6° for abaxial side) except for leaves from *Populus tremula* in abaxial side. With several species (*Acer pseudoplatanus*, *Fraxinus excelsior*, *Tilia cordata*, *Aesculus hippocastanum*, and *Salix cinerea*), the decrease involved a change of wettability class. This was the case for both adaxial and abaxial side of the leaves, but the decrease was more pronounced in the adaxial side. The most significant changes were observed for *Tilia cordata* where the wettability classification changed from non-wettable to wettable. Furthermore, CA values for several other species presented a decrease of around 20° (see Figs. 3b, 4b). However, the classes did not change, because all these species belonged to the “highly wettable” class and that class encompass a broad range of CA values (40° < CA < 90°, Aryal and Neuner 2010). Moreover, three of the other wettability classes (wettable, non-wettable, and highly non-wettable) are 20° range classes. In that case, if a

20° of CA occurs, it will automatically lead to a change of wettability class. It should be noted that half of CA changes are taking place during the first 10 s of measurements (e.g., 11.5° for adaxial side and 18.2° for abaxial side of *Acer negundo*; 15.0° for adaxial side and 8.1° for abaxial side of *Corylus avellana*). This seems important to take into account when reporting CA<sub>in</sub>. We believe that a standardization of the methodological procedure related to time of CA measurement is required, since water droplet placement on the leaf surface is required for proper comparison of CA results from different studies. It should be noted that standardization procedure was preconized by Matos and Rosado (2016) in regards to the volume of the droplet, since the droplet volume does matter when measuring leaf wetness traits.

The PCA analysis showed that, under identical measurement conditions, it is possible to compare leaf wettability basis on both initial and final CA, because those CA's have a high positive correlation for the adaxial and abaxial leaf sides. Furthermore, laboratory analysis revealed that the CA's changes during the measurement time (2 min) for all investigated species are variable. In some species, we observed CA stability for, e.g., the adaxial side of *Acer pseudoplatanus* (less than 5°) in contrast to *Acer negundo* (more than 20°). Therefore, we propose that the behavior of drops during the measurement should be taken into account in the evaluation of leaf wettability. Rosado and Holder (2013) also noticed that the methodology is not unambiguous for goniometric studies and showed that, in the literature, there are more than five different methodologies for CA measurements. Moreover, the leaf wettability classification based on the CA values proposed by Aryal and Neuner (2010) is widely used. Applying the same CA scale with different measurement methodology may not be correct.

The studied plant species have been classified into five groups (Fig. 8) by applying cluster analysis based on CA values (abaxial and adaxial leaf surface). The results were confirmed by the PCA analysis (Fig. 6) run on CA<sub>in ad</sub>, CA<sub>fin ad</sub>, CA<sub>in-fin ad</sub>, CA<sub>in-fin ad</sub>, DA<sub>ad</sub>, and DA<sub>ab</sub>. These groups represent species of eight different plant families: *Betulaceae*, *Fagaceae*, *Sapindaceae*, *Malvaceae*, *Oleaceae*, *Rosaceae*, *Salicaceae*, and *Ulmaceae* (The Plant List 2010). However, differences in CA values were observed not only between these groups but also among species within the same family. *Acer negundo* (I group), *Acer platanoides* (II group), *Acer pseudoplatanus* (IV group) from *Sapindaceae* family like *Betula pubescens* (II group) and *Betula pendula* (III group) from *Betulaceae* family due to CA differences have been clustered in different groups. These results could be explained by the differences in leaf morphology. Leaves of *Acer negundo* consist of three to five leaflets which are about 5–10 cm long and 3–7 cm wide. The leaves of *Acer platanoides* are palmately lobed with five lobes (7–14 cm long and 10–18 cm wide) and the leaves of *Acer pseudoplatanus*

are 10–25 cm long, broad, and palmated (Johnson and More 2009; van Gelderen and van Gelderen 1999; Rushforth 1999). In turn, the leaves of *Betula pubescens* are slightly smaller, thicker, and more serrated compared to the leaves of *Betula pendula* (Johnson and More 2009; Rutkowski 2004). These morphological differences between the leaves of different species are probably closely related to the leaf surface microstructure, e.g., the presence or absence of cuticular wax and trichomes, stomatal density, or the shape of epidermal cells.

