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# Accelerators Increase Permeability of Cuticles for the Lipophilic Solutes Metribuzin and Iprovalicarb but Not for Hydrophilic Methyl Glucose

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Effects of diethylsuberate (DESU), tributyl phosphate (TBP), and monodisperse ethoxylated alcohols (EAs) on rate constants of penetration ($k$) of model solutes across astomatous cuticular membranes isolated from Madagascar ivy (*Stephanotis floribunda*) and pear (*Pyrus communis*) leaves were studied. Model solutes (selected on the basis of their octanol/water partition coefficients, $K_{ow}$) were iprovalicarb (log $K_{ow} = 3.18$), metribuzin (log $K_{ow} = 1.60$), and methyl glucose (MG) (log $K_{ow} = -3.0$). $K_{ow}$ varied by more than 6 orders of magnitude. Accelerators had wax/water partition coefficients (log $K_{ww}$) ranging from 1.75 (DESU) to 4.32 ($C_{12}E_2$), and their equilibrium concentrations in *Stephanotis* wax varied from 0 to about 160 g kg$^{-1}$. Accelerators increase solute mobility in cuticles by increasing fluidity of cutin and waxes. This effect was quantified by plotting log $k$ versus the accelerator concentration in wax. With the lipophilic solutes metribuzin and iprovalicarb, these plots were linear. Slopes of these plots characterize the intrinsic activities of the accelerators, and they decreased in the order DESU (0.029) > TBP (0.015) > EAs (0.01). Using these intrinsic activities, the effects of accelerators on rate constants of penetration can be calculated for any accelerator concentration in wax. For instance, at 50 g kg$^{-1}$, rate constants for lipophilic solutes increased by factors of 28 (DESU), 5.6 (TBP), and 3.2 ($C_{12}E_n$), respectively. Permeability of cuticles for the hydrophilic MG was not increased by DESU, TBP, $C_{12}E_2$, and $C_{12}E_4$, while $C_{12}E_6$ and $C_{12}E_8$ increased it. Small hydrophilic solutes such as MG can access aqueous pores in cuticles, and this pathway is not affected by changes in fluidity of amorphous waxes. After rate constants of penetration of ionic $CaCl_2$ were compared with those for nonionic MG, it was concluded that 60% of the MG diffused across aqueous pores, while 40% used an alternative pathway. Because the solubility of MG in wax is extremely low, it is unlikely that MG diffused along the lipophilic pathway used by metribuzin and iprovalicarb. This agrees with the observation that DESU and TBP had no effect on rate constants for MG. An alternative pathway of unknown properties is suggested. It is speculated that $C_{12}E_6$ and $C_{12}E_8$ sorbed in cuticles might have generated a polar pathway for MG.

**KEYWORDS: Adjuvant; cuticular permeability; foliar uptake; pesticide efficacy; partition coefficient**

## INTRODUCTION

Intrinsic activities of a number of selected accelerator adjuvants have recently been measured (*1*, *2*). Cuticular membranes (CMs) from *Stephanotis floribunda* leaves served as model membranes. 2,4-Dichlorophenoxybutyric acid (2,4-DB) was the model solute, and the UDOS (unilateral desorption from the outer surface) method was used. 2,4-DB is a relatively lipophilic compound, with the non-ionized form having a cuticle/water partition coefficient of 1122 (*3*). This raises the question if these results can be generalized and are valid for all nonionic active ingredients. The UDOS method is not suitable to test this, because it is limited to relatively lipophilic solutes with $K > 100$ (*4*).

A mechanistic analysis of accelerator action in cuticles is difficult when accelerators and active ingredients are simultaneously applied to plant cuticles (*5*, *6*). Accelerators are small lipophilic solutes. They increase mobility of lipophilic solutes, but they also can act as solvents for active ingredients, which affects partition coefficients. The driving force for cuticular penetration is proportional to the solute concentration in the applied solution and to the partition coefficient wax/formulation (*7*). Thus, accelerators used as formulants invariably affect the driving force. In addition, accelerators penetrate into and across the cuticle and are sorbed in the cuticle. This results in

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**Table 1.** Properties and Sources of Radio-Labeled Model Compounds

| common name | chemical name | molecular weight (g mol$^{-1}$) | log $K_{cw}$ | specific activity (MBq mg$^{-1}$) | concentration in donor (mg L$^{-1}$) |
|---|---|---|---|---|---|
| MG | 3-*O*-[$^{14}$C-methyl]-D-glucose[a] | 194.2 | –0.89[b] | 10.50 | 9.5 |
| metribuzin | 4-amino-6-(1,1-dimethylethyl)-3-methylthio)-1,2,4-triazin-5 (4H)-one[c] | 214.3 | 1.60 | 0.029 | $3.45 \times 10^3$ |
| iprovalicarb | carbamic acid, [2-methyl-1-[[[1-(4-methylphenyl) ethyl] amino]carbonyl] propyl]-1-methylethyl ester[c] | 320.4 | 3.18 | 1.00 | 100 |
| 2,4-DB | [U-phenyl-$^{14}$C]-2,4-dichlorophenoxybutyric acid[d] | 249.0 | 3.05[e] | 1.60 | 62.5 |
| $CaCl_2 \times 2H_2O$ | $^{45}CaCl_2$[f] | 111.0 | | 396 | $1.00 \times 10^3$ |

[a] Sigma, Taufkirchen, Germany. [b] log $K_{cuticle/water}$ from Baur (*11*). [c] Bayer, Leverkusen, Germany. [d] International Isotopes, München, Germany. [e] log $K_{cuticle/water}$ from Buchholz (*3*). [f] NEN, Boston, MA.

