Surfactant Effects on Cuticular Penetration of Neutral Polar Compounds: Dependence on Humidity and Temperature

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Effects of poly(ethylene glycol) (PEG 400) and polydisperse fatty alcohol ethoxylates ("Genapols") on methylglucose penetration across cuticles of *Pyrus communis* were studied under different humidities and temperatures. All surfactants increased methylglucose penetration better than PEG 400. The efficacy was proportional to their own penetration. Genapol C-100 (C12.5E8.4; C, average number of carbon atoms in the alkyl chain; E, average number of ethoxy groups) effects were strongly concentration dependent (0.2−5 g/L). At 2 g/L distinct differences between surfactants were observed. Effects of Genapol C-050 (C12.5E5.8) did not depend on humidity (11−93% RH) and temperature (15−35 °C). They were maximal after application but decreased rapidly due to surfactant penetration. Genapol C-200 (C12.5E17) effects increased strongly with humidity and temperature both of which prevented its solidification. Genapol C-100 was superior under most conditions and never failed to increase penetration. There was no simple relationship between ethylene oxide content and surfactant effect.

Keywords: *Adjuvants; foliar uptake; humectants; polar compounds; surfactants*

INTRODUCTION

The efficient use of many foliar applied crop protection agents requires rapid uptake of the active ingredient (a.i.) into the plant. Foliar uptake is a complex process with several possibilities for loss of active ingredient, e.g. due to unfavorable conditions of drift, retention, wash-off of a preformed deposit, or degradation of a.i. on the leaf surface, and the biological activity is often very low (Hartley and Graham-Bryce, 1982; Hall, 1993; Holloway, 1994).

Usually penetration of a.i. during evaporation of water and other solvents present is low and uptake proceeds mainly from the formulation residue. If a.i.'s are applied as simple solutions of aqueous acetone or other solvents, uptake into leaves of most species is often very low and not sufficient for the intended biological effect (Chamberlain et al., 1987; Stevens et al., 1988; Baker et al., 1992). It has been shown by many studies that adjuvants can increase rates of foliar penetration, and surfactants are probably the most intensively adjuvants studied (McWorther, 1982; Foy, 1992). Such compounds are often included in formulations since they have other beneficial functions such as those as emulsifiers or wetting agents (Cross and Scher, 1988; Foy, 1992). Nonionic surfactants have been found to increase uptake of a.i.'s differing widely in physicochemical properties. In many studies the influence of the degree of ethoxylation of nonionic surfactants and the lipophilicity of the a.i. for penetration were studied (e.g. Stevens and Bukovac, 1987; Knoche and Bukovac, 1991; Kirkwood et al., 1992; Schönherr, 1993; Holloway et al., 1992; Stock et al., 1993; Baur et al., 1997a). It was suggested that surfactants with a low degree of ethoxylation are better in increasing penetration of lipophilic a.i., while for polar compounds a high degree of ethoxylation is more beneficial (Stock et al., 1993; Stock and Holloway, 1993). Evidence for such a relationship was obtained for several compounds, but it was obvious that surfactant effects depend also on concentration and the period of time investigated or useful for practical purposes. In addition, there is scarce information on the dependence of surfactant effects on humidity and temperature. This is surprising since it is known that these compounds can act as humectants (e.g. Anderson and Girling, 1983) and some surfactants are solids at ambient temperatures. Humidity and temperature vary greatly, and microclimate effects over leaf surfaces often cause additional differences among species (Burrage, 1971).

It is clear that adjuvants can influence cuticular penetration mainly by two ways. They can act as nonvolatile (co)solvents on the surface, or they can affect transport properties of the cuticle by simultaneous penetration into the cuticle (e.g. Van Valkenburg, 1982; Stock and Holloway, 1993; Schönherr and Baur, 1994). Since cuticular penetration resembles passive transport by diffusion, it is advantageous to distinguish surfactant effects on driving force and mobility of the a.i. in the cuticle (Schönherr and Baur, 1994; Baur et al. 1997a, 1998).

In particular for polar compounds that have low solubility in the cuticle and often form solid residues on the surface, both properties will significantly affect rates of penetration. There are some reports showing that penetration of polar compounds can be enhanced by addition of humectants that keep the a.i. in a dissolved or at least not immobile form without simultaneous effects on transport properties (Babiker and Duncan, 1975; Cook et al., 1977; Matsumoto et al., 1992).

The aim of the present study was to examine the

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Table 1. Physicochemical Properties of Adjuvants Used for Penetration Studies

adjuvant	physical state at 25 °C	pour point (°C)	viscosity mPa s (50°)	range of EO[a] groups	weighted mean of EO groups	HLB[b]
Genapol C-050[c]	liquid	12	17.6	0−15	5.8	11.4
Genapol C-100	paste-like	26	30.4	0−18	8.4	13.2
Genapol C-200	solid	43	61.5	9−23	17	15.9
C12E8	paste-like	30−33[d]		8	8	13.1
PEG 400	liquid	1−5[c]	35.7	3−17	9	

[a] EO = ethylene oxide. [b] HLB (hydrophile-lipophile balance) was calculated as weight % ethylene oxide/5 (Myers, 1991). [c] All Genapol C surfactants have an alkyl lengths from 10 to 18 carbon atoms with an weighted mean of 12.5 (data from Hoechst, Germany). [d] Melting points according to data sheet of Fluka (Neu-Ulm, Germany).

potential of polydisperse ethoxylated fatty alcohol surfactants to increase cuticular penetration of the hydrophilic model compound methylglucose under different humidities and temperatures. The test adjuvants include polydisperse fatty alcohol ethoxylates, monodisperse octaethylenglycol monododecyl ether, and a polydisperse poly(ethylene glycol). These are adjuvants which can (surfactants) or cannot penetrate the cuticle. Cuticles of pear were used since preliminary experiments showed a (relatively) high permeability to lipophilic and hydrophilic organic compounds under proper conditions and the negligible penetration of methylglucose when applied alone. This enables one to identify differences in penetration under defined conditions and what conditions ensure rapid penetration. Experiments lasted usually 1 day or less which is a relevant time scale for rapid foliar uptake of polar compounds under practical conditions.

MATERIALS AND METHODS

Plant Material. For the penetration studies adaxial leaf cuticles from mature leaves of pear (*Pyrus communis* L.) trees growing in an orchard of the institute in Lower Saxony were used. Two different cultivars of pear trees were used, cv. Conference (1995) and cv. Bartlett (1996), and in both cases leaves were harvested in the first half of July. No differences in the results were found between these cultivars, and no distinction is made in the presentation of the results below. Preliminary experiments were carried out also with pear cuticles cv. Bartlett isolated in June 1992 in an orchard in Bavaria. Further studies were carried out with cuticles from mature leaves of *Malus domestica* Borkh. trees growing in the same orchard as pear and from glasshouse plants of *Citrus grandis* L., *Ilex paraguariensis* St.-Hil., and *Stephanotis floribunda* Brongn. The enzymatic isolation of cuticular membranes (CM) is described in Schönherr and Riederer (1986). Due to changes of manufacturer supply a different technical product of pectinase from Nordisk, Denmark was used. According to manufacturer information this enzyme had an higher purity and activity, and the concentration was only 2% (w/v).

