Enhancement of the Diffusion of Active Ingredients in Barley Leaf Cuticular Wax by Monodisperse Alcohol Ethoxylates

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Rates of uptake of active ingredients (ai) across the plant cuticle are enhanced by the action of alcohol ethoxylate (AE) adjuvants. The partitioning of monodisperse AE between aqueous solutions and isolated cuticular wax from barley (*Hordeum vulgare* L.) leaves was investigated. Quantitative structure−property relationships for wax/water partition coefficients ($K_{wax/w}$) and maximum AE concentrations in the wax (c_{wax}^{max}) were established. In the presence of AE, the diffusion coefficients of six ai in cuticular wax increased by factors of up to 125. AE effects were linearly related to their respective c_{wax}^{max}, suggesting a common intrinsic activity. AE had higher effects on the diffusion coefficients of large ai than on those of smaller ones. Conclusions are drawn concerning the mechanism of AE action on the physical structure of cuticular waxes.

Keywords: *Alcohol ethoxylate adjuvants; cuticular wax; diffusion coefficient; foliar uptake; Hordeum vulgare*

INTRODUCTION

The cuticle covers the largest part of the above-ground surface of crop and weed plants and, consequently, often plays an important role during the foliar uptake of agrochemicals (Hull, 1970; Bukovac, 1976; Bayer and Lumb, 1979; Baur and Schönherr, 1996). This extracellular lipoid membrane consists of the biopolymer cutin (Holloway, 1982) and associated cuticular waxes (Baker, 1982; Bianchi, 1995). Cuticular waxes constitute the main barrier controlling the rate of transcuticular diffusion of active ingredients (ai) (Riederer and Schreiber, 1995). Waxes are semicrystalline solids (Reynhardt and Riederer, 1991, 1994; Merk et al., 1998) and, therefore, solubility and mobility of organic solutes in cuticular waxes are fairly low (Schreiber and Schönherr, 1992, 1993b).

Alcohol ethoxylates (AE) are used as adjuvants in a wide variety of agrochemical formulations to enhance ai effectiveness (Kirkwood, 1993). Numerous studies have demonstrated that, in addition to improving spray retention and leaf wetting, ethoxylated adjuvants may also increase cuticular permeability (Riederer and Schönherr, 1990; Chamel et al., 1992; Tan and Crabtree, 1992; Stock and Holloway, 1993; Knoche and Bukovac, 1994; Schönherr and Baur, 1996). Increasing solute mobility in the cuticular transport barrier has been recognized as the main mechanism leading to enhanced cuticular permeability. Adjuvants having this effect have been called accelerators (Schönherr, 1993a,b; Riederer et al., 1995).

The commercially employed AE are polydisperse technical products consisting of a wide range of compounds of varying molecular structure and physical−chemical properties. This compositional complexity of polydisperse preparations renders mechanistic studies on the structure−property relationships of adjuvants very difficult (Schönherr et al., 1991). In contrast, monodisperse AE are chemically pure compounds and therefore much more suitable for basic studies on adjuvant behavior and effects on cuticular transport properties (Riederer et al., 1995; Schreiber, 1995; Schreiber et al., 1996c).

The sorption of AE in plant cuticular waxes and their effects on solute diffusion can be investigated using an in vitro test system developed by Schreiber and Schönherr (1993b). It has been shown repeatedly that reconstituted cuticular wax films on inert supports reflect basic transport properties (Schreiber and Schönherr, 1993b; Schreiber et al., 1996b; Schreiber and Riederer, 1996; Kirsch et al., 1997) of the wax barrier of cuticular membranes. This system also allows one to single out the processes of adjuvant partitioning and acceleration of solute mobility without interference by other adjuvant-related variables such as wetting (Knoche and Bukovac, 1992), spray retention (Gauvrit and Dufour, 1990; Stevens et al., 1993), and solubilization of ai (Shafer and Bukovac, 1989; Nassetta et al., 1991).

The processes and properties of adjuvants leading to the acceleration of the transcuticular movement of ai are not yet fully understood. The objective of the present study therefore is to quantitatively relate the effects of monodisperse AE on ai mobility to their concentrations in the cuticular wax. The latter is governed by the partitioning of adjuvant molecules between an aqueous (micellar) solution and the waxes covering the cuticular surface. Thus, the distribution of AE in the system aqueous solution/cuticular wax has been studied. As far as possible, the results will be formulated as quantitative structure−property relationships which may be generally applicable to the prediction of AE effects on foliar uptake. The findings of the present study will also provide new insights into the physical mechanisms leading to the acceleration of ai

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Table 1. Molar Masses (MW), Critical Micelle Concentrations (cmc) at 25 °C, and Cuticular Polymer Matrix (MX)/Water Partition Coefficients ($K_{MX/w}$) at 25 °C of Monodisperse AE

compound	abbrev	MW (g/mol)	cmc[a] (mmol/kg)	$K_{MX/w}$[a]
diethylene glycol monobutyl ether	C_4E_2	162.23	590	0.91
triethylene glycol monohexyl ether	C_6E_3	234.34	60	6.8
tetraethylene glycol monooctyl ether	C_8E_4	306.45	6.2	50
pentaethylene glycol monodecyl ether	$C_{10}E_5$	378.56	0.63	370
octaethylene glycol monodecyl ether	$C_{10}E_8$	510.72	1.2	120
diethylene glycol monododecyl ether	$C_{12}E_2$	274.45	0.028	13000
triethylene glycol monododecyl ether	$C_{12}E_3$	318.50	0.035	8900
tetraethylene glycol monododecyl ether	$C_{12}E_4$	362.56	0.043	6000
pentaethylene glycol monododecyl ether	$C_{12}E_5$	406.61	0.053	4100
hexaethylene glycol monododecyl ether	$C_{12}E_6$	450.66	0.066	2800
heptaethylene glycol monododecyl ether	$C_{12}E_7$	494.72	0.079	1900
octaethylene glycol monododecyl ether	$C_{12}E_8$	538.77	0.098	1300
heptaethylene glycol monotetradecyl ether	$C_{14}E_7$	522.77	0.0066	20000
octaethylene glycol monotetradecyl ether	$C_{14}E_8$	566.83	0.0081	14000
octaethylene glycol monohexadecyl ether	$C_{16}E_8$	594.88	0.00068	150000

[a] Calculated according to prediction equations from Riederer et al. (1995).

mobility in cuticular wax and identify structural attributes of adjuvant molecules required for accelerating activity.