**Table 2.** Properties of Accelerators

| chemical name | molecular weight (g mol$^{-1}$) | log $K_{cw}$ | log $K_{wax/rec}$ | concentration in wax (g kg$^{-1}$) |
|---|---|---|---|---|
| diethyl suberate (DESU)[a,b] | 230.1 | 1.96[c] | 1.75[d] | variable |
| tributyl phosphate (TBP)[a,b] | 266.3 | 2.54[c] | 2.39[d] | variable |
| diethyleneglycol monododecyl ether ($C_{12}E_2$)[b,e] | 274.5 | | 4.32[d] | $163 \pm 22$ |
| tetraethyleneglycol monododecyl ether ($C_{12}E_4$)[b],e | 362.6 | | 3.90[d] | $123 \pm 9$ |
| hexaethyleneglycol monododecyl ether ($C_{12}E_6$)[b],e | 450.7 | | 3.42[d] | $78 \pm 11$ |
| octaethyleneglycol monododecyl ether ($C_{12}E_8$)[b],e | 538.8 | | 2.98[d] | $51 \pm 11$ |

[a] Merck-Schuchardt, Germany. [b] Purity > 98%. [c] Schönherr et al. (*1*). [d] Simanova et al. (*14*). [e] Fluka, Neu-Ulm, Germany.

nonconstant accelerator concentrations both in the residue on the cuticle and in the cuticle itself. Because accelerator effects are proportional to the concentration (*8*−*10*), their effects vary with time. Hence, both the driving force and accelerator effects are time-dependent, and contributions of accelerators to the solute mobility and driving force cannot be separated quantitatively (*5*, *6*, *11*) when they are simultaneously applied to the cuticle. Some accelerators are surface-active, and in those cases, wetting and plasticizing activities also play a role (*5*, *6*).

Effects of a homologous series of esters of dicarboxylic acids as well as tributyl phosphate on the mobility of 2,4-DB in CMs from *Stephanotis* leaves have been studied recently (*1*, *2*). Their effects on 2,4-DB mobility increased in the order dibutyl sebacate < dibutylsuberate < diethylsebacate < diethylsuberate. Rate constants were linearly related to the accelerator concentration in CM and wax, and when efficacy was related to the concentration in wax, it was about 3 times higher (*2*). Accelerators increase fluidity of amorphous cuticular waxes, and this is the main reason that they increase solute mobility (*5*, *9*). We have tested whether accelerator activity depends on the polarity of solutes by using the lipophilic pesticides metribuzin and iprovalicarb and the hydrophilic compound methyl glucose (MG).

## MATERIALS AND METHODS

**Plant Material.** Astomatous adaxial CMs of mature leaves from greenhouse grown Madagascar jasmine (*Stephanotis floribunda* Brongn.) and field grown pear (*Pyrus communis* L. cv. Conference) were isolated enzymatically (*12*), dried, and stored at 8 °C until used. These CMs were selected as model systems to facilitate comparisons with previous UDOS studies.

**Chemicals.** The following chemicals (source and purity in parentheses) were used: citric acid (>99%, Merck, Darmstadt, Germany), L-(+)-lactic acid (Roth, Karlsruhe, Germany), propane-1,2-diol (>98%, Fluka, Neu-Ulm, Germany), calcium chloride dehydrate (reagent grade, Merck, Darmstadt, Germany), MG (3-*O*-methyl-D-glucopyranose, >98%, Sigma−Aldrich, Germany), and sodium azide (>99%, Merck). All donor solutions contained Plantacare 1200 UP (Fluka, Neu-Ulm, Germany) at 0.2 g L$^{-1}$ as the wetting agent. This is a $C_{12/14}$-polyglycoside (APG) with an average of 1.4 glucose units. Properties of radio-labeled model compounds and accelerators are listed in **Tables 1** and **2**, respectively. With MG and $CaCl_2$, concentrations of 1 g L$^{-1}$ were obtained by mixing radio-labeled solutes with the respective nonradioactive compounds.

