Arch. Environ. Contam. Toxicol. 17, 13–19 (1988)

Archives of Environmental Contamination and Toxicology

Desorption of Chemicals from Plant Cuticles: Evidence for Asymmetry

Jörg Schönherr and Markus Riederer

Institut für Botanik und Mikrobiologie, Technische Universität München Arcisstraße 21, D-8000 München 2, Federal Republic of Germany

Abstract. Isolated cuticular and polymer matrix membranes from four plant species (*Citrus, Ficus, Lycopersicon, Capsicum*) were preloaded with ^{14}C-(2,4-dichlorophenoxy)acetic acid (2,4-D) by sorption from solutions and subsequently subjected to simultaneous bilateral desorption. With cuticular membranes, only 2 to 3% of the 2,4-D initially contained in the cuticles could be desorbed from the outer surface, while 86 to 92% were desorbed from the inner surfaces within 6 hr. Initial desorption rates where 50 to 80 times higher from the inner surfaces than from the outer surfaces. This asymmetrical desorption is mainly due to the presence of apolar and crystalline soluble lipids (waxes) on the surface and in the outer layers of the cuticles. These constituents drastically decrease the mobility of 2,4-D in the outer layers of of the leaf cuticles. In fruit cuticles, extensive cutinization of anticlinal and periclinal walls increases the inner surface area and thus contributes significantly to asymmetry. The fate of chemicals sorbed in cuticles depends on the presence or absence of a sink. If a sink exists (metabolization, translocation away from the epidermis) all cuticles will exhibit a pronounced inward permeability and accumulation in the cuticles is not likely to occur. Accumulation in the cuticles will occur only in absence of a sink. Ecotoxicological implications such as monitoring environmental pollution history by analyzing contents of sorbed chemicals in cuticles and detoxification by leaching are discussed.

Airborne pollutants which are intercepted by leaf surfaces will be sorbed in the cuticle. Equilibrium sorption can be predicted quantitatively from the octanol/water partition coefficients of the chemicals (Shafer and Schönherr 1985; Kerler and Schönherr 1987a). Equilibrium is not established instantaneously, because cuticles are of finite thickness and mobility of chemicals in cuticles is much lower than in water (Riederer and Schönherr 1985).

The fate of the chemicals once sorbed in the cuticles from the environment is of great ecotoxicological importance. Some compounds may be bound covalently to epoxide groups of the cuticles (Riederer and Schönherr 1986a). These will be immobile and no longer subject to redistribution. All other compounds that are only sorbed in the cuticle are mobile and can diffuse out of the cuticle, depending on the directions and the magnitudes of the driving forces.

Pollutants may be lost from cuticles to the environment either by volatization or they may be washed out by rain, mist or fog. Alternatively, they may diffuse into the cell walls and cells. If driving forces of equal magnitudes act simultaneously on the outer and inner surfaces of the cuticle one might expect that equal amounts leave the cuticle through the outer and inner surfaces, provided that cuticles are chemically and structurally homogeneous. However, there is ample evidence, that this is not the case (Holloway 1982). Unequal effluxes and influxes are therefore to be expected even if equal driving forces act on both surfaces of the cuticle. The magnitude of the asymmetry is of practical importance as it determines the success of removing pollutants from cuticles by washing and leaching.

Materials and Methods

Membranes

Cuticles were isolated enzymatically from mature leaves of *Citrus aurantium* L. and *Ficus elastica* Roxb. var *decora* and from ripe tomato (*Lycopersicon esculentum* Mill.) and green

pepper (*Capsicum annuum* L.) fruits. The procedures described earlier were followed (Schönherr and Riederer 1986). Membrane structure and integrity are not adversely affected by the isolation procedures.

The fruit cuticles have no stomata and from the leaf cuticles only the upper, astomatous cuticles were used. Isolated cuticles will be referred to as cuticular membranes (CM). A portion of the cuticular membranes was exhaustively extracted with chloroform in a Soxhlet apparatus to remove soluble cuticular lipids. These extracted cuticular membranes will be called polymer matrix (MX) membranes. To eliminate covalent binding activity, cuticular membranes and MX-membranes (from *Ficus* and *Capsicum*) were treated with 1M HCl for 24 hr (Riederer and Schönherr 1986a).

Sorption

Cuticular membranes and polymer matrix membranes were equilibrated with aqueous solutions of (2,4-dichlorophenoxy)-[2-^{14}C]lacetic acid (specific activity 1.04 TBq/mol, radiochemical purity >97%, Amersham Buchler) for 8 days. During incubation, the solutions were kept in a thermostated waterbath (25°C) and were slightly agitated. The solutions were buffered (0.01M citric acid) at pH 3.0 and they contained NaN_3 (0.001M) to prevent growth of microorganisms.

Membranes loaded with 2,4-D were finally picked up from the radioactive solutions on pieces of PTFE (polytetrafluoroethylene) and excess solution was blown off with pressurized air. After air drying, the membranes were stored for four weeks at room temperature before they were used in desorption studies. Since the cuticles had been pretreated with HCl to eliminate epxoy groups, the 2,4-D sorbed in the cuticles could not be bound covalently during storage. Other reactions that might modify 2,4-D sorbed in cuticles during storage in dry air have not been observed.

Desorption

To measure simultaneous bilateral desorption, the membranes were inserted in the transport apparatus described earlier (Kerler *et al.* 1984). The apparatus was thermostated at 25°C and the solutions facing the outer and inner surfaces of the membranes were stirred at 1200 rpm. Desorption was started by adding 0.8 ml borax buffer (0.01M, pH 9.18) simultaneously to the outer and inner chambers of the apparatus. The solutions were withdrawn quantitatively at predetermined intervals (1, 3, 7, 15, 30, 60, 120, 240, 360 min.) and immediately replaced by fresh, non-radioactive borax buffer. The radioactivity of the samples was determined by scintillation counting with a 2 σ error of 1%.

At the end of each experiment, the half-cells of the transport apparatus were carefully separated, the portion of the membrane that had been exposed to the borax buffers was cut out and its residual radioactivity was determined by scintillation counting.

The total radioactivity initially contained in the membrane area subjected to desorption (0.38 cm^2) was calculated as the sum of the radioactivities of the desorption solutions on both sides and the residual radioactivity in the membrane. This sum will be denoted as M_∞. The radioactivity desorbed from the membrane at a given time will be denoted as M_t. The concentration of 2,4-D initially contained in the membranes ranged from 5 to 20 $\times$ 10^{-7} mol/kg. Within this narrow range of concentrations the desorption curves (Figure 1) are essentially independent of concentration and results are therefore given as fractional desorption, that is M_t/M_∞. For example, $M_t/M_\infty = 0.5$ means, that 50% of the 2,4-D initially contained in the cuticles have been desorbed.

Scanning Electron Microscopy

To get an impression of the inner surfaces (cell wall side) of the membranes, pieces of cuticular membranes from each species were mounted upside down on aluminium stubs, sputter coated with gold/paladium and viewed with a Jeol 35C scanning electron microscope (secondary electron mode) using an accelerating voltage of 25 kV.

Results

All desorption curves show an initial steep rise during the first few minutes followed by a leveling off at about 1 hr (Figure 1). Desorption continues at a much slower rate from the inner surfaces of the membranes, while very little additional 2,4-D was desorbed from the outer surfaces of the cuticular membranes. This leveling off is due to the rapid decrease in 2,4-D concentration in the membranes. After only 15 min., 44 (*Capsicum*) to 66% (*Lycopersicon*) of the initial amount of 2,4-D had already diffused out of the cuticular membranes (Table 1), most of it through the inner surfaces (Figure 1). After 60 min, total desorption ranged from 59% (*Ficus*) to 82% (*Lycopersicon*) and after 6 hr only 5% of the initial amount of 2,4-D was left in *Citrus* and *Lycopersicon* cuticular membrane, while 28% and 30% were left in the cuticular membrane of *Ficus* and *Capsicum*, respectively (Table 2).

Desorption from MX-membranes followed the same pattern as in cuticular membranes but desorption from the outer surface proceeded much more rapidly compared to the cuticular membranes (Figure 1). From cuticular membranes only 3 (*Ficus*) to 6% (*Citrus*) were desorbed through the outer surface, while from MX-membranes 12 (*Capsicum*) to 25% (*Ficus*) of the initial amount of 2,4-D were desorbed (Figure 1). Since with MX-membranes desorption rates from the outer surfaces were higher, less was desorbed from the inner surfaces, when compared to the cuticular membranes. The total amounts desorbed from the inner and outer surfaces of the MX-membranes were 97% (*Lycopersicon*), 97% (*Ficus*), 93% (*Citrus*) and 72% (*Capsicum*), respectively (Table 2).

Scanning electron micrographs of the inner sur-

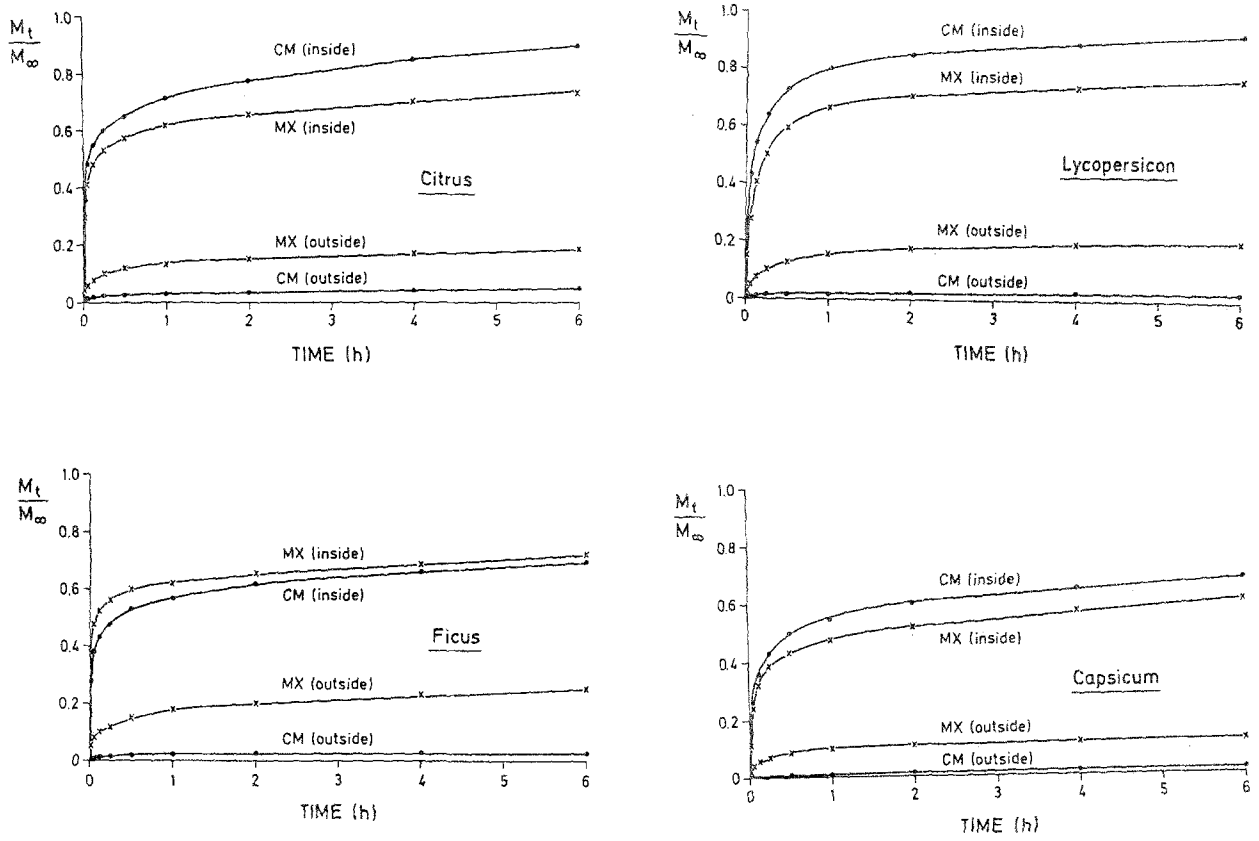

Fig. 1. Time courses of simultaneous bilateral desorption of 2,4-D from cuticular membranes (CM) and polymer matrix-membranes (MX). Data are averages of 5 membranes each. Error bars were omitted for clarity. Standard deviations are given in Tables 1 to 3

Table 1. Initial fractional desorption of 2,4-D from cuticular membranes[a]

Time min.	Species			
	Citrus M_t/M_∞	*Ficus* M_t/M_∞	Lycopersicon M_t/M_∞	Capsicum M_t/M_∞
3	0.493 (0.063)	0.390 (0.041)	0.440 (0.062)	0.262 (0.040)
15	0.620 (0.081)	0.499 (0.035)	0.655 (0.061)	0.439 (0.025)
60	0.746 (0.059)	0.588 (0.034)	0.824 (0.012)	0.654 (0.033)

[a] Total desorption from the outer and inner surfaces of 5 cuticular membranes each. Standard deviations given in parenthesis. M_t is the amount of 2,4-D desorbed at a given time t. M_∞ is the total amount of 2,4-D initially contained in the cuticle, when t = 0

faces of cuticular membranes show the characteristic anticlinal ledges generally observed with isolated leaf cuticles (Holloway 1982). The fruit cuticles, however, exhibited a pronounced cutinization even of periclinal and anticlinal walls of subepidermal cell layers. This gives them a spongy appearance and causes a drastic increase in the inner surface area (Figure 2).

Discussion

In a homogeneous membrane, the shape of a desorption curve depends only on the thickness of the membrane (l) and the diffusion coefficient (D) of the species in the membrane (Crank 1975). When desorption proceeds on both sides simultaneously, identical curves will be obtained for both sides, which approach $M_t/M_\infty = 0.50$ as a limiting value. With cuticular membranes, desorption is much faster from the inner surface and it must be concluded, that cuticular membranes are heterogeneous. To a lesser degree this is also true for polymer matrix membranes (Figure 1).

Heterogeneity is pronounced. After 6 hr, 68% (*Capsicum*) to 92% (*Lycopersicon* of the total amount of 2,4-D were desorbed from the inner sur-

Table 2. Total fractional desorption of 2,4-D from the inner and outer surfaces of cuticular membranes and polymer matrix membranes[a]

	Fraction desorbed (M_t/M_∞) from			
	Cuticular membranes		Polymer matrix membranes	
Species	Inner surface	Outer surface	Inner surface	Outer surface
Citrus	0.90 (0.05)	0.05 (0.02)	0.74 (0.03)	0.19 (0.03)
Ficus	0.69 (0.01)	0.03 (0.01)	0.71 (0.05)	0.26 (0.05)
Lycopersicon	0.92 (0.09)	0.03 (0.01)	0.76 (0.02)	0.21 (0.02)
Capsicum	0.68 (0.07)	0.02 (0.01)	0.60 (0.04)	0.12 (0.02)

[a] Average desorption after 6 hr from 5 membranes each, standard deviations given in parenthesis. M_t is the amount of 2,4-D desorbed after 6 hr. M_∞ is the total amount of 2,4-D initially contained in the cuticle, when $t = 0$

faces of cuticular membranes, while only 2 (*Capsicum*) to 5% (*Citrus*) were desorbed from the outer surfaces (Table 2). With polymer matrix membranes, 12 (*Capsicum*) to 26% (*Ficus*) were desorbed from the outer surfaces and desorption from the inner surfaces was reduced, except for *Ficus*, where the total amount desorbed increased strongly as a consequence of extraction of soluble cuticular lipids (Table 2). In the other three species total desorption after 6 hr was similar in cuticular membranes and MX-membranes.

The data of Figure 1 solely reflect the properties of the membranes studied. 2,4-D has a pK_a value of 2.78 (Rippen 1984) and since the desorption medium had a pH of 9.18 the concentration of non-ionized species of 2,4-D in the outer media was always essentially zero, even though the volumes of the receiver compartments were small. Only non-ionized 2,4-D molecules are sorbed in the lipophilic regions of the cuticles where they reside in an essentially nonaqueous environment (Riederer and Schönherr 1984, 1986b). In this environment, they remain undissociated, even though the cuticles are submerged in a buffer of pH 9.18.

Desorption curves identical to those observed here would also have been obtained with water as desorption medium. In this case, however, it would have been necessary to use much larger receiver volumes and to make frequent changes of the receiver solutions, in order to keep the 2,4-D concentration practically zero, otherwise efflux from the cuticles would be limited by solubility of 2,4-D in water and by an increasing 2,4-D concentration. These considerations show, that our experimental set-up mimics a situation, where a leaf is rinsed with rain that contains no 2,4-D and where 2,4-D molecules in the cell walls are quickly removed by uptake into cells followed by metabolization. In this case, the magnitudes of the two effluxes will be determined exclusively by the properties of the cuticles.

The initial efflux rates represent the properties of the boundary layers of the cuticles and the ratio of the initial slopes of the desorption curves are a measure of asymmetry. Asymmetry factors of cuticular membranes ranged from around 50 (*Citrus, Ficus, Lycopersicon*) to 80 (*Capsicum*) (Table 3). This means, that in the first few minutes 50 to 80 times more 2,4-D leaves the cuticle through its inner surface than through its outer surface. During the first three minutes 26 to 50% of the total amount sorbed in the cuticular membranes were already desorbed (Table 1).

Asymmetry factors for the polymer matrix membranes range from 6 to 7. Thus, by about a factor of 10, they are smaller than those for the cuticular membranes. This demonstrates that soluble cuticular lipids play a major role in asymmetry, but they are by no means the sole determinant. Extracted cuticles still exhibit a pronounced asymmetry (Figure 1, Table 3).

On the basis of the above data, a simple model of cuticles, that includes heterogeneity explicitly, can be developed (Figure 3). Cuticular membranes are composed of an outer and an inner volume element (V_o and V_i). Each volume element is characterized by its thickness (l), its partition coefficient (K) and its diffusion coefficient (D). The inner surface area (A_i) is much larger than the outer surface area (A_o) by virtue of the pronounced cutinization of anticlinal (*Citrus, Ficus*) and even periclinal walls (*Lycopersicon, Capsicum*).

Unfortunately, no values can be assigned to these eight variables. To make things worse, the model is most certainly an oversimplification, for it is unlikely that there is a sharp boundary between the inner and outer volume elements. Furthermore, it is known that partition coefficients depend on concentration (Riederer and Schönherr 1984, 1986b; Shafer and Schönherr 1985) and this may also apply to diffusion coefficients. A quantitative analysis of the data of Figure 1 is therefore not possible at this time. Only a qualitative discussion can be attempted.

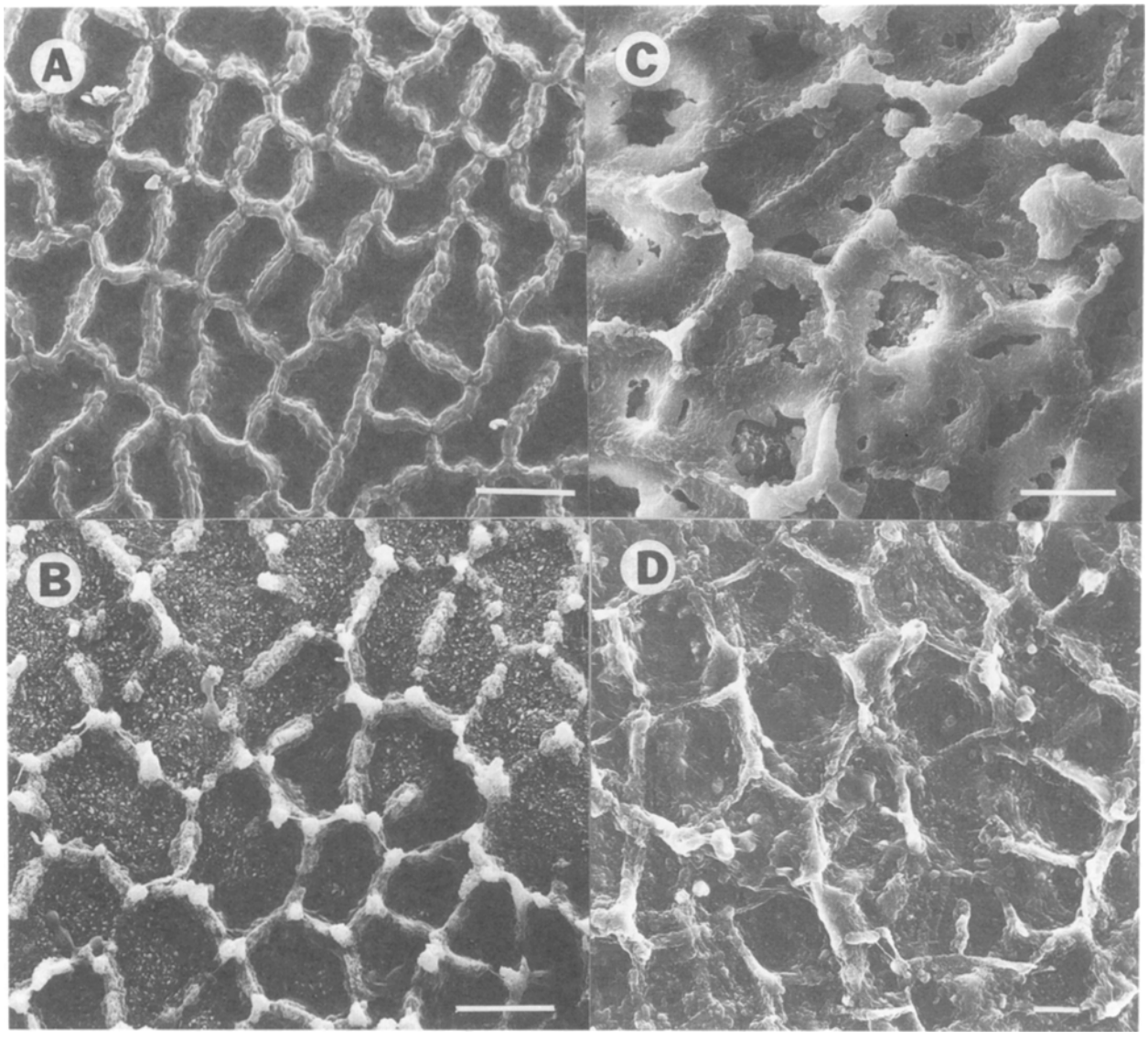

Fig. 2. Scanning electron micrographs of the inner surfaces of isolated cuticular membranes form *Citrus* (A), *Ficus* (B), *Lycopersicon* (C) and *Capsicum* (D). Bars at the lower right corners represent 20 μm

The cuticular membranes of the four species tested have an inner volume element that is characterized by high sorption capacitance and high mobility of 2,4-D. High sorption capacitance is due to high partition coefficients and to the fact that the inner volume element is much larger than the outer one ($l_i \gg 1_o$). The outer volume element of cuticular membranes is thin and has a low sorption capacitance. This can, at least in part, be attributed to the presence of soluble cuticular lipids, which ar both non-polar and crystalline. For these reasons, the mobility of 2,4-D in the outer volume element is low.

While it is clear that the presence of soluble cuticular lipids in the outer volume element is responsible for the transport resistance both in desorption studies and in transmembrane diffusion (Riederer and Schönherr 1985; Kerler and Schönherr 1987b) the individual contributions of epicuticular and intracuticular soluble lipids cannot be distinguished. We did not succeed in selectively removing surface lipids without simultaneously affecting amounts and structure of embedded lipids.

With the leaf cuticular membranes, asymmetrical desorption is probably mainly a consequence of large differences in mobility (t_e) of 2,4-D in the outer and inner volume elements. Extraction of soluble lipids increased mobility of 2,4-D in leaf cu-

Table 3. Parameters describing the transport of 2,4-D across cuticular membranes and polymer matrix membranes from various plant species

Species		Mass/area[a] g/m²	Asymmetry[a] factor	Permeance m/s	P(MX)/P(CM)	t_e(CM)/t_e(MX)
Citrus	CM	2.61 (0.30)	55 (25)	2.8×10^{-10}		53
	MX	2.77 (0.35)	6.7 (1.0)		1767	
Ficus	CM	6.71 (0.48)	49 (13)	1.0×10^{-10}		321
	MX	5.25 (0.80)	6.9 (0.6)		9192	
Lycopersicon	CM	16.0 (1.81)	56 (9)	2.6×10^{-8}		1.5
	MX	22.3 (1.90)	5.6 (1.2)		29	
Capsicum	CM	21.9 (5.13)	80 (14)	2.7×10^{-8}		3.2
	MX	25.6 (2.14)	6.2 (1.6)		46	

[a] Mass per unit area and asymmetry factors are averages from 5 cuticular membranes (CM) and 5 polymer matrix membranes (MX), respectively. Standard deviations are given in parenthesis. The asymmetry factor is the ratio of the initial (up to 3 min.) desorption rates from the inner and outer surfaces of the membranes. Permeances (P) and extrapolated hold-up times (t_e) were taken from Riederer and Schönherr (1985). P(MX)/P(CM) is the ratio of the permeances of polymer matrix and cuticular membranes, respectively. t_e(CM)/t_e(MX) is the ratio of the extrapolated hold-up times observed with cuticular membranes and polymer matrix membranes, respectively

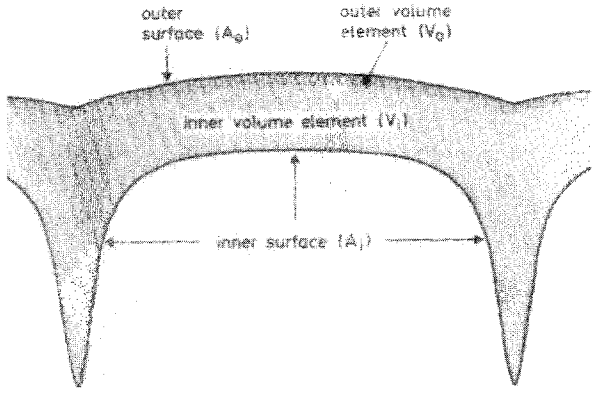

Fig. 3. Schematic drawing of a cross-section of a leaf cuticular membrane (not to scale)

ticles by 53- to 321-fold (Table 3) while it had little effect in fruit cuticular membranes. The presence of soluble lipids in the outer volume elements imparts to them a high transport resistance and a low partition coefficient (Riederer and Schönherr 1984, 1985) and most 2,4-D molecules, therefore, leave the cuticular membranes through the inner surfaces.

Extensive cutinization of the cell walls (Figure 2) increases the inner surface of cuticles, especially with the fruit cuticles studied. Desorption from the inner surface, therefore, proceeds from a much larger surface area as compared to the outer surface. This fact is probably responsible for the high asymmetry factors of *Capsicum* and *Lycopersicon* cuticular membranes. The relatively high permeances of fruit cuticular membranes as compared to leaf cuticular membranes (Table 3) and the relatively small effect of extraction of soluble lipids on permeance indicate that the contents of soluble cuticular lipids cannot be the sole factor contributing to the high degree of asymmetry observed for fruit cuticular membranes.

Even though asymmetrical desorption cannot be explained quantitatively in terms of K, D and l of the inner and outer volume elements, asymmetry is a fact and has important ecotoxicological consequences. Cuticular membranes consist of a thin outer skin, which is the transport limiting barrier. Underneath this skin is the bulk of the cuticle and this inner volