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Leaf Wettability in Bilberry *Vaccinium myrtillus* L. as Affected by Altitude and Openness of the Growing Site

By

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With 9 Figures

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Summary

ARYAL B. & NEUNER G. 2012. Leaf wettability in bilberry *Vaccinium myrtillus* L. as affected by altitude and openness of the growing site. – Phyton (Horn, Austria 52 (2): 245–262, with 9 figures.

Leaf wettability of *Vaccinium myrtillus* L. (*Ericaceae*) leaves was investigated on plants naturally growing along an altitudinal gradient from 690 m to 2030 m a.s.l. and in open and understorey habitats in the Tyrolean Central Alps of Austria in order to investigate the role of environmental factors for the development of highly non-wettable or wettable leaf surfaces. Leaf wettability was assessed by measurement of the water droplet contact angle, θ. θ was positively related with increasing altitudes, i.e. leaves from subalpine individuals were non-wettable ($\theta > 110°$) and that from montane sites were highly wettable ($\theta < 90°$). Analysis of variance also indicated that leaf wettability was significantly different along increasing elevation. Leaf surfaces of plants from open habitats were more water repellent, than that from leaves grown in the adjacent understorey. In the understorey, droplet retention was much higher as compared to open places. Steep angled leaves from the subalpine region had also a low leaf wettability, which repels water droplets away from the leaf surface immediately after contact. Stomatal density increased from lowland (99.7 $\pm$ 3.6) to

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highland (153.4 ± 5.1) and from the understorey to open growing places. Leaves were amphistomatous but had much more stomata on abaxial than adaxial leaf surfaces. Leaf surface wettability of *V. myrtillus* decreases with increasing altitude and with increasing openness of the habitat. In both gradients the probability of wetting events increases having major effects on gas exchange but additionally promotes infection and formation of diseases that in turn can increase leaf surface wettability.

Zusammenfassung

Aryal B. & Neuner G. 2012. Leaf wettability in bilberry *Vaccinium myrtillus* L. as affected by altitude and openness of the growing site. [Blatt-Benetzbarkeit von *Vaccinium myrtillus* L. unter dem Einfluss der Seehöhe und der Exponiertheit des Standortes]. – Phyton (Horn, Austria) 52 (2): 245–262, mit 9 Abbildungen.

Die intra-spezifische Variabilität der Blatt-Benetzbarkeit von *Vaccinium myrtillus* L. (*Ericaceae*) wurde an Blättern untersucht, die entlang eines Höhentransekts von 690 m bis 2030m a.s.l. bzw. in offenem Gelände oder im Unterwuchs beprobt worden sind. Dadurch sollte die Wirkung von natürlich auftretenden Umweltfaktoren auf die Ausbildung unterschiedlicher Benetzbarkeiten von Blatt-Oberflächen erfasst werden. Die Blatt-Benetzbarkeit wurde mit Hilfe eines Tropfenkonturanalysesystems und der Bestimmung des Wassertropfen – Kontaktwinkles (θ) erfasst. Die Benetzbarkeit der Blätter nahm mit zunehmender Seehöhe signifikant ab. Blätter von subalpinen Individuen waren nicht-benetzbar ($\theta > 110°$), die von im montanen Bereich gewachsenen Individuen waren hoch benetzbar ($\theta < 90°$). Blatt-Oberflächen von Individuen, die auf offenen Flächen gewachsen waren, waren wasserabstoßender, als jene die sich an Pflanzen im Unterwuchs entwickelt haben. Bei Pflanzen aus dem Unterwuchs war die Tropfen-Retention viel höher als bei jenen von offenen Flächen. Durch die zudem steile Blattstellung bei Pflanzen im subalpinen offenen Gelände dürften hier Wassertropfen rasch abperln. Die Stomata Dichte nahm vom Tiefland (99.7 ± 3.6) in die alpine Stufe hin zu (153.4 ± 5.1), aber auch vom Unterwuchs hin zu offenen Flächen. Die Blätter waren amphistomatisch, aber hatten deutlich mehr Stomata auf der abaxialen als auf der adaxialen Blattseite. Die Blatt-Benetzbarkeit von *V. myrtillus* nimmt mit zunehmender Seehöhe und mit zunehmender Offenheit des Standortes ab. In beiden Gradienten steigt die Häufigkeit von Ereignissen bei denen die Pflanzen benetzt werden können. Häufiges Benetzen könnte sich auf den Gasaustausch negativ auswirken, und hat zusätzlich eine förderliche Wirkung für das Auftreten mikrobieller Infektionen und damit der Manifestation von Krankheiten, die in Folge die Benetzbarkeit noch vergrößern, was saisonale Befunde zu bestätigen scheinen.