**Simulation of Foliar Penetration (SOFU).** This method was used to study effects of accelerators on rates of penetration across CM (*7*). CM were inserted into the desorption chambers with the morphological outer surface facing outward. A 5 $\mu$L droplet of radioactive donor solution containing about 500 Bq radio-labeled solute was applied to the center of the CM. All aqueous donor solutions contained 0.2 g L$^{-1}$ Plantacare 1200 UP as the wetting agent. With metribuzin and iprovalicarb, donor solutions contained 50% ethanol to enhance dissolution. After the solvents (water and ethanol) had evaporated at room temperature (about 1 h), the donor compartment was sealed using Tesafilm (Beiersdorf, Hamburg, Germany). This was done to ensure 100% humidity over the donor residues (radio-labeled solutes and surfactant). Next, 0.6 mL of desorption medium was pipetted into the receiver chambers, and the chambers were placed with the CM facing down into the wells of a thermostated aluminum block (20 ± 1 °C), which was shaken at 80 rpm to keep the desorption medium well mixed. Aqueous receiver solutions were buffered with citric acid adjusted to pH 6.0, and propane-1,2-diol (100 g L$^{-1}$) was added to aid dissolution of DESU and TBP. Various concentrations of DESU or TBP were added to these receiver solutions. This is the same mixture as used previously for studying solute mobility and wax/receiver partition coefficients for DESU and TBP (*1*, *2*). Propane-1,2-diol has no accelerator properties (*2*). With the ethoxylated alcohols (EAs), propane-1,2-diol was omitted because they were sufficiently soluble in aqueous citric acid buffer. Effects of DESU and TBP on solute mobility were studied by adding various concentrations of these accelerators to the desorption media (receiver). The concentrations used in the previous study (*2*) were employed. With monodisperse EAs, concentrations in the receiver were 5 times higher than their critical micelle concentrations (cmc's) (*10*). Desorption media were withdrawn quantitatively in predetermined intervals and replaced by fresh media. After three or four successive samples had been taken, the CMs were cut out and residual radioactivity was extracted with a scintillation cocktail. In a few experiments with pear leaf CM and MG, CMs were not cut out at the end of the experiment. Instead, the residual donor was washed off with three changes of deionized water (300 $\mu$L), and the cocktail was added to the combined washes. Recovery was better than 95%. This

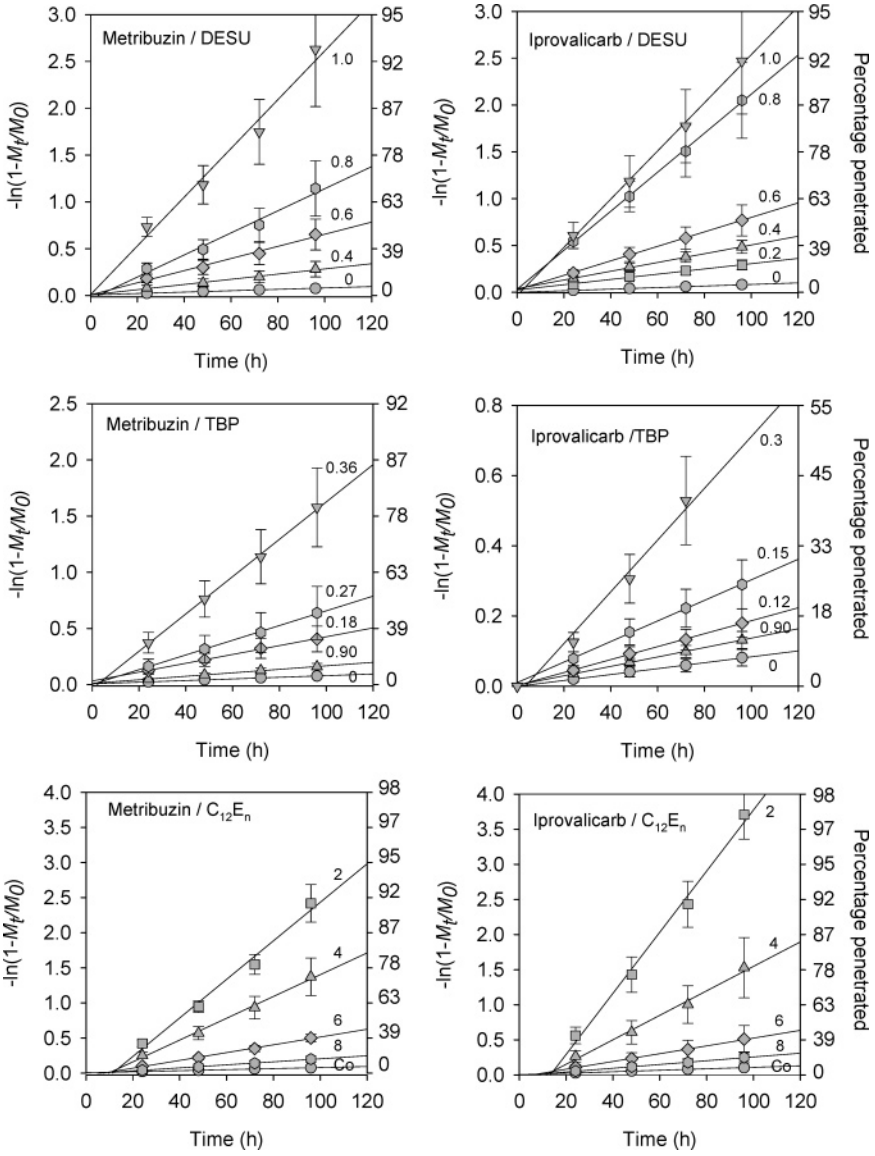


**Figure 1.** SOFU first-order plots showing the time course of penetration of metribuzin and iprovalicarb across *Stephanotis* CM as affected by the accelerators DESU, TBP, and EAs ($C_{12}E_n$). DESU and TBP concentrations (g $L^{-1}$) in the receiver are given in the graphs. With EAs, the numbers in the graphs refer to the number of ethoxy groups (*n*), and the concentrations in wax are given in **Table 2**. Plots show means and 95% confidence intervals.

permitted using the same set of CMs for two or more experiments (paired comparisons). Scintillation cocktail was also added to the desorption media, and radioactivity was determined at a $2\sigma$ error of 3% using a Wallac 1409 Liquid Scintillation Counter (Wallac Oy, Turku, Finland).

Data analysis consisted of plotting $-\ln(1 - M_t/M_0)$ versus *t*, where $M_0$ is the total amount of radioactivity applied to the outer surface of the CM ($t = 0$) and $M_t$ is the amount of radiolabel desorbed from the inner surface at time *t*. These plots are linear, and the slopes of the plots are the first-order rate constants of penetration (*k*). Slopes were calculated for each CM separately, and 30−40 replicate CMs were used. Because the distribution of rate constants is often not normal (*13*), average rate constants were calculated from log-transformed data. Thus, geometric means with 95% confidence intervals will be reported.

**Determination of Partition Coefficients.** Partition coefficients for the system *Stephanotis* wax/aqueous citric acid buffer were determined following the method described by Simanova et al. (*14*). EAs (**Table 2**) were dissolved in citric acid buffer (2 g $L^{-1}$ at pH 6.0) at concentrations above the cmc, and reconstituted *Stephanotis* wax samples were equilibrated in these solutions at 20 °C. The surfactant concentration in the wax was determined by GC, and partition coefficients were calculated using the cmc published by Burghardt et al. (*10*).

## RESULTS

SOFU penetration plots obtained with metribuzin and iprovalicarb are shown in **Figure 1**. Plots of $\ln(1 - M_t/M_0)$ vs time were linear at all accelerator concentrations. This demonstrates that the solute concentration in the residue on the CM decreased exponentially with time; that is, penetration was a first-order process. Linearity is evidence that equilibrium accelerator concentrations in the CM were established rapidly and were constant throughout the experiments. With DESU and TBP, no significant hold-up times (*x*-axis intercepts) were observed as lines intersect with the origin. The slopes of the lines are the first-order rate constants of penetration (*k*). They increased with increasing accelerator concentration (DESU and TBP) in the receiver. With EA ($C_{12}E_n$), the steepest slopes were obtained with $C_{12}E_2$ and slopes decreased with increasing number of ethoxy groups (**Figure 1**).

Permeability of *Stephanotis* CM to MG was very low, and rate constants amounted to only $3.2 \times 10^{-3}$ $h^{-1}$ (**Figure 2**). Variability among individual CMs was very large, as can be seen from the 95% confidence intervals. Penetration plots were

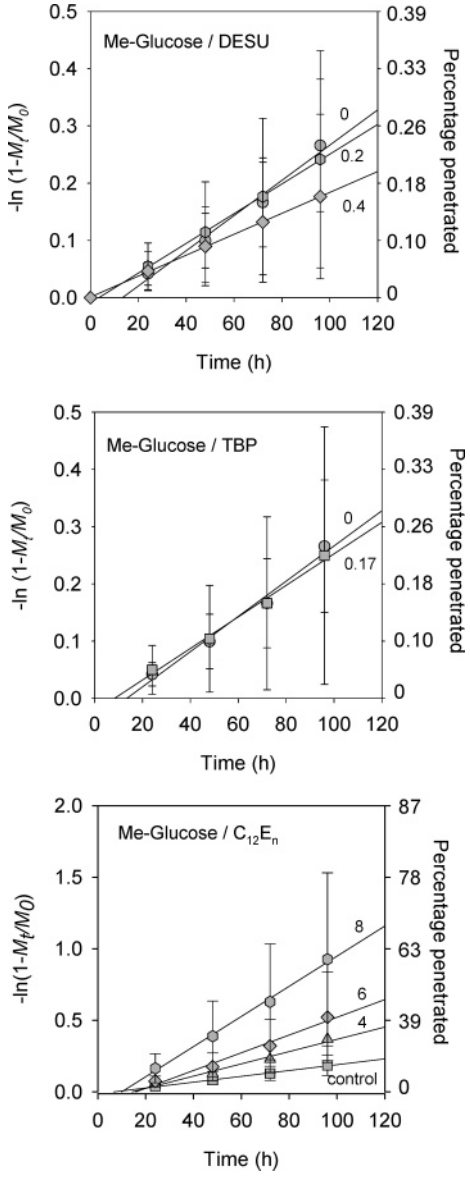


**Figure 2.** SOFU first-order plots showing the time course of penetration of MG across *Stephanotis* CM as affected by the accelerators DESU, TBP, and EAs ($C_{12}E_n$). DESU and TBP concentrations (g $L^{-1}$) in the receiver are given in the graphs. With EAs, the numbers in the graphs refer to the number of ethoxy groups (*n*), and the concentrations in wax are given in **Table 2**. Plots show means and 95% confidence intervals.

linear, and in most cases, significant hold-up times are evident. When compared to the control (no accelerator), DESU and TBP did not significantly increase the slopes of the plots, whereas with EAs, slopes increased moderately with an increasing number of ethoxy groups in contradiction to the effects observed with metribuzin and iprovalicarb (**Figure 1**).

The effects of EA on MG permeability of pear leaf CM were also investigated to test if this unexpected result was a characteristic of *Stephanotis* CM. In the absence of a wetting agent (neither Plantacare 1200 UP nor $C_{12}E_n$), rate constants were only $3.44 \times 10^{-3}$ $h^{-1}$. Adding 0.2 g $L^{-1}$ Plantacare 1200 increased *k* to $22.1 \times 10^{-3}$ $h^{-1}$, which is a factor of 6.4. Adding EAs to the donors containing MG and the APG surfactant further increased rate constants, but the increases were small. The steepest slope of $38.1 \times 10^{-3}$ $h^{-1}$ was obtained with $C_{12}E_8$ (**Figure 3**). Thus, with pear CM, absolute values of the rate constants were higher than with *Stephanotis* but the effects of the EAs were the same.

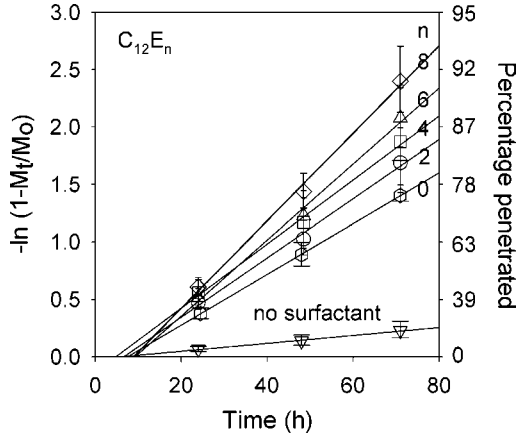


**Figure 3.** SOFU first-order plots showing the time course of penetration of MG across pear leaf CM as affected by EAs ($C_{12}E_n$). With EAs, the numbers in the graphs refer to the number of ethoxy groups (*n*), and the concentrations in wax are given in **Table 2**. In the "no surfactant" control, the donor consisted of MG dissolved in water. All other donor solutions contained 0.2 g $L^{-1}$ Plantacare 1200 UP. In the treatment $n = 0$, the receiver did not contain any EAs. Plots show means and 95% confidence intervals.

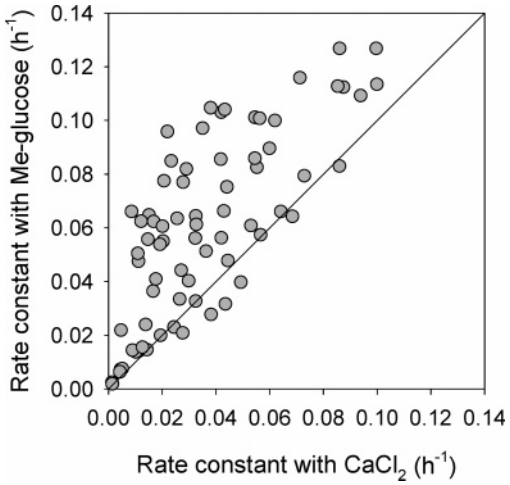


**Figure 4.** Comparison of permeability of 74 pear leaf CMs to MG and $CaCl_2$. With each CM, permeability to $CaCl_2$ was measured first followed by MG (paired comparisons). Solute concentration was 1 g $L^{-1}$.

Permeability of pear leaf CM to nonionic MG and ionic $CaCl_2$ was studied using paired comparisons. At first, permeability was measured using $^{45}CaCl_2$ (1 g $L^{-1}$). After the CM was thoroughly washed with deionized water, the same set of CM was used to measure permeability to MG (1 g $L^{-1}$). Both donors contained 0.2 g $L^{-1}$ Plantacare 1200 UP as the wetting agent. Rate constants measured with MG were either equal or higher than those for $CaCl_2$ (**Figure 4**). The mean *k* obtained with 74 CM amounted to $5.97 \times 10^{-2}$ and $3.59 \times 10^{-2}$ $h^{-1}$ for MG and $CaCl_2$, respectively.

## DISCUSSION

**Validation of Procedures.** The present work was conducted to compare the efficacy of the accelerators DESU, TBP, and EAs in increasing rate constants of penetration of lipophilic (metribuzin and iprovalicarb) and hydrophilic (MG) model compounds. Such a comparison requires that adjuvant effects on partitioning and spreading are absent or constant and concentrations of accelerators in cuticles are constant and known. Any possible effects of accelerators on partitioning were

eliminated by adding the accelerators to the receiver and not to the donor. Constant droplet spreading was ensured by adding the wetting agent Plantacare 1200 UP to all donor solutions. This APG surfactant has no accelerator properties (*2*). This strategy avoided confounding accelerator effects on spreading with effects on solute mobility. This is essential when accelerators are surface-active, as is the case with EA.