Leaves play key roles in plant function and long-term adaptation to the environment. Although leaves are basically an assemblage of epidermis, stomata, and mesophyll, they exhibit apparent differences in area, thickness, and shape among different species as a result of phylogenetic relationships and adaptation to specific environments (Royer et al. 2008). In our study, we observed differences in CA values not only between the five groups but also among species within the same family. These results could be explained by leaf morphology differences which could be correlated with leaf surface microstructure and leaf anatomy. Tian et al. (2016) conducted an integrative investigation of leaf morphological and anatomical traits, including leaf area, leaf dry weight, stomatal length, stomatal density, stomatal pore area index, leaf thickness, palisade-leaf mesophyll thickness ratio, and spongy-leaf mesophyll thickness ratio, in 99 tree species. They identified positive correlations between leaf area and leaf dry weight, leaf thickness and palisade mesophyll thickness, and negative correlations between stomatal length and stomatal density. Moreover, these authors pointed out that climate was the main factor that regulated leaf morphological and anatomical traits (Tian et al. 2016). Leaf morphology correlates with water and light availability (Xu et al. 2009). In hot environments, leaves could adapt to climatic conditions by favoring the selection of small, narrow, or dissected leaves to help thermal regulation (Leigh et al. 2017). Other factors like low and high wind rates and leaf surface thermal regulation can also influence leaf shape (Vogel 2009).

The leaves of higher plants are covered by a protective lipophilic membrane called cuticle which consists of cutin matrix and waxes, including epicuticular wax (exterior to cutin that can be mechanically peeled off) and intracuticular wax residing within the mechanically resistant layer of cutin (Müller and Riederer 2005; Buschhaus and Jetter 2011). The physicochemistry of cuticular wax (e.g., wax content, composition and microstructure) affects the leaf surface wettability (Neinhuis and Barthlott 1998; Wang et al. 2015). The cuticular waxes vary considerably between and among different species. Cuticular waxes change during leaf developmental stages and between leaf sides (Wang et al. 2013, 2014, 2015). The CAs of *Nelumbo nucifera* and *Colocasia esculenta* were significantly lower than 90° when the wax

was removed with acetone from the leaf surface by Burton and Bhushan (2006). Neinhuis and Barthlott (1998) demonstrated that high CAs (130°–140°) for *Ginkgo biloba* leaves can be attributed to the density of wax crystals covering the leaf during the whole period of growth, while leaves of *Quercus robur*, which are only partially covered by amorphous wax, have hydrophobic properties only a few weeks after the wax crystals were produced. Therefore, our findings of different CA values between the five groups and between species within the same plant family could be related to differences in cuticular wax content between species and during different leaf developmental stages. Thus, despite the fact that leaves were collected at the same time, they could have been at different stage of their development and present different wettability.

Hairs called trichomes are an additional important factor affecting leaf wettability. The trichomes have different structures and functions. There are glandular and non-glandular trichomes which originate from epidermal cells (Werker 2000). The covering by non-glandular trichomes and substances secreted by glandular trichomes (e.g., waxes and lipids) provides a barrier against unfavorable environmental factors. In some plants, the growth of leaf trichomes begins very early and their final number is established already during leaf differentiation, although, in other species, new hairs are formed during the entirety of leaf development (Valkama et al. 2004; Werker 2000; Turner et al. 2000). In many plant species, trichome density is very high in young leaves and decreases with leaf expansion (Valkama et al. 2004; Werker et al. 1993). It is suggested that, in young leaves, trichomes act as an epidermis, while, in the next stages of leaf development, their protective role is taken over by the epidermis (Valkama et al. 2004). High trichome density is also particularly characteristic for young leaves of *Salix cinerea* and *Populus tremula* belonging to the fifth study group (Johnson and More 2009).

The location of trichomes on plant organs, their size, and their density are variable among species (Wang et al. 2015). Differences in trichome structure and density among leaves of *B. pendula*, *B. pubescens* ssp. *pubescens*, and *B. pubescens* ssp. *czerepanovii* have been observed by Valkama et al. (2003, 2004). The total number of glandular trichomes per leaf did not change during leaf development of the examined species but varied significantly among species and between leaf sides. On the adaxial leaf side of *B. pendula*, the total number of glandular trichomes was higher than in both subspecies of *B. pubescens*. On the abaxial side of *B. pendula* and *B. pubescens* ssp. *pubescens*, it was higher than *B. pubescens* ssp. *czerepanovii* (Valkama et al. 2004). Pandey and Nagar (2002) indicated that leaves with trichomes, and particularly those for which density is greater than 25 mm<sup>-2</sup>, are more hydrophobic. The results of Wang et al. (2015) showed that the adaxial side of *Callistephus chinensis*

leaves characterized by a CA of  $139^\circ$  was densely covered with conoid trichomes, while the adaxial side of *Cucurbita pepo* leaves, sparsely covered by villous, had a CA of  $70^\circ$ .

The wettability of leaf surface with trichomes is also dependent on the presence of wax crystals on the trichomes. Wang et al. (2015) indicated that the adaxial surface of *Nelumbo nucifera* with waxy trichomes presented a high CA of  $150^\circ$ , but the abaxial surface of *Calyptrea chinensis* with non-waxy trichomes had a CA of  $97^\circ$ . Leaves with non-waxy trichomes are only water repellent for a short period of time after application of the drop of water, but leaves with waxy trichomes were highly resistant to water (Wang et al. 2015). Engel et al. (1993) indicate that glandular trichomes of *Quercus robur* leaves contain an essential oil which dissolves wax crystalloids after it is released on the leaf surface. In turn, the results of Nikolić et al. (2003) showed that, on both *Quercus robur* leaf surfaces, trichomes were present, but they were plentiful in terms of density and type on the abaxial side. On the adaxial leaf surface, only lonely non-glandular trichomes were observed, while, on the abaxial surface, glandular and non-glandular trichomes were present. Therefore, the observed differences between abaxial and adaxial sides of the initial and final leaf CAs (Figs. 3, 4) could be connected to trichome structure and to the presence or absence of wax on them. The initial CA values on the abaxial side were 10–56% higher than on adaxial leaves side.

Wettability is also affected by the structure of epidermal cells. Wang et al. (2014) observed that convex epidermal cells with wax crystals of *Cynanchum chinense* and *Anemone vitifolia* had higher CAs than leaves with smooth epidermal cells of *Populus simonii* and *Cynanchum komarovii*.

Our research also demonstrated that, for a majority of species, the abaxial side of the leaf is characterized by greater CA values as compared to the adaxial side (Fig. 5). The initial CA values on the abaxial side were 10–56% higher than on the adaxial side. Many studies have postulated that the higher CA on the abaxial leaf surface rather than on adaxial side is due to stomatal distribution. Brewer

and Nuñez (2007) showed that a leaf surface with a greater concentration of stomata is less wettable, and Brewer and Smith (1997) indicated that higher stomatal density is combined with higher CA. It can also be caused by the presence of both trichomes and cuticular wax, as described above. However, many studies postulated that the higher CA on the abaxial leaf surface rather than on adaxial side is combined with stomatal distribution. Bačić (1981) indicates that differences of stomatal number among *Q. robur*, *Q. cerris*, and *Q. fraineto* species are dependent on location, tree age, and the part of the leaf. Ashton and Berlyn (1994) observed that stomatal density was higher in the leaves exposed to full sun than those growing in the shade. Brewer and Nuñez (2007) then proved that a greater concentration of stomata means decreased wettability of the leaf surface and is related to a higher CA value.

The species *Acer negundo*, *Acer platanoides*, *Aesculus hippocastanum*, *Alnus glutinosa*, and *Fraxinus excelsior* were characterized with the initial adaxial CA values that were higher by 4–13% than the abaxial CA. Similar results were observed by Kardel et al. (2012). Those authors examined five species. Two species had less hydrophobic abaxial sides (*Alnus glutinosa* and *Sambucus nigra*), and three had abaxial sides that were more hydrophobic (*Acer pseudoplatanus*, *Betula pendula*, and *Quercus robur*). The CA values obtained were slightly lower than those observed in our research. This is the result of the type of habitat. According to Kardel et al. (2012), the habitat type significantly affects leaf wettability. In our study, the samples were taken from a natural habitat, which confirms the thesis that leaves have higher CA values in their natural habitat. This means that depending on the habitat conditions, the wettability is variable even among plants of the same species. Combining our results with Kardel et al. (2012), the leaf CAs are increasing from industrial to semi-natural and finally to natural (present study) habitats (Table 2). The increase of CA values with the decrease of air pollution is on that particular situation and particular plant species may be a rule, but further