Introduction

Leaf wettability must be considered as an important plant functional trait whose significance seems to be highly varied (Brewer & al. 1991). Intra-specific differences indicate environmental adaptability of leaf wettability (Neinhuis & Barthlott 1997, Brewer & Nuñez 2007). Leaf wettability decreases intra-specifically with increasing altitude in leaves of evergreen tree species (*Eucalyptus urginera:* Thomas & Barber 1974, *Picea abies* and *Pinus sylvestris*: Cape & al. 1989, *P. abies* and *Pinus cembra*: An-

FODILLO & al. 2002) which even seems to hold true inter-specifically (ARYAL & NEUNER 2010). Altitudinal reductions of leaf wettability have been traced to structural surface modifications as high altitudinal needles of *P. abies* and *P. cembra* had a dense structure of wax crystals while epicuticular waxes were degraded in low altitude needles (ANFODILLO & al. 2002).

Vaccinium myrtillus L. is one of the most frequent and abundant vascular plant species in Northern Europe and in the Middle and Southern parts of Europe the species is usually found in mountainous environments (COUDUN & GÉGOUT 2007) commonly growing in association with *P. abies* (Norway spruce) at least at the subalpine level (GENSAC 1970). *V. myrtillus* plants have enough developmental plasticity to adapt to a wide range of environmental conditions, particularly with respect to environmental changes commonly observed along altitudinal gradients. The species can grow from the deep shade on the forest floor of montane spruce forests to sun exposed places in the subalpine dwarf shrub heath. These microhabitat differences and environmental altitudinal changes induce many changes in anatomical features and the growth habit of individual plants (BILLINGS & MOONEY 1968, KÖRNER & LARCHER 1988, JONES 1992, LARCHER 2003). This peculiar plasticity of *V. myrtillus* plants appeared to be particularly suitable to study effects of changing environmental conditions along the broad altitudinal gradient where *V. myrtillus* can grow but additionally in contrast to trees (THOMAS & BARBER 1974, CAPE & al. 1989, ANFODILLO & al. 2002) of microhabitat peculiarities such as sun or shade adaptation on the specific formation of leaf wettability.

With increasing elevation numerous environmental variables change-namely temperature, leaf-to-air vapour pressure gradient, solar radiation, exposure to wind, and soil characteristics (BARRY 1992, KÖRNER 2000). In temperate mountains such as the Austrian Alps also precipitation increases with elevation and fog events are more frequent (KÖRNER 1999). Hence, leaf surfaces of plants from high altitude can be expected to get more frequently wetted by rain and fog. Plants from open places are more exposed to dew formation. Without large temperature gradients droplets may stay longer on the surface at high altitude than at low altitude. Evaporation depends on the vapour pressure gradient that due to colder air temperatures at higher elevation can be reduced. Water droplets on leaf surfaces were additionally often discussed to form water films that may occlude the stomata and could affect gas exchange properties (TERASHIMA & al. 1993, BREWER & SMITH 1994, 1995, ISHIBASHI & TERASHIMA 1995, NEINHUIS & BARTHLOTT 1997).

The purpose of the current study was to characterize wettability of leaves of *V. myrtillus* grown in different microhabitats along an altitudinal gradient in the European Central Alps to assess the question what effect the change in environmental conditions has on the repletion of water droplets from it's leaf surfaces.

Material & Methods

Plant Material

The investigations were carried out on bilberry (*Vaccinium myrtillus* L.), which was selected as it grows frequently from lower montane altitudes (about 700 m) up to the alpine regions (2845 m, POLATSCHEK & al. 1999) of the Tyrolean Central Alps of Austria. Leaf samples were taken randomly on 12^{th} July 2006 at 13 different altitudinal sites (see Table 1) from montane to the subalpine region (2030 m a.s.l.). Additional measurements were conducted on leaf samples collected on 26^{th} August 2006. At lower altitudes *V. myrtillus* usually grows in the understorey of coniferous forests (commonly growing in association with *P. abies* at least at the subalpine level (GENSAC 1970) at higher altitudes in the subalpine region and above the timberline it can also be found in open places as part of the dwarf shrub heath. The vegetation that accompanies dense *V. myrtillus* carpets changes with altitude, bryophytes that are common at the montane level are replaced by forbs at the subalpine level (FRAK & PONGE 2002). Sampling sites were selected to cover a wide variety of altitudes and mircohabitats (open places and shaded habitats underneath of closed coniferous forests) from the montane to the subalpine zone. Measurements were made on five randomly selected, fully expanded healthy leaves per site with five replicates per leaf. The contact angle measurements were made for both adaxial and abaxial leaf surfaces.

Table 1. Leaves were collected at 13 different sites from montane to subalpine ecological zone of Tyrolean Central Alps of Austria.