Wetting increased rate constants of MG by a factor of 6.4 (**Figure 3**). This is much more than the effect of ethoxylated surfactants, which amounted to only a factor of 1.72 ($k$ was $3.81 \times 10^{-2}$ h$^{-1}$ for $C_{12}E_8$ and $2.21 \times 10^{-2}$ h$^{-1}$ for the surfactant control). If the surfactant-free treatment had served as a control, the accelerator action of EAs would have been confounded with their wetting effects. This had been overlooked in a previous study (*11*). Very large effects of wetting on rate constants of penetration of ionic compounds have been reported before (*15*, *16*).

The desorption media contained propane-1,2-diol to aid dissolution of DESU and TBP. Neither Plantacare 1200 UP nor propane-1,2-diol have accelerator properties, and they had no effect on 2,4-DB mobility (*2*). This is assumed to hold for all nonionic model solutes used. Accelerators were added to the receiver and penetrated into the CM from the inner surface. Equilibration between receiver and CM was rapid. With DESU and TBP, no significant hold-up times were discernible (**Figure 1**). Equilibration with EAs took about 10 h (**Figures 1** and **2**). After the equilibration accelerator concentration was constant throughout the experiment, as with every exchange of receiver solution, the accelerator was replenished. This experimental setup differs from the situation in the field but allows accelerator effects on solute penetration to be studied without interference from wetting, dissolution, or partitioning.

From the specific activity and molecular weights, concentrations in the donor solution can be calculated. They ranged from 9.5 mg L$^{-1}$ (MG) to 3.45 g L$^{-1}$ (metribuzin) (**Table 1**). For the comparison of penetration rates of $CaCl_2$ and MG (**Figure 4**), the concentrations were 1 g L$^{-1}$. Amounts deposited on the outer surface of the CM ranged from 0.05 $\mu$g (MG) to 17.2 $\mu$g (metribuzin). Surfactant coverage was 1 $\mu$g. The mass of CM beneath a droplet area of 0.5 cm$^{-2}$ amounted to about 150 $\mu$g. Hence, the percentage by weight of solutes and surfactants applied ranged from approximately 0.033 (MG) to 11.5 (metribuzin) (**Table 1**). MG was used at concentrations of 9.5 mg L$^{-1}$ (**Figure 2**) or 1 g L$^{-1}$ (**Figure 3**), and there are no indications that coverage or donor concentrations had an influence on accelerator efficacy.

After droplet drying, a concentrated solution containing solutes and surfactant could be seen on the surface of the CM. This residue was in the liquid state and had a shiny appearance. At 100% humidity, the hydroxyl groups of the sugar moieties of Plantacare 1200 UP and MG are hydrated, but the exact concentration of the solutes is not known. However, penetration plots were linear in all instances (**Figures 1**−**3**). This is good evidence that the sole process involved was first-order penetration of the radio-labeled solutes from the donor into the receiver, while other conditions were constant. Dissolution of solutes on the surface of the CM was secured because relative humidity was 100%. At lower humidity, EAs serve as humectants and are needed to dissolve MG (*11*).

**Performance of Accelerators.** Permeability of *Stephanotis* CM was very low. In the absence of accelerators, rate constants ($k$) ranged from $7 \times 10^{-4}$ h$^{-1}$ (iprovalicarb) to $10 \times 10^{-4}$ h$^{-1}$ (metribuzin). This amounts to half times of penetration ($t_{1/2} = -\ln 0.5/k$) of 990 and 693 h, respectively. For the surfactant control, MG half times were 266 and 31 h in *Stephanotis* and pear leaf CM, respectively (**Figures 1** and **2**). For systemic active ingredients, these half times would be far too long, and the need for shortening them through the application of accelerators is obvious.

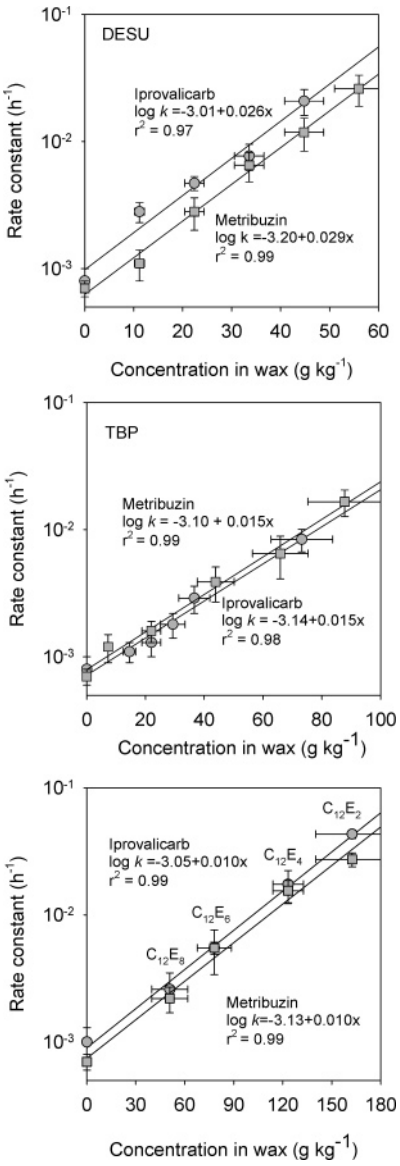


**Figure 5.** Rate constants of penetration of metribuzin and iprovalicarb as affected by the accelerator concentration in *Stephanotis* wax. DESU and TBP concentrations in wax were calculated from partition coefficients (**Table 2**) and receiver concentrations (**Figure 2**). EA concentrations in wax were taken from **Table 2**.