**Table 2** Comparison of the initial CA values and standard errors for adaxial and abaxial leaf surfaces, depending on species and habitat types

Species	Contact angle ( $^\circ$ )		
	Natural habitat Own results	Semi-natural Kardel et al. 2012	Industrial Kardel et al. 2012
<i>Acer pseudoplatanus</i>			
Ad	$83 \pm 3.2$	$70 \pm 3.3$	$69 \pm 10.4$
Ab	$130 \pm 2.1$	$122 \pm 11.4$	$115 \pm 8.3$
<i>Betula pendula</i>			
Ad	$80 \pm 2.6$	$74 \pm 4.3$	$70 \pm 3.1$
Ab	$88 \pm 2.4$	$84 \pm 9.3$	$88 \pm 6.2$
<i>Quercus robur</i>			
Ad	$110 \pm 1.7$	$79 \pm 2$	$65 \pm 4.5$
Ab	$129 \pm 3.3$	$118 \pm 4.4$	$102 \pm 9$

investigation are needed to test that hypothesis. Kardel et al. (2012) also hypothesized that drop asymmetry (DA) will increase with exposure time to pollution because of damage to the leaf surface. Their findings were not, however, conclusive, probably due to the microscopic scale of leaf heterogeneity. The DA from the industrial area was not automatically higher than that in the semi-natural area. However, our findings tend towards their hypothesis, since, in our natural habitats, the DA values are smaller than in Kardel et al. (2012).

This is confirmed by other papers. Pinon et al. (2006), e.g., reported that in the case of poplar leaves, the CA values ranged from 42 to 97°. In our study, three species from the *Aceraceae* family were characterized by several CA. *Acer platanoides* was characterized by similar CA values as *Acer negundo*, with adaxial leaf sides that were slightly more hydrophobic (CA greater by about 10–13°) than abaxial leaf sides. *Acer pseudoplatanus* has leaves characterized by more hydrophobic abaxial sides (CA  $\geq$  130°) as compared to adaxial leaf sides (83°). This is similar to Holder (2007) for *Acer saccharinum* (73.2° for the adaxial leaf surface, and 134.6° for the abaxial leaf surface).

As described above, many internal factors can affect the wettability of the leaf surface. However, to explain thoroughly all the differences between the examined tree and shrub species, the microstructure of these leaves should be analysed. This will be investigated in the near future. Such a comprehensive analysis of leaf surfaces (a measurement of the CA value and microstructure analysis) could have very important ecological significance, because leaves are multi-functional interfaces between plants and their environment.

## Conclusions

In this study, we investigated leaf wettability of 19 deciduous trees and shrubs commonly found in a temperate zone. The presented results confirm that wetting CA values depend on the plant species but also of the leaf side. CA differences were observed among species even within the same family. The CA values of the analysed leaves ranged from roughly 60° to about 140°. These leaves are classified from highly wettable to highly non-wettable for both sides of the leaves. The CA values changed during measurement time for all tested species with a rapid CA decrease in the first 10 s of measurement. We advise that not only the initial CA value is important when assessing wettability, but also what happens with the drop with time. We observed that these changes of CA with time were different among species and could, sometimes, generate wettability class changes.

The leaves originated from trees and shrubs in a natural habitat, and the measured CA values were higher than the ones presented in the literature for industrial or semi-natural habitats where leaves are exposed to air pollution which may

affect the amount and quality of waxes and damage the leaf surface. Moreover, the drop asymmetry (DA) values were also lower than what can be found for the same species in industrial habitats. For the vast majority of species (14), the abaxial side of the leaf was characterized by higher CA values (less wettability) compared to the adaxial side of the leaf surface, which is more exposed to environmental factors as well as to water loss. The knowledge of leaf wettability and the understanding of water retention processes on leaf surface are very important and complex, and need further analysis.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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