Ecological zone	Sites	Locations	Altitudes (m)
Montane	47°16’14.30”N, 11°11’21.36”E	Inzing, Prantl	690
	47°12’15.10”N, 11°25’02.57”E	Mt Patscherkofel	1020
	47°12’16.73”N, 11°25’15.87”E	Mt Patscherkofel	1120
	47°12’16.42”N, 11°25’28.94”E	Mt Patscherkofel	1220
	47°12’20.15”N, 11°25’54.22”E	Mt Patscherkofel	1440
	47°12’21.46”N, 11°26’14.07”E	Mt Patscherkofel	1560
	47°12’25.25”N, 11°26’30.41”E	Mt Patscherkofel	1700
	47°12’25.97”N, 11°26’42.55”E	Mt Patscherkofel	1800
Subalpine	47°12’26.98”N, 11°26’50.46”E	Mt Patscherkofel	1860
	47°12’26.41”N, 11°26’58.99”E	Mt Patscherkofel	1920
	47°12’37.89”N, 11°26’07.23”E	Mt Patscherkofel	1950
	47°12’38.98”N, 11°27’09.88”E	Mt Patscherkofel	1960
	46°59’25.63”N, 11°37’59.54”E	Pfitsch, Grieplalpe	2030

Contact Angle Measurement

Leaf wettability can be expressed by the contact angle of a water droplet with the leaf surface. The contact angle is determined by the balance between adhesive forces between the liquid and solid, and cohesive forces in the liquid. Adhesive forces cause a liquid drop to spread. Cohesive forces cause the drop to ball up (BERG 1993). The test consists of depositing a drop of water on a surface and determining the contact angle between the surface and the tangent to the drop at the solid-liquid-air meeting point (MARTIN & JUNIPER 1970, BERG 1993) (Fig. 1). Contact angle measurement is a very sensitive indicator for the repellency of a surface and has been applied in various leaves, since the degree of surface wetting by water gives information on the characteristics of the outermost layer of the interphase between the liquid water and the atmosphere (HOLMES-FARLEY & WHITESIDES 1987). High contact angle (θ) shows low wettability and low contact angle shows high wettability of surfaces by water droplets and theoretically can vary from 0° (completely flat) to 180°.

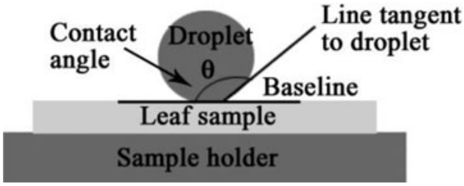

Fig. 1. The contact angle (θ) is the angle between the surface of the leaf (baseline) and the tangent plane of a water droplet at the point of contact between air, water and the leaf surface.

The wettability of both leaf surfaces (adaxial and abaxial) were determined by measuring the contact angle (θ) of a 5 μl droplet of distilled water placed by a micropipette on each leaf. The leaves were mounted on the sample stage of a Drop Shape Analyser (DSA100, Krüss, Hamburg, Germany) using double sided tape. The contact angle (θ) of a line tangent to the droplet through the point of contact between the droplet and leaf surface was measured by using Tangent 1 default method according to BREWER & al. 1991 with the Drop Shape Analyser. The criteria for judging surface wettability were as follows: Leaves were termed as highly wettable if θ was less than 90° based on earlier reports (FOGG 1948, CHALLEN 1960, ADAM 1963, CRISP 1963, WARBURTON 1963, HOLLOWAY 1970, SCHÖNHERR & BUKOVAC 1972) and wettable if θ less than 110° (CRISP 1963). If contact angle (θ) exceeded 110° the leaves were considered non-wettable. Highly non-wettable leaves had θ values greater than 130° (CRISP 1963).

Leaf angle was estimated in situ before leaf sampling in two angular classes: 0°–45° termed flat and 45°–90° termed steep.

Stomatal Density

Stomatal counts were made using Collodium surface impressions of the adaxial and abaxial leaf surfaces. The Collodium impressions were digitized and counting of the number of stomata within a 250 mm^2 leaf area was conducted by use of software Adope Photoshop (Version 7.0, Adope Systems Inc., USA). Care was taken to avoid veins. One replicate on 12 leaves were counted from 1020 m a.s.l. to 1960 m a.s.l.

Water Retention

Water retention of leaves of *V. myrtillus* was studied by use of the method described by HANBA & al. 2004. Pre-weighed leaves were sprayed with a cone nebuliser with distilled water until run off. The water is then left to settle horizontally for approximately 30 s. Adhering water is then collected from both leaf surfaces (adaxial and abaxial) with filter paper fragments that had previously been oven dried and pre-weighed. Water applied to the leaf surface during spraying may be absorbed by the leaf. Therefore we checked whether leaves absorbed water directly from the leaf surface or not, and values for adhering water were corrected accordingly. The amount of water retained, i.e. water retention, by the leaf surface was then calculated as mg water retained/mg leaf fresh weight.