**Lipophilic Solutes Metribuzin and Iprovalicarb.** When the logarithms of the rate constants (log $k$) were plotted against the accelerator concentrations in wax (**Figure 5**), linear plots were obtained for metribuzin and iprovalicarb with all three accelerators [DESU, TBP, and the EA ($C_{12}E_n$)]. Concentration in the wax is the product of the partition coefficient and the accelerator concentration in the receiver. Partition coefficients for EA, DESU, and TBP were taken from the literature (*1*, *2*, *14*) and from (**Table 2**). The simple equation

$$\log k = bC_{aw} - a \qquad (1)$$

accounts for accelerator effects on mobility of lipophilic solutes, where $k$ is the rate constant (h$^{-1}$), $b$ is the regression coefficient,

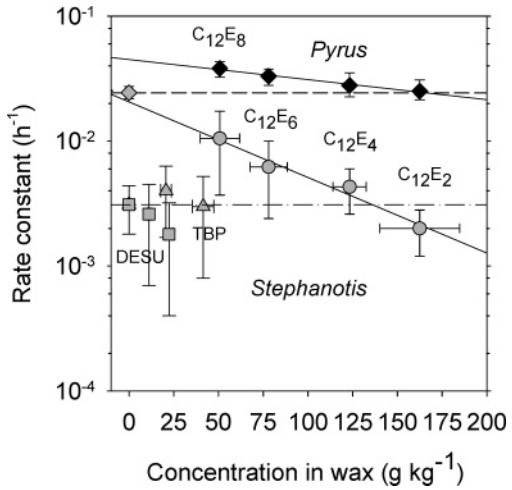


**Figure 6.** Rate constants of penetration of MG as affected by the accelerator concentration in *Stephanotis* and pear leaf wax. DESU and TBP concentrations in wax were calculated from partition coefficients (**Table 2**) and receiver concentrations (**Figure 2**). EA concentrations in wax were taken from **Table 2**. Data for pear CM were plotted using EA concentrations in *Stephanotis* wax.

$C_{aw}$ is the accelerator concentration in wax (g kg$^{-1}$), and $a$ is a constant. Accelerator concentration in CM might have been used, but in a previous study (*2*), it was shown that accelerator effects are larger when referred to waxes because waxes constitute the major barrier to diffusion in cuticles (*7*, *9*).

The constants $a$ of eq 1 are the $y$ intercepts of the regression equations shown in **Figure 5**. They ranged from 3.01 to 3.20 (log scale) with an average of 3.11 ± 0.07. The average rate constant of the two solutes in the absence of an accelerator amounted to $7.76 \times 10^{-4}$ h$^{-1}$. The slopes of the regression plots ($b$) are measures of the intrinsic activity of the different accelerators. Slopes did not significantly differ among the two model solutes, but they decreased in the order DESU (0.028), TBP (0.015), and C12En (0.010). The intrinsic activity of DESU was higher than that of TBP and EAs by factors of 1.9 and 2.9, respectively. Using these slopes and an average $y$ intercept, rate constants in the presence of 50 g kg$^{-1}$ accelerator can be estimated as $21.8 \times 10^{-3}$ h$^{-1}$ (DESU), $4.36 \times 10^{-3}$ h$^{-1}$ (TBP), and $2.45 \times 10^{-3}$ h$^{-1}$ ($C_{12}E_n$), respectively. For the accelerator efficacy, defined as the ratio of rate constants in the presence or absence of the accelerator, values of 28.1 (DESU), 5.6 (TBP), and 3.16 ($C_{12}E_n$) are obtained. Thus, at a given accelerator concentration in wax, DESU was most effective, because it increased rate constants 8.9 times more strongly than $C_{12}E_n$ and 5.1 times more strongly than TBP. We conclude that neither intrinsic activity nor efficacy of accelerators depended on molecular weights and partition coefficients of model compounds within the range of values used here (**Table 1**). For a given plant species, intrinsic activity is a property of the accelerators only. It shows the extent to which fluidity of the amorphous wax fraction (which varies with plant species) can be increased by the sorbed accelerator.

**Hydrophilic MG.** A different picture evolved with the highly water-soluble compound MG (**Figure 6**). Neither DESU nor TBP increased rate constants in *Stephanotis* CM. EAs increased rate constants slightly, both in *Stephanotis* and pear leaf CM, provided that they had more than four ethylene oxide units. Of the EAs tested, $C_{12}E_2$ was the most lipophilic accelerator. Partition coefficients decrease with an increasing number of ethoxy groups, but even $C_{12}E_8$ was more lipophilic and had a higher partition coefficient than DESU and TBP (**Table 2**). The effects of EA increase with an increasing number of ethoxy groups and a decreasing concentration in wax (**Figure 6**). This is exactly the opposite of what was observed with lipophilic solutes (**Figure 5**).

MG is much more soluble in water than in cuticles (**Table 1**), but as a nonelectrolyte, it can enter into and diffuse within lipid phases. The cuticle/water partition coefficient is 0.13 (*11*); i.e., the concentration in water is about 7.7 times higher than in cuticles. A wax/water partition coefficient is not available, but its value might be considerably lower because the octanol/water partition coefficient is only $1 \times 10^{-3}$ (*17*). Aqueous pores traverse some cuticles, albeit to different degrees (*15*). These pores are accessible to polar solutes and ions with molecular weights of less than 500 g mol$^{-1}$ (*4*). Therefore, MG can diffuse across these pores. Paired comparisons of rate constants measured for $CaCl_2$ and MG revealed that with some CMs both rate constants were very similar, while with others, rate constants were higher for MG (**Figure 4**). When rate constants for MG are higher than for $CaCl_2$, an alternative diffusion path must have been available for MG, which hydrated ionic $CaCl_2$ could not access. The contributions of the two pathways can be estimated if it is assumed that rate constants are additive. If rate constants measured with MG ($k$) are the sum of the aqueous ($k_{aq}$) and the alternative ($k_{alt}$) pathways

$$k = k_{aq} + k_{alt} \qquad (2)$$

the contribution of the alternative pathway to total flux of MG can be calculated by subtracting the rate constant measured with $CaCl_2$ from that obtained with MG. For the data shown in **Figure 4**, this would be 0.060 h$^{-1}$ − 0.036 h$^{-1}$ = 0.024 h$^{-1}$, which suggests that 40% of MG used the alternative pathway, while 60% diffused across the aqueous pathway. These are average figures for 74 CMs. The calculation could have been performed for each individual CM, but for some CMs, $k$ was smaller than $k_{aq}$ (**Figure 4**). This is physically impossible. We suggest that it results from the fact that the molecular weight of MG is 1.75 times larger than that of $CaCl_2$, hence hindrance of MG in aqueous pores is larger. Size selectivity of pores in pear leaf CM is not known, and a correction cannot be made at the present time. With poplar CM, $k_{aq}$ for MG would have been only 57% of that measured with $CaCl_2$ (*4*).

These considerations may account for the fact that accelerator effects on rate constants of highly polar solutes are small. With ionic solutes, surfactants increased rate constants by improved wetting but accelerator effects were absent (*14*, *16*). This is to be expected because accelerators increase fluidity of amorphous waxes but hydrated ionic solutes are excluded from the waxy or lipophilic pathway, while polar nonelectrolytes (which can shed their hydration shells) can access them.

Permeability of aqueous pores in CM decreases with decreasing humidity (*15*, *16*). This is probably a consequence of deswelling. With decreasing humidity, the contribution of the aqueous pathway decreases and might even become insignificant, causing the alternative pathway to dominate penetration. In fact, it has been shown that surfactants can sometimes increase rates of penetration of polar solutes (*5*, *6*, *11*, *17*). However, neither DESU nor TBP increased rate constants for MG and *Stephanotis* CM (**Figure 6**) nor were they affected by highly lipophilic EAs with 2 or 4 ethoxy groups. Small but significant increases were observed only with EAs having 6 or 8 ethoxy groups, and these accelerators had much lower concentrations in *Stephanotis* wax than the more lipophilic homologues (**Table 2** and **Figure 6**).

The ineffectiveness of TBP and DESU suggests that MG did not diffuse along the same paths used by metribuzin and iprovalicarb. The partition coefficient given in **Table 1** for MG is not the wax/water but the cuticle/water partition coefficient. A small amount of MG may have been dissolved in the wax. CMs also contain polar domains made up of polysaccharides and cutin, which sorb water and affect the partition coefficient. Hence, solubility of MG in amorphous waxes might be much lower than suggested by the partition coefficient given in **Table 1**. Extremely low concentrations of MG in amorphous wax domains could account for the ineffectiveness of DESU and TBP. This leaves the question as to the nature of the alternative pathway for MG, besides aqueous pores and random diffusion through waxy domains. Is there a third pathway in cuticles? Possibly, sorption of $C_{12}E_6$ and $C_{12}E_8$ in amorphous waxes is not random. These amphiphilic compounds might be oriented in waxes such that the dodecanol moieties are in contact with waxes, while ethylene oxide chains form a continuous polar phase in which MG can diffuse. It requires further attention.

## ABBREVIATIONS USED

a.i., active ingredient; APG, alkyl polyglycoside; CM, cuticular membrane; cmc, critical micelle concentration; EA, ethoxylated alcohol; DESU, diethyl suberate; MG, methyl glucose; SOFU, simulation of foliar uptake; TBP, tributyl phosphate; UDOS, unilateral desorption from the outer surface.

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