Partition Coefficients of Active Ingredients between Plant Cuticle and Adjuvants As Related to Rates of Foliar Uptake

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After spraying, water evaporates and foliar penetration proceeds from a formulation residue of active ingredients and adjuvants which may contain water depending on relative humidity and hygroscopic compounds present. Rates of uptake depend on solute mobility in and driving force across cuticles, which are proportional to the cuticle/formulation residue partition coefficient. Partition coefficients cuticle/glycerol (K_{CGly}) and cuticle/poly(ethylene glycol) 400 (PEG400) (K_{CPEG}) for seven organic compounds differing 6 orders of magnitude in octanol/water (K_{OW}) or cuticle/water (K_{CW}) partition coefficients have been measured. K_{CGly} can be estimated from K_{CW} and K_{OW} values ($r^2 = 0.95$). A plot of log K_{CGly} versus log K_{CW} had a slope of 0.69, indicating that glycerol is a better solvent for lipophilic solutes than water. In contrast, PEG400 (PEG) was a good solvent for polar and nonpolar solutes. All K_{CPEG} values were below 1, differing <10-fold without correlation with lipophilicity. PEG400 sorbs water from air, and log K of the lipophilic compound, bifenox, increased linearly toward the value of log K_{CW} with decreasing mass fraction of PEG400. Rates of penetration of bifenox differed drastically if K_{CPEG} was modified by different humidities in the ambient air.

Keywords: *Adjuvants; foliar uptake; glycerol; humectants; partition coefficient; penetration; polyethylene glycol*

INTRODUCTION

Foliar-applied pesticides are seldom used without adjuvants. For polar compounds humectants such as glycerol or poly(ethylene glycol)s are often used (Hartley and Graham-Bryce, 1980; McWhorter, 1982), and most formulations contain surfactants. Some surfactants can penetrate the cuticle rapidly (Anderson and Girling, 1983; Stock et al., 1992), and if they are sorbed in substantial amounts, surfactants can increase solute mobilities in the cuticle (Schönherr, 1993; Schönherr and Baur, 1994). In addition, they may affect the solubility of active ingredients in the cuticle (Baur and Schönherr, 1996) and the physical state of the active ingredient in the surface residue. Thus, they influence both the permeability of the cuticular membrane and the driving force for the solute uptake (Schönherr and Baur, 1994; Baur and Schönherr, 1996).

Other organic liquids, like the humectants and solvents glycerol or ethylene and propylene glycols and certain surfactants with a high number of ethylene glycol groups, do not or hardly penetrate into an intact cuticle [e.g., Hartley and Graham-Bryce (1980), Anderson and Girling (1983), and Holloway and Edgerton (1992)] and manifest their effect only on the leaf surface. At constant temperature such liquids influence rates of cuticular penetration by the differential solubilities of the active ingredients in cuticle and adjuvant, i.e. by their partition coefficients in such two phase systems. This partition coefficient will limit uptake rates as long as the concentration of the active ingredient is near saturation or has not decreased substantially. There

are not many reports on the mode of action of humectants on penetration, but, for instance, Cook et al. (1977) found a clear dependence of the performance of glycerol and polyoxyethylene 20 sorbitan monolaurate (Tween 20) for its effect on the uptake of 3-amino-1,2,3-triazole (amitrole) into leaves of bean (*Phaseolus vulgaris* L.) on relative humidity; this dependence varied with the amount of humectant. Both increasing the amount of humectant on the leaf surface and the uptake of water due to high humidity caused a dilution of the concentration of this hydrophilic active ingredient, which decreased rates of uptake. Similar qualitative results were obtained for the action of glycerol on the efficiency of some herbicides and fungicides (Matsumoto et al., 1992). Poly(ethylene glycol)s are also used as solvents for lipophilic actives in emulsion concentrates (Ogava et al., 1991) and act similarly on the leaf surface due to the fact that humectants not only improve dissolution of hydrophilic compounds but are producing a continuous liquid phase, avoiding crystallization.

Due to the large spectrum in lipophilicity of active ingredients and adjuvants, partition coefficients between cuticle and adjuvants will vary widely. Partition coefficients between cuticle and water have been reported repeatedly [e.g., Riederer and Schönherr (1984), Kerler and Schönherr (1988), and Schönherr and Baur (1994)]. Cuticle/water partition coefficients are useful to interpret uptake and accumulation of many xenobiotics from aqueous solutions which appear, for instance, due to wet deposition by fog or rain (Glotefelty et al., 1987; Valsaraj et al., 1993). For the foliar uptake of plant protection agents cuticle/water or octanol/water partition coefficients are useful only for the early state of application where excess water is present, but usually the liquid phase in contact with the cuticle is not simply water and partitioning between adjuvant residue and cuticle occurs. These partition coefficients are not relevant for aqueous formulations which contain sur-

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Table 1. Chemical Names, Specific Activities, Water Solubilities (S_{H_2O}), and Sources of the Radiolabeled Compounds

compound (source)[a]	chemical name	specific activity (MBq mmol^{-1})	S_{H_2O} (25 °C) (g L^{-1})
methylglucose (1)	3-*O*-methyl-α-D-glucose	318	∞
phenylurea (2)	phenylcarbonyl[^{14}C]diamide	148	4.1
cyanazine (2)	6-chloro-*N*-ethyl-[1-^{14}C]-*N'*-(2-methylpropionitrile)-1,3,5-triazine-2,4-diamine	507	0.17
chlorfenvinphos (2)	2-chloro-1-(2,4-dichloro-[U-^{14}C]phenyl)vinyl diethyl phosphate	303	0.145
WL110547 (2)	1-(3-fluoromethylphenyl)-5-[U-^{14}C]phenoxy-1,2,3,4-tetrazole	503	3×10^{-3}
bifenox (3)	methyl 5-(2,4-dichlorophenoxy)-2-nitrobenzoate [U-^{14}C]	1288	0.3×10^{-3}
permethrin (2)	±*cis,trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane 1-carboxy-3-phenoxybenzyl ester	68	0.2×10^{-3}

[a] Sources: 1 = Amersham Buchler, Braunschweig, Germany; 2 = Shell Research, Sittingbourne, United Kingdom; 3 = International Isotopes Munich, Germany.

factants above the critical micellar concentration, which form a lipophilic phase (Baur and Schönherr, 1996).

For a comprehensive analysis and model for the uptake of crop protection agents the values of such partition coefficients must be known. To our knowledge such values have not been reported before. In the present paper we report partition coefficients (*K*) between cuticle and glycerol and poly(ethylene glycol) 400 (PEG400) of seven organic compounds which differ in K_{OW} by >6 orders of magnitude.

MATERIALS AND METHODS

Plant Material. Cuticle/water partition coefficients of organic solutes are quite similar for different species and cuticles from both leaves and fruits (Riederer and Schönherr, 1984; Kerler and Schönherr, 1988). Therefore, rather thick and strong fruit cuticles of green pepper (*Capsicum annuum* L. cv. Bell Boy) were used for the experiments. The cuticles were isolated enzymatically (Schönherr and Riederer, 1986) and cut in small pieces prior to use.

Cuticular penetration was studied with adaxial astomatous leaf cuticles of *Stephanotis floribunda* Brongn., which were grown in a glasshouse. Cuticles were also enzymatically isolated, air-dried, and stored in a refrigerator at 8 °C prior to use.

Chemicals. Seven organic nonelectrolytes, including some active ingredients, differing widely in octanol/water partition coefficients were used for the sorption experiments (Table 1). No compound was surface active, and all were used as ^{14}C-labeled isotopes. The liquid phases were deionized water in equilibrium with CO_2 (pH 5.6), glycerol (Sigma, Deisenhofen, Germany), and PEG400 with an average molecular mass of 400 g/mol (Roth, Karlsruhe, Germany). PEG400 is a mixture of ethylene glycols with 3−17 ethylene glycol units, and an average mass of 400 g/mol (ca. 9 ethylene glycol groups) is a weighted mean. Glycerol and poly(ethylene glycol) were dried over silica gel for some days before use.

Partition Coefficients. Partition coefficients between cuticle and water (K_{CW}) can be measured in a similar manner as for two immiscible liquids (Kerler and Schönherr, 1988). The partition coefficients refer to the masses of the cuticle (m_C) and liquids (m_l) according to

$$K_{CW} = (M_C/m_C)/(M_l/m_l) \qquad (1)$$

where *M* is the amount of radioactivity (Bq) of the solute in the cuticle or the solution, respectively. The radioactivity in the two phases is measured directly. The mass of the cuticle samples varied in most cases between 1 and 10 mg and was measured exactly with a microbalance (±1 μg; Sartorius, Göttingen, Germany). The liquids had a mass of ca. 1 g. These are only rough order of magnitude estimates since the actual mass ratios were adjusted to (i) the solubilities (Table 1) and expected partition coefficients of the compounds in the respective liquid, (ii) the radiochemical purity, and (iii) the specific activity of the radiochemicals. Since no data existed for the partition coefficients between cuticle and glycerol or PEG400, preliminary experiments were carried out to choose the adequate masses and radioactivities for the actual experiments. The highest ratio of masses was 30 mg of cuticle /0.6

g of H_2O (with methylglucose), and the lowest was 1 mg of cuticle/5 g of H_2O (with permethrin). The highest amount of solute (permethrin) sorbed in the cuticle was 7% (m/m). For obtaining sorption equilibrium cuticles and liquids were put into glass scintillation vials (6 mL) or smaller screw cap glass bottles (1 mL; Wheaton, Millville, USA) and mixed during the experiment on a roller bench at ∼40 rpm.

In all cases but one the solute was dissolved in the respective liquid or solution for 1 day and measured, and a piece of the cuticle was added. With permethrin and glycerol cuticle pieces were first loaded with permethrin from an aqueous solution, air-dried, and placed into glycerol. After 48 h or later, cuticles were taken from the bottle and the adhering liquid was removed by pressing them on filter paper or laboratory paper (PEG400). Sorption equilibrium with water was obtained after 1 day (data not shown), but the duration was prolonged in this study due to the higher viscosities of glycerol (954 mPa·s at 25 °C) and PEG400 (120 mPa·s at 20 °C) to compensate for poorer mixing with low volumes. After the experiments were finished, the radioactivity in the cuticles was extracted using scintillation cocktail (Aquasafe 500, Zinsser, Frankfurt, Germany), which completely extracted the radioactivity within 1 h. Radioactivities were assayed using a liquid scintillation counter (Packard CA 2000 counter, Downers Grove, IL) with a 2σ error of 2%. The radioactivity in the solution was measured twice by taking one sample and measuring the remaining radioactivity in the vials or by taking two samples from the screw cap glass bottles. This was done to detect errors by undissolved particles of the radiochemicals in the liquids and by tiny pieces of the cuticles which may have detached from the remaining cuticle. In partitioning experiments with PEG400 and in those cases in which a significant fraction (>5%) of radioactivity was attributed to the adhering liquid, a correction for this was calculated from

$$M_C = M_t - (m_{ad}M_L/m_L) \qquad (2)$$

where M_C is the amount of radioactivity in the cuticle (Bq), M_t is the total measured radioactivity (Bq), m_{ad} is the mass of adhering liquids/solutions (mg), and M_L/m_L is the radioactive concentration of compound in solution (Bq mg^{-1}).

For instance, in one sample the radioactive concentration of bifenox in PEG400 (M_l/m_l) at equilibrium was 2.53 Bq mg^{-1}. The mass of the corresponding cuticle taken after equilibration with the PEG400 solution was 13.9 mg, while the previously measured dry mass was 7.355 mg. The difference was due to adhering PEG400, and the radioactive amount was calculated by multiplying this mass (m_{ad}) by 2.53 Bq mg^{-1}. Subtracting this value from the total radioactivity (19.25 Bq) in the cuticle yielded the true amount per mass of the cuticle (0.36 Bq mg^{-1}) and a partition coefficient of *K* = 0.14.

With PEG400, partition coefficients ranged between 0.1 and 1 (see Results), indicating similar solubilites in the cuticle and in PEG400; therefore, radiochemical impurities cause no errors. The very lipophilic permethrin had the lowest radiochemical purity, and water was exchanged until its ^{14}C concentration was constant to remove water soluble impurities. All partition coefficients refer to a temperature of 25 ± 0.5 °C. At this temperature viscosities of glycerol and PEG400 are sufficiently low for good mixing. Partition coefficients

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between water and cuticle (K_{CW}) are normally distributed and had coefficients of variation between 5 and 35%. For all compounds 10 replications were used.

Penetration Experiments. Penetration of bifenox (Table 1) through cuticles of *S. floribunda* as influenced by PEG400 was studied by simulating foliar uptake (Schönherr and Baur, 1994). Bifenox was chosen as a lipophilic involatile model compound that penetrates the cuticle easily and does not change its transport properties. Cuticles were mounted in steel chambers covered by a lid with the morphological inner surface of the cuticle facing the chamber interior. The chambers were completely filled with 1.5 mL of a 1% (w/v) aqueous soybean lecithin suspension [phospholipid suspension (PLS), Roth, Karlsruhe, Germany) through a sampling port, which was closed with an adhesive tape (Tesafilm, Beiersdorf, Hamburg, Germany). Penetration was measured immediately after application of 5 μL droplets of solutions of bifenox to the outer surface of the cuticles. In the control experiments a mixture of acetone and water at a ratio of 1+2 by volume and containing 25 μg of PEG400 was used. During penetration the chambers were rocked horizontally while standing in wells of a thermostated (25 °C) aluminum block. At predetermined time intervals the receiver solution (PLS) was withdrawn and replaced by a fresh one. After the last sample (48 h), the cuticles were cut out and the residual radioactivity in the cuticles was extracted using scintillation cocktail (Aquasafe 500, Zinsser, Frankfurt, Germany) and counted as stated above.

From the samples drawn the total amount penetrated (M_t) at each time was calculated and the amount applied (M_0) was calculated from the sum of the amount penetrated and the residual radioactivity in the cuticle. Data were analyzed by plotting the logarithm of the relative unpenetrated amount, $\ln(1 - M_t/M_0)$, versus time, t. Under constant conditions with the concentration of the penetrated compound in the receiver being zero, penetration follows first-order kinetics as indicated by a straight line in this plot. The slope of the line gave the rate constant (k) of penetration. Zero concentration is fulfilled if PLS is used as receiver solution for lipophilic neutral solutes (Schönherr and Baur, 1994), which completely partition into the liposomes (Kennedy and Harvey, 1972). Data were analyzed by calculating the geometric mean with 95% confidence intervals, since permeabilities of cuticles were found to be lognormally distributed (Baur, 1997). Between 15 and 20 replications were used.

The bulk of the solvents (acetone and water) of the 5 μL donor droplets had evaporated after 30−40 min, leaving a solute/liquid residue when PEG400 was added. After the first sample (2 h), the chambers were inverted so that they faced the aluminum block for the remainder of the experiment. The side with the outer surface of the cuticle exposed was then either closed with Tesafilm (closed donor) or not (open donor). In the first case saturation humidity was obtained due to the remaining water of the PEG400 residue and the water which permeates through the cuticle from the receiver solution into the air layer above the cuticle (ca. 200 μL). With the open donor the air above the cuticle is in contact with the ambient air above the aluminum block and the humidity is assumed to be slightly above this value, again due to permeation of water from the receiver solution. The realtive humidity in the laboratory was about 70% at 20 °C. An accurate value of humidity in the open donor conditions was not available, and a distinction in the experimental conditions is made only qualitatively between closed donor (high humidity) and open donor (low humidity).

RESULTS AND DISCUSSION

The flux J (mol m^{-2} s^{-1}) of active ingredients into leaves is proportional to the solute mobility in the cuticle k^* (s^{-1}) and the driving force for penetration, which can often be reduced to the partitioning from the formulation or formulation residue (fr) into the cuticle (Schönherr and Baur, 1996) by

$$J = k^* l_{\text{ls}} (K_{Cfr} C_{\text{fr}}) \tag{3}$$

where l_{ls} is the thickness of the limiting skin, K_{Cfr} is the partition coefficients of the active ingredient between cuticle and residue, and C_{fr} is the concentration in the residue. If the formulation does not change the transport properties of the cuticle, only the partition coefficient changes during droplet drying and, after evaporation of solvents, uptake of active ingredients is limited by the differential solubility in the formulation residue and the cuticle (K_{Cfr}).

Cuticle/Water Partition Coefficients. Usually the only available parameter characterizing the differential affinity to solvents is the octanol/water partition coefficient. Partition coefficients between cuticle and water correlate well with octanol/water partition coefficients (Table 2). In this study pepper fruit cuticle was used and the slope of a linear regression line in a plot of log K_{CW} versus log K_{OW} has a value of 1.01 [±0.2, 95% confidence interval (CI)] with a coefficient of regression of 0.98 ($n = 6$). A negative value (−0.32) for the intersection with the ordinate indicates a lower solubility of the model compounds in the cuticle than in octanol. For a higher number of 21 compounds and pepper cuticle the slope was exactly 1.00 (±0.12), while the point of intersection was lower (−0.15, data not shown). Thus, the K_{CW} of pepper cuticles amounts to about 70% of the K_{OW}. The solid state restricts the validity of such correlations to moderate amounts sorbed where K_{CW} is below 10 % mass of the cuticle (Schönherr and Riederer, 1989).

Cuticle/Glycerol Partition Coefficients. After evaporation of solvent water, a concentrated formulation residue is produced and, if an adjuvant that does not penetrate the cuticle such as glycerol or poly(ethylene glycol)s (Hartley and Graham-Bryce, 1980; Anderson and Girling, 1983; Holloway and Edgerton, 1992) is present, a different two-phase system develops. A comparison of cuticle/glycerol partition coefficients (K_{CGly}) for the model compounds with the values for K_{CW} showed the same trend (Table 2). Partition coefficients increased with increasing lipophilicity of the compounds, and a good linear correlation of log-transformed partition coefficients for the systems cuticle/glycerol and cuticle/water was obtained (Figure 1). About 95% of the variation in K_{CGly} with water-free glycerol for the compounds can be explained by the variation in K_{CW}. Due to the strong correlation of K_{CW} and K_{OW} this holds also for the octanol/water partition coefficient. The slope of 0.69 decreases the differences of partition coefficient between permethrin and methylglucose compared to K_{CW} by approximately 2 orders of magnitude. It shows that glycerol is a better solvent for lipophilic solutes and, if extrapolated below the value for methylglucose, a poorer one for more polar solutes. From the equation (Figure 1) equal values are obtained at $K = 0.18$; that is, below this value K_{CGly} is higher than K_{CW}. The good correlation for these predominantly cyclic compounds is indicated by the results of Collander (1950, 1951), who got significant differences in selectivity (slope of the log/log plot) and y-intercept for a homologues series of aliphatic compounds for partitioning between the alcohols butanol and octanol and water. Selectivity and y-intercept in plots of log K_{CW} against log K_{OW} or log K_{CGlyc}, respectively, are therefore average values.

The enthalpy of sorption is proportional to absolute temperature (Frumkin, 1925), and therefore partition

Table 2. Octanol/Water Partition Coefficients and Partition Coefficients between Green Pepper Fruit Cuticles and Water (log K_{CW}), Glycerol (log K_{CGly}), or PEG400 (log K_{CPEG}) of the Organic Solutes

compound	log K_{OW}	log K_{CW} ±95%[a]	log K_{CGlyc} ±95%[a]	log K_{CPEG} ±95%[a]
methylglucose		−0.87 ± 0.10	−0.76 ± 0.25	−0.40 ± 0.06
phenylurea	0.8	0.87 ± 0.08	0.19 ± 0.04	−0.60 ± 0.13
cyanazine	2.1	1.80 ± 0.10	0.77 ± 0.07	−1.00 ± 0.30
chlorfenvinphos	3.5	3.04 ± 0.02	2.16 ± 0.11	−0.28 ± 0.13
WL110547	3.6	3.25 ± 0.08	2.40 ± 0.18	−0.33 ± 0.18
bifenox	4.6	4.44 ± 0.04	2.30 ± 0.13	−0.85 ± 0.11
permethrin	6.1	5.51 ± 0.08	3.67 ± 0.18	0.04 ± 0.03

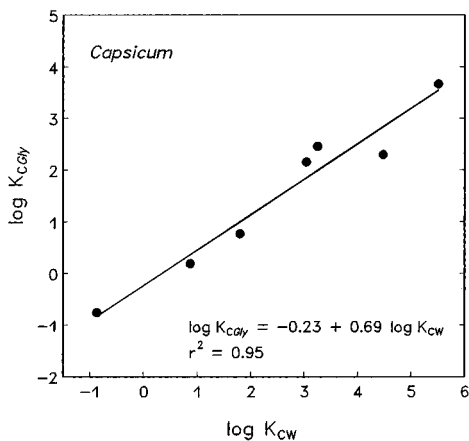[a] Maximum confidence interval of log-transformed values.

Figure 1. Correlation of cuticle/glycerol partition coefficients and cuticle/water partition coefficients for the model compounds and green pepper fruit cuticles.

coefficients differ only slightly with temperature and can be calculated for any temperature. This is possible also for sorption into cuticular membranes at low solute concentrations (Schönherr and Riederer, 1989). The correlation of K_{CGly} and K_{CW} is analogous to the correlation of partition coefficients for different alcohol/water systems (Collander, 1950). Since the K_{CW} is correlated with the K_{OW} (Table 2), the cuticle/glycerol partition coefficient can be calculated from the equation in Figure 1 for any compound for which the K_{OW} is known. For example, for the nonsystemic fungicide prochloraz, log K_{OW} is 4.0 (Hartley and Graham-Bryce, 1980) and the log K_{CGly} is calculated to be 2.53. For other liquids that behave like glycerol, partition coefficients can be calculated for any solute and two-phase system if the values of at least two compounds are known. However, there is a limitation since these values refer to pure glycerol, neglecting the variable water content of such liquid residues due to the effects of humidity and temperature under practical conditions (see below).

Cuticle/PEG400 Partition Coefficients. The situation is completely different with poly(ethylene glycol). Partition coefficients between cuticle and PEG400 (log K_{CPEG}) varied for the different model compounds only by a factor of 10 and no systematic trend with lipophilicity was observed (Table 2). Even methylglucose and permethrin, which vary in K_{CW} by 7 orders of magnitude, have very similar values for K_{CPEG} (0.4 and 1.1, respectively). Except for methylglucose partition coefficients are lowest with PEG400 compared with glycerol and water. In a phase system with a lipid phase like octanol or the cuticle the partition coefficient is determined by the other phase. This is known for systems with water, in which the hydrophobic effect determines the phase equilibrium (Tanford, 1973), and it holds also

for glycerol and poly(ethylene glycol). The low values for the cuticle/PEG400 partition coefficients are exclusively caused by the good solvent power of PEG400. It has been shown that poly(ethylene glycol)s with average molecular masses above 200 g/mol can be miscible with certain active ingredients like fenpropathrin (log K_{OW} = 5.1) up to 20% (m/m) or greater, and for that they are often used as solvents in emulsifiable concentrates (Ogawa et al., 1991). Thus, poly(ethylene glycol)s are used for both hydrophilic and lipophilic active ingredients, since they have the solvent power for both and can attract and hold water. Poly(ethylene glycol)s belong to the group of humectants, and this term refers not to a preferential dissolution of hydrophilic compounds but to the fact that usually the time for evaporation of aqueous droplets to dryness is prolonged and a liquid state is kept. PEG400 is a mixture of ethylene glycols with 3−17 ethylene glycol units and a weighted mean of 9 ethylene glycol groups (product data sheet). Due to the extremely different behaviors of glycerol and PEG400, it seems promising to study partition coefficients of a series with decreasing chain length from poly(ethylene glycol)s to oligo(ethylene glycol)s and ethylene glycol. This will indicate if there is a threshold to the chain length at which ethylene glycols become solvents for lipophilic compounds.

The similarity of K_{CPEG} values and the widely differing K_{CW} values lead to marked changes in the nature of the solvent system and the permeation driving force after such solutions have been sprayed. With a diluted aqueous solution of active ingredient and PEG400, the driving force is proportional to the partition coefficient K_{CW} and the concentration (eq 3). After this short initial state during evaporation of volatile solvents, the formulation becomes a better solvent for lipophilic solutes. With the exception of methylglucose, partition coefficients changed from values above 1 to lower values (Table 2) and sorption changes from an energetically favored process with spontaneous sorption into the cuticle from aqueous solution to an energy-consuming one with PEG400. In this case sorption in the cuticle increases with an increase in temperature. The change in K during evaporation of water is more pronounced with lipophilic compounds as visible by the larger differences (Table 2). Log K decreased reasonably linearly with increasing mass fraction of PEG400 from pure water to pure PEG400 (Figure 2A). The partition coefficient K decreased about 14% with an increase of the mass fraction by 1%.

Penetration As Influenced by PEG400 and Humidity. PEG400 is a slightly hygroscopic liquid (Israellachvili, 1992), completely miscible with water, and the difference between log K_{CW} and log K_{CPEG}, especially for lipophilic solutes, suggests that under practical conditions humidity will influence the partition coefficient. The penetration of bifenox applied at t =0 in a mixture of acetone plus water without PEG400 was fastest, and >90% had penetrated after 1 day into the PLS receiver solution (Figure 3). Adding PEG400 had almost no influence on the penetration if the outer surface was covered after the first sample time (2 h), thereby increasing humidity above the surface residue toward 100%. In contrast, under lower humidity conditions, when the donor side was kept open, penetration was significantly lower after 2 h. If bifenox was applied without PEG400, no influence of humidity or covering the outer side was measurable (data not shown). This is not surprising because bifenox is very lipophilic and
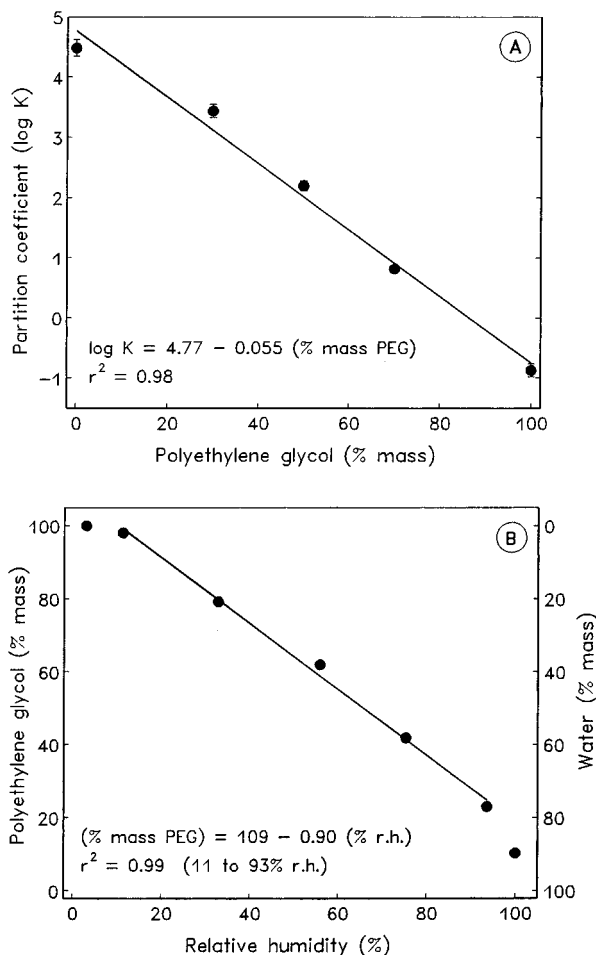
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Figure 2. (A) Dependence of partition coefficients for bifenox and green pepper fruit cuticles on the percent mass fraction of PEG400 in mixtures with water. (B) Dependence of the percent mass fraction of PEG400 on relative humidity in the ambient air (see text).
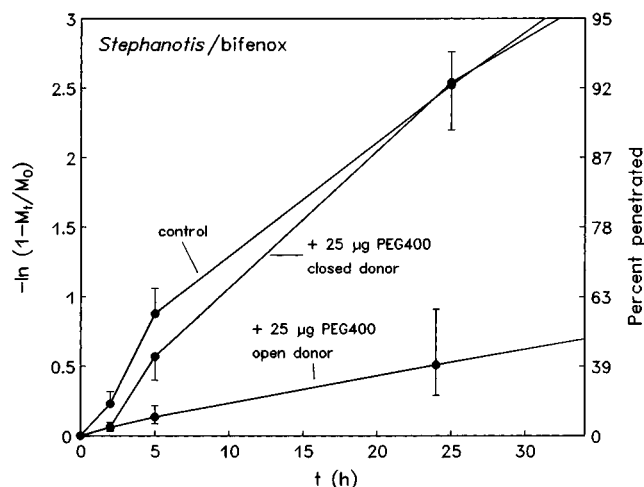

Figure 3. Penetration of bifenox through *S. floribunda* leaf cuticles in the presence of PEG400 under high (closed donor) or low (open donor) humidity conditions. In the absence of PEG400 (control) the time courses of penetration under high and low humidities were equal (see text).

most of the compound will be sorbed rapidly in the surface wax of *Stephanotis* cuticles during droplet drying as suggested by the almost complete and rapid penetration. Penetration of bifenox in an open donor situation followed first-order kinetics, and we interpret this as indicating that the PEG400 residue served as

donor. Penetration was slower under these circumstances since the partition coefficient is much lower owing to the lower water content in the residue after the first sample was drawn and dissolution in (mainly) PEG400 slowed sorption into the cuticle.

We do not know accurately the humidity of the air on the open donor side in contact with the ambient air. To get estimates of the mass fraction of PEG400 (in mixtures with water) under different humidities, PEG400 was brought to equilibrium with air above saturated salt solutions of LiCl (11.3% relative humidity), $MgCl_2$ (32.8%), $Ca(NO_3)_2$ (55.9%), NaCl (75.3%), and KNO_3 (93.6%) (Greenspan, 1977; Apfelblat, 1992) as well as above silica gel (<3%) at 25 °C. The amount of water sorbed was measured gravimetrically with an analytical balance (±0.1 mg accuracy) in a PEG400 droplet of 35 mg covering a surface of ca. 1 cm^2. The mass fraction of PEG400 was measured over 2 weeks until values were constant. If pure PEG400 was brought to equilibrium with air of 100% relative humidity, a mixture was obtained with <20% (m/m) PEG400 after only 1 day but, due to the slight hygroscopy, decreased further even after 2 weeks. A fairly linear relationship was obtained between the mass fraction of PEG400 and relative humidity between 11.3 and 93.6% (Figure 2B). For comparison, Matsumoto et al. (1989) measured a value of approximately 25% (m/m) PEG400 at 90% relative humidity and 20 °C with a Karl Fisher moisture meter. In Figure 3 the slopes from 2 to 25 h with PEG400 under high humidity (closed donor) and low humidity differed 5-fold. The results of Figure 2A,B suggest that, for example, a difference in humidity of 15% between open and closed donor would result in a 7-fold lower partition coefficient with the open chamber and corresponding (eq 3) lower rates of penetration. This assumes equilibrium of the PEG400 residue with the air above the donor. Although there was a time lag of some days in the assay in this work, fast equilibrium on a leaf surface is likely since (i) the amount and thickness of a humectant residue would be much lower than in this assay and (ii) water is initially present and will be more rapidly lost to the air from an exposed leaf surface.