Statistical Data Analysis

The differences between means of contact angle (degrees), i.e. leaf wettability, on leaves surfaces with increasing altitudes were tested by one-way and between leaf sides (abaxial or adaxial), between habitat types (open or understorey) were assessed with two-way analysis of variance and the Duncan's multiple range test. A given effect was assumed significant at $P<0.05$ using SPSS software (*SPSS* Inc., Chicago, IL, USA).

Results

Significant differences ($P<0.05$, Table 2) in the wettability of leaves of *V. myrtillus* taken from 13 sampling sites along an altitudinal gradient (690 m–2030 m a.s.l.) were observed (Fig. 2A). High contact angles (θ) indicate water repellency and low θ-values show a high wettability by water droplets. The general trend in leaf wettability along the measured altitudinal gradient was from lower mean θ-values and flattened droplets in the low altitudes to high θ-values and spherical droplets for leaf surfaces from high altitude. Most leaves from the montane ecological region were highly wettable to wettable indicated by θ-values of less than 90°–110°. In leaves from subalpine individuals θ-values were significantly higher (median $>110°$, non-wettable) i.e. the leaves had more repellent surfaces. Complementary measurements on leaves of *V. myrtillus* sampled in August 2008 revealed that at all investigated altitudes the wettabilty of the leaves had significantly increased (Fig. 2B). On the adaxial surface the mean reduction of the θ-value was significantly higher (-26.1 ± 7.4 SE) than on the abaxial surface (-7.9 ± 4.9 SE). A similar trend of a decrease in leaf wettability with increasing altitude was obtained on leaves of the accompanying tree species *P. abies* and *P. cembra* (Fig. 3). Contact angles were significantly different between leaves of *V. myrtillus* plants from open places and the forest understorey (Table 3). Leaves of *V. myrtillus* developed in open habitats from the montane to the subalpine region had significantly increased contact angles, i.e. had non-wettable (median $\theta>110°$) leaf surfaces (Fig. 4). Leaves from open places were mostly steeply arranged, by contrast leaves from understorey were flatly arranged (data not shown).

Leaves with steep leaf angles from the subalpine environment were non-wettable which was not true in the montane region (Fig. 5). Both flatly

Table 2. One-way analysis of variance on differences in contact angle of leaves of *V. myrtillus* between altitudes, leaf surfaces (adaxial & abaxial) and habitats (open & understorey). Values indicate statistical significance differences at $P < 0.05$. In the last column, symbols 'S' and 'NS' represent significant and non-significant respectively.

Variable	Source of variation	MS	df	F	P	Remarks
Contact	Altitude	2314.5	12	11.7	0.000	S
angle (θ)	Error	196.2	127			
	Leaf surface	1445.7	1	3.8	0.052	0.052
	Error	371.3	138			
	Habitat	17426.6	1	68.1	0.000	S
	Error	255.5	138			
	Leaf angle	6532.0	1	19.5	0.000	S
	Error	334.4	138			
Leaves in open habitats						
Contact	Altitude	726.2	3	12.8	0.000	S
angle (θ)	Error	56.5	36			
	Leaf surface	1.01	1	0.009	0.924	NS
	Error	110.8	38			
Leaves in understorey habitats						
Contact	Altitude	1021.1	8	4.0	0.000	S
angle (θ)	Error	251.4	91			
	Leaf surface	2081.7	1	7.0	0.009	S
	Error	295.6	98			

and steeply arranged leaves were slightly more water repellent on the adaxial leaf surface (Fig. 6). While no difference was found in wettability of the two leaf sides (abaxial-adaxial) in the subalpine region (Fig. 7) and in open places (Fig. 8), the abaxial leaf side of leaves from montane sampling sides and from the understorey was highly wettable ($\theta < 90°$) than the adaxial side. Only between adaxial and abaxial surfaces of leaves from understorey places significant differences ($P < 0.05$) in contact angle of water droplets were found (see Table 2 & 3).

Table 3. Overall effects of leaf surface (adaxial or abaxial) and habitat type (open or understorey) on Leaf wettability (contact angle) of leaves of *V. myrtillus* assessed with a two-way analysis of variance. Values indicate statistical significant differences at $P < 0.05$. In the last column, symbols 'S' and 'NS' represent significant and non-significant respectively.

