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RESEARCH

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Interaction of surfactants with *Prunus laurocerasus* leaf surfaces: time-dependent recovery of wetting contact angles depends on physico-chemical properties of surfactants

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Abstract

Background Surfactants are added to spray solutions because they significantly improve foliar uptake of active ingredients (AIs) into the leaves. It was intended to investigate whether surfactant solutions forming a dried deposit on Prunus leaf surfaces after they were sprayed, lead to structural and functional changes of the cuticle/atmosphere interface. This could potentially result in irreversibly enhanced leaf surface wetting, which should be of major disadvantage. Enhanced wetting could promote leaching of ions and promote leaf surface colonization with microorganisms.

Results *Prunus laurocerasus* leaf surfaces were sprayed with aqueous solutions of non-ionic alcohol ethoxylates, a cationic, an anionic and one large polar surfactant. Directly after spraying and drying of the different surfactant solutions, wetting contact angles of deionized water (without surfactant) were significantly lower (between 6 and 54°) compared to wetting contact angles on untreated leaves (77°). Leaf surface wettability with deionized water was more pronounced with non-ionic alcohol ethoxylates (wetting contact angles ranging between 6 and 22°) compared to the other 3 surfactants (wetting contact angles ranging between 42 and 54°). Wetting contact angles of deionized water on leaf surfaces treated with non-ionic alcohol ethoxylates continuously increased again over time resulting in final wetting contact angles not different from untreated leaf surfaces. The time-dependent recovery of wetting contact angles was dependent on the degree of ethoxylation of the non-ionic alcohol ethoxylates. The wetting contact angle recovery rate was lower the higher the degree of ethoxylation of the alcohol ethoxylates was. With the cationic, anionic and large polar surfactant a recovery of wetting contact angles was not observed. In addition, on fully dehydrated and dead leaves wetting contact angle recovery was not observed for any of the tested surfactants after spraying and drying. Analytical determinations of the amounts of alcohol ethoxylates on the leaf surfaces after spraying and drying showed that amounts of alcohol ethoxylates decreased over time on the surface (24–72 h).

Conclusion Our results indicate that non-ionic alcohol ethoxylates diffused within hours from the leaf surface into the leaf over time and thus fully disappeared from the leaf surface. This was not the case with the cationic, anionic and the large polar surfactants remaining on the leaf surface.

Keywords Cuticle, Foliar uptake, Leaf surface, Surfactant, Wetting contact angle

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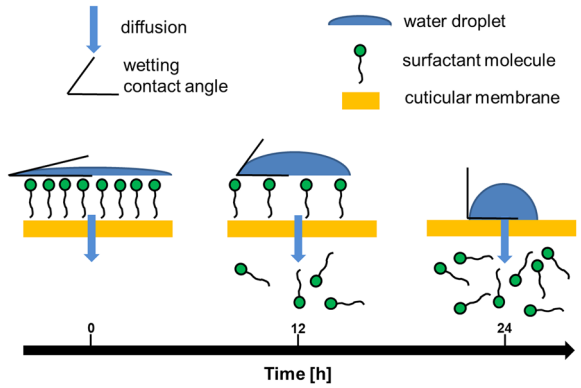
Graphical Abstract



Background

The main barrier against water loss of leaves is established by the water repellent hydrophobic plant cuticle. Due to their lipophilic nature, plant cuticles without epicuticular wax crystallites have wetting contact angles of water around 90° [36]. With a pronounced formation of epicuticular waxes crystallites leaf surfaces can become superhydrophobic having wetting contact angles of 140° or higher [7, 23]. This hydrophobic waxy barrier, protecting plants from uncontrolled water loss, represents also an efficient barrier for plant protecting agents, which have to diffuse into leaves [14, 32]. Thus, surfactants are frequently added to spray solutions because they significantly improve foliar uptake of active ingredients (AIs) into the living plant leaves [4, 9, 10, 16]. This enhancement of AI uptake is mainly due to (i) an improved retention of the spray droplets on leaf surfaces [36], (ii) an enhanced leaf surface wettability, increasing the contact area between spray droplets and the plant cuticle [12] and (iii) a plasticizing effect of surfactants on the cuticluar transport barrier established by wax [9, 18, 26]. Furthermore, surfactants can act as humectants, keeping the spray deposits fluid [3, 34].

Amphiphilic surfactants can be classified in three major groups [15]: non-ionic surfactants consisting of a hydrophobic tail and non-ionic uncharged head group, cationic surfactants having a positively charged head group and anionic surfactants having a negatively charged head group. Amphiphilic surfactants reduce the surface tension of water and thus improve wetting of hydrophobic surfaces by aqueous solutions [2]. Furthermore, it is known that they can form micelles in water [28]. Both properties could affect the structure and function of waxy leaf surfaces. Micelles can efficiently solubilise hydrophobic lipids in water and it can be speculated that they might also solubilise cuticular waxes [35]. Since waxes form the transport limiting barrier of the plant cuticle [31], this could irreversibly damage and reduce barrier properties of leaf surfaces. This would lead to an unwanted, negative side effect of surfactants when used in spray solutions, since transpiration might be enhanced [27] and crops might become more drought sensitive. Furthermore, droplets sprayed to leaf surfaces, will dehydrate within minutes and the spray deposit consisting of the AI and surfactants will remain on the leaf surface [13, 21]. Thus the leaf/surface interface is chemically altered (polar spray deposit vs. hydrophobic untreated leaf surface). This will result in an enhanced wetting of leaf surfaces different from untreated leaves and as a consequence it might lead to enhanced leaching of ions and solutes [37] leading to nutrient imbalances. Furthermore, it could promote leaf surface colonization by microorganisms potentially including plant pathogens [25].

In a recent study, investigating the interaction of surfactants with barley leaf surfaces, it could be shown that complete leaf surface wettability with deionized water (wetting contact angle of 0°), which was obtained after spraying and drying of an aqueous solution of alcohol ethoxylates to the leaf surface, was fully reversible. Final wetting contact angles of 144°, which are characteristic for untreated super-hydrophobic barley leaf surfaces, were obtained again several hours after treatment [6]. Based on these results, the interaction of 4 different types of surfactants (non-ionic, cationic, anionic and large polar surfactants), varying in their physicochemical properties, with *Prunus laurocerasus* leaf surfaces was studied in more detail. It was our intention to investigate whether surfactant solutions forming a dried deposit on *Prunus* leaf surfaces after they were sprayed, lead to structural and functional changes of the cuticle/

atmosphere interface, and potentially result in irreversibly enhanced leaf surface wetting. Leaves of *Prunus laurocerasus* were chosen for this study for 2 main reasons. (1) It is a well-established model species for studying the interaction of surfactants with plant cuticles in agrochemical research [14]. (2) Prunus, as perennial species with a thick and fairly impermeable cuticle, represents an interesting and strongly contrasting species compared to previously investigated barely, being an annual species with thin very permeable cuticle [6].

Time-dependent changes of wetting contact angles of dionized water on *Prunus* leaf surfaces, which were covered by a dried surfactant deposit, were recorded over time. Our results show that effects of surfactants on *Prunus* leaf surface wetting properties and its potential reversibility are strongly dependent on the physicochemical properties of the corresponding surfactants.

Methods

Chemicals

All chemicals were of high analytical purity (p.a.). Four monodisperse alcohol ethoxylates ($C_{12}E_2$: diethylene glycol monododecyl ether; $C_{12}E_4$: tetraethylene glycol monododecyl ether; $C_{12}E_6$: hexaethylene glycol monododecyl ether; $C_{12}E_8$: octaethylene glycol monododecyl ether; Fluka) with increasing degrees of ethoxylation, one polydisperse alcohol ethoxylate (Brij L4: polyoxyethylen(4)-laurylether; Fluka), one cationic surfactant (CTAB: cetyltrimethylammonium bromide; Fluka), one anionic surfactant (SDS: sodium dodecyl sulfate; Fluka) and one polar alkyl polyglycoside (Glucopon 215 CSUP: C8C10-alkyl polyglucoside; Fluka) with high molecular weight were used in the experiments (Table 1). The monodisperse alcohol ethoxylates used in this work are monomeric constituents of the polydispers Brij L4, which has a mean calculated molecular structure given as $C_{12}E_4$.

Plant material

Leaves of cherry laurel (*Prunus laurocerasus*) were harvested from plants growing near the institute. In most experiments fresh and healthy *Prunus* leaves were harvested on a daily base. During the experiments they were still attached to the shoots and supplied with water. In some experiments dead *Prunus* leaves, which were fully dehydrated at 60 °C for 3 days in an incubator, were used. During the experiment shoots were kept in a growth chamber at 23/20 °C (day/night), 50–65% relative humidity and a 16 h light period (150 µmol m^{-2} s^{-1}).

Foliar application of surfactants

Aqueous surfactant solutions of 0.1% (w/v) concentrations were sprayed on the intact leaf surfaces of living and dead leaves using an airbrush system (Start Single Action Airbrush-Pistole, Conrad, Germany) as described in detail in Baales et al. [6]. In a series of preliminary experiments, spraying was standardized (3 × 1 s, distance to the leaf surface 10 cm), resulting in an average surfactant coverage of the leaf surfaces of 1 µg cm^{-2}. This represents a realistic surfactant coverage used in agrochemical spray applications [6].

Wetting contact angle measurements

Wetting contact angle measurements were performed by applying droplets of dionized water on surfactant treated and non treated *Prunus* leaves. Pieces of *Prunus* leaves (dead, living, treated and untreated) were carefully placed on clean microscopic slides using double sided adhesive tape. Care was taken, that the surfaces were not disturbed. Droplet shapes of deionized water

Table 1 Molecular structures and physicochemical properties of the different types of surfactants (non-ionic, cationic, anionic and large polar surfactants). The size of the carbon chain length (C-ratio), ethoxylation degree (EO-ratio), molecular weight (MW) and the corresponding partition coefficient ($logK_{ow}$) are given

Type of surfactant	Name	Hydrophobic tail [C-ratio]	Polar head group [EO-ratio]	Molecular weight [g mol^{-1}]	$LogK_{ow}$ [a]
Non-ionic	Diethylene glycol monododecyl ether ($C_{12}E_2$)	C12	E2	274,44	3.30
	Tetraethylene glycol monododecyl ether ($C_{12}E_4$)	C12	E4	362,54	2.43
	Hexaethylene glycol monododecyl ether ($C_{12}E_6$)	C12	E6	450,64	2.30
	Octaethylene glycol monododecyl ether ($C_{12}E_8$)	C12	E8	538,74	2.01
	Polyoxyethylen(4)-laurylether (Brij L4)	C10/C12/C14	E1-E15	362[b]	2.43
Cationic	Cetrimonium bromide (CTAB)	C16	-	364,45	3,18
Anionic	Sodium dodecyl sulphate (SDS)	C12	-	288,37	2.03
Large polar polyglucoside	Glucopon 215 UP	C8/C10	-	778–806	1.77

[a] Octanol water partition coefficients taken from the safety data sheet of the corresponding chemical

[b] Mean calculated molecular weight

(10 µl) were measured using a drop shape analyzer (DSA 25E; Krüss, Germany). Static equilibrium wetting contact angles were measured shortly after adding the droplet of water, when the drop had stabilized on the leaf surface after a few seconds. Time-dependent changes of wetting contact angles were measured on treated *Prunus* leaves every hour up to 7 h. This allowed to calculate rates of wetting contact angle recovery from regression lines fitted to slopes of the wetting contact angles as function of time (Figs. 1 and 5). After 24 (Fig. 1) and sometimes 48 h

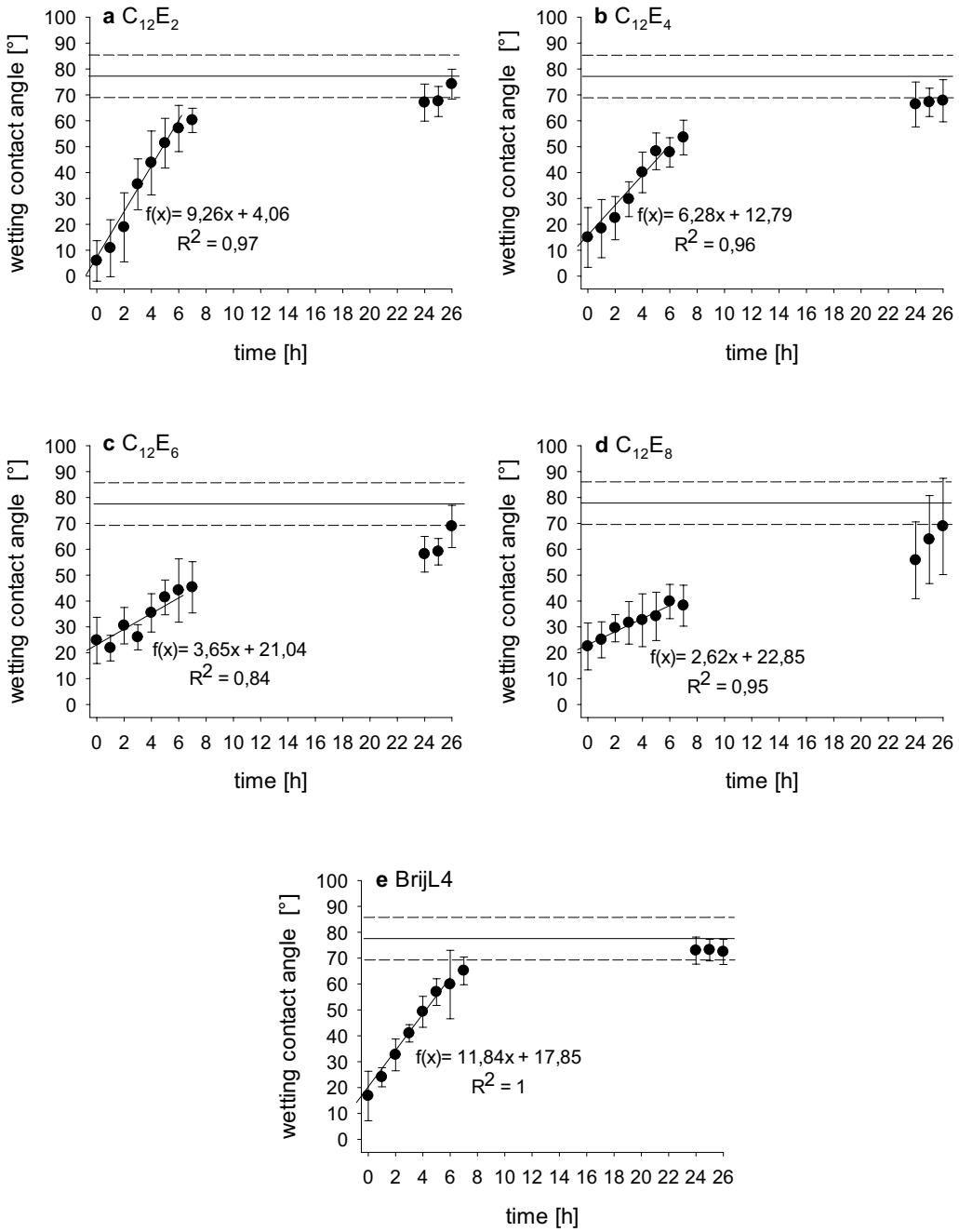


Fig. 1 Time-dependent recovery of wetting contact angles of water on *Prunus* leaf surfaces after spraying with non-ionic alcohol ethoxylates. Wetting contact angles were measured after drying of the sprayed surfactant solutions. Leaf surface coverages of $C_{12}E_2$ (**a**), $C_{12}E_4$ (**b**), $C_{12}E_6$ (**c**), $C_{12}E_8$ (**d**) and Brij L4 (**e**) were 1 µg cm^{-2}. Wetting contact angles on *Prunus* leaves previously treated with deionized water represent the control (dotted black lines). Rates of recovery were calculated from regression lines fitted to the time interval between 0 and 6 h. Data points represent means with standard deviations ($n = 15$)

(Fig. 5) further measurements were taken to see if the wetting contact angels of water could fully recover or not. Wetting contact angle measurements were performed on independent leaf samples to ensure that always fresh and non-dehydrated leaf material was used. Measurements were taken in the laboratory at an average air temperature of 20 °C (± 2 °C) and an average air humidity of 50% (± 10%).

Analytical quantification of monodisperse alcohol ethoxylates

The sorption of the different monodisperse surfactants into the cuticle was investigated by chemical analysis using GC-FID (gas chromatography coupled to flame ionization detection) and GC–MS (gas chromatography coupled to mass spectrometry). Surfactant deposits sprayed on *Prunus* leaf surfaces were extracted after 0, 24, 48 and 72 h using rolled edge vials filled with 3 ml methanol. For extraction the vials were gently pressed onto the leaf surface and the vial was carefully reverted for 1 s. Methanol was completely evaporated from the extracts. Dry extracts were redissolved in 1 ml chloroform, spiked with tetracosane (10 µg per sample) as internal standard, and reduced to a final volume of 200 µl under a gentle stream of nitrogen gas at 60 °C. Extracts were analysed using gas chromatography and mass spectrometry as described recently in detail [5]. Prior to gas chromatography samples were derivatized using BSTFA (N,O bis-(trimethylsilyl)-trifluoroacetamide, Merck, Germany) and pyridine at 70 °C for 45 min.

Quantification was performed by on-column injection, analysing 1 µl sample in a gas chromatograph connected to a flame ionization detector (GC-FID: Agilent 5980; column: 30 m DB-1 with an inner diameter of 0.32 mm and inner coating 0.2 µm, Agilent, USA). Amounts of detected surfactant monomers were related to the internal standard and the extracted areas given by the diameter of the rolled edge vials (inner diameter: 1.3 cm). Identification of molecules was achieved by mass spectrometry (GC–MS: Agilent 6890N; MS: Agilent 5973N mass selective detector; column: 30 m DB-1MS with an inner diameter of 0.32 mm and coating 0.2 µm). Identification of the individual peaks was based on fragmentation patterns of the peaks and by comparing obtained mass spectra with stored mass spectra in a homemade library.

Statistical analysis

Data analysis and statistical test were carried out with OriginPro 9. Normal distribution of the data was tested with the Shapiro–Wilk test. Significant differences between means were tested with a one-way ANOVA at a significance level of $p = 0.05$. Corresponding sample sizes are given in the respective figure legends.

Results

Time-dependent recovery of wetting contact angles after surfactant treatments

Time-dependent recovery of wetting contact angles of deionized water on leaf surfaces with a surfactant coverage of 1 µg cm^{-2} was measured when leaf surfaces appeared visually dry (10 min after spraying with 0.1% surfactant solution). The wetting contact angle of a 10 µl droplet of deionized water on an untreated *Prunus* leaf was 77° ± 8° (Fig. 1). In the case of leaf surfaces carrying dried deposits of the different alcohol ethoxylates, wetting contact angles of deionized water varied between 10 and 20° at time 0 h (Fig. 1). However, within 24 h wetting contact angles approached the values of 70 to 80°, characteristic for untreated leaf surfaces (Fig. 1). A rate of wetting contact angle recovery (° h^{-1}) was calculated from regression lines fitted to those parts of Fig. 1 a–e, where wetting contact angles were linearly increasing (0 to 6 h).

Rates of wetting contact angle recovery decreased with increasing degree of ethoxylation (Fig. 2). Brij L4, the only polydisperse surfactant with a mean ethoxylation

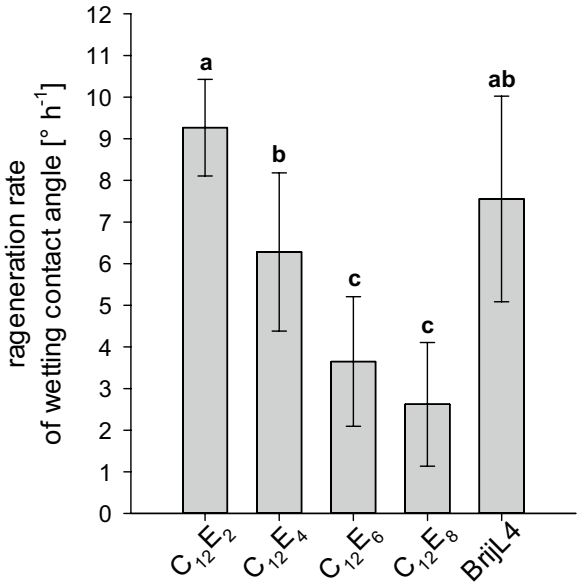


Fig. 2 Rate of wetting contact angle recovery (° min^{-1}) after treatment of *Prunus* leaf surfaces with $C_{12}E_2$, $C_{12}E_4$, $C_{12}E_6$, $C_{12}E_8$ and Brij L4. Surfactant coverage of leaves were 1 µg cm^{-2}. Rates of wetting contact angle recovery decreased with increasing degree of ethoxylation. Differential letters indicate significant differences between the different alcohol ethoxylates at $p < 0.05$. Bars represent means with standard deviations ($n = 15$)

degree of 4, showed a rate of wetting contact angle regeneration between the rates of $C_{12}E_2$ and $C_{12}E_4$ (Fig. 2).

Directly after drying of leaf surfaces, wetting contact angles of deionized water were lowest with the lowest degree of ethoxylation and wetting contact angles increased with increasing degrees of ethoxylation (Fig. 3). However, when dried surfactant deposits on the leaf surfaces were washed off again with water, values of wetting contact angles of deionized water were again identical to those of an untreated leaf surface (Fig. 3).

On fully dehydrated and dead *Prunus* leaves, wetting contact angles of Brij L4 and $C_{12}E_4$ were 104° ± 7° (Fig. 4) and thus were significantly higher compared to fully hydrated living leaves (Figs. 1 and 3). Moreover, dead leaf surfaces covered with Brij L4 and $C_{12}E_4$ had significantly higher wetting contact angles between 60° and 70° (Fig. 4) compared to fully hydrated living leaves (Figs. 1 and 3). Moreover, on dead leaves recovery of wetting contact angles of deionized water could not be observed within 8 h (Fig. 4).

Living and fully hydrated leaf surfaces treated with CTAB, SDS or Glucopon revealed wetting contact angles between 42° and 54° (Fig. 5). Wetting contact angle values nearly identical to untreated leaf surfaces could not be observed with, CTAB, SDS or Glucopon within 24 h (Fig. 5). With CTAB, there was a slight increase of the wetting contact angle between 0 and 8 h from 42 and 55° (Fig. 5a), but a full recovery of the wetting contact angles could not be observed.

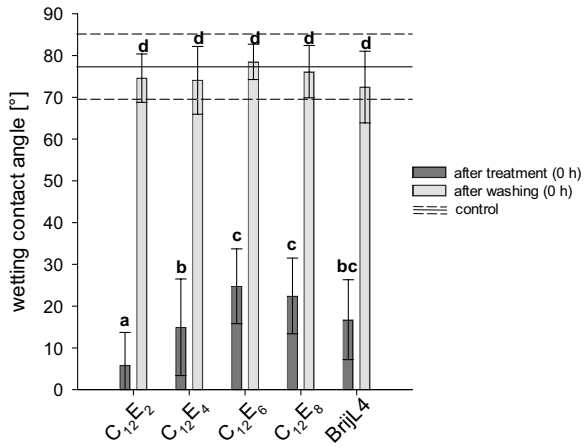


Fig. 3 Wetting Contact angles of deionized water on *Prunus* leaf surfaces. Wetting contact angles were measured directly after the sprayed alcohol ethoxylates had completely dried (black bars) and after dried alcohol ethoxylate deposits had again been washed off from the leaf surface (grey bars). Alcohol ethoxylates were sprayed leading to a leaf surface coverage of 1 µg cm^{-2}. After washing off the alcohol ethoxylates from the leaf surfaces, wetting contact angles fully recovered and were not different from control leaves treated with deionized water instead of alcohol ethoxylates (dotted black line). Differential letters indicate significant differences between the different alcohol ethoxylates and the different treatements at $p < 0.05$. Bars represent means with standard deviation ($n = 15$)

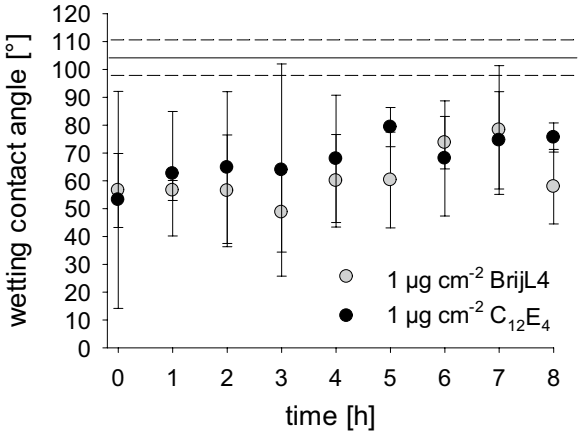


Fig. 4 Time-dependent wetting contact angle measurements of deionized water on fully dehydrated and dead *Prunus* leaf surfaces. Surfactant coverages of leaf surfaces with $C_{12}E_4$ and Brij L4 were 1 µg cm^{-2}. Wetting contact angles were measured every hour for 8 h after the sprayed surfactant solutions had dried off. Wetting contact angles of deionized water on fully dehydrated and dead *Prunus* leaf surfaces represent controls (dotted black line) of untreated dead *Prunus* leaf surfaces. Data points represent means with standard deviations ($n = 4$)

Wetting contact angles of water on leaf surfaces treated with CTAB, SDS or Glucopon were still at about 58° after 24 h (Fig. 6). When leaf surfaces were rinsed with deionized water 24 h after surfactant treatments, wetting contact angles of water reached again the initial values of untreated leaf surfaces (control values, Figs. 5 and 6).

Time-dependent chemical analysis of alcohol ethoxylates residues on leaf surfaces after surfactant treatment

Chemical analysis by gas chromatography showed that amounts of $C_{12}E_2$, $C_{12}E_4$, $C_{12}E_6$ and $C_{12}E_8$ deposited on the leaf surfaces were decreasing over a time interval of 0 to 72 h (Fig. 7). With all 4 monodisperse alcohol ethoxylates the decrease was most pronounced within the first 24 h. Between 48 and 72 h the decrease of the alcohol ethoxylates was significantly slower reaching final values between 0.1 µg cm^{-2} for $C_{12}E_2$ and 0.25 µg cm^{-2} for the other 3 surfactants (Fig. 7). At time point 0 h, after spraying and drying, the amounts that could be detected by gas chromatography were lowest for $C_{12}E_2$ and they increased with increasing degrees of ethoxylation (Fig. 7).

Discussion

Wetting contact angles of deionized water on barley leaf surfaces, covered with a dried layer of the monodisperse alcohol ethoxylate $C_{12}E_4$ and the polydisperse alcohol

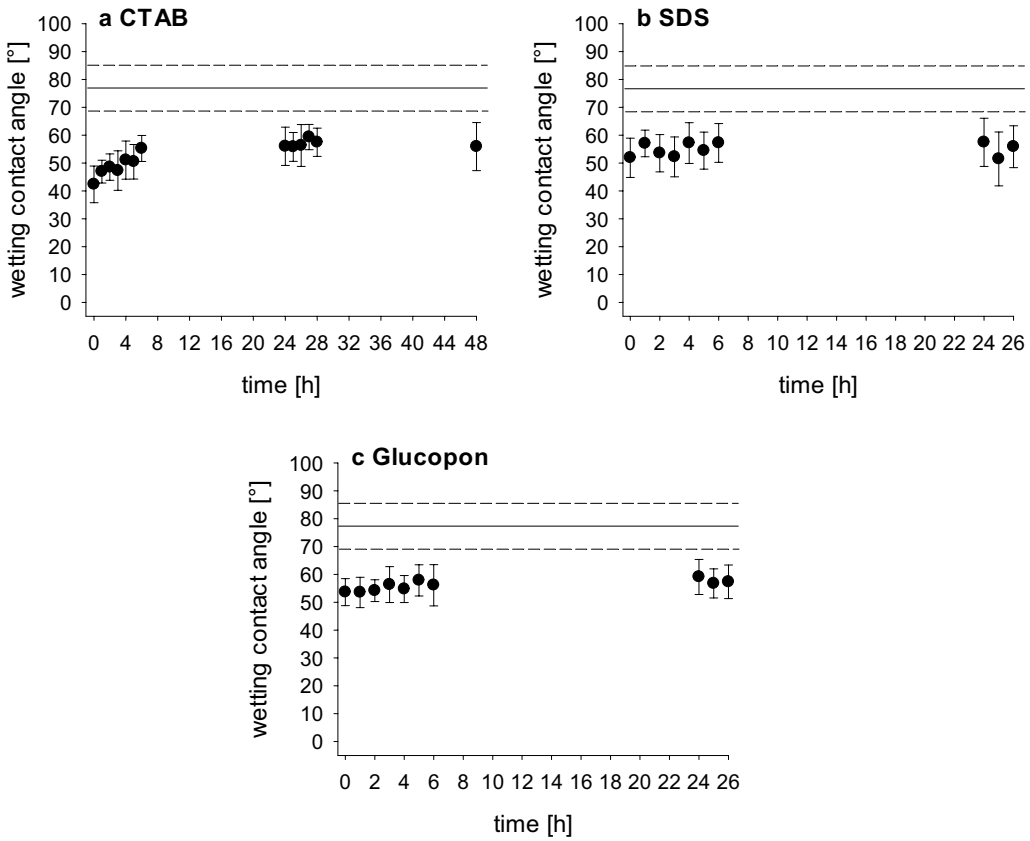


Fig. 5 Time-dependent recovery of wetting contact angles of water on *Prunus* leaf surfaces sprayed with CTAB (**a**), SDS (**b**) and Glucopon (**c**). Surfactant coverage of leaf surfaces were 1 µg cm^{-2}. Wetting contact angle measurements were started after sprayed surfactant solution had fully dried. Wetting contact angles on *Prunus* leaves treated with deionized water represent the control (dotted black lines). With SDS and Glucopon there was no regeneration of wetting contact angles. With CTAB there was a partial regeneration of the wetting contact angles within the first 8 h. Data points represent means with standard deviations ($n = 15$)

ethoxylate Brij L4, having a thickness of 1 µg cm^{-2}, were 0°. Thus barley leaf surfaces covered with alcohol ethoxylates were 100% wettable [6]. However, within 1 h wetting contact angles of water were fully regenerating reaching values of about 144°, which are characteristic for intact barley leaves serving as control. Compared to barley, several differences in wetting and regeneration can be observed with *Prunus* leaf surfaces and when covered (1 µg cm^{-2}) with 4 different monodisperse alcohol ethoxylates and Brij L4 as polydisperse alcohol ethoxylate (Fig. 1).

Wetting contact angles of deionized water on *Prunus* were in general much lower (77°) compared to the superhydrophobic barley leaf surface [7]. This difference in wetting between native *Prunus* and barley can best be explained by the very different leaf surface morphology. *Prunus* has a more or less flat and unstructured but hydrophobic leaf surface of cutin and wax constituents, which are to the largest extent composed of methyl- and methylene groups with few functional groups like acids, aldehydes and alcohols [19]. A surface like polyethylene, exclusively composed of deionized methyl and methylene groups, results in a wetting contact angle of water of about 90 to 100° [2]. Wetting contact angles will decrease with increasing amounts of interfacial functional polar groups, like alcohols or acid [17]. This explains best the wetting contact angles close to 80° observed with untreated *Prunus* leaf surfaces (Fig. 1).

Very different from *Prunus* the barley leaf surface is characterized by a pronounced bloom of epicuticular wax crystallites rendering the leaf superhydrophobic with wetting contact angles higher than 140° [6]. Besides the typical water-repellent hydrophobic wax molecules, leading to wetting contact angles between 90 and 100° this epicuticular wax layer of barley leads to a rough surface allowing water droplets only to sit on the tips of the wax crystallites. Between the leaf surface and the water droplets a pronounced air layer is still present limiting an efficient wetting [20]. Most interestingly, superhydrophobic barley leaf surfaces, when treated with a 0.1% solution of

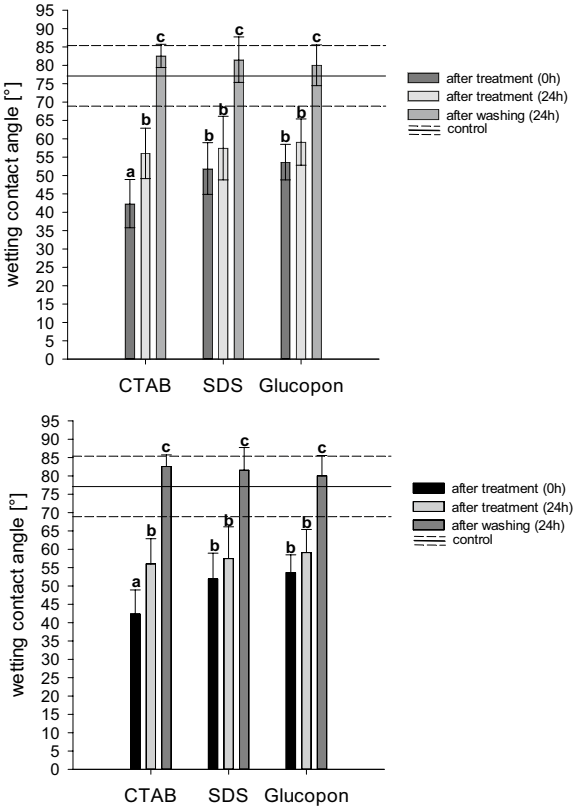


Fig. 6 Wetting contact angles of deionized water on *Prunus* leaf surfaces directly after aqueous solutions of SDS, CTAB and Glucopon were sprayed to the leaf surfaces and had dried off (black bars). Wetting contact angles were again measured 24 h after the treatment (light gray bars) and on leaves where surfactant deposits had been washed off after 24 h (dark grey bars). Surfactants were sprayed leading to a coverage of 1 µg cm.$^{-2}$. After washing off the dried surfactants from the leaf surfaces, wetting contact angles fully recovered (grey bars) and were not different from control leaves treated with deionized water instead of surfactants (dotted black line). Differential letters indicate significant differences between the different alcohol ethoxylates and the different treatements at $p < 0.05$. Bars represent means with standard deviations ($n = 15$)

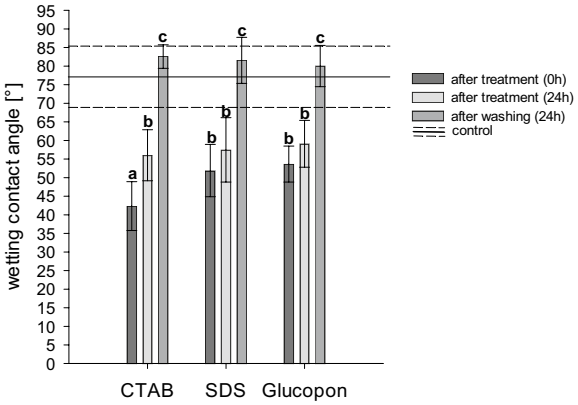


Fig. 7 Time-dependent changes in the amounts (µg cm^{-2}) of the monodisperse alcohol ethoxylates $C_{12}E_2$, $C_{12}E_4$, $C_{12}E_6$ and $C_{12}E_8$ remaining on *Prunus* leaf surfaces after spraying aqueous solutions of 0.1% leading to a surfactant coverage of 1 µg cm^{-2}. Amounts of the alcohol ethoxylates rapidly decreased within the first 24 h, whereas the rates of decrease levelled off between 24 and 72 h. Differential letters indicate significant differences between the different times at $p < 0.05$. Bars represent means with standard deviations ($n = 4$)

the 2 alcohol ethoxylates $C_{12}E_4$ and Brij L4, leading to a dry surface deposition of 1 µg cm^{-2}, were rendered fully wettable and wetting contact angles of 0° were observed [6]. This can be explained by capillary forces [22] dragging water droplets with a very low surface tension of around 30 mN m^{-1}, as it is the case with a 0.1% aqueous solution of alcohol ethoxylates between the epicuticular wax crystallites [28]. Very different from barley, in the case of *Prunus* leaf surfaces, when treated with the different alcohol ethoxylates leading to a deposit of 1 µg cm^{-2}, wetting contact angles of water were still measurable and they ranged between 6 and 25° (Fig. 1). Interestingly, wetting contact angles of water on *Prunus* leaf surfaces treated with alcohol ethoxylates were also regenerating within 24 h reaching again a value of 68 to 74°, which was nearly identical to wetting contact angles of water of 77° on untreated *Prunus* control leaves (Fig. 1). However, the initial rates of wetting contact angle regeneration were much faster with barley (about 3° min^{-1}), whereas with *Prunus* they varied between 3 and 10° h^{-1} (Fig. 2).

The other 3 surfactants behaved very different compared to the alcohol ethoxylates. Wetting contact angles of water on *Prunus* leaf surfaces, carrying a dried layer of these surfactants of 1 µgcm^{-2}, were between 42 and 54° (Fig. 5). Thus, compared to alcohol ethoxylates, the cationic CTAB, the anionic SDS and the large and polar, but non-ionic, Glucopon were less efficient in modifying the cuticle/atmosphere interface of the *Prunus* leaf surface and were hindered in entering the cuticle due to the charge and size of the molecule itself. Furthermore, a complete regeneration of the wetting contact angle reaching the values of the untreated *Prunus* leaf surface within 24 h, as it was the case with the monodisperse alcohol ethoxylates, could not be observed here, even not within 48 h (Fig. 5). Depending on the surfactants there were trends that wetting contact angles were slightly regenerating to some extent within the first few hours, most pronounced with CTAB, but final values were still round 56° and thus still significantly lower compared to control leaves.

After spraying of 0.1% aqueous surfactant solutions to *Prunus* leaf surfaces, they needed about 10 min until they were completely dried off, finally resulting in 1 µg cm^{-2}

coverage. During that time irreversible surfactant induced changes of wetting properties with water of the cuticle/atmosphere interface, which might be caused by wax solubilization [34] or rearrangement of leaf surface wax molecules [24], could potentially be induced. However, with all 4 different types of surfactants investigated here, there was no indication that irreversible changes of *Prunus* leaf surface wetting properties were induced. After washing off the alcohol ethoxylates with water directly after drying, wetting contact angles of deionized water were not different from untreated leaf surfaces (Fig. 3). In the case of the alcohol ethoxylates, it was anyway rather unlikely that leaf surfaces properties would be significantly changed directly after surfactant application, since wetting contact angles were fully regenerating within 24 h (Fig. 1). With the other 3 surfactants, where a full regeneration of wetting contact angles was not occurring, leaf surfaces were washed 24 h after surfactant treatments, since here it seemed more likely expecting irreversible changes of *Prunus* leaf wetting surface properties with time. However, this was also not the case since wetting contact angles of water were again identical to untreated control leaf surfaces of *Prunus* (Fig. 6). Thus, observed increases in *Prunus* leaf surface wetting after surfactant application and drying are exclusively due to the fact that droplets of deionized water are in contact with the dry surfactant layer and not due to altered physicochemical properties of the *Prunus* leaf surface itself.

The fact that wetting contact angles of water on *Prunus* leaf surfaces were regenerating within 24 h with the non-ionic alcohol ethoxylates but not with the cationic CTAB, the anionic SDS and the large polar Glucopon can again be best explained by diffusion of the alcohol ethoxylates into the leaf interior, whereas this is not at all or at least at a far slower rate taking place with the other three types of surfactants. This is also confirmed by the observation that wetting contact angle regeneration of alcohol ethoxylates did not happen on fully dehydrated and dead leaves (Fig. 4). Obviously, with fully dehydrated leaves the necessary aqueous sorption compartment in the epidermal and the mesophyll cells, where surfactants can dissolve after diffusing across the cuticle, is missing. Consequently, surfactants are trapped on the leaf surface after spraying and drying as it is also the case with the big and charged surfactants tested.

Gas chromatographic analysis showed that the amounts of monodisperse alcohol ethoxylates remaining on the *Prunus* leaf surface after spraying and drying are decreasing over time (Fig. 8), indicating that the surfactants are diffusing into the leaf interior. This was previously described for barley [6]. However, alcohol ethoxylates did not fully disappear within 24 to 72 h. Interestingly even after full regeneration of the wetting contact angle (24 h after spraying) there were still 20 to 40% of the applied alcohol ethoxylates left on *Prunus* leaf surfaces (Fig. 8).

This fact might be explained either by the fact, that the surfactant molecules rearrange themselves in a way not affecting wetting anymore, or alternatively they are sorbed and thus hidden in the most outer wax layer of the transport barrier of the cuticle. Washing off the leaf surface for 1 s with MetOH, to extract and quantify the surfactants from the leaf surface, might in turn also dissolve surfactant molecules from the most outer wax layer. (Fig. 7). Methanolic extracts of the leaf surface in fact contained also small amounts (less than 1%) of wax molecules, indicating that methanol as organic solvent also removed traces of wax molecules and thus maybe as well alcohol ethoxylates sorbed between these wax molecules.

The observations reported here, including (i) the regeneration of wetting contact angles on living hydrated leaves previously treated with alcohol ethoxylates, (ii) the lack of wetting contact angle regeneration on fully dehydrated dead leaves and (iii) the decreasing amounts of alcohol ethoxylates on the leaf surface clearly indicate that they diffuse into the leaf with time. The diffusion rate in *Prunus* leaves is 60 to 200-fold slower in comparison to the recently described diffusion into barley leaves [6]. This is best explained by pronounced differences in the cuticular barrier properties within the two plant species. Comparing the water permeance of both species, indicate that barley had a higher permeance of about 10^{-9} m s^{-1} [6], in comparison to *Prunus* with a 10-times lower permeance (10^{-10} m s^{-1}) [33].

It is obvious that the rates of wetting contact angle regeneration of the 4 monodisperse alcohol ethoxylates investigated were continuously decreasing with an increasing degree of ethoxylation (Fig. 2). Plotting the logarithms of the rates of wetting contact angle regeneration versus various molecular parameters (degree of ethoxylation, molecular weight, molecular volume and molecular diameter) resulted in highly linear correlations with correlation coefficients (r) higher than 0.98 (Fig. 9).

All these parameters are describing in a way the size of the different monodisperse alcohol ethoxylates investigated. It was shown in the past that rates of the diffusion of organic molecules across cuticles are strongly dependent on their molecular size. Increasing the molar volume by a factor of two reduced the rates of diffusion in cuticles of other plant species than *Prunus* by a factor of nearly 9 [8]. Here, the rate of wetting contact angle regeneration was nearly fourfold faster with $C_{12}E_2$ compared to $C_{12}E_8$, which is about 2-times larger, clearly indicating a faster diffusion of $C_{12}E_2$ into the leaf compared to $C_{12}E_8$. This faster diffusion of smaller alcohol ethoxylates compared to the larger ones is obviously already taking place during

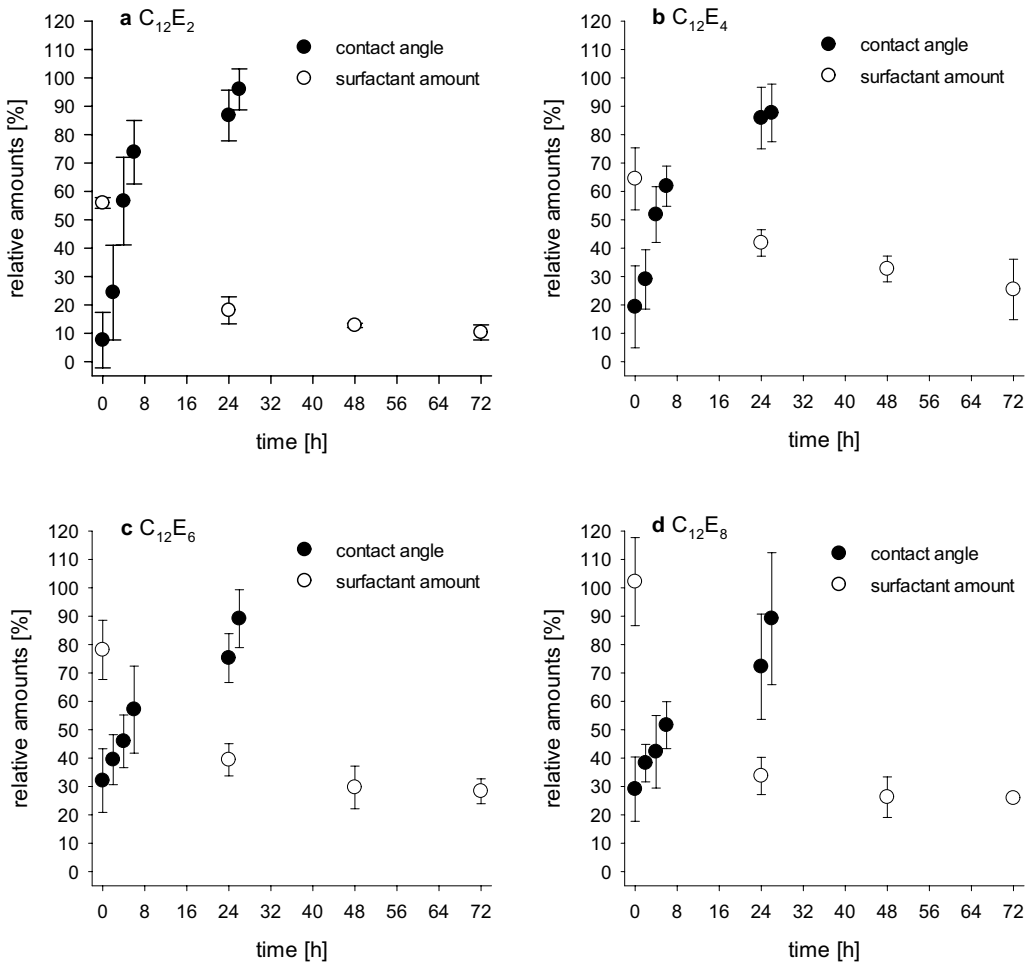


Fig. 8 Time-dependent recovery of the relative wetting contact angles (%) on *Prunus* leaf surfaces (black circles) in comparison to the disappearance of relative amounts (%) of the monodisperse alcohol ethoxylates (white circles) $C_{12}E_2$ (**a**), $C_{12}E_4$ (**b**), $C_{12}E_6$ (**c**) and $C_{12}E_8$ (**d**) from *Prunus* leaf surfaces. Data points represent means with standard deviations

the initial drying period of the sprayed surfactant solutions [11]. The amounts of alcohol ethoxylates remaining on the leaf surface directly after drying (0 h) were only about 60% with $C_{12}E_2$ and increased with the degree of ethoxylation, whereas 100% of the $C_{12}E_8$ were still present on the leaf surface directly after drying (Fig. 7).

The result that the other 3 surfactants did obviously not rapidly diffuse into the leaf within 24 h can be explained by the fact that they are either positively (CTAB) or negatively (SDS) charged or fairly large (Glucopon). Plant cuticles are polyelectrolytes and they carry fixed positive as well as negative charges within the cutin polymer [29, 30]. This affects the "free" diffusion of the charged CTAB and SDS molecules across cuticles. With charged molecules it is not the concentration gradient alone driving the diffusion, but the electro-chemical potential gradient acting across the cuticle [34]. This hinders or at least significantly reduces the rates of diffusion of charged molecules compared to non-ionic molecules. The polar Glucopon is non-ionic, but compared to the non-ionic alcohol ethoxylates investigated here, much larger with a molecular weight of about 800 g mol^{-1}. It has been shown in the past that the rates of diffusion of organic molecules in cuticles, having molecular weights higher than 650, are becoming more and more infinite and thus cuticles can be considered nearly impermeable for molecules with molecular weights higher than 650 [32, 33]. This explains best why the fairly large non-ionic Glucopon does not rapidly diffuse into the leaf and remains on the cuticle surface.

Conclusion

Our study shows that non-ionic monodisperse alcohol ethoxylates can diffuse into the cuticle and in the leaf, whereas charged and very large surfactants essentially remain on the cuticle surface. Thus, measuring wetting

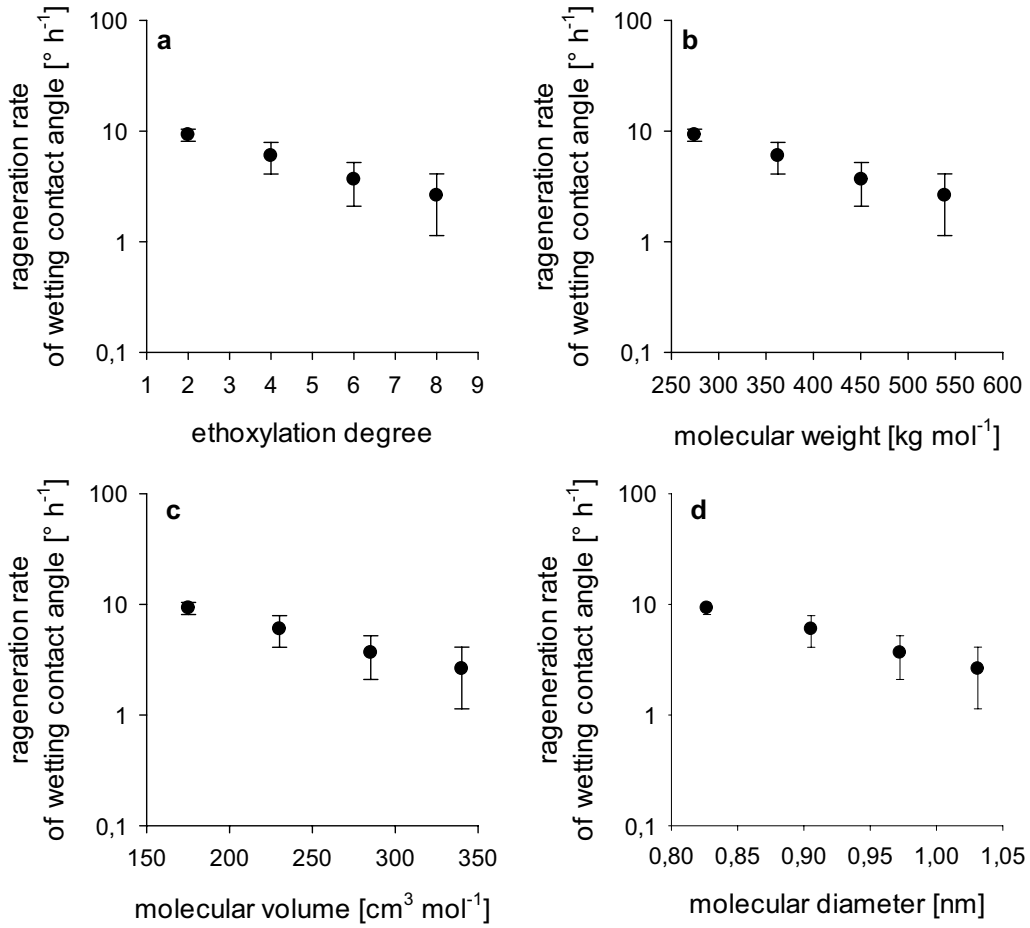


Fig. 9 Correlations between slopes of wetting contact angle recovery (° h^{-1}) of 4 monodisperse alcohol ethoxylates and different molecular parameters [degree of ethoxylation (**a**), molecular weight (**b**), molecular volume (**c**) and molecular diameter (**d**)]. Correlation efficient (r) for the regression lines fitted to the plots were always better than 0.98. Molecular volume (**c**) and molecular diameter (**d**) were calculated as described by Abraham and McGowan in 1987 [1]. Data points represent means with standard deviations

contact angles of deionized water on surfactant treated leaf surfaces represents a fast and easy method to decide, which surfactants will disappear with time in the leaf and which will stay on the surface. This knowledge is essential to decide which surfactants are best added in the spray mixture of different agrochemicals. Surfactants diffusing into the cuticle and inducing a plasticizing effect on the transport barrier will more efficiently contribute to the uptake of AIs into the leaf, which may be of benefit for AIs with a systemic mode of action. Surfactants mainly staying for a longer period at the plant/atmosphere interface will contribute to the formation of a deposit of AIs on the leaf surface, which may be of benefit for AIs with a mode of action based on a direct contact between AI and pathogen (e.g., fungicides, insecticides). Our observation reported here, may also have ecotoxicological implications, since surfactants staying on the leaf surface will enhance leaf surface wetting for a much longer time compared to surfactants disappearing within the leaf. This could promote colonization with microorganisms including pathogens and it could lead to an enhanced leaching of nutrients from the leaf interior. Finally, the observation that alcohol ethoxylates are rapidly diffusing into the leaf lead to the question of their fate inside the living tissue and to what extent they are accumulating and/or metabolised or detoxified by plants. This is currently under investigation.

Abbreviations

$C_{12}E_2$	Diethylene glycol monododecyl ether
$C_{12}E_4$	Tetraethylene glycol monododecyl ether
$C_{12}E_6$	Hexaethylene glycol monododecyl ether
$C_{12}E_8$	Octaethylene glycol monododecyl ether
Brij L4	Polyoxyethylen(4)-laurylether
CTAB	Cetrimonium bromide
SDS	Sodium dodecyl sulphate
Glucopon 215 UP	C8C10-alkyl polyglucoside

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