For the most hydrophilic compound in this study, methylglucose, the situation should be reversed. Penetration should be higher from PEG400 solution than from the same mass of an aqueous solution since log K_{CPEG} is higher than log K_{CW}. The dependence on humidity of partition coefficients in the system cuticle/ glycerol is probably less since partition coefficients between this system and cuticle/water differ less (Table 2). However, differences will increase with the lipophilicity of the solutes. Surfactants with very long poly-(ethylene glycol) chains which do not change solute mobilities in the cuticle can similarly act as humectants for active ingredients (Stock et al., 1992; Schönherr and Baur, 1994), and differences in their effects on uptake of actives should depend mainly on the length of the poly(ethylene glycol) moiety. This means that solute partition coefficients between cuticle and ethoxylated surfactants may be very low and similar to that of PEG400, as was found to be the case for chlorfenvinphos and a polydisperse ethoxylated fatty alcohol surfactant (Baur and Schönherr, 1996). The model compounds in this study are all nonelectrolytes, but active ingredients of pesticides and growth regulators may also be electrolytes and applied as salts. With such compounds crystallization is likely and the most unfavorable process for penetration since the crystals will be much less

sorbed in surface wax than lipophilic compounds during evaporation of droplets (Kerler et al., 1984). Holloway and Edgerton (1992) found that the uptake of the salt difenzoquat (log K_{OW} = −0.62 at pH 7) into wild oat and bean leaves was most improved by Tween 20 and less so with glycerol or pol(yethylene glycol)s. Uptake rates into field bean leaves were rather constant over a period of 5 days, similar to the penetration observed for bifenox in this work. For both plants a fatty alcohol surfactant, which can increase solute mobility in the cuticle, was superior. The differences of the humectant effect between species let them conclude that some further mechanism acts with nonpenetrating surfactants like Tween 20 compared to humectants. One would expect that the very hydrophilic difenzoquat would be less soluble in Tween 20 compared with glycerol and poly-(ethylene glycol), and therefore the cuticle/residue partition coefficient would be larger, thereby favoring uptake. Additionally, partition coefficients of Tween 20 residues would also depend on humidity, as indicated by the decrease in water content from 30 to 19% (m/m) when the relative humidity decreases from 90 to 84% (Anderson and Girling, 1983). Humidity was not controlled in these experiments but could also account for the observed differences since humidities in the microclimate at the leaf surface can vary between species (Burrage, 1971).

The results indicate that the partition coefficient is the most variable transport parameter (compared to area and diffusion coefficient) during the uptake from a droplet containing adjuvants and that with lipophilic compounds the humectant action is identical with this partitioning effect. For solvents such as glycerol, such partition coefficients can easily be estimated from relationships like Figure 1 and a calibration plot. For nonselective, hygroscopic liquids such as PEG400, with good solvent power for both hydrophilic and lipophilic compounds, K depends strongly on relative humidity and subsequent water sorption into the residue. The value of K can be estimated from the water content in the residue, which depends on the relative humidity in the ambient air.

It should be emphasized that poly(ethylene glycol) and glycerol do not change solute mobility in cuticles (Baur et al., 1996) since they are not sorbed into the cuticle (Hartley and Graham-Bryce, 1982); they simply affect the driving force and partitioning of solutes. In contrast, surfactants such as monodisperse alcohol ethoxylates can be sorbed in substantial amounts even from aqueous micellar solutions (Riederer et al., 1995) and can change solute solubility and mobility in the cuticle. Partition coefficients of active ingredients between cuticle and surfactants will be reported in a further contribution.

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