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Variability and Extremes in Leaf Wettability and Run-Off Properties in Plants from Various Habitats

Biva Aryal and Gilbert Neuner*

Institute of Botany, University of Innsbruck, Sternwartestraße 15, 6020 Innsbruck, Austria

Research Article

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***For Correspondence**

Gilbert Neuner, University of Innsbruck, Institute of Botany, Sternwartestraße 15, 6020 Innsbruck, Austria, Tel: +43/512/507-51026

E-mail: Gilbert.Neuner@uibk.ac.at

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ABSTRACT

The natural variability and extremes of surface wettability properties of leaves was investigated in 396 plant species out of 85 families growing in three different continents at various elevations (186-5268 m) and in all major ecological zones. This should yield the range of leaf surface wettablility properties but also allow detecting extreme hydrophobic and hydrophilic leaf surfaces. Surface wettability properties were measured by contact angle θ of defined water droplets applied to the leaves and their run off angle.

Wettability of leaf surfaces differed significantly between species (16.7° <θ<169.7°). 72.6% of the species investigated showed extreme surface properties, being either highly wettable or extreme-hydrophilic (37.8 %) or highly non-wettable or extreme-hydrophobic (34.8%). Leaf surface wettability was in some families specific. Oxalidaceae and Fabaceae showed hydrophobic properties, Ericaceae and Moraceae were rather hydrophilic. Many families comprised all wettability classes. Sixteen leaf surfaces were extreme-hydrophilic (θ=27-40°) and with one exception found in the tropical to subtropical ecological zones. Extreme-hydrophilic properties were detetcted more frequently on glabrous and adaxial leaf surfaces. Twenty-four leaf surfaces were super-hydrophobic (θ>150°), were from virtually all ecological zones and observed mostly abaxial and on pubescent leaves. On extreme-hydrophilic leaf surfaces water droplet run off occurred at a significantly lower mean angle of inclination (8°) as compared to super-hydrophobic (19°) leaf surfaces. Within each leaf wettability category, significant differences between species with respect to run off angle were found.

Leaf surface wettability properties in the 396 species tested were highly divers. Extreme surface properties were only partly related to the ecological zone or a peculiar family and run off and leaf surface wettability turned out to be independent functional traits.

INTRODUCTION

The wettability of leaves, i.e. the amount of water captured and retained on leaf surfaces differs significantly between plant species: as studied by so far all types of leaves can be found from completely wettable leaves that become covered by a film of water to virtually non-wettable leaves that bead water into small spherical droplets [1-7]. Leaf wettability is determined by the measurement of the contact angle θ between 5 µl water droplets applied to the leaf surface [8].

On a hydrophobic surface water droplets have little contact with the leaf surface due to higher cohesive than adhesive forces [9]. The combination of hydrophobicity and special micro-scale physio-chemical surface properties such as microscopic pillars that reduce surface energy and make water droplets hardly stick to the surfaces can lead to the phenomenon known as super-hydrophobicity [10-12]. Super-hydrophobicity has been observed on natural and synthetic surfaces [13-16]. A super-hydrophobic surface has a water droplet contact angle greater than 150° and also a small angle of water droplet run off (<5°: [10,11,17-20], <10°:

[17, 21,22]). Super-hydrophobicity combined with fast run off properties was responsible for the removal of dust and microorganisms from leaf surfaces [10]. These self-cleaning properties have been described for *Nelumbo nucifera* and have been termed 'Lotus-effect' [10]. Super-hydrophobic surface properties attract great interest in material science because artificially copied there is a range of potential applications, such as the development of self-cleaning surfaces [10,16,21,23]. Similarly, extreme-hydrophilic leaf surfaces with low contact angle, good contact of the water droplet to the surface may be also interesting for applied sciences **(Supplementary Tables 1-5).**

The aim of the present study was to find out the natural variability but particularly also extremes in leaf wettability and water droplet run off properties within a broad range of plant species. Leaf samples were taken out of divers habitats of three continents, at various elevations (186-5268 m) and from all major ecological zones yielding in total information on 792 leaf surfaces obtained on adaxial and abaxial leaf surfaces of 396 species out of 85 families [24,25].