Chemicals. Radiochemicals were ^{14}C-labeled compounds of methylglucose (3-*O*-methyl-α-D-glucose) from Sigma, Deisenhofen, Germany (spec. activity 318 MBq mmol^{-1}) and octaethyleneglycol-dodecyl ether (C12E8) from CEA, Grenoble, France (2090 MBq mmol^{-1}). About 50000 dpm/5 μL of both methylglucose or C12E8 were applied per cuticle (see below) which corresponds to amounts of 0.5 and 0.2 μg, respectively. The fatty alcohol ethoxylates of the Genapol series (Hoechst, Frankfurt, Germany) were polydisperse and of technical grade (Table 1). The radiolabeled compounds were mixed with the surfactants, nonlabeled C12E8 or poly(ethylene glycol) 400 (both from Fluka, Neu-Ulm, Germany) (Table 1) in aqueous systems with deionized water throughout. Adjuvant concentrations varied between 0.2 and 5 g/L which corresponds to absolute amounts between 1 and 25 μg per droplet.

Penetration Experiments. Penetration of methylglucose (and C12E8) was studied after applying the radiolabeled substances with adjuvants to the outer surface of cuticles and measuring their appearance in a receiver medium in contact with the inner side. Details of the method can be found elsewhere (Schönherr and Baur, 1996; Baur and Schönherr, 1997; Baur et al., 1997). In the experiments the cuticles were mounted on a steel chamber with a lid. The test solutions were applied as 5 μL droplets to the morphological outer surface of the cuticle. Penetration was usually followed after the droplet water has evaporated (start) or (in preliminary experiments) immediately after application of droplets. In the latter case the chambers stood upright in a thermostated aluminum block with the chamber interior completely filled with the receiver medium which was usually water if not stated otherwise. At predetermined time intervals the receiver was withdrawn and replaced by a fresh one. After the last sample the CM were cut out, and the residual radioactivity associated with the CM was extracted using scintillation cocktail (Aquasafe 500, Zinsser, Frankfurt, Germany). Radioactivity in the desorption media and in the CM were assayed using a liquid scintillation counter (Packard CA 2000 counter, Downers Grove, IL). The total amount penetrated (M_t) at each time was calculated, and the amount applied (M_o) was calculated from the sum of the amount penetrated and the residual radioactivity in the CM. Data were analyzed by plotting the logarithm of the unpenetrated fraction, $-\ln(1 - M_t/M_o)$, versus time, t. Under constant conditions, with a negligible concentration of the penetrated compound in the receiver, penetration follows first-order kinetics as indicated by a straight lines in this plot. The slope of the line gives the rate constant (k) of penetration. Deviation from linearity is often observed in the presence of surfactants and reflects the influence of surfactants on (methylglucose) penetration.

Control of Humidity. The humidity above the cuticle was controlled by using saturated salt solutions with excess salt crystals that cause constant relative humidities in the surrounding air in a closed system. A closed system was established by sealing the lid over the outer side of the cuticles with an adhesive tape (Baur and Schönherr 1997). Three relative humidity levels were tried with LiCl (11% relative humidity, RH), $Ca(NO_3)_2$, (56% RH), and KNO_3 (93% RH) (see Apelblat, 1992; Greenspan, 1977). The saturated salt solution was introduced by sticking the salt crystals to the adhesive tape (Tesafilm, Beiersdorf, Hamburg) before sealing the lid. The (dry) salt crystals were spread to layer, and a Teflon stencil with a circle area of 1 cm^2 was overlayed. During the experiment the solution volume did not exceed about 5−10 μL (estimate), and crystals were visible until the end of the experiment. In longer experiments the adhesive tape with salt was exchanged daily. During the experiments the chambers stood in a thermostated aluminum block with an insulating cover. Penetration was usually studied at constant temperature within 1 day. In one experiment temperature was changed, and it was constant at least after the first sample at the new temperature was drawn. Temperature was monitored by measuring temperature directly in the chambers and was within ±0.5−1.0 °C of the desired value. The relative humidity above saturated salt solutions is constant in the studied temperature range between 15 and 35 °C (Apfelblat, 1992).

Statistics. Due to the high variability of permeabilities between individual cuticles (Baur, 1997) sample sizes between 10 and 20 CM were used. From data of single cuticles mean

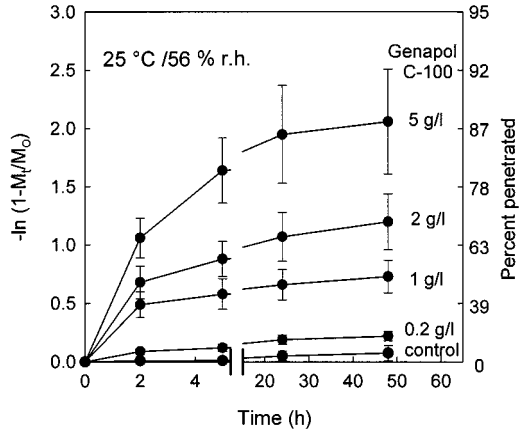

Figure 1. Concentration dependence of the effect of Genapol C-100 on the penetration of methylglucose across leaf cuticular membranes (CM) of *Pyrus communis* (means of 15−20 CM with 95% confidence intervals, CI).

values of $\ln(1 - M_t/M_o)$ with 95% confidence intervals were calculated and are given in all figures.

RESULTS

In preliminary experiments with pear cuticles penetration was measured immediately after droplet application. It was found that penetration of methylglucose during evaporation of water was negligible. In the experiments reported below penetration was measured after water has evaporated, i.e., about 0.5−1 h after application. Evaporation refers to bulk water only since depending on surfactant and humidity the residue contained water.

Concentration Dependence of the Genapol C-100 Effect. The effect of concentration was studied with Genapol C-100 at an intermediate relative humidity of 56%. Methylglucose penetration increased with Genapol C-100 concentration over the whole range of 0.2−5 g/L studied (Figure 1). At all concentrations the rate constants of penetration (slopes in Figure 1) of methylglucose were highest immediately after evaporation of droplet water but ceased early after 2 h. After 5 h slopes became almost constant for 3 days (only 2 days shown in Figure 1) and did not differ significantly between concentrations. After 2 h only 9% methylglucose had penetrated at 0.2 g/L (1 μg) Genapol C-100, while at 5 g/L already 65% had penetrated. In the control experiment without surfactant total methylglucose penetration after 48 h was below 5% of the amount applied.

Surfactant Effects on Penetration As Affected by Humidity. In further experiments a concentration of 2 g/L was used with all surfactants. The surfactant effects were studied at extreme humidity levels of 11 and 93% RH. If methylglucose was applied in aqueous solution without any adjuvant added, penetration was below 2% after 24 h at 11% RH and about 11% at 93% RH (Figure 2A). PEG 400 was used as a control for solvent effects only as it has no effects on transport properties of the cuticle due to negligible penetration (Hartley and Graham-Bryce, 1980; Baur et al., 1997). Penetration was significantly increased by PEG 400 and quite similar under such different humidities as 11 and 93%. One day after application 30−40% methylglucose has penetrated, and rate constants of penetration did not (93% RH) or slightly (11% RH) decrease during the experiment.

Penetration of methylglucose in the presence of Genapol C-050 showed a different pattern (Figure 2B). Rate constants of penetration were highest during the first sampling interval and decreased thereafter to values even below those obtained with PEG 400. Humidity had no effect at all. In contrast humidity was of considerable significance for the effect of Genapol C-200 to increase penetration of methylglucose (Figure 2D). At low humidity rate constants of methylglucose penetration with this surfactant were low throughout and compared to those with PEG 400 more than 3-fold lower. However, at 93% RH rate constants were higher than with PEG 400 and more than 50% of methylglucose had penetrated after 24 h. Compared to Genapol C-050 there is no rapid effect immediately after application, but rate constants of penetration are about 5-fold higher after 5 h. As might be expected from these results the effect of Genapol C-100 is intermediate (Figure 2C). At 11% RH the time course of methylglucose penetration showed the same pattern as obtained with Genapol C-050 although rate constants were slightly higher up 5 h. In contrast at 93% RH rate constants were lower at the beginning and decreased only slightly, similar to the situation with Genapol C-200 at this humidity. At 93% RH and this surfactant concentration penetration of methylglucose occurred at maximum rates. Results obtained with a further surfactant, Genapol C-080 (C12.5E8.1), which is similar to Genapol C-100 (C12.5E8.4) were identical at both humidities (data not shown).

Comparison of Surfactant Efficacy at Intermediate Humidity. The results shown in Figure 2 relate to rather extreme humidities. Therefore the surfactants were tested also at an intermediate relative humidity of 56% at 2 g/L (Figure 3). In absence of surfactants methylglucose penetration at 56% was below 6% after 1 day. Methylglucose penetration in the presence of Genapol C-050 at 56% RH was identical to the other humidities in both time course and the amount penetrated. Genapol C-200 showed the same effect as at 93% RH, the rate constants from 5 to 24 h being somewhat lower than at 93%. Genapol C-100 showed the largest effect on methylglucose penetration, and this is clearly caused by the extremely high rates of methylglucose penetration at the beginning. Such after 2 h more than 50% had penetrated while less than 10% methylglucose penetrated at 5−24 h.

Temperature Dependence of Surfactant Effects. The dependence of surfactant effects on temperature at 56% RH was studied with Genapol C-100 and C-200. Temperature had a great influence on the differential effects of these surfactants on methylglucose penetration (Figure 4). With Genapol C-100 penetration of methylglucose was identical at 25 and 35 °C. At 15 °C rate constants of penetration were lower up to 5 h but about 5-fold higher from 5 to 24 h compared to the higher temperatures. Even at 15 °C Genapol C-100 increased methylglucose penetration within 24 h more than the other surfactants (Figures 2 and 3). In contrast to Genapol C-100 the efficacy of Genapol C-200 depended strongly on temperature (Figure 4B). At 15 °C (and 56% RH) rate constants of methylglucose penetration were very low and almost equal to those observed at 11% RH and 25 °C. An increase in temperature to 25 and 35 °C proportionally increased rate constants, and, most strikingly, at 35 °C penetration of methylglucose in the presence of Genapol C-200 was as rapid

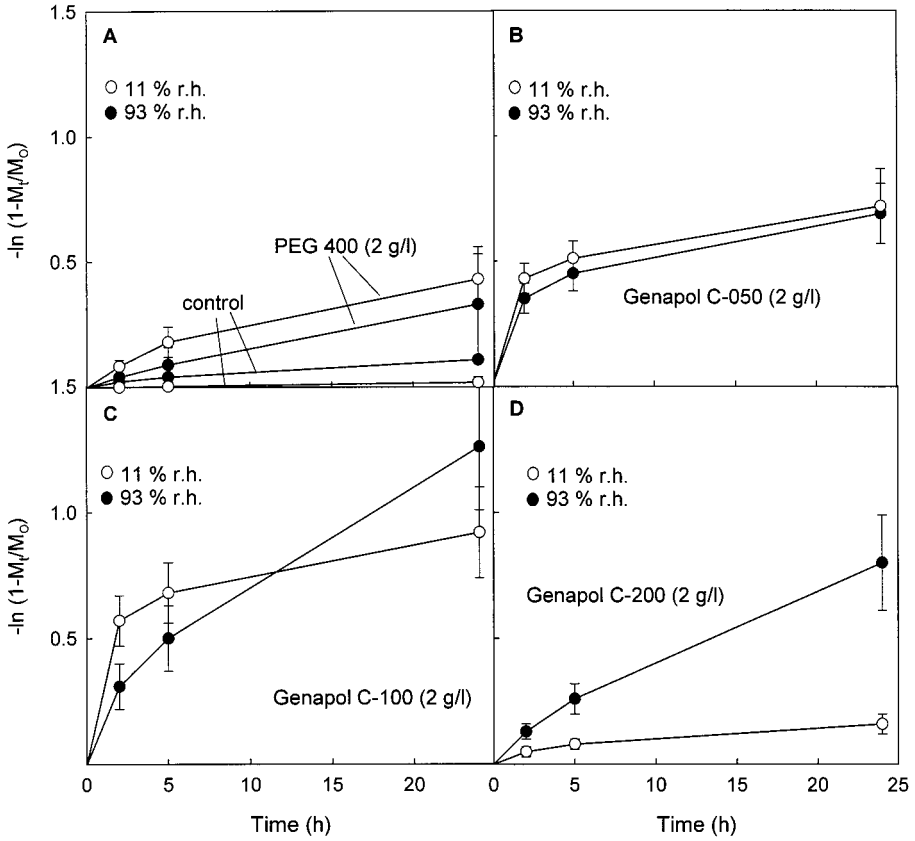

Figure 2. Effect of PEG 400 (A) and Genapol C surfactants (B−D) on the penetration of methylglucose across leaf CM of *Pyrus communis* at relative humidities of 11 and 93%. In the control treatment (A) methylglucose was applied in deionized water (means of 13−20 CM with 95% CI).

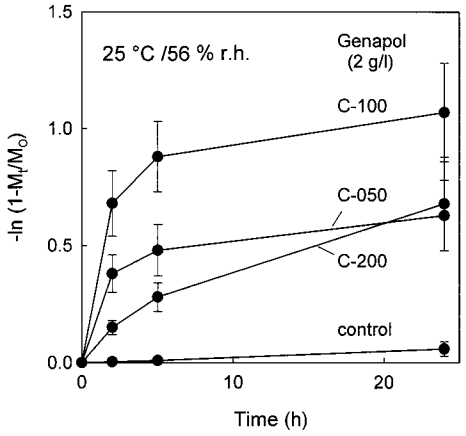

Figure 3. Effect of Genapol C surfactants on the penetration of methylglucose across leaf CM of *Pyrus communis* at a relative humidity of 56% (means of 17−20 CM with 95% CI).

as with Genapol C-100. The effect of temperature was maximum at 0−2 h where rate constants increased 24-fold from 15 to 35 °C.

Short time experiments showed that the effect of temperature caused an immediate response with a delay of only 0.5−1 h (Figure 5). However, there was no effect of temperature on methylglucose penetration in the control or in the presence of Genapol C-050. In presence of PEG 400 increasing the temperature in 10 °C steps increased rate constants of penetration greatly, but the absolute rates of penetration were still very low. Temperature influenced particularly methylglucose penetration in the presence of Genapol C-100 and C-200. The effect was very similar between the surfactants and was in agreement with the long-term experiment (Figure 4).

Methylglucose and ^{14}C-C12E8 Penetration in the Presence of C12E8. Penetration of methylglucose was also increased by C12E8 (Figure 6) but at comparable surfactant concentrations the effect of Genapol C-100 was significantly greater (see Figure 1). Figure 6 shows the time course of penetration of radiolabeled C12E8 (0.04 g/L). The presence of an excess amount of methylglucose (5 g/L) had no effect on ^{14}C-C12E8 penetration. After 2 h mean penetration was more than 60% ^{14}C-C12E8, and 1 day after application about 80% had penetrated. Penetration of ^{14}C-C12E8 applied alone was very rapid and was even greater when additional C12E8 was added (Baur and Schönherr, 1997). Figure 7A shows that the rate constants of penetration of methylglucose and ^{14}C-C12E8 change similarly over time at 2 and 8 g/L C12E8 (calculated from slopes of Figure 6 and from data of Baur and Schönherr, 1997). Rate constants of penetration of methylglucose depend in a linear fashion on the amount of C12E8 not penetrated (Figure 7B). At 15 °C rate constants of penetration of ^{14}C-C12E8 (no addition of nonlabeled C12E8) were approximately half of the values at 25 °C and after 1 day only 60% had penetrated.

Surfactant Effects on Methylglucose Penetration across CM of Other Species. The most efficient surfactant Genapol C-100 (at 5 g/L) was also tested with CM of *Citrus grandis*, *Ilex paraguariensis*, *Malus domestica*, and *Stephanotis floribunda*. Leaf cuticles of these are better transport barriers than that *Pyrus communis* cuticles. In the presence of Genapol C-100 at least 55% (*Citrus grandis*) of the applied amount of methylglucose had penetrated after 5 h and at least 87% (*Stephanotis floribunda*) after 1 day at 25 °C and 56% relative humidity (Figure 8). While rate constants of

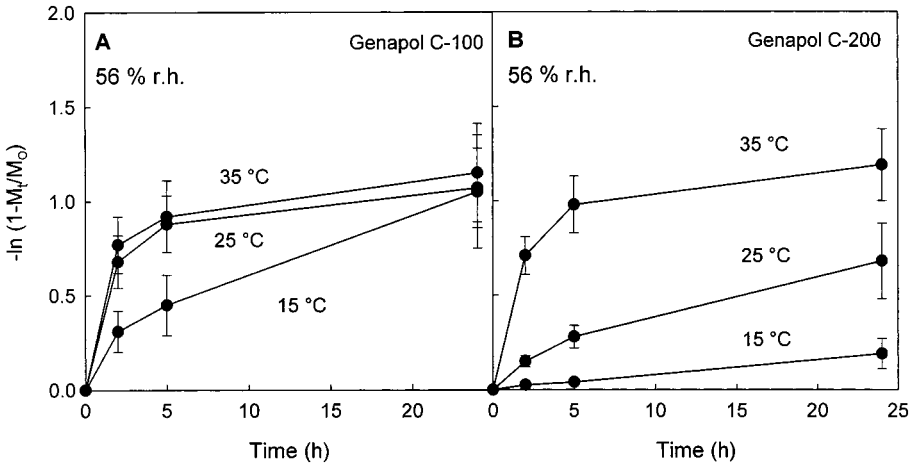

Figure 4. Temperature dependence of the effects of Genapol C-100 and C-200 on the penetration of methylglucose across leaf CM of *Pyrus communis* at 56% RH (means of 15−20 CM with 95% CI).

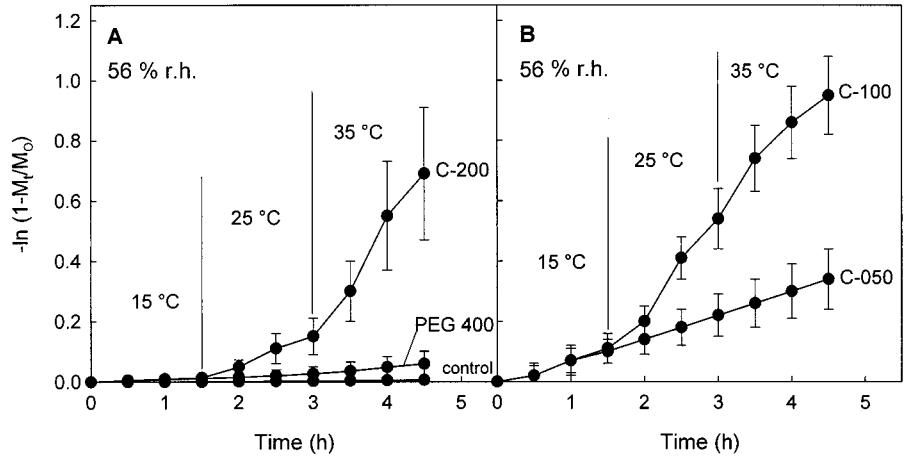

Figure 5. Effect of increasing temperatures at a constant relative humidity of 56% on penetration of methylglucose across leaf CM of *Pyrus communis* in absence (control) or presence of Genapol C surfactants and PEG 400 (means of 9−10 CM with 95% CI).

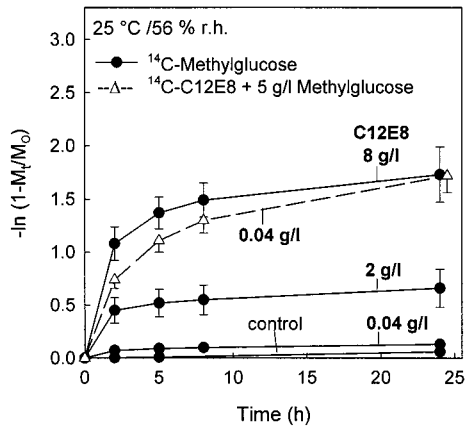

Figure 6. Penetration of methylglucose across leaf CM of *Pyrus communis* at various concentrations of C12E8. The dashed line shows the penetration of radiolabeled C12E8 (0.04 g/L) in the presence of an excess amount of methylglucose (means of 14−15 CM with 95% CI).

penetration were highest at the beginning with *Malus domestica*, they became maximum after 2 h with *Ilex paraguariensis* and *Stephanotis floribunda*, or they were lower but constant with *Citrus grandis*.