EXPERIMENTAL PROCEDURES

Samples of Cuticular Waxes. Cuticular wax was isolated from leaves of 4-week-old barley (*Hordeum vulgare* cv. Igri) plants grown in a greenhouse. Cuticular wax was extracted by dipping the leaves for 5 s in chloroform (purity 99%; Riedel de Haën, Seelze, Germany) at room temperature. As shown previously, this procedure assures the quantitative extraction of both epicuticular and intracuticular waxes from barley leaves (Schreiber and Schönherr, 1993b). The wax extract was filtered, adjusted to a concentration of 50 mg/mL, and stored at −18 °C. The chemical composition of the surface extract from barley leaves is dominated (>80 mass %) by 1-hexacosanol (Reynhardt and Riederer, 1994).

Wax samples were prepared as described earlier in detail (Schreiber and Schönherr, 1993b). Briefly, cuticular waxes were recrystallized on aluminum disks (diameter = 8 mm) by dipping the disks in a chloroform solution of the wax and evaporating the solvent. The wax samples were heated for 5 min to 100 °C to produce a homogeneous and smooth wax film adhering to the aluminum surface. Wax coverage was determined gravimetrically (Microbalance D3, accuracy ± 1 μg; Sartorius, Göttingen, Germany).

Monodisperse Alcohol Ethoxylates. A series of monodisperse AE (Fluka, Neu-Ulm, Germany) was selected with regard to a wide variation of alkyl chain length and degree of ethoxylation (Table 1). In the following, individual homologues will be designated by abbreviations giving the number (x) of carbon atoms in the alkyl chain (C) and the number (y) of ethoxy units (E) according to C_xE_y. The monodisperse AE chosen occur in many commercial polydisperse products, where they make up the low-ethoxylated range of the monomer distribution. The purity (>97%) and identity of the compounds used were checked by gas chromatography/mass spectrometry.

The AE were dissolved in deionized water, and sodium azide (1 mM, purity 99%; Merck-Schuchardt, Hohenbrunn, Germany) was added to prevent microbial growth.

Active Ingredients. Six ^{14}C-labeled herbicidal and fungicidal ai were selected as model compounds for mobility studies. They were chosen to cover an extended range of molar volumes (Table 2). Salicylic acid was included because of its comparably low molar volume. It is a naturally occurring compound playing an essential role in the systemic acquired resistance of plants (Durner et al., 1997). Radiochemical purities of the model compounds were checked regularly by radio-thin-layer chromatography (Berthold Dünnschichtscanner, Wildbad, Germany) and were always >97%.

Sorption Isotherm of $C_{12}E_3$. Aluminum disks carrying amounts of cuticular wax between 150 and 250 μg were added to aqueous solutions of $C_{12}E_3$ (0.005−0.5 mmol/kg) in 100- or 20-mL glass vials. The vials were closed with Teflon-lined septum caps and agitated on a rotating bench (60 rpm) at 25 °C (±0.5 °C). When equilibrium had been established after 24 h (as shown by preliminary tests), the wax samples were removed from the $C_{12}E_3$ solutions, dipped for a few seconds in deionized water to remove superficially adsorbed AE molecules, and blotted carefully with filter paper for removing adhering solution.

The wax films were removed from the aluminum disks by extraction with chloroform (30 min at 70 °C) in 1-mL Reactivials. Afterward, the disks were removed and the solvent was evaporated under a gentle stream of nitrogen at 40 °C. The amounts of $C_{12}E_3$ present in the wax samples were determined by gas chromatography (GC). GC was also used for determining the equilibrium concentrations in the aqueous phase. Aliquots were taken from the AE solutions, taken to dryness under a gentle stream of nitrogen at 40 °C, and prepared for GC analysis. The sorption isotherm was obtained by plotting the equilibrium concentrations (millimoles per kilogram) in the wax versus those in the aqueous supernatant (Figure 1).

Wax/Water Partition Coefficients of Monodisperse AE. Wax/water partition coefficients were determined essentially as described above for the sorption isotherm of $C_{12}E_3$. In this case, however, aluminum disks carrying wax amounts between 100 and 1000 μg were added to aqueous AE solutions that were 5−10 times above the respective critical micelle concentrations (cmc; Table 1). To distinguish between adsorption of AE molecules to the wax surface and sorption within the wax film, the amounts of AE (millimoles) detected in the samples of varying mass (and, consequently, surface-to-volume ratios) were plotted versus the corresponding masses of wax (kilograms). The concentration of AE sorbed within the wax (millimoles per kilogram) was obtained from the slope of the regression line (Schreiber and Schönherr, 1992, 1993a).

Wax/water partition coefficients ($K_{wax/w}$) were calculated from the AE concentrations in the wax (c_{wax}^{max}) in equilibrium with 5−10-fold cmc and the corresponding cmc according to

$$K_{wax/w} = c_{wax}^{max}/\text{cmc} \quad (1)$$

Critical micelle concentrations for individual homologues (Table 1) were estimated using a quantitative structure−property relationship established previously (Riederer et al., 1995). Since comparably small amounts of AE were associated with the cuticular wax, the large supernatant reservoir ensured that their aqueous concentrations remained practically constant during the course of the experiment.

GC. GC analysis of AE associated with the wax samples essentially followed the procedure described previously in detail (Riederer et al., 1995). Analysis was carried out using a gas chromatograph equipped with a flame ionization detector and an on-column-injector (HP 5890 II; Hewlett-Packard, Avondale, PA). Separation of the AE from wax constituents was achieved on a fused silica column (DB-1, 30 m × 0.32 mm inner diameter, 1 μm coating; J&W Scientific, Folsom, CA).

Table 2. Specific Activities, McGowan's Characteristic Volumes (V_x), Molar Masses (MW), and Experimentally Determined Wax/Water Partition Coefficients ($K_{wax/w}$) (Leaf Cuticular Wax of *H. vulgare* L.) at 25 °C of Selected ^{14}C-Labeled ai

compound	common name[a]	specific activity (GBq/mol)	V_x[b] (cm^3/mol)	MW (g/mol)	$K_{wax/w}$
2-hydroxybenzoic acid	salicylic acid (1)	2100	99.04	138.12	30[c]
(2,4-dichlorophenoxy)acetic acid	2,4-D (2)	581	137.61	221.04	46[d]
4-amino-6-*tert*-butyl-4,5-dihydro-3-(methylthio)-1,2,4-triazin-5-one	metribuzin (3)	769	161.96	214.28	3.5[c]
1-(4-chlorophenoxy)-3,3-dimethyl-1(1*H*)-1,2,4-triazol-1-yl)butan-2-ol	triadimenole (3)	586	218.82	295.77	32[d]
1-(4-chlorophenyl)-4,4-dimethyl-3(1*H*)-1,2,4-triazol-1-ylmethyl)pentan-3-ol	tebuconazole (3)	1068	241.13	307.82	640[c]
1-(biphenyl-4-yloxy)-3,3-dimethyl-1(1*H*)-1,2,4-triazol-1-yl)butan-2-ol	bitertanole (3)	624	267.36	337.42	1000[d]

[a] Sources: (1) NEN, Dreieich, Germany; (2) Sigma Chemie, Deisenhofen, Germany; (3) Bayer AG, Leverkusen, Germany. [b] Calculated according to the method of Abraham and McGowan (1987). [c] Own determination. [d] From Schreiber and Schönherr (1992).

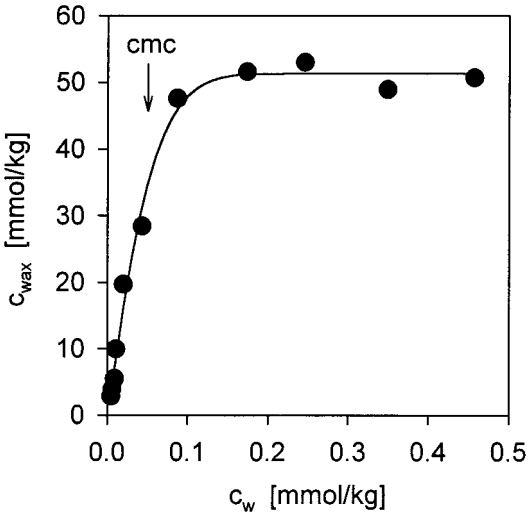

Figure 1. Isotherm for the partitioning of $C_{12}E_3$ between an aqueous solution and reconstituted cuticular wax from barley leaves. The equilibrium concentrations in the wax (c_{wax}) are plotted versus those in the supernatant (c_w).

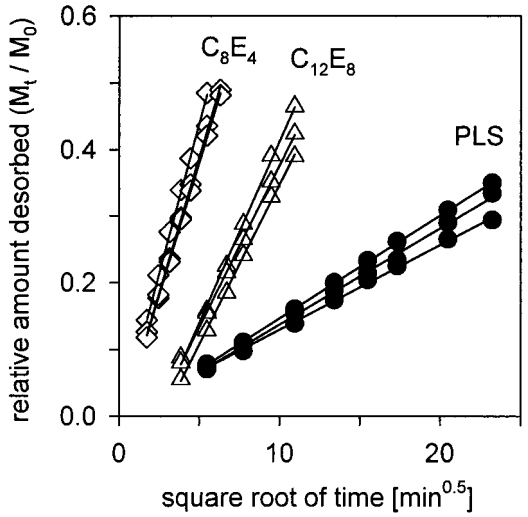

Figure 2. Desorption kinetics of bitertanole from reconstituted cuticular wax of barley leaves using an inert (PLS) and two "active" desorption media (micellar aqueous solutions of $C_{12}E_8$ and C_8E_4). The time courses of desorption were linearized by plotting the relative amounts of bitertanole desorbed versus the square root of time (three representative samples for each desorption medium shown).

The temperature program was as follows: injection at 50 °C, 2 min at 50 °C, 40 °C/min up to 200 °C, 2 min at 200 °C, 3 °C/min up to 300 °C. The inlet pressure of the hydrogen carrier gas was adjusted to 40 kPa. For derivatization, the samples were treated with 10 μL of *N*,*N*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA; Machery-Nagel, Düren, Germany) in 10 μL of dry pyridine (purity 99%; Merck) for 30 min at 70 °C. Silylation of the samples was necessary for reducing the interaction of the terminal hydroxyl groups with the column. For quantification, *n*-dotriacontane (purity 99%; Sigma, St. Louis, MO) was added to the samples as an internal standard. Correction factors for the quantitative analysis of monodisperse AE were determined by analyzing known amounts of each homologue together with the internal standard.

Determination of Diffusion Coefficients. Diffusion coefficients of the ai in barley leaf cuticular wax were determined as described previously in detail (Schreiber and Schönherr, 1993b; Schreiber and Riederer, 1996). In short, wax samples were doped with the ^{14}C-labeled compounds either by adding the compounds (tebuconazole, bitertanole) to the wax solution used for coating the aluminum disks ("internal loading", typical mass ratio compound/wax 1:2000) or by equilibrating coated aluminum disks with aqueous solutions of the compounds (salicylic acid, 2,4-D, metribuzin, triadimenole). In the case of salicylic acid (pK_a = 2.98; Albert and Serjeant, 1962) and 2,4-D (pK_a = 2.73; Rippen, 1992), citrate buffer (0.01 M) adjusted to pH 3 with KOH was used instead of water. It has been shown earlier that both methods of loading give identical results when used for lipophilic compounds (Schreiber, 1993b). On the other hand, using the method of internal loading with more polar compounds may exceed the solubility of the compound in the wax to an unknown degree and, consequently, result in an overestimation of its sorptive capacity.

The ai were desorbed from the wax samples into a desorption medium that was exchanged regularly. An aqueous phospholipid suspension (PLS) prepared from deionized water and soybean lecithin (10 g/kg; purity 98%; Roth, Karlsruhe, Germany) was used as an inert desorption medium for control experiments (Bauer and Schönherr, 1992). Sodium azide (1 mmol/kg) was added to prevent growth of microorganisms in PLS. Effects of AE on the diffusion coefficients of ai in the cuticular wax were obtained from experiments in which aqueous AE solutions with concentrations 5−10 times above the corresponding cmc were used instead of PLS. The dissolution of the desorbed molecules in either phospholipid vesicles or micelles of AE kept the external concentration of the compounds in the aqueous phase of the desorption media at practically zero. Linear graphs obtained by application of eq 2 (below) were taken as evidence that kinetics were not limited by the capacity of the desorption medium during the sampling intervals chosen.

The amounts of radioactivity in the desorption medium were determined by liquid scintillation counting (Tri Carb 2500; Canberra Packard, Frankfurt, Germany) after addition of equal volumes of scintillation cocktail (Ultima Gold XR; Canberra Packard). The radioactivity remaining in the wax films after termination of the experiment was measured after the wax was removed from the aluminum disks by immersion into scintillation cocktail.

The relative amounts desorbed were plotted versus the square root of time (Figure 2). Linear plots were obtained up to 50% desorption. The diffusion coefficients of the ai in barley leaf cuticular wax were calculated from (Felder and Huvard, 1980)

$$\frac{M_t}{M_0} = \frac{4}{\Delta x}\sqrt{\frac{D}{\pi t}} \quad (2)$$

where M_t (dpm) is the amount of radioactivity desorbed up to time t (s), M_0 (dpm) the total amount of radioactivity in the wax sample, M_t/M_0 the relative amount of radioactivity desorbed at time t, Δx (m) the thickness of the wax layer, and D (m^2/s) the diffusion coefficient. The thickness of the wax layer was estimated from the wax coverage determined gravimetrically by assuming a wax density of 0.9 g/cm^3 (Büscher, 1960). The aluminum disks used for desorption experiments carried amounts of wax from 150 to 250 μg each corresponding to wax films with a thickness of 1.7−2.8 μm.

The acceleration of the diffusion of ai by AE is quantitatively expressed by the ratio of the diffusion coefficients measured in the presence and absence of AE, respectively, according to

$$\text{effect} = D_{C_xE_y}/D_{PLS} \quad (3)$$

Statistics. Statistical tests were carried out using SPSS for Windows (version 5.02; SPSS Inc., Chicago, IL). The wax/water partition coefficients and the diffusion coefficients presented are based on 5−10 replications each. Arithmetic means and, where appropriate, the parameters of regression equations are given together with 95% confidence intervals.

RESULTS

Sorption of AE in Cuticular Wax. At aqueous concentrations of AE below their cmc, the equilibrium concentrations in cuticular wax from barley leaves (c_{wax}) were linearly proportional to the concentrations in the supernatant (c_w). When c_w exceeded the cmc, the concentration of AE sorbed in the wax was independent of the external concentration. Thus, the sorption isotherm of $C_{12}E_3$ from an aqueous micellar solution in barley leaf wax was characterized by an initial linear portion and a plateau at c_w well below and above the cmc, respectively (Figure 1).

The plateau values can be considered as the maximum concentrations of monodisperse AE in the wax (c_{wax}^{max}) attainable under given conditions (see Discussion). For the range of AE homologues included into the present study, c_{wax}^{max} varied from 6.5 mmol/kg for $C_{10}E_8$ and $C_{12}E_8$ to 55 mmol/kg for $C_{12}E_2$ (Table 3). Maximum concentrations in the wax systematically decreased with increasing numbers of ethylene oxide units (E) attached to the alkyl chain of the AE.

Wax/water partition coefficients ($K_{wax/w}$) estimated according to eq 1 ranged from 5.6 for $C_{10}E_8$ to 2000 for $C_{12}E_2$. They consistently increased with increasing alkyl chain length and decreased with the number of ethylene oxide units (Table 3).

Effects of AE on the Diffusion Coefficients of ai. The diffusion coefficients of the model ai in reconstituted barley leaf cuticular wax ranged from 1.9×10^{-18} (bitertanole) to 2.4×10^{-17} m^2/s (salicylic acid) when the inert desorption medium PLS was used as control. Diffusion coefficients of ai in the cuticular wax were size-dependent and decreased with increasing characteristic volumes (Table 4).

The kinetics of ai desorption from cuticular wax were drastically accelerated in the presence of AE (Figure 2). Diffusion coefficients of all ai studied were significantly higher when aqueous solutions of monodisperse AE (5−10-fold cmc) were used instead of the inert desorption medium PLS (Table 4). Both $C_{12}E_8$ and C_8E_4 caused the highest effects on the diffusion coefficient of bitertanole (factors of 20 and 83, respectively), whereas the two AE increased the diffusion coefficient of salicylic acid by factors of only 3.4 and 11.3, respectively.

With bitertanole, the effects of additional homologues of monodisperse AE were studied. The diffusion coefficient of this compound was enhanced by factors of between 9.8 ($C_{16}E_8$) and 130 ($C_{12}E_2$), leading to a maximum value of 2.4×10^{-16} m^2/s (Table 5). Among homologous AE with equal alkyl chain lengths but various degrees of ethoxylation, the effects on bitertanole diffusion in cuticular wax increased with decreasing numbers of ethylene oxide groups.

Table 3. Maximum Concentrations in Reconstituted Cuticular Wax of Barley Leaves (c_{wax}^{max}) and Wax/Water Partition Coefficients ($K_{wax/w}$) at 25 °C of Monodisperse AE (Means ± 95% ci)

compound	c_{wax}^{max} (mmol/kg)	c_{wax}^{max} (g/kg)	$K_{wax/w}$
a, C_4E_2	58[a]	9.3	0.09[b]
b, C_6E_3	42[a]	9.8	0.66[b]
c, C_8E_4	30[a]	9.3	4.7[b]
d, $C_{10}E_5$	22[a]	8.3	33[b]
e, $C_{10}E_8$	6.5 ± 0.9	3.3 ± 0.46	5.6 ± 0.7
f, $C_{12}E_2$	55 ± 6.7	15 ± 1.8	2000 ± 240
g, $C_{12}E_3$	46 ± 9.5	15 ± 3.0	1300 ± 270
h, $C_{12}E_4$	28 ± 8.9	10 ± 3.2	670 ± 210
i, $C_{12}E_5$	21 ± 3.0	8.6 ± 1.2	400 ± 57
j, $C_{12}E_6$	16 ± 2.7	7.0 ± 1.2	240 ± 42
k, $C_{12}E_7$	13 ± 1.8	6.2 ± 0.89	160 ± 22
l, $C_{12}E_8$	6.5 ± 1.1	3.5 ± 0.59	67 ± 11
m, $C_{14}E_7$	12[a]	6.0	1700[b]
n, $C_{14}E_8$	9.5 ± 3.0	5.4 ± 1.7	1200 ± 370
o, $C_{16}E_8$	8.3[a]	4.9	12000[b]

[a] Estimated according to eq 6. [b] Estimated according to eq 4.

Table 4. Diffusion Coefficients (D, at 25 °C) of Selected ai in Reconstituted Cuticular Wax from Barley Leaves and Effects of $C_{12}E_8$ and C_8E_4 (Means ± 95% ci)

		$C_{12}E_8$		C_8E_4	
compound	control $D \times 10^{16}$ (m^2/s)	$D \times 10^{16}$ (m^2/s)	effect	$D \times 10^{16}$ (m^2/s)	effect
salicylic acid	0.24 ± 0.055	0.82 ± 0.21	3.4	2.7 ± 0.79	11
2,4-D	0.19 ± 0.067	0.88 ± 0.21	4.3	2.2 ± 0.76	12
metribuzin	0.12 ± 0.038	0.63 ± 0.12	5.2	1.8 ± 0.23	15
triadimenole	0.036 ± 0.011	0.33 ± 0.098	9.2	1.8 ± 0.64	50
tebuconazole	0.058 ± 0.016	0.50 ± 0.083	8.7	2.0 ± 0.33	34
bitertanole	0.019 ± 0.0028	0.37 ± 0.074	20	1.6 ± 0.23	83

Table 5. Effects of the Monodisperse AE on the Diffusion Coefficient (D) of Bitertanole in Reconstituted Cuticular Wax from Barley Leaves (Means ± 95% ci)

desorption medium	$D \times 10^{16}$ (m^2/s)	effect	desorption medium	$D \times 10^{16}$ (m^2/s)	effect
control	0.019 ± 0.0028	1.0	$C_{12}E_4$	1.2 ± 0.24	64
C_4E_2	1.5 ± 0.32	80	$C_{12}E_5$	1.1 ± 0.11	57
C_6E_3	2.3 ± 0.35	120	$C_{12}E_6$	0.66 ± 0.16	35
C_8E_4	1.6 ± 0.23	83	$C_{12}E_7$	0.43 ± 0.10	23
$C_{10}E_5$	0.85 ± 0.19	45	$C_{12}E_8$	0.37 ± 0.074	20
$C_{10}E_8$	0.51 ± 0.15	27	$C_{14}E_7$	0.45 ± 0.14	24
$C_{12}E_2$	2.4 ± 0.24	130	$C_{14}E_8$	0.30 ± 0.064	16
$C_{12}E_3$	1.7 ± 0.28	88	$C_{16}E_8$	0.19 ± 0.046	9.8

DISCUSSION

When agrochemicals are applied to the foliage of crop or weed plants, the diffusion of ai across the cuticle is a decisive step during the chain of events leading to biological activity. To achieve the desired physiological effects, the kinetics of cuticular penetration have to be accelerated in many cases (Bukovac and Petracek, 1993; Kirkwood, 1993; Steurbaut, 1993).

AE are a class of adjuvants widely used in formulations of agrochemicals. Their effects on the rates of

foliar uptake of ai may differ according to the physical–chemical properties and the concentrations of adjuvants and ai and, in addition, may depend on the target plant (Holloway et al., 1992; Stock et al., 1992; Stock and Holloway, 1993). These results were obtained from experiments studying foliar uptake after application of small droplets of formulation. Even though this approach is close to the real situation, it provides only limited information on the mechanisms by which the rates of foliar uptake of ai may be activated by adjuvants. The factors affecting the different processes cannot be separated and controlled.

This experimental disadvantage can be circumvented by a reductionist approach using reconstituted cuticular waxes. They allow one to study the accelerating effects of AE or any other adjuvants on ai mobility in the cuticular wax barrier under closely controlled conditions. This information helps to assess the whole process of uptake because it has been clearly shown that cuticular wax is the main barrier against the transcuticular movement of organic solutes (Schönherr et al., 1991; Riederer and Schreiber, 1995).

The objectives of the present study were to achieve a quantitative analysis of the factors underlying the accelerating effects of monodisperse AE on the diffusion coefficients of ai in cuticular waxes and to derive tools for predicting AE action. Therefore, the results obtained in this work will be discussed primarily in terms of the dependence of accelerating effects on (1) the sorption of AE in cuticular wax and (2) the properties of the ai. In addition, the adjuvancy of AE will be interpreted in context with knowledge and hypotheses on the physical structure of plant cuticular waxes.

Partitioning of Monodisperse AE between an Aqueous Solution and Cuticular Wax. The isotherm for the partitioning of a monodisperse AE between reconstituted cuticular wax of barley leaves and an aqueous solution is linear at low concentrations in the aqueous phase and reaches a plateau at high concentrations. This behavior, demonstrated here for $C_{12}E_3$ as a model compound (Figure 1), is equivalent to sorption isotherms observed in the system cuticular polymer matrix (MX)/water (Riederer et al., 1995). The transition between the two branches of the isotherm occurs in the range of the cmc. Below the cmc, only monomeric AE molecules are present in the solution. Above the cmc, a more or less extensive fraction of the total of the AE molecules present aggregates into micelles. However, the activity of the free dissolved monomers remains constant (Rosen, 1989).

The latter is the driving force for the partitioning of AE molecules between a micellar solution and cuticular wax. This is demonstrated by the relatively sharp inflection of the sorption isotherm at the cmc (Figure 1). It follows from this argument that above the cmc the maximum equilibrium concentration of a given AE (c_{wax}^{max}) will be reached in cuticular wax. This interpretation of the sorption isotherm justifies the calculation of wax/water partition coefficients according to eq 1 from experimentally determined values of c_{wax}^{max} (Table 3) and values of cmc (Table 1). The cmc are considered the maximum concentrations of free monomers in solution.

Wax/Water Partition Coefficients of AE. Experimentally determined wax/water partition coefficients ($K_{wax/w}$) for a series of monodisperse AE varied from 5.6 ($C_{10}E_8$) to 2000 ($C_{12}E_2$; Table 3). This variation systematically depends on chemical structure and follows

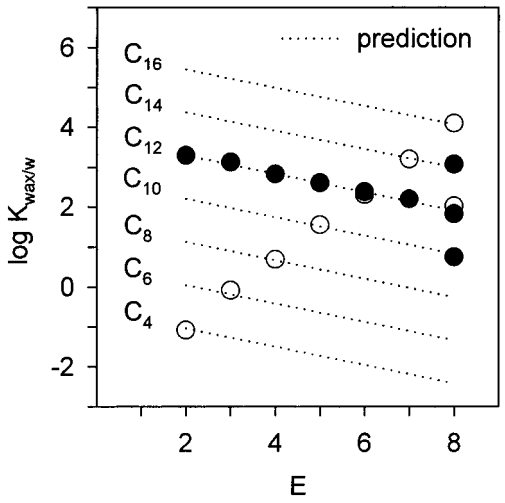

Figure 3. Dependence of the wax/water partition coefficients ($K_{wax/w}$) of monodisperse AE on the carbon number in the alkyl chain (C) and the number of ethoxy units (E). Open symbols (○) indicate data taken from Schreiber et al. (1996c).

the general rule that, in a series of homologues, an additive group contributes with a constant value to the logarithm of the partition coefficient (Leo et al., 1971). Consequently, the logarithm of wax/water partition coefficients of AE is linearly dependent on the alkyl chain length (C) and the degree of ethoxylation (E):

$$\log K_{wax/w} = -2.73\ (\pm 0.13) + 0.54\ (\pm 0.01) \times C - 0.23\ (\pm 0.02) \times E \quad (4)$$

$$n = 17;\ r^2 = 0.998$$

When eq 4 was calculated, additional values of $K_{wax/w}$ for barley leaf wax taken from the literature ($n = 8$; Schreiber et al., 1996c) were included. Equation 4 is a straightforward quantitative structure–property relationship based on two simple descriptors of chemical structure. It accurately predicts cuticular wax/water partition coefficients of monomeric AE in the range from C_4 to C_{16} and from E_2 to E_8 (Figure 3). Wax/water partition coefficients increase by ≈1 order of magnitude with the addition of two methylene groups to the alkyl chain and decrease by almost a factor of 10 when the number of ethoxy groups is increased by four units.

Wax/water and MX/water partition coefficients ($K_{MX/w}$; Table 1) are linearly related (Figure 4), resulting in the following linear free energy relationship:

$$\log K_{wax/w} = -1.06\ (\pm 0.18) + 1.00\ (\pm 0.05) \times \log K_{MX/w} \quad (5)$$

$$n = 17;\ r^2 = 0.991$$

The slope of this equation equals 1.0, indicating that, in both systems, the sensitivities to changes in lipophilicity of the solutes are the same (Leo et al., 1971). The intercept of eq 5 is not significantly different from −1.00, suggesting that $K_{wax/w}$ values of AE are, on the average, 1 order of magnitude lower than the corresponding values of $K_{MX/w}$. This reflects the reduced sorption capacity of cuticular wax, which can tentatively be attributed to its semicrystalline structure. Crystalline domains of the wax are considered to be not accessible to solutes (Riederer and Schreiber, 1995). The observation that sorption in cuticular waxes is reduced in

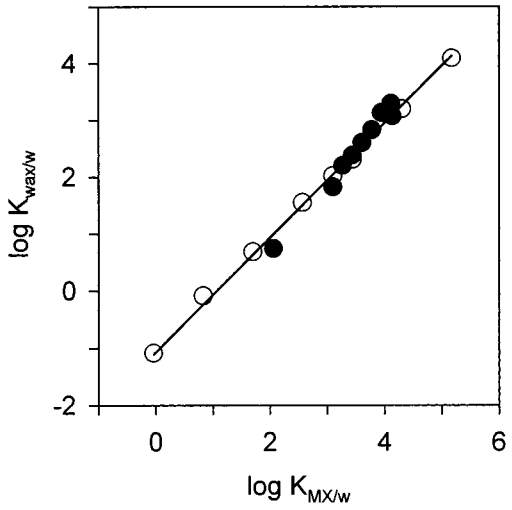

Figure 4. Correlation between wax/water ($K_{wax/w}$) and cuticular polymer matrix/water partition coefficients ($K_{MX/w}$) of monodisperse AE. Values for $K_{MX/w}$ were estimated according to a prediction equation reported by Riederer et al. (1995). Open symbols (○) indicate data taken from Schreiber et al. (1996c).

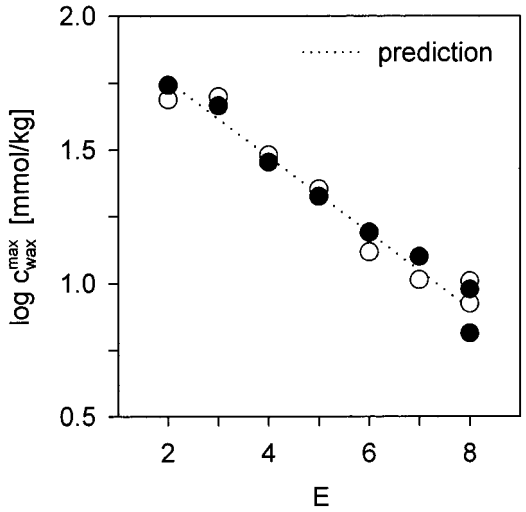

Figure 5. Dependence of maximum concentrations of monodisperse AE in reconstituted barley leaf wax (c_{max}^{max}) on the number of ethoxy units (E). Open symbols (○) indicate data taken from Schreiber et al. (1996c).

comparison to other parts of plant cuticles agrees with reports using additional compounds and plant species (Riederer and Schreiber, 1995; Kirsch et al., 1997).

Maximum Concentrations of AE in Cuticular Wax. The maximum concentrations in the wax (c_{wax}^{max}) of the monodisperse AE studied here (Table 3) varied from 6.5 ($C_{10}E_8$) to 55 ($C_{12}E_2$) mmol/kg. Including again data from the literature (Schreiber et al., 1996c), c_{max}^{max} (in mmol/kg) of monodisperse AE can be directly related to the degree of ethoxylation (E) by

$$\log c_{wax}^{max} = 2.04\ (\pm 0.09) - 0.14\ (\pm 0.01) \times E \quad (6)$$

$$n = 17;\ r^2 = 0.965$$

Maximum AE concentrations in the wax estimated from eq 6 are in good agreement with experimental values (Figure 5). The addition of seven ethoxy groups to an AE will reduce c_{wax}^{max} by almost 1 order of magnitude. Regression analysis showed that, in contrast to $K_{wax/w}$, c_{wax}^{max} was not significantly influenced by the alkyl chain length (C). This behavior can be explained by counteracting effects of alkyl chain lengths on $K_{wax/w}$ and cmc, respectively. In both cases the regression coefficient for C is 0.54 but with opposite signs (eq 5; Riederer et al., 1995). According to eq 1, c_{wax}^{max} is the product of $K_{wax/w}$ and cmc and, therefore, the contributions from alkyl chain length will cancel.

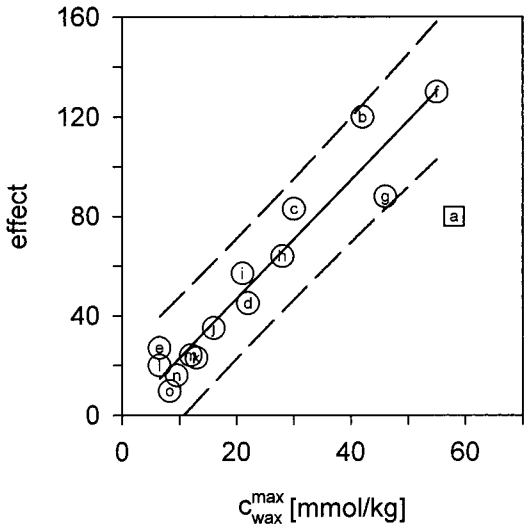

Figure 6. Dose−effect curve for effects of monodisperse AE on the diffusion coefficients of bitertanole in reconstituted cuticular wax of barley leaves. The effects are plotted versus the maximum surfactant concentrations in the wax (c_{wax}^{max}) calculated according to eq 6. Small letters refer to Table 3.

Activation of ai Mobility and AE Concentrations in Cuticular Wax. When ai were desorbed from reconstituted films of barley leaf cuticular wax in the presence of micellar solutions of monodisperse AE, the diffusion coefficients of the solutes in the wax significantly increased in comparison to control (Tables 4 and 5). These effects were caused by adjuvant molecules partitioning into the wax according to the external monomer activities and their respective $K_{wax/w}$ values (Table 3). In all experiments, the AE concentrations in the desorption medium exceeded the cmc by 5−10-fold. Thus, the AE concentration in the wax was equal to c_{wax}^{max} and, consequently, the effects on the mobility of ai observed can be considered as the maximum under the given experimental conditions.

The maximum effects of monodisperse AE on bitertanole diffusion in cuticular wax are directly related to AE structure by

$$\log \text{effect} = 2.46\ (\pm 0.17) - 0.15\ (\pm 0.03) \times E$$

$$n = 14;\ r^2 = 0.919 \quad (7)$$

The effect decreases with increasing number of ethoxy moieties in the AE molecule and is again independent of the alkyl chain length. This makes eq 7 similar to eq 6 and suggests that the maximum effect on the diffusion coefficient is only a function of c_{wax}^{max} of the AE in the wax.

Indeed, maximum effects of monodisperse AE on the diffusion coefficient of bitertanole linearly increased with c_{wax}^{max} (Figure 6). For a c_{wax}^{max} range from 8 to 58 mmol/kg (estimated according to eq 6), the following linear dose−effect relationship can be deduced:

$$\text{effect} = 2.12\ (\pm 0.14) c_{\text{wax}}^{\max} \qquad (8)$$

$$n = 14;\ r^2 = 0.935$$

Regression equations were also calculated using mass- and volume fraction-based concentrations instead of $c_{\text{wax}}^{\max}$ as independent variables, respectively. Linear relationships were obtained even though r^2 values were smaller. Equation 8 demonstrates that the activating effect of a monodisperse AE on the diffusion coefficient of an ai in cuticular wax linearly depends on AE concentration in the wax barrier. In the system micellar aqueous solution/wax the maximum concentration in the wax, in turn, is solely a function of the degree of ethoxylation, as shown by eq 6. This leads to the conclusion that all AE studied here have the same intrinsic accelerating activity on ai diffusion in cuticular wax. This finding agrees well with earlier reports that there are no specific differences in the activating properties of monodisperse AE (Riederer et al., 1995; Schreiber et al., 1996c). Equation 8 also provides evidence that alkyl and polyoxyethylene chain lengths are not descriptors for the still unknown intrinsic property of the AE molecules causing the activating effect. This observation is important as it suggests that the interfacial activity of AE is not essential for activating solute diffusion in the cuticular transport barrier. Both surface−activity and detergency depend on alkyl chain length and degree of ethoxylation (Schick, 1987).

The increase of the diffusion coefficients of solutes in the presence of these compounds is evidence for their plasticizing effect on cuticular wax. Generally, the addition of a plasticizer to an organic solid results in an increase in molecular or segmental mobility (Vroskesenskii and Orlova, 1964; Cadogan and Howick, 1992). It is clear that this should result in an increased rate of diffusion or penetration (Crank and Park, 1968; Vieth, 1991). The structural attributes of AE contributing to their intrinsic plasticizing properties are unknown.

Diffusion coefficients of molecules are proportional to the viscosity of the medium (Jost, 1960). This conclusion agrees with findings from recent differential scanning calorimetric (Coret and Chamel, 1994, 1995), electron spin resonance (Schreiber et al., 1996c), and nuclear magnetic resonance studies (Schreiber et al., 1997), which had provided conclusive evidence for an increased fluidity of cuticular waxes in the presence of AE.

There is, however, one compound used in the present study deviating from the general behavior described. The effect of C_4E_2 on bitertanole diffusion was significantly smaller than expected from the overall trend (Figure 6), suggesting that this compound has reduced plasticizing activity. Therefore, C_4E_2 was excluded when eqs 7 and 8 were calculated.

AE Effects and Size of ai. The diffusion coefficients of solutes in isolated cuticular membranes (Schönherr and Baur, 1994; Baur et al., 1996, 1997a) and reconstituted cuticular waxes (Schreiber, 1995; Schreiber et al., 1996a; Kirsch et al., 1997) exponentially decrease with molecular size:

$$D = D_0 \times e^{-\beta V_x} \qquad (9)$$

In eq 9, D is the diffusion coefficient measured for a molecule having the characteristic volume V_x, D_0 is the diffusion coefficient of a hypothetical compound having zero volume, and β is the size selectivity of the medium where diffusion takes place (Potts and Guy, 1992). The characteristic volume V_x estimated according to McGowan (Abraham and McGowan, 1987) is used as an easily accessible descriptor for molecular size. Plotting the natural logarithms of the diffusion coefficients versus the McGowan's characteristic volumes of six ai (Table 4) in the absence and presence of $C_{12}E_8$ and C_8E_4 gives three straight lines (Figure 7). The intercepts with the y-axis (D_0) are not affected significantly by the treatments (Table 6).

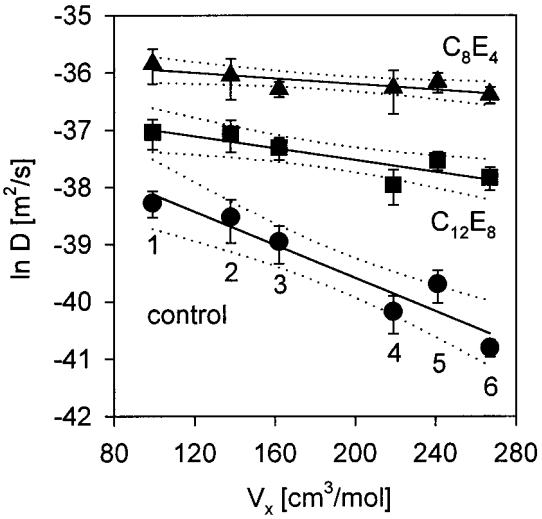

Figure 7. Plots of the diffusion coefficients of ai in cuticular wax (D) obtained from desorption experiments using an inert (PLS) and two active desorption media (micellar solutions of $C_{12}E_8$ and C_8E_4) versus McGowan's characteristic volumes (V_x) of the test compounds salicylic acid (1), 2,4-D (2), metribuzin (3), triadimenole (4), tebuconazole (5), and bitertanole (6).

Table 6. Size Selectivity (β), Mean Free Volume Available for Diffusion ($1/\beta$), Diffusion Coefficient of a Hypothetical Molecule Having Zero Molar Volume (D_0) (±95% ci), and Correlation Coefficients (r^2) of the Linear Regression Equations Obtained from Plots of ln D versus V_x[a]

desorption medium	β (mol/cm³)	$1/\beta$ (cm³/mol)	ln D_0 (m²/s)	$D_0 \times 10^{16}$ (m²/s)	r^2
control	0.015 ± 0.0064	69	−36.7 ± 1.3	1.2	0.909
$C_{12}E_8$	0.0052 ± 0.0040	190	−36.5 ± 0.79	1.4	0.761
C_8E_4	0.0025 ± 0.0024	410	−35.7 ± 0.47	3.1	0.671

[a] Diffusion coefficients of selected ai of different molecular size were measured in reconstituted cuticular wax of barley leaves in the absence and presence of the monodisperse AE $C_{12}E_8$ and C_8E_4.

Both coefficients of eq 9 are related to the physical structure of cuticular waxes. D_0 is considered as a measure for the tortuosity of the diffusive pathway across the wax barrier, which should primarily depend on the volume fraction and the aspect ratio of the crystalline domain of the wax. Tortuosity factors in the order of 200 and 2000 have been estimated for pear and *Citrus* leaf cuticular waxes, respectively (Schönherr and Baur, 1994; Baur et al., 1996). As the estimates of D_0 remain unchanged in the presence of $C_{12}E_8$ and C_8E_4, tortuosity (and crystallinity) of the wax seem to be unlikely to be affected by the plasticizing action of the AE. The slopes β of the graphs, however, systematically decrease from the control to the $C_{12}E_8$ and C_8E_4 treatments (Table 6). The differences between the effects of $C_{12}E_8$ and C_8E_4 are obviously due to different values of $c_{\text{wax}}^{\max}$ (eq 6).

The slope β of the regression line is interpreted as a measure for the size selectivity of the medium where

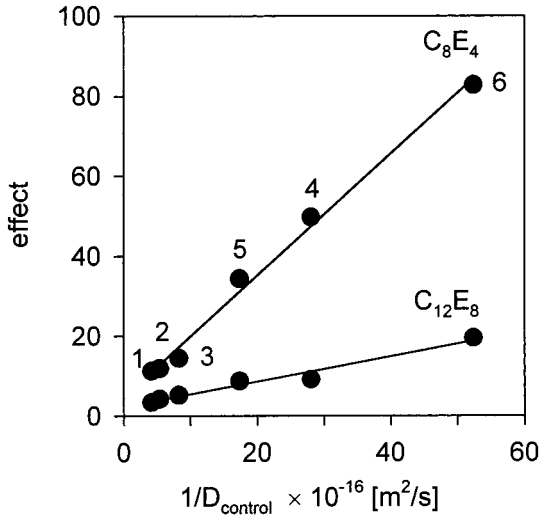

Figure 8. Effects of $C_{12}E_8$ and C_8E_4 on the diffusion coefficients of the ai salicylic acid (1), 2,4-D (2), metribuzin (3), triadimenole (4), tebuconazole (5), and bitertanole (6) in cuticular wax as a function of the reciprocal diffusion coefficient of the respective compound obtained with the inert control desorption medium PLS ($D_{control}$).

diffusion takes place and, therefore, reflects transport properties of the amorphous wax fraction. The experimental evidence presented demonstrates that size selectivity is reduced in the presence of $C_{12}E_8$ and C_8E_4. This finding agrees with evidence obtained in studies using isolated cuticular membranes instead of reconstituted cuticular wax (Baur, 1993; Schönherr and Baur, 1997; Baur et al., 1997a).

The reduction of size selectivity can be discussed from the point of view of the free volume theory of diffusion. The reciprocal of size selectivity ($1/\beta$) is a measure for the free volume available for diffusion (Schönherr and Baur, 1994). The theory of free volume states that diffusion takes place in voids formed by the thermal motion of the molecules making up the medium. The diffusion coefficient of a given solute depends on the probability that there is a free volume adjacent to it which is sufficiently large for accommodating it (Crank and Park, 1968; Vieth, 1991). At a given temperature, this probability increases with the fluidity of the medium. The acceleration of ai diffusion in cuticular wax that, in the previous section, has been ascribed to plasticization by AE can now be related to a quantitative measure of free volume in the amorphous wax fraction: without any AE present in the wax, the mean free volume available for diffusion in the amorphous wax phase is 69 cm^3/mol, while it increases to values 2.8 and 5.9 times higher when $C_{12}E_8$ and C_8E_4, respectively, are added (Table 6).

The data shown here may not only be interpreted in terms of changes in size selectivity of the wax induced by AE. They also indicate that the effects of $C_{12}E_8$ and C_8E_4 on the diffusion coefficients of ai in cuticular wax depend on the molecular size of the solutes. The maximum effects on diffusion caused by both AE are proportional ($r^2 = 0.969$ for $C_{12}E_8$; $r^2 = 0.993$ for C_8E_4) to the reciprocal of the diffusion coefficients measured under control conditions (Figure 8). Thus, the activation of the diffusion of large molecules (having low initial diffusion coefficients) is much more pronounced than that of small solute (having high initial diffusion coefficients), which agrees with similar results obtained with intact cuticular membranes (Schönherr and Baur, 1997). This conclusion helps one understand earlier results concerning AE effects on the mobility of 2,4-D in isolated cuticular membranes (Schönherr, 1993a,b) and on the permeability of isolated cuticular membranes to 4-nitrophenol (Schreiber et al., 1995). In all cases, low initial mobilities and permeabilities, respectively, were correlated with high effects of AE treatment.

Activation of Foliar Uptake by AE. The main objectives of this study were to analyze quantitatively the relationships between AE structure and their effects on the diffusion of ai in cuticular waxes and to further the physical understanding of the processes involved. The present work clarified some basic principles necessary for the understanding and prediction of the activating effects of AE. However, the limitations of the approach chosen must be addressed openly. In three major aspects the model system investigated deviated from the real situation. First, micellar aqueous solutions of adjuvants were used in lieu of the concentrated or even neat residues formed by spray droplets intercepted by leaf surfaces. Second, in the systems used AE were allowed to reach equilibrium partitioning between the wax and the adjacent solution, whereas, during foliar uptake, this will not happen for a number of reasons. Most important are time-dependent variations of residue composition and driving forces that will result in complicated time courses of AE concentrations in the cuticular wax (Baur et al., 1997a,b). Finally, monodisperse AE were studied instead of the technical polydisperse products.

Current research in the authors' laboratory aims at bridging the gap between the real situation during foliar uptake of ai and the experimental systems accessible to a stringent quantitative analysis of the processes and factors involved.

ABBREVIATIONS USED

2,4-D, (2,4-dichlorophenoxy)acetic acid; AE, alcohol ethoxylate(s); ai, active ingredient(s); ci, confidence interval; CM, cuticular membrane; cmc, critical micelle concentration; GC, gas chromatography; MX, polymer matrix membrane; PLS, phospholipid suspension.

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