Variable	Source of variation	MS	df	F	P	Remarks
Contact	Habitat	17426.6	1	71.4	0.000	S
angle (?)	Leaf surface	553.9	1	2.2	0.134	NS
	Interaction	637.0	1	2.6	0.108	NS
	Error	243.9	136			

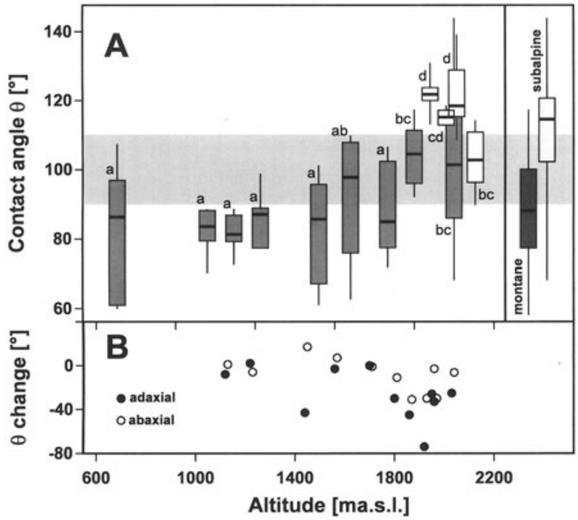

Fig. 2. (A) Contact angle (θ) (degrees) of water droplets on leaves of *V. myrtillus* (data from adaxial and abaxial leaf surface included) taken from 13 different sampling sites along an altitudinal gradient and from different mircohabitat types: understorey of coniferous forests (medium grey box plots) and open places (white box plots) in the Tyrolean Alps of Austria. The leaves from subalpine individuals (light grey box plot) had significantly higher contact angles, i.e. non-wettable as compared to leaves from montane altitudes (dark grey box plot) that are highly wettable. The boxplots show the median (line inside the box), 25%-, 75%-percentile, maximum, minimum values). The shaded area from θ-values 90°–110° indicates the wettable leaves. Non-wettable leaf surfaces have a θ-values >110°. Different letters indicate significant differences ($P < 0.05$) between mean contact angles and increasing altitudes (tested with Duncan's multiple range tests). (B) Change of θ-values from July to the end of August 2007.

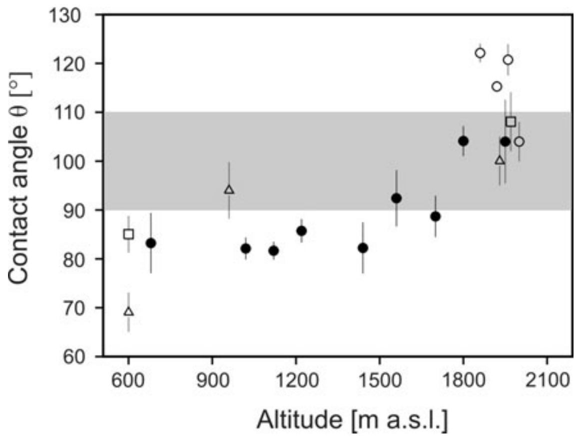

Fig. 3. Altitudinal increases of θ-values determined on leaves of (● = understorey, ○ = open place) *V. myrtillus* as compared to leaves of (Δ) *P. cembra* and (□) *P. abies*.

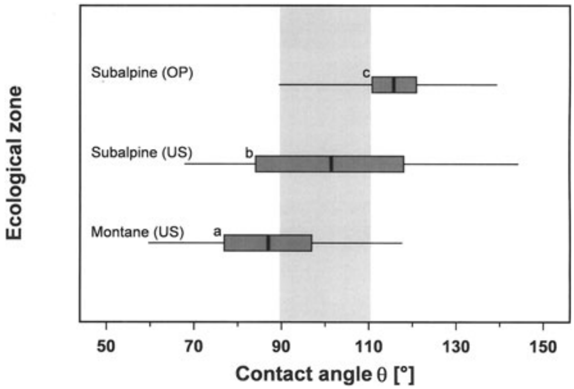

Fig. 4. The leaves taken from open places (OP) had significantly higher contact angles than the leaves sampled from the understorey (US) in both the montane and subalpine region. The boxplots show the median (line inside the box), 25%-, 75%-percent, maximum, minimum. The shaded area from θ-values 90°–110° indicates the wettable leaves and $\theta > 110°$ were considered as non-wettable leaf surfaces. Different letters indicate significant differences ($P < 0.05$) between mean contact angles and habitats (tested with Duncan's multiple range tests).

In leaves of *V. myrtillus* from montane to subalpine ecological zones, there were significantly more stomata on the abaxial surfaces than the adaxial surfaces and there was an increase of stomatal density in leaves of plants from the understorey (Fig. 9). The most significant differences were detected between leaves from understorey plants that had much less stomata (99.7 ± 3.6) than leaves taken from individuals grown in open places (153.4 ± 5.1) (Table 4). Droplet retention was much higher on leaves from understorey sites when compared with open places. The abaxial leaf side retained higher amounts of water than the adaxial leaf side.

Table 4. Mean stomatal density (number per mm^2) and droplet retention (mg H_2O retained/mg leaf fresh weight) on leaves of *V. myrtillus* collected on open and understorey growing sites. Data are mean ± SE.