Interaction of Surfactant with $CaCl_2$. It was recently shown that $CaCl_2$ and other compounds can decrease rates of penetration of fatty alcohol ethoxylates (Baur and Schönherr, 1997). The addition of $CaCl_2$ at a concentration of 1 g/L greatly reduced the effect of Genapol C-100 (2 g/L) on methylglucose penetration at 25 °C and 56% RH (Figure 9). In contrast to the usual time course of methylglucose penetration in the presence of Genapol C-100 rate constants of penetration were constant under these conditions. After 1 day about 15% had penetrated which is still more than in the control where methylglucose was applied in water without surfactant (<2%, Figure 1).

DISCUSSION

After evaporation of volatile constituents the physicochemical properties of the formulation residue control rates of penetration by affecting the driving force across and the mobility of the a.i. in the cuticle (Schönherr and Baur, 1996). If the concentration of a.i. in the aqueous phase of the epidermal cell wall is negligible, the flux J (mol m^{-2} s^{-1}) is given by

$$J = k^* \, l_{ls} \, (K_{Cfr} \cdot C_{fr}) \qquad (1)$$

where k^* (s^{-1}) is the solute mobility, l_{ls} is the (constant) thickness of the limiting skin in the cuticle, K_{Cfr} is the partition coefficient of a.i. between cuticle and residue, and C_{fr} is the concentration in the residue. Since C_{fr} decreases with time the flux decreases as well even if the other variables are constant. If only C_{fr} changes with time a straight line would be obtained in plots such as

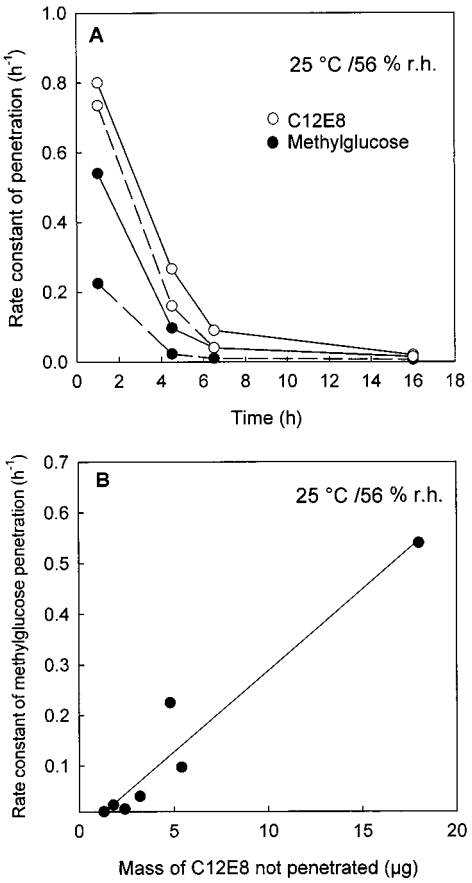

Figure 7. (A) Rate constants of penetration of methylglucose and C12E8 at concentrations of C12E8 of 2 g/L (dashed lines) and 8 g/L (solid lines). (B) Rate constants of penetration of methylglucose as dependent on C12E8 concentration. (calculated from Figure 6 and from data of Baur and Schönherr, 1997).

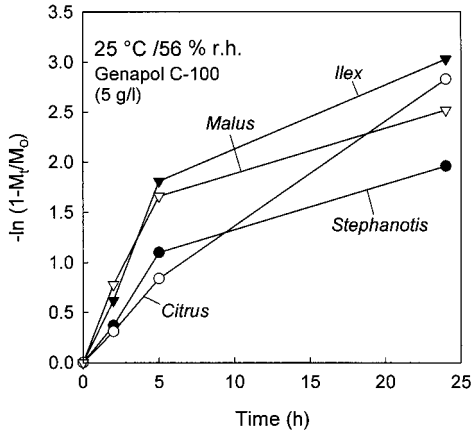

Figure 8. Effect of Genapol C-100 (5 g/L) on the penetration of methylglucose across leaf CM of *Citrus grandis*, *Ilex paraguariensis*, *Malus domestica*, and *Stephanotis floribunda* (means of 12−15 CM, CI omitted for clarity).

that of Figure 1. The slope of the curves in such plots gives the rate constant of penetration (k) which is proportional to k^* and K_{Cfr}.

The selected adjuvants act to a different extent and in a time dependent manner on mobility and driving force across. Adjuvants that do not penetrate into the cuticle can influence a.i. penetration only by effects on driving force. Genapol C surfactants can penetrate into the cuticles, and it is not possible to separate effects on mobility and driving force in penetration experiments.

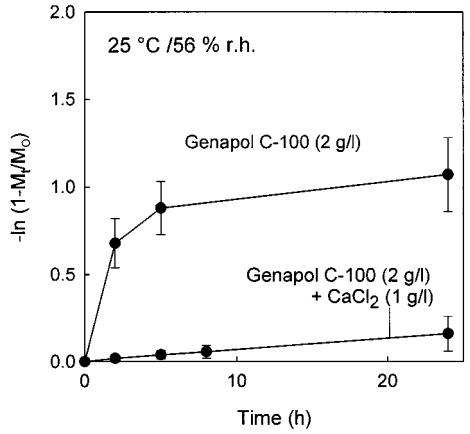

Figure 9. Antagonistic effect of $CaCl_2$ on the penetration of methylglucose across leaf CM of *Pyrus communis* in the presence of Genapol C-100 surfactants (means of 15−20 CM with 95% CI).

Investigating the differential performance and mode of action of commercial polydisperse surfactants such as the Genapol C series is often difficult due to a large degree of similarity of the products. For example Genapol C-100 has more than 70% homologues in common with Genapol C-050 and about 30% homologues in common with Genapol C-200 (Baur et al., 1997a). Even Genapol C-050 and C-200 have 14% common homologues. However, both physicochemical properties (Table 1) and the potential of these surfactants to increase rates of methylglucose penetration differ significantly.

In absence of adjuvants methylglucose penetration was negligible although a slight effect of humidity was detectable (Figure 2A). In this case solidification of methylglucose resulted in a near zero driving force. In contrast, at 100% RH (closed with adhesive tape) rate constants of methylglucose penetration across *Pyrus* CM were more than 10-fold higher and almost 90% has penetrated after 1 day (data not shown). A similar result was obtained if the CM were covered with a steel cover (Schönherr and Baur, 1996). Under these conditions the driving force is very high since penetration occurs from a saturated solution. The saturation concentration corresponds to the water solubility of methylglucose which is ca. 900 g/L (Stock et al., 1993). The partition coefficient cuticle/water (K_{CW}) is 0.13 (Baur et al., 1997b). The product of K_{CW} and saturation concentration gives the driving force and has a value of 117 g/L which is similar to the driving force of more lipophilic compounds such as cyanazine (10.7 g/L) and tebuconazole (75 g/L), and similarly high rates of penetration are obtained at 100% RH with such compounds (Schönherr and Baur, 1996). This shows that the driving force can be high under these conditions, and the rates of penetration depend then only on the fraction of penetrant that is actually dissolved.

If PEG 400 was added at 2 g/L, rate constants of penetration were significantly increased at both humidities. There was no significant difference between the two humidities although during the first hours penetration at 11% RH was somewhat faster (Figure 2). PEG 400 does not affect cuticular transport properties (Baur et al., 1997a) due to its poor penetration, and its beneficial effect on methylglucose penetration was only via driving force, i.e., it serves as a solvent for otherwise solid methylglucose at relative humidities below 100%. Increasing the amount of PEG 400 will decrease rate constants due to dilution and the con-

comitant decrease of the driving force. At 11% RH PEG 400 is almost water free, while it contains almost 80% water at 93% RH (Baur et al., 1997b). The partition coefficients cuticle/water and cuticle/PEG 400 are very similar, 0.13 and 0.4, respectively, and methylglucose (at 0.1 g/L) is probably easily dissolved in PEG 400 (2 g/L) under both humidities. Therefore the driving force is only slightly lower at 93% RH due to lower partition coefficients and dilution of methylglucose due to water sorption. That methylglucose penetration is still not rapid indicates that there is no polar route through the cuticle continuous to the surface. Such a route should result in rapid penetration since the film of PEG 400 on the surface produces a liquid bridge between the residue and the cuticle.

In contrast to PEG 400 Genapol C surfactants in particular with a low degree of ethoxylation have the potential to penetrate the cuticle (Anderson and Girling, 1983; Stock et al., 1992; Baur and Schönherr, 1997). This will cause time dependent effects on both mobility and driving force, and the results indicate that distinct differences exist among the selected surfactants. If Genapol C-050 is sorbed into the CM from an aqueous solution under equilibrium conditions, it increases the mobility in the cuticle and this effect depends on the molar volume of the diffusing molecule (Baur et al., 1997a). Methylglucose has a molar volume of 134 cm^3/mol, and for *Pyrus* CM an approximately 5.5-fold increase in mobility is expected. This estimate is based on experiments where Genapol C-050 was sorbed under equilibrium conditions, and it depends on the volume fraction sorbed (Riederer et al., 1995). In the present penetration experiments similar or even higher amounts were sorbed and the 5.5-fold increase of mobility is a conservative estimate (Baur and Schönherr, 1997). Increased mobility and a high driving force for methylglucose due to penetration of surfactant resulted in the observed rapid initial rate constants of penetration. Since most of the surfactant had penetrated after 5 h, methylglucose was left behind on the surface, and this explains why rate constants were even lower than with PEG 400 thereafter. However, some constituents have a degree of ethoxylation up to 15, and a small fraction of Genapol C-050 was probably still present and caused greater rate constants of methylglucose penetration than in the control.

Genapol C-200 affected methylglucose penetration completely differently. At 11% RH it had almost no effect, while at 93% it was even better than Genapol C-050. The lack of any effect of this surfactant at low humidity is probably related to the physical state of this surfactant. With a pour point of 43 °C (Table 1) this surfactant forms a water free solid residue on the surface of the cuticle at low relative humidities (11% RH), and the driving force for both methylglucose and surfactant is very low. This residue was visible as a whitish deposit at low humidity. The effect of Genapol C-200 was also highly temperature dependent (at 56% RH), and at 15 °C methylglucose penetration was very similar to the conditions 25 °C/11% RH (Figures 2D and 4B). A relative humidity of 56% is probably sufficient to avoid a rigid solid residue of Genapol C-200 since the time course of methylglucose penetration was almost equal to that at 93% RH (Figures 2D and 3). Effects on temperature on the partition coefficient of methylglucose can be neglected since they are almost constant between 15 and 35 °C (Marzouk et al., 1998). In contrast solute mobility in the cuticle depends strongly on temperature due to increased mobility of segmental chains in cutin and waxes (Baur et al., 1998). Therefore mobilities of methylglucose and surfactants increase with temperature, and even with PEG 400 rate constants of methylglucose penetration increased relatively (Figure 5).

At 93% RH rate constants of methylglucose penetration in the presence of Genapol C-200 at 2 g/L were about 3-fold higher than in the presence of the same amount PEG 400 (Figure 2A,C). Since the driving force is expected to be very similar to that with PEG 400 this indicates that a fraction of the surfactant penetrates into the cuticle. Evidence for the potential of penetration and concomitant increase of mobility is given by a comparison of Genapol C-100 and C-200 at 35 °C and 56% RH (Figure 4). Under these conditions both surfactants influence methylglucose penetration in the same fashion suggesting that they penetrate at the same velocity. It was observed in previous work that foliar uptake of ethoxylated aliphatic alcohols into field been and wild oat (Stock et al., 1992) and of ethoxylated octylphenoxy (Triton X series) surfactants into maize (Stevens and Bukovac, 1987) is poor if the number of ethylene oxide (EO) groups is high (>16 EO). These studies were carried out at about 20 °C. The results with Genapol C-200 show that temperature can drastically affect uptake of such compounds.

The partition coefficient for methylglucose in the system cuticle/pure Genapol C-050 (pure ≈ 11% RH) is about 3.6 (Baur et al., 1998) which is more than 20-fold higher than the K_{CW}. The exact difference in the partition coefficient cuticle/Genapol C-100 at 11 and 93% RH is unknown, but it is expected from these values that it is lower at the higher relative humidity due to a much higher water content. It is known that ethoxylated fatty alcohols can sorb high amounts of water at high (>80% RH) humidities (Anderson and Girling, 1983). We have recently determined the dependence of the mass fraction (m/m) of water sorbed in PEG 400 on the relative humidity in the ambient air (Baur et al., 1997b). At 11% RH less than 1% (m/m) water was sorbed, while at 93% RH almost 75% (m/m) was sorbed. Since water sorption decreases the concentration of methylglucose and increases the solubility in the residue the driving force is lower.

Genapol C-100 and C-200 have about 28% constituents in common, and it is suggested that the presence of higher ethoxylates (>8 EO groups) account for the higher rate constants after 5 h. This means that only the lower ethoxylates (<8 EO groups) penetrated the cuticle rapidly. In fact it was shown previously that the penetration of C12E8 was increased by adding 2 g/L Genapol C-100, while Genapol C-200 had no effects (Baur and Schönherr, 1997). The kinetic of C12E8 penetration at different humidities suggested that the monomers of Genapol C surfactants penetrated independently, but rate constants of penetration can be increased by those homologues (<8 EO groups) that increase mobility in the cuticle (see below).

The pour point of water free Genapol C-100 is about 26 °C (Table 1), and this may affect the differential time course of methylglucose penetration at 15 °C and 56% RH while no difference was found at 25 and 35 °C (Figure 4A). At 15 °C only the initial rate constants of penetration are decreased, while after 5 h rate constants are about 3-fold higher. The observation that rate

constants of penetration of ^{14}C-C12E8 are lower at 15 °C compared to 25 °C suggests that the longer lasting effect at this temperature is caused by decreased penetration of Genapol C-100 relative to that of methylglucose.