MATERIALS AND METHODS

Study Sites

The first study area was located in Asia, Nepal (80°04'-88°12' E-26°22'-30°27' N) extending from 186 m-5268 m (a.s.l.) within 150-200 km. Sampling was conducted along this extreme altitudinal gradient on plant species found within the tropical, subtropical, temperate, subalpine, alpine and nival region of Central and Western part of the country [24]. The Southern slopes of the Himalaya are humid as they fall within the monsoon system of the Indian subcontinents; the Northern slopes of the Himalaya are semi-arid.

The second study area was in Europe in the Tyrolean Central Alps in Austria (48°12' N, 16°22' E) where plants from the montane, subalpine, alpine and nival region were investigated. The European Alps exhibit a humid temperate continental climate. The montane sampling site was the Botanical garden of the Institute of Botany of the University of Innsbruck (600 m a.s.l., 47°16'N, 11°22'E). Investigations at subalpine sites were carried out in the surroundings of the summit of Mt. Patscherkofel (600 m-2100 m a.s.l., 47°12'N, 11°27'E), and in Obergurgl (2350 m-2680 m a.s.l., 46°49'N 11°02'E). Plants from higher alpine sites were collected in the glacier foreland of the Hintertux Glacier (2581 m-2660 m a.s.l., 47°04' N, 11°40'E and at Mt. Wildgrat (2973 m a.s.l, 47°08'N, 10°49'E) [25].

The third study area was in South America in the Andean mountains in Chile. Two study sites were selected. One site was located in the east of Santiago of Central Chile (33°24' S, 70°40' W) which has a Mediterranean type climate without remarkable rainfalls during summer and another further south in Corralco (1430 m-1484 m a.s.l., 38°23' S, 71° 35' W). In Corralco sampling was conducted in the subalpine ecological zone (1430 m-1484 m). At the site close to Santiago at various altitudes plant samples were taken: Militar village (2600 m a.s.l., 33°20' S, 70°18'W), La Parva (2985 m-3088 m a.s.l., 33°19' S, 70°16'W), Valle Nevado (2410 m-3050 m a.s.l., 33°21' S, 70°14'W), San Franciscano (3440 m-3600 m a.s.l., 33°19' S, 70°15'W) and Farellones (2470 m a.s.l., 33°20' S, 70°18'W).

A total number of 396 plant species (for detail see supplementary material) from 85 families were surveyed. Only fully developed and healthy looking leaf samples were taken. Measurements were made both on adaxial and abaxial leaf surfaces of twenty leaves per species in Nepal and ten per species in Austria and also in Chile. Random sampling was conducted on dominant plant species during the main growth period (Himalaya: November 2006 until August 2007, Austrian Alps: June-July 2006, Chilean Andes: January 2007 and February 2008).

Contact Angle Measurement

Leaf wettability was determined by measuring the contact angle θ of a 5 µl water droplet deposited on the leaf surface [8]. The contact angle (θ) of a line tangent to the water droplet through the point of contact between the water droplet and the leaf surface was measured by Tangent 1 default method according to Brewer et al. with a drop shape analyser (DSA100, Krüss, Hamburg, Germany). A high θ indicates beading of the water droplet which minimizes the contact area with the leaf surface [3]. In contrast a small θ is measured on flat water droplets that have a good contact with the leaf surface **(Figure 1).**

Photographic digital images of 5 µl water droplets deposited on leaf surface were taken by the integrated digital camera of the drop shape analyser (DSA1, Krüss, Hamburg, Germany) or for Chilean species and in remote sites in Nepal by a digital camera with a macro function (Canon, Power Shot A710IS). The digital photographs were further processed with the software of the DSA100 (DSA1, Krüss, Hamburg, Germany). In preliminary tests images of water droplets on leaves of the same species were taken with the two cameras. When processed with the DSA100 similar contact angles θ were determined.

The criteria for judging leaf surface wettability were based on various earlier reports [8,14,22,26-31]. In the current study the following terminology is used **(Figure 1)**: If contact angles were between 27°-40°, leaf surfaces are termed extreme-hydrophilic, between 40°-90° highly wettable and between 90°-110° wettable. If θ-values exceeded 110° the leaves are classified as being non-wettable. Highly non-wettable leaves have surfaces with water droplet contact angles greater than 130°. The term super-hydrophobic is used for leaf surfaces with θ-values greater than 150°.