	Stomatal density (Number per mm^2)			Droplet retention (gH_2O/gfresh weight)		
Microenvironment	Total	Adaxial	Abaxial	Total	Adaxial	Abaxial
OP	153.4 ± 5.1	13.0 ± 3.2	140.0 ± 5.8	1.33 ± 0.69	0.5	0.8
US	99.7 ± 3.6	6.2 ± 1.9	91.0 ± 4.9	2.25 ± 0.85	1.05	1.2

Discussion

Leaf surface wettability of *V. myrtillus* significantly decreased with increasing altitude. In the montane region mean θ-values of flattened water droplets were lower than 90° indicating high wettability. In the sub-

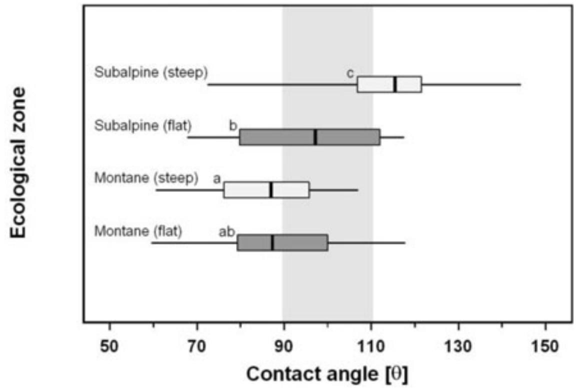

Fig. 5. Contact angles were generally low (highly wettable) on leaves from montane sampling sites independent from leaf orientation. In contrast high values of contact angle were found in steeply arranged leaves from the subalpine region as indicated by a significant difference between means (Boxplots: median, 25%-, 75%-percent, maximum, minimum). The shaded area from θ-values 90°–110° indicates the wettable leaves and $\theta > 110°$ were considered as non-wettable leaf surfaces. Significant differences ($P < 0.05$) between mean contact angles are indicated by different letters and were tested with Duncan's multiple range tests.

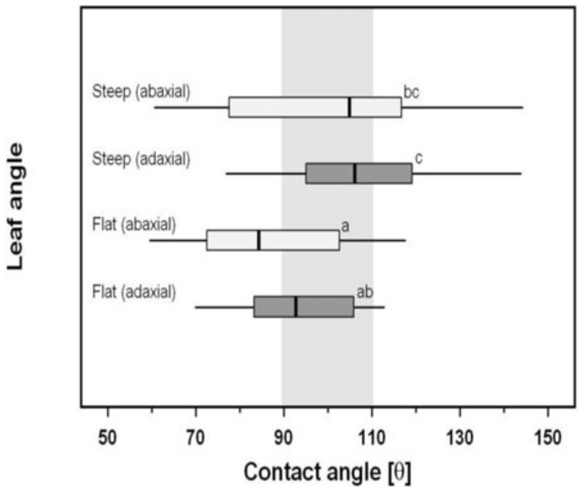

Fig. 6. Both flatly and steeply arranged leaves showed adaxial surface had less wettability (higher θ) than abaxial are displayed as boxplots (median, 25%-, 75%-percent, maximum, minimum). The shaded area from θ-values 90°–110° indicates the wettable leaves. Non-wettable leaf surfaces have a θ-values $>110°$. Significant differences ($P < 0.05$) between mean contact angles are indicated by different letters and were tested with Duncan's multiple range tests.

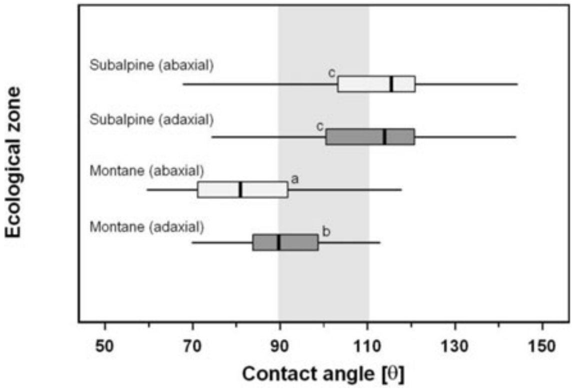

Fig. 7. No specific trend was observed in adaxial and abaxial leaf surfaces from montane and subalpine region. The boxplots show the median, 25%-, 75%-percent, maximum, minimum. The shaded area from θ-values 90°–110° indicates the wettable leaves. Non-wettable leaf surfaces have a θ-values >110°. Significant differences ($P<0.05$) between mean contact angles are indicated by different letters and were tested with Duncan's multiple range tests.