These results show that the effect of Genapol C surfactants depends on the time course of penetration of surfactant relative to that of methylglucose. Since Genapol C are polydisperse and contain at least 30 different homologues varying in alkyl and ethoxy chain length, it is not possible to compare "surfactant" and methylglucose penetration. A comparison of rate constants of penetration of methylglucose and monodisperse C12E8, which is a constituent of all Genapol C surfactants, confirms the above arguments (Figure 7). The data on penetration of C12E8 were measured in absence of methylglucose, but this makes no difference since even a 100-fold excess of methylglucose had no effect on C12E8 penetration (Figure 6). The penetration of methylglucose in the presence of 2 g/L C12E8 was most rapid up to 2 h and almost identical to that with 2 g/L Genapol C-050. This is reasonable since about 80% of Genapol C-050 constituents have a lower EO content than 8.

Both effects of adjuvants that affect only driving force by a humectant effect (e.g. Cook et al., 1977; Matsumoto et al., 1992) and greater effects with surfactants that can penetrate into the cuticle and affect transport properties have also been observed with other active ingredients and species (e.g. Holloway et al., 1992; Stock et al., 1992). Due to penetration of surfactant it is reasonable that surfactant effects increase over a wide range of surfactant concentration (Figure 1). This was previously observed for uptake and dose−response curves of various active ingredients and plant species (Holloway and Edgerton, 1992; Coret and Chamel, 1994; Grayson et al., 1995). At a Genapol C-100 concentration of 5 g/L almost quantitative methylglucose penetration was observed with all species studied (Figure 8). In contrast to the other species the time course of methylglucose penetration across *Citrus grandis* CM was high until the end of the experiment. Notably, penetration of ^{14}C-C12E8 across CM of *Citrus aurantium* was also constant, while rate constants decreased with all other species studied (Baur and Schönherr, 1997). The threshold for a beneficial effect on penetration is low as indicated by the effect of the lowest surfactant concentration (0.2 g/L). Similar observations were made by other investigators and neither the surfactant effect (Holloway and Edgerton, 1992; Stock et al., 1993) nor surfactant penetration (Anderson and Girling, 1983) could be related to the wetting properties of the formulations.

The enhancement of methylglucose penetration by 2 g/L Genapol C-100 was abolished by the addition of 1 g/L $CaCl_2$ (Figure 9). Notably this effect was established immediately after evaporation of water, and rate constants of methylglucose penetration were almost as low as for methylglucose in the absence of any adjuvants. Compounds such as $CaCl_2$, $MgCl_2$, phenylurea, and urea decrease penetration of ^{14}C-C12E8 (Baur and Schönherr, 1997; Baur et al., 1998). For urea and derivatives this effect results from adducts with ethoxy groups and less so with aliphatic chains, and sometimes urea is added to fatty alcohol ethoxylates and poly(ethylene glycol)s to solidify them (Barker and Ranauto, 1955). A similar effect was suggested for $CaCl_2$ and $MgCl_2$, and it was observed that the adverse effect on surfactant penetration was neutralized by high humidities (Baur and Schönherr, 1997). The situation resembles that of solid Genapol C-200 at 11% RH or 15 °C in that the driving force for surfactant penetration and concomitant effects on solute mobility decreases strongly and the solvent effect of Genapol C-100 disappears.

CONCLUSIONS

The penetration of polar compounds across plant cuticles is limited by low solubility in the cuticle. However, high rates of penetration can be obtained if the driving force for penetration across the cuticle is large. This can be managed by high partition coefficients of a.i. between cuticle and formulation residue or by maintaining a high concentration in the outer phase, i.e. by maximizing the product of partition coefficient and concentration. High partition coefficients result from a relatively lower solubility in the formulation residue. If the formulation contains adjuvants that can penetrate into the cuticle, this may also change the solubility in the cuticle. Rates of penetration can be further improved by increased diffusivity in the cuticle due to the action of accelerator adjuvants such as Genapol C-100. Diffusivity in the cuticle depends strongly on temperature (Baur et al., 1998), while there is no evidence for an effect of humidity. Therefore the penetration of polar compounds and surfactants into the cuticle will be influenced by differential environmental effects on mobility (temperature) and driving force (humidity).

It appears that surfactants with an intermediate degree of ethoxylation, like Genapol C-100, are most suitable for a wide range in humidity and temperature. In fact, this surfactant was very efficient with other compounds such as NAA, cyanazine, and more lipophilic compounds such as bifenox (Baur and Schönherr, unpublished results). Similar observations were made for other a.i.s and other classes of surfactants (Holloway et al., 1992; Kirkwood et al., 1992) though differences among species exist. The time course of penetration of surfactants seems to be more important than compatibility with hydrophilic or lipophilic compounds. The choice of a surfactant can be adopted to the most probable environmental conditions. At low temperatures and humidities or if cuticular permeability of the target species is low, rapid penetration will be achieved with Genapol C-050. Under favorable conditions or if penetration should take place for some days or longer, surfactants with high degree of ethoxylation or a poor penetrating adjuvant like humectants or Tween 80 should be preferred.

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LITERATURE CITED

Anderson, N. H.; Girling, J. The uptake of surfactants into wheat. *Pestic. Sci.* **1983**, *35*, 399−405.

Apfelblat, A. The vapour pressures of water over saturated aqueous solutions of barium chloride, magnesium nitrate, calcium nitrate, potassium carbonate, and zinc sulphate, at temperatures from 283 K to 313 K. *J. Chem. Thermodyn.* **1992**, *24*, 619−626.

Babiker, A. G. T.; Duncan, A. T. Penetration of bean leaves by asulam as influenced by adjuvants and humidity. *Pestic. Sci.* **1975**, *6*, 1655−664.

Baker, E. A.; Hayes, A. L.; Butler, R. C. Physicochemical properties of agrochemicals: Their effects on foliar penetration. *Pestic. Sci.* **1992**, *34*, 167−182.

Barker, G. E.; Ranauto, H. J. Solid nonionic surface-active agents. *J. Am. Oil Chem. Soc.* **1955**, *32*, 249−252.

Baur, P. Log normal Distribution of Water Permeability and Organic Solute Mobility in Plant Cuticles. *Plant Cell Environ.* **1997**, *20*, 167−177.

Baur, P.; Schönherr, J. Penetration of an ethoxylated fatty alcohol surfactant across leaf cuticles as affected by concentration, additives, and humidity. *J. Plant Dis. Prot.* **1997**, *104*, 380−393.

Baur, P., Grayson, B. T., Schönherr, J., Polydisperse ethoxylated fatty alcohol surfactants as accelerators of cuticular penetration. 1. Effects of ethoxy chain length and the size of the penetrants. *Pestic. Sci.* **1997a**, *51*, 131−152.

Baur, P.; Marzouk, H.; Schönherr, J.; Grayson, B. T. Partition coefficients between plant cuticle and adjuvants as related to foliar uptake. *J. Agric. Food Chem.* **1997b**, *45*, 3659−3665.

Baur, P.; Schönherr, J.; Grayson, B. T. Polydisperse ethoxylated fatty alcohol surfactants as accelerators of cuticular penetration. 2. Separating effects on driving force and mobility and reversibility of surfactant action. *Pestic. Sci.* **1998**, submitted for publication.