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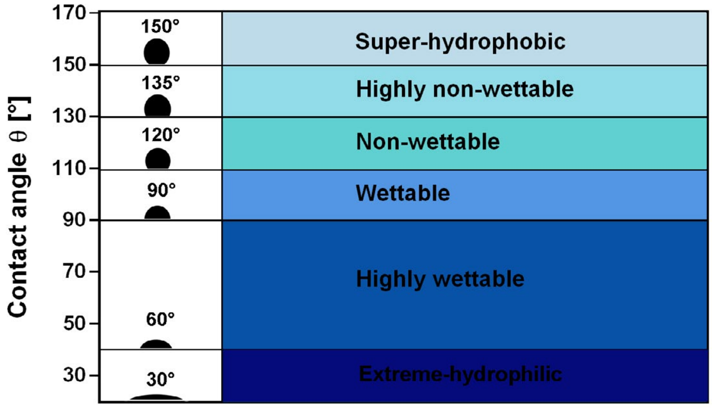

Figure 1. Wettability of the leaf surfaces increases with decreasing contact angle θ (°) of water droplets. Terminology and judgment criteria for leaf wettability is based upon the measurement of θ after various authors (Fogg, 1948, Challen, 1960, Adam, 1963, Crisp, 1963, Warburton, 1963, Holloway, 1970, Schönherr and Bukovac, 1972, Yoshimitsu et al., 2002, Sun et al., 2005).

Run off Angle Measurement

Run off angles (tendency to retain water droplet on leaf surface) were determined by the goniometer method of Brewer and Smith [5]. Leaves were collected from growing sites and run off angle measurements were recorded on the same day at the field site. Leaves were taped to a horizontal metal plate that was fixed to a goniometer (R&A Rost, Viennia). A 50 µl water droplet which is the recommended standard volume was then deposited on the initially horizontal leaf surface by a micropipette (SGC, Australia Pvt. Ltd., Melbourne) [32]. The leaf inclination was then successively increased and the angle at which the water droplet first began to move was recorded. Ten replicates were taken. Slight angles of inclination <5° indicate a slippery surface where water droplets run off very easily without leaving any residue, while high values (>10°) indicate a sticky leaf surface [10]. Additionally, the presence or absence of trichomes was determined by naked eyed.

Statistical Analysis

The significance of differences of means of leaf wettability and run-off angles were evaluated by ANOVA and the Bonferroni post-hoc test using SPSS software (SPSS Inc., Chicago, IL, USA).

RESULTS

Leaf wettability measured on 792 surfaces of 396 species collected at various elevations (186 m- 5268 m) on three continents differed significantly. Contact angle θ ranged between an absolute minimum of 16.7° and an absolute maximum of 169.7° **(Figure 2).** Remarkably in species that were found to show extreme surface properties concerning leaf wettability, i.e. that were either super-hydrophobic or extreme-hydrophilic, variation in θ was lowest. Similarly the frequency distribution of the leaf wettability classes indicates that 72.6% of the species investigated show extreme surface properties **(Figure 3)**, being either highly wettable or extreme-hydrophilic (37.8%) or highly non-wettable or super-hydrophobic (34.8%).

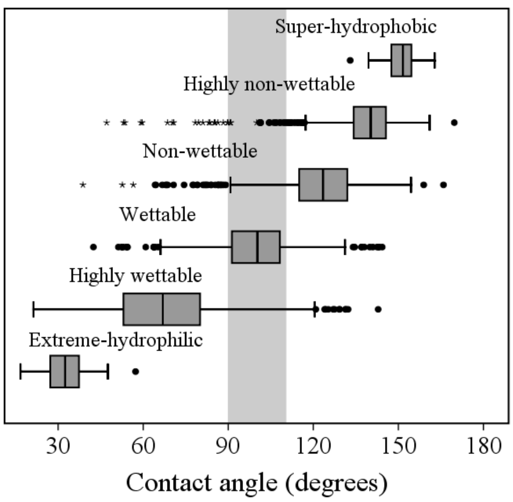

Figure 2. Variability of contact angles θ (°) of species from various ecological zone of the Himalaya, the European Alps and Chilean Andes within different leaf wettability categories: extreme-hydrophilic, highly wettable, wettable, non-wettable, highly non-wettable and super-hydrophobic. The box plots show the (median, range from 25%-, 75%-percentile (grey horizontal box), maximum, minimum (whiskers)). The shaded area from θ-values 90°-110° indicates the wettable leaves and θ>110° were considered as non-wettable leaf surfaces.

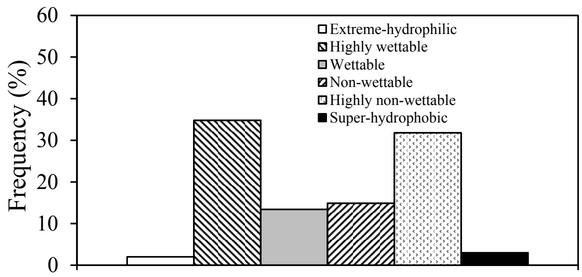

Figure 3. Relative frequency (%) of extreme-hydrophilic and super-hydrophobic, highly non-wettable and highly wettable and wettable and non-wettable leaf surfaces within the investigated leaf surfaces (N=792).

Thirteen families out of 85 were selected where we had sampled a representative number of at least 10 species **(Figure 4).** Significant differences in leaf wettability between these families were observed. Highly non-wettable to super-hydrophobic families were the Oxalidaceae and Fabaceae, highly wettable to extreme-hydrophilic were Ericaceae and Moraceae. Several families comprised all wettability types, e.g. Asteraceae and Euphorbiaceae.

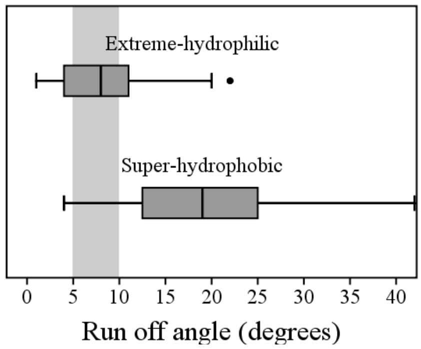

Figure 4. Comparison of mean water droplet run off angle (°) in extreme-hydrophilic and super-hydrophobic leaf surfaces. Box plots show the (median, range from 25%-, 75%-percentile (grey horizontal box), maximum, minimum value (whiskers)). The vertical grey bar (5°-10°) indicates the limit for low run off angles.

Out of 792 leaf surfaces only 16 extreme-hydrophilic and 24 super-hydrophobic surfaces were detected **(Table 1).** The majority of extreme-hydrophilic leaf surface properties were found in the tropical to subtropical ecological zones, only one species of the alpine zone showed extreme-hydrophilicity. In contrast super-hydrophobicity could be recorded on leaves out of virtually all ecological zones. Extreme-hydrophilic surfaces were more frequently adaxial (11 as compared to 5) while a slightly higher number of species showed extreme-hydrophobicity on abaxial leaf surfaces.

Table 1. Super-hydrophobic and extreme-hydrophilic leaf surface of 30 plant species from 21 families collected at various elevations in three continents. Leaf wettability is indicated by contact angle θ (° ; mean ± SD) and water droplet retention (° ; mean ± SD). Absence (-) or presence (+) of trichomes is additionally indicated.