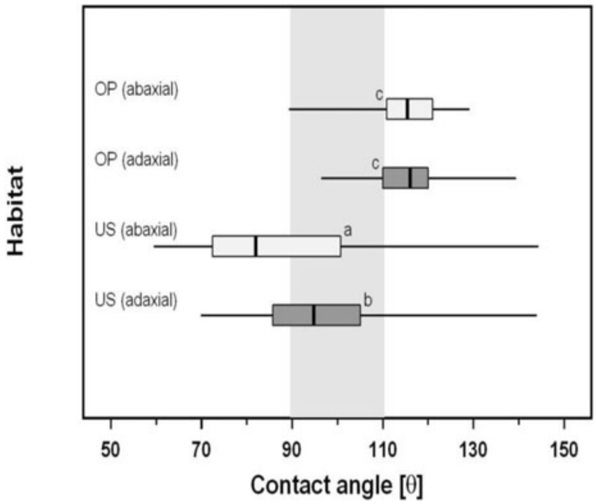

Fig. 8. Leaf surface characteristics of *V. myrtillus* plants grown in open places as compared to understorey habitats from the whole altitudinal range showed significantly higher contact angles (less wettability) in leaves taken from open places. (Boxplots: median, 25%-, 75%-percent, maximum, minimum). The shaded area from θ-values 90°–110° indicates the wettable leaves. Non-wettable leaf surfaces have a θ-values >110°. Significant differences ($P<0.05$) between mean contact angles are indicated by different letters and were tested with Duncan's multiple range tests.

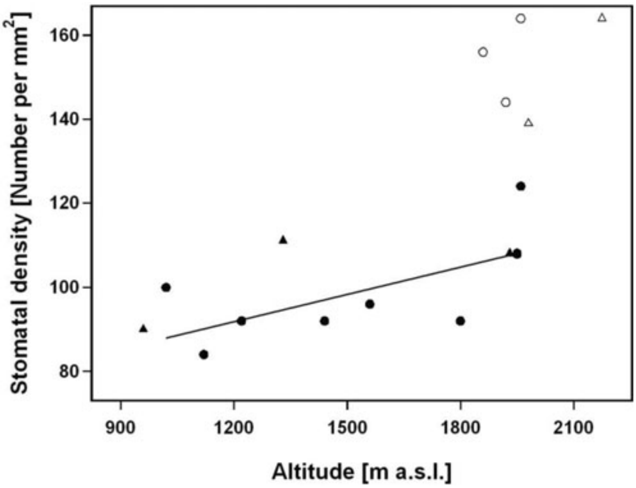

Fig. 9. Stomatal density (number per mm^2) of *V. myrtillus* increases in understorey leaves (closed symbol) with altitude and is distinctly higher in leaves collected from plants grown in open places (open symbols). (●,○) Current measurements are compared to data (▲,△) obtained by KÖRNER & al. 1979.

alpine region water repellency was high as validated by high mean θ-values of the spherical water droplets that formed on these leaves. This intraspecific altitudinal effect on leaf wettability is consistent with earlier observations on leaves of evergreen tree species in Tasmania (*Eucalyptus urginera*: THOMAS & BARBER 1974) and Italy (*Picea abies* and *Pinus cembra*: ANFODILLO & al. 2002) which is additionally corroborated by our results on *P. abies* and *P. cembra* in the Austrian Alps. Similarly in *Pinus sylvestris* and *Picea abies* leaf wettability increased consistently from low altitude sites in Britain and the Netherlands to high altitude sites in Germany where forest show decline symptoms (CAPE & al. 1989). The observed intraspecific increase of water repellence with increasing altitude appears to follow the more general inter-specific tendency observed along an extreme altitudinal gradient in Nepalese Himalayas (186 m – 5268 m, ARYAL & NEUNER 2010) underlining the general significance of a low wettability at higher altitudes.

Leaf surface wettability of *V. myrtillus* was significantly lower in plants grown in open habitats as compared to understorey individuals. This observation corroborates earlier findings that water repellence is more commonly found in plants from open habitats as compared to understorey habitats (BREWER & SMITH 1997, PANDEY & NAGAR 2002, 2003, ARYAL & NEUNER 2010). In open places leaves are exposed to radiational cooling that is particularly increased at higher altitudes that drives leaf temperatures below air and dewpoint temperatures (JORDAN & SMITH 1994) resulting in formation of dew droplets on the leaf surface. In understorey habitats, dew formation is negligible because of night time leaf temperature do not drop below dew point temperature as they usually have more or less a similar temperature as the surrounding air. A comparison between open and understorey sites in tropical to temperate forests revealed that leaves taken from the understorey were more wettable but had a better droplet run off than species in open places (ARYAL & NEUNER 2010). This is not the case in leaves of *V. myrtillus* that show a much higher retention in leaves taken from the understorey.

Wettability of *V. myrtillus* leaves was significantly increased at the end of the summer growing period. One explanation is pathogen infection. BRADLEY & al. 2003 showed that both the amount of water captured on a leaf surface and the length of time that water was held on the leaf surface after wetting were excellent predictors of the extent of pathogen infection. Generally, the persistence of water on leaves appears to promote the infection and formation of plant diseases.