Burrage, S. W. The micro-climate at the leaf surface. In *Ecology of leaf surface microorganisms*; Preece, T. H., Dickinson, C. H., Eds.; Academic Press: London, 1971.

Chamberlain, K.; Briggs, G. G.; Bromilow, R. H.; Evans; A. A.; Fang, C. Q. The influence of physicochemical properties of pesticides following foliar application. *Asp. Appl. Biol.* **1987**, *14*, 293−304.

Cook, G. T.; Babiker, A.; Duncan, H. J. Penetration of bean leaves by aminotriazole as influenced by adjuvants and humidity. *Pestic. Sci.* **1977**, *8*, 137−146.

Coret, J.; Chamel; A. Effect of some ethoxylated alkylphenols and ethoxylated alcohols on the transfer of (^{14}C)chlorotoluron across isolated plant cuticles. *Weed Res.* **1994**, *34*, 445−451.

Pesticide Formulations: Innovations and Developments; Cross, B., Scher, H. B., Eds.; ACS Symposium Series 371; Washington, DC, 1988.

Adjuvants for agrochemicals, Proceedings of the 2nd International Symposium on Adjuvants for Agrochemicals 1989; Foy, C. L., Ed.; CRC Press: Boca Raton, FL, 1992.

Grayson, B. T.; Boyd, S. L.; Walter, D. Reinvestigating adjuvants for the wild oat herbicide, flam-M-isopropyl. I: Glashouse trials. *Pestic. Sci.* **1995**, *43*, 147−155.

Greenspan, L. Humidity fixed points of binary saturated aqueous solutions. *J. Res. Nat. Bur. Stand. A* **1977**, *81A*, 89−96.

Hall, F. R.; Chapple, A. C.; Downer, R. A.; Kirchner, L. M.; Thacker, J. R. M. Pesticide application as affected by spray modifiers. *Pestic. Sci.* **1993**, *38*, 123−33.

Hartley, G. S.; Graham-Bryce, I. L. *Physical principles of pesticide behaviour*; Academic Press: London, 1980.

Holloway, P. J. Physicochemical factors influencing the adjuvant-enhanced spray deposition and coverage of foliage-applied agrochemicals. In *Interactions between adjuvants, agrochemicals and target organisms*. Ernst Schering Research Foundation: Holloway, P. J., Rees, R. T., Stock, D., Eds.; Springer-Verlag: Berlin, 1994.

Holloway, P. J.; Edgerton, B. M. Effects of formulation with different adjuvants on foliar uptake of difenzoquat and 2,4-D: model experiments with wild oat and field bean. *Weed Res.* **1992**, *32*, 183−195.

Holloway, P. J.; Wong, W.; Partridge, H. J.; Seaman, D.; Perry, R. B. Effects of some nonionic polyoxyethylene surfactants on uptake of ethrimol and diclobutrazol from suspension formulations applied to wheat leaves. *Pestic. Sci.* **1992**, *34*, 109−118.

Kirkwood, R. C.; Knight, H.; McKay, I.; Chandrasena, J. P. N. R. The effect of a range of nonylphenol surfactants on cuticle penetration, absorption, and translocation of water-soluble herbicides. In *Adjuvants for agrochemicals*, Proceedings of the 2nd International Symposium on Adjuvants for Agrochemicals 1989; Foy, C. L., Ed.; CRC Press: Boca Raton, FL, 1992.

Knoche, M.; Bukovac, M. J. Studies on octylphenoxy surfactants: IX. Effect of oxyethylene chain length on GA_3 absorption by sour cherry leaves. *J. Plant Growth Regul.* **1991**, *10*, 173−177.

Marzouk, H.; Baur, P.; Schönherr, J. Relative solubilities of bifenox and naphthylacetic acid (NAA) in plant cuticles and in selected pure or aqueous glycol additives. *Pestic. Sci.* **1998**, *53*, 278−284.

Matsumoto, S.; Suzuki, S.; Tomita, H.; Shigematsu, T. Effect of humectants om pesticide uptake through plant leaf surfaces. In *Adjuvants for agrochemicals*, Proceedings of the 2nd International Symposium on Adjuvants for Agrochemicals 1989; Foy, C. L., Ed.; CRC Press: Boca Raton, FL, 1992.

Myers, D. *Surfaces, interfaces and colloids*; VCH Publishers: New York, 1991.

McWorther, C. G. The use of adjuvants. In *Adjuvants for herbicides*; Hodgson, R. H., Ed.; Weed Science Society of America: Champaign, IL, 1982.

Riederer, M.; Burghardt, M.; Mayer, S.; Obermeier, H.; Schönherr, J. Sorption of monodisperse alcohol ethoxylates and their effects on the mobility of 2,4-D in isolated plant cuticles. *J Agric. Food Chem.* **1995**, *43*, 1067−1075.

Schönherr, J. Effects of monodisperse alcoholethoxylates on mobility of 2,4-D in isolated cuticles. *Pestic. Sci.* **1993**, *38*, 155−164.

Schönherr, J.; Baur, P. Modelling penetration of plant cuticles by crop protection agents and effects of adjuvants on their rates of penetration. *Pestic. Sci.* **1994**, *42*, 185−208.

Schönherr, J.; Baur, P. Effects of temperature, surfactants and other adjuvants on rates of uptake of organic compounds. In *The plant cuticle − an integrated functional approach*; Kerstiens, G., Ed.; BIOS: 1996.

Schönherr, J.; Riederer, M. Plant cuticles sorb lipophilic compounds during enzymatic isolation. *Plant Cell Environ.* **1986**, *9*, 459−466.

Stevens, P. J. G.; Baker, E. A.; Anderson, N. H. Factors affecting the foliar absorption and redistribution of pesticides. 2. Physicochemical properties of the active ingredient and the role of surfactant. *Pestic. Sci.* **1988**, *24*, 31−53.

Stevens, P. J. G.; Bukovac, M. J. Studies on octylphenoxy surfactants. 2. Effects on foliar uptake and translocation. *Pestic. Sci.* **198**7, *20*, 37−52.

Stock, D.; Holloway, P. J. Possible mechanisms for surfactant-induced foliar uptake of agrochemicals. *Pestic. Sci.* **1993**, *38*, 165−177.

Stock, D.; Edgerton, B. M.; Gaskin, R. E.; Holloway, P. J. Surfactant-enhanced foliar uptake of some organic compounds: Interactions with two model polyoxyethylene aliphatic alcohols. *Pestic. Sci.* **1992**, *34*, 233−242.

Stock, D.; Holloway, P. J.; Grayson, B. T.; Whitehouse P. Development of a predictive uptake model to rationalise selection of polyoxyethylene surfactant adjuvants for foliage-applied agrochemicals. *Pestic. Sci.* **1993**, *37*, 233−245.

Van Valkenburg, W. A. Terminology classification and chemistry. In *Adjuvants for herbicides*; Hodgson, R. H., Ed.; Weed Science Society of America: Champaign, IL, 1982.

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