Species	Site (alt)	Families	Contact angle (degrees)		Retention (degrees)		Trichome	
			AD	AB	AD	AB	AD	AB
Aconogonum molle	Nepal (1958)	Polygonaceae	..	150.0 ± 4.8	..	9.2 ± 3.0	..	+
Alstroemeria hookeri	Chile (2600)	Alstroemeriaceae	144.0 ±13.3	..	..	..	-	..
Androsace sp2.	Nepal (4940)	Primulaceae	..	149.1 ± 3.8	..	25.2 ± 3.2	..	+
Artemisia sp.	Tyrol (600)	Asteraceae	..	152.3 ± 3.2	..	..	..	+
Astragalus sp.	Tyrol (2581)	Fabaceae	144.8 ± 9.2	..	..	..	+	..
Bauhinia vahlii	Nepal (230)	Fabaceae	..	147.9 ± 3.9	..	13.7 ± 2.9	..	+
Bombax ceiba	Nepal (186)	Malvaceae	30.1 ± 7.7	..	10.9 ± 1.3	..	-	..
Bridelia retusa	Nepal (186)	Phyllanthaceae	41.6 ± 5.8	..	12.1 ± 3.7	..	+	..
Calceolaria arachnoidea	Chile (3050)	Scrophulariaceae	..	146.6 ± 6.1	..	..	..	+
Cassia fistula	Nepal (186)	Fabaceae	27.9 ± 5.0	..	4.2 ± 0.7	..	-	..
Castanopsis indica	Nepal (1467)	Fagaceae	40.5 ± 10.6	..	1.8 ± 0.7	..	+	..
Chaetanthera pusilla	Chile (2985)	Asteraceae	152.6 ± 3.8	..	..	..	+	..
Chuquiraga oppositifolia	Chile (1430)	Asteraceae	149.6 ± 11.6	..	..	..	-	..
Epilobium sp.	Tyrol (1950)	Onagraceae	..	145.8 ± 5.0	..	..	..	+
Ficus auriculata	Nepal (186)	Moraceae	40.6 ± 7.6	..	6.8 ± 0.6	..	-	..
Hydrocotyle sibthorpioides	Nepal (1322)	Apiaceae	32.5 ± 4.8	28.8 ± 5.8	9.0 ± 1.4	8.0 ± 3.6	-	-
Iris decora	Nepal (4205)	Iridaceae	..	151.1 ± 2.5	..	12.4 ± 6.8	..	-
Lantana camara	Nepal (1322)	Verbenaceae	44.1 ± 8.1	..	4.1 ± 0.6	..	-	..
Lupinus polyphyllus	Tyrol (600)	Fabaceae	..	149.1 ± 4.8	..	..	..	-
Mallotus philippinensis	Nepal (186)	Euphorbiaceae	34.0 ± 3.4	45.9 ± 8.2	2.7 ± 0.5	6.6 ± 2.9	-	-

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Montiopsis sericea	Chile (3050)	Portulacaceae	144.9 ± 6.2	..	..	..	+	..
Oxalis compacta	Chile (3600)	Oxalidaceae	148.0 ± 5.1	149.2 ± 1.6	..	..	+	+
Oxalis compacta	Chile (3050)	Oxalidaceae	144.2 ± 5.8	145.0 ± 5.5	..	..	+	+
Oxalis latifolia	Nepal (1322)	Oxalidaceae	147.5 ± 4.7	148.3 ± 4.9	18.3 ± 9.9	11.1 ± 1.1	+	+
Persicaria hydropiper	Nepal (1322)	Polygonaceae	31.9 ± 4.2	..	15.5 ± 4.7	..	+	..
Polygonum viviparum	Tyrol (2581)	Polygonaceae	..	148.6 ± 6.6	..	..		-
Potentilla argyrophylla	Nepal (4205)	Rosaceae	148.6 ± 6.8	..	27.2 ± 4.0	..	+	..
Potentilla fulgens	Nepal (3868)	Rosaceae	149.3 ± 4.5	..	23.2 ± 6.3	..	+	..
Rhododendron anthopogon	Nepal (4205)	Ericaceae	42.4 ± 9.7	..	14.0 ± 3.4	..	-	..
Rubus paniculatus	Nepal (1759)	Rosaceae	..	149.8 ± 2.7	..	23.1 ± 5.6	..	+
Sambucus hookeri	Nepal (1322)	Sambucaceae	..	42.0 ± 7.0	..	8.9 ± 2.6	..	+
Tectona grandis	Nepal (186)	Verbenaceae	34.7 ± 7.4	..	9.3 ± 4.5	..	-	..
Thunbergia coccinea	Nepal (186)	Acanthaceae	..	37.2 ± 3.5	..	7.7 ± 3.7	..	-
Trewia nudiflora	Nepal (186)	Euphorbiaceae	..	40.2 ± 9.1	..	7.5 ± 2.5	..	+
Tropaeolum polyphyllum	Chile (2410)	Tropaeolaceae	147.2 ± 6.6	..	..	..	-	..

On extreme-hydrophilic leaf surfaces water droplet run off occurred at a significantly lower angle of inclination (8°) as compared to super-hydrophobic (19°) surfaces **(Figure 5).** For the species from Nepal Himalaya where additionally information about water droplet run off properties were recorded within the extreme-hydrophilic species some were found to readily repel water droplets at angle of inclination as low as 1.8° such as *Castanopsis indica* **(Figure 6.1)**. Similarly *Cassia fistula, Mallotus philippinensis* and *Lantana camara* combine extreme-hydrophilic properties with fast run off (2.7-4.2°). Within the group of extreme-hydrophilic species different run off properties, e.g. 15.5° of *Persicaria hydropiper*, were recorded indicating that run off and leaf wettability are two independent traits.

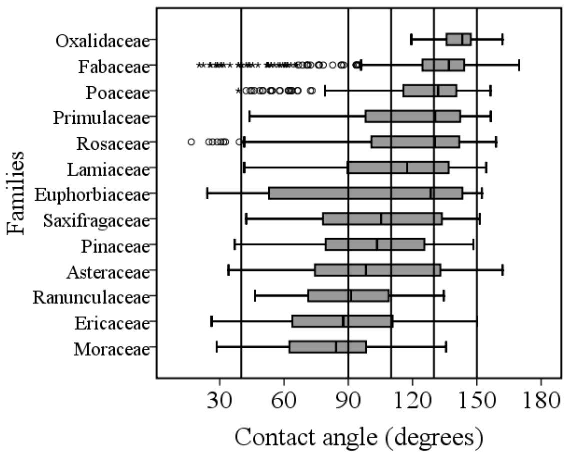

Figure 5. Comparison of mean contact angles (°) in different families. Box plots show the (median, range from 25%-, 75%-percentile (grey horizontal box), maximum, minimum value (whiskers)). The vertical lines indicate the borders between different wettability categories.

On super-hydrophobic leaf surfaces water droplet run off was observed to occur under slightly higher but still low angles (9.2°: **Figure 6.2**). *Aconogonum molle* and Iris decora are super-hydrophobic and with this low angles showed Lotus-effect properties. Also within the group of super-hydrophobic species significant differences in run off properties were observed, on the adaxial leaf surface of *Potentilla argyrophylla* run off occurred at mean at an angle of inclination of 27.2°. Super-hydrophobic leaf surfaces were less frequently glabrous **(Figure 7)**, i.e. trichomes are an important feature for the repellence of water. In contrast, extreme-hydrophilic leaf surfaces were more often glabrous.

DISCUSSION

Fourty leaf surfaces (5.1%) out of 792 tested were found to show super-hydrophobic (24) or extreme-hydrophilic (16) properties. Our leaf wettability results show a representative cross section from low to high wettability. However, the values obtained for each single species may not mirror the potential maximum or extreme as intra-specific differences in leaf wettability indicate environmental adaptability [6,25,33]. For instance, it has been shown that species from open places possess a lower wettability than species from the understorey [5,24,34,35]. Plants from wet environments had more wettable leaf surfaces than plants from dry environments and generally leaf wettability decreased with increasing elevation [6,24]. From this it can be expected that by alteration of the growth conditions also the wettability properties can be significantly changed and hence the leaf surface characteristics of the encountered species can be expected to be improvable with respect to quality under optimum growth conditions.

Among the super-hydrophobic surfaces (θ ± 150°) three were found on leaves of *Aconogonum molle*, *Iris decora, and Oxalis* *latifolia* that additionally had a run off angle ±10°, i.e. fulfilling the criteria for a super-hyrdophobic self-cleaning surface (*in sensu*: [17,21, 22]). While in *A. molle* and *O. latifolia* leaf surface properties are influenced by trichomes, *I. decora* is glabrous and surface wettability characteristics are solely affected by the structural peculiarities of the cuticle and epidermal outer cell wall. Hence, this species appears to be particularly promising with respect to self-cleaning properties **(Supplementary Tables 6-10).**

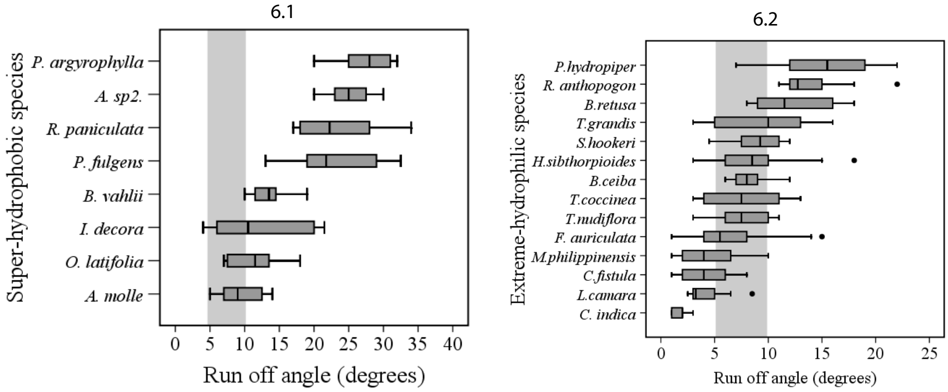

Figure 6. Comparison between mean run off angles (°) in species with a (6.1) super-hydrophobic or (6.2) extreme-hydrophilic leaf surface. Box plots show the (median, range from 25%-, 75%-percentile (grey horizontal box), maximum, minimum value (whiskers)). The vertical grey bar (5°-10°) indicates the limit for low run off angles.

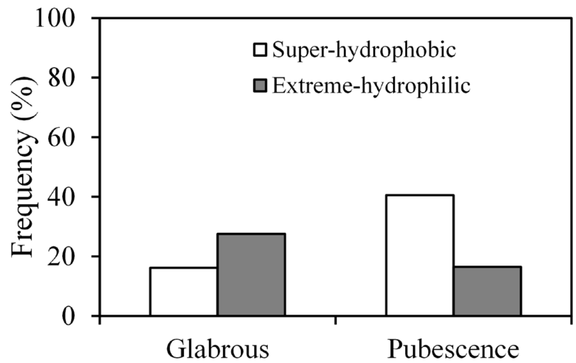

Figure 7. Super-hydrophobicity (open bar) was more frequent in pubescent leaves. Extreme-hydrophilicity (grey bars) was more frequently observed in glabrous leaves.

The self-cleaning effect of the leaves of *N. nucifera* (θ=160.4°) was explained by a wax crystal covered leaf surface and very rough papillose epidermal cells that provide hydrophobicity but also greatly influence the motion of moving water droplets [10]. Lotus like behaviour has been observed on other biological surfaces, silver ragwort, and also in the wings of insects, especially those with large wings, which cannot be cleaned by legs, have water repellent wing surfaces and exhibit self-cleaning ability [36,37]. In the latter case, not only the removal of particles is of interest, but also the maintenance of flight capability.

Sixteen extreme-hydrophilic (θ=27-40°) leaf surfaces were detected. Four of them were adaxial and exhibited particularly low run off angles: *Castanopsis indica* (1.8 ± 0.7°), *Cassia fistula* (4.2 ± 0.7°), *Lantana camara* (4.1 ± 0.6°) and *Mallotus philippinensis* (2.7 ± 0.5°). Except for *C. indica*, the species have glabrous leaves. In these species water droplets spread very quickly but water runs off the leaves at very low inclinations with considerable speed which may be important for the removal of deposited particles from the leaf surface. In contrast to super-hydrophobic surfaces on these leaves the water droplet has good contact with the surface and hence potentially touches and cleans a more expanded stripe on the leaf surface. Already earlier low contact angles combined with low sliding angles were presumed to be responsible for the removal of micro-organisms, i.e. to have surface cleaning properties [38]. By contrast Barthlott and Neinhuis considered only water repellent surfaces to be keystones in the self-cleaning mechanism [10].

Pubescence was found to promote hydrophobicity. Similar results were observed by Brewer et al. that trichomes decrease leaf wettability significantly but this effect depends additionally strongly on the presence or absence of wax crystals on the trichomes [3,33]. Non-waxy trichomes were only water repellent for a short time while waxy trichomes were extremely water repellent. Additionally trichome morphology can affect hydrophobicity Koch et al. [21]. Pubescence seems additionally to slow down water droplet run off.

Our results clearly demonstrate that a considerable diversity with respect to leaf surface wettability and water droplet run off exists which corroborates earlier findings. Leaf surface wettability and water droplet run off appear independent traits. Several new plant species with extreme surface properties could be detetced. Their leaf surface peculiarities could potentially be of great interest in applied sciences that have the challenge to engineer artificial surfaces with specific wettability and run off properties.

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