Another explanation is alteration of leaf surface characteristic due to frequent leaf washing events that can cause cuticular wax erosion (NEINHUIS & BARTHLOTT 1997). Wax erosion alters wax morphology and can significantly alter leaf wettability properties during leaf aging (NEINHUIS & BARTHLOTT 1997, ANFODILLO & al. 2002, SHEPHERD & GRIFFITHS 2006). In flat leaves of understorey *V. myrtillus* plants θ obtained on the adaxial leaf side got more reduced during leaf aging than that on the abaxial leaf side. The adaxial leaf side is more exposed to wetting. In the steep angled leaves of the subalpine *V. myrtillus* plants of the dwarf shrub heath both leaf sides that are more or less similarly exposed to wetting showed similar increases in leaf wettability with leaf aging.

Low leaf wettability appears to be a common adaptation among plant species in habitats exposed to frequent daily precipitation during the summer growth period (SMITH & MCCLEAN 1989, BREWER & SMITH 1997, PIERCE & al. 2001, MÜLLER & RIEDERER 2005). In the European Alps precipitation commonly increases with elevation (KÖRNER 1999) and open places are more prone to wetting events than understorey places. Leaves of *V. myrtillus* from habitats with more frequent wetting events showed an increased stomatal density. Frequent wetting events can cause occlusion of stomatas and by this be restrictive to photosynthetic gas exchange. CO_2 diffuses approximately 10 000 times slower in water than air (NOBEL 1991). Beading of water on leaf surfaces reduces the contact area of the water droplet with the leaf surface and hence may reducing the wettability of leaf surfaces is thought to be physiologically driven (SMITH & MCCLEAN 1989, BREWER & al. 1991, BRADLEY & al. 2003, HANBA & al. 2004, DIETZ & al. 2007). Stomatal density on leaves of *V. myrtillus* increased with altitude but even more strikingly from the understorey to open places which corroborates earlier findings (KÖRNER & al. 1979, WOODWARD 1986). This may allow the maintenance of high photosynthetic rates as less leaf area including stomates is covered by water droplets (SMITH & MCCLEAN 1989, BREWER & al. 1991, BRADLEY & al. 2003) causing selective pressure for high water repellence and increased water shedding rates. The absence of hypostomaty in low land plants is consistent with observations of WOODWARD 1986 but was not found by KÖRNER & al. 1979.

Gas exchange depends on stomatal conductance (affected by number, size and orientation of stomates) which has been shown to increase in *V. myrtillus* with altitude corresponding to changes in stomatal density (KÖRNER & al. 1979, WOODWARD 1986). However, growing *V. myrtillus* plants in CO_2 concentrations below ambient at low altitude similar to them expected at high altitude resulted in a similar increase in the adaxial stomatal density and hence indicated that stomatal density in *V. myrtillus* leaves may be controlled by ambient CO_2 concentrations (WOODWARD 1986).

Occlusion of stomata by water droplets may not be problematic for the majority of flatly arranged (less than 45°) leaves of montane distributed *V. myrtillus* plants found usually in the forest understorey although they have shown to be completely wettable. The flat orientation provides umbrella like protection to the stomata of the abaxial surface and by this can hardly affect gas exchange. Steeply (upwards or downwards) arranged leaves of plants from the subalpine region were found to be strongly water repellent with decreased water retention. This may be important to minimize the duration of water droplets on the leaf surfaces to prevent interruption of gas exchange.

Our results show that it is helpful to include leaf orientation data into the functional discussion of leaf wetatbility. We found that the adaxial leaf surface had less number of stomata and was less wettable than the abaxial surface. This corroborates the findings of PANDEY & NAGAR 2002 but is in contrast to others (PANDEY & NAGAR 2003, BREWER & NUÑEZ 2007) were abaxial surfaces were usually stomatous and more water repellent.

Stomatal density is further important in the view of the potential number of ice entrance sites on leaf surfaces by which extrinsic ice nucleation could occur. Potential sites for ice penetration from the surface into the leaf tissue are stomates, cracks in the cuticle, wounds, broken epidermal hairs or other lesions (CHEN & al. 1995, WISNIEWSKI & FULLER 1999, PEARCE & FULLER 2001, WISNIEWSKI & al. 2002b, WISNIEWSKI & al. 2002a, HACKER & NEUNER 2007, 2008). The question of extrinsic ice nucleation, however, would only be important for a supercooling species. Supercooling is not the frost survival mechanism of *V. myrtillus* leaves that tolerate ice formation in their tissue. Ice usually forms in *V. myrtillus* leaves around –1.5°C but they get frost damaged not before –4.1°C (LT_i, TASCHLER & NEUNER 2004). However, a low wettability combined with a reduced probability of extrinsic ice nucleation could be an important point for survival of supercooling species and tissues of alpine plants such as alpine grasses (TASCHLER & NEUNER 2004) or reproductive units of cushion plants (HACKER & al. 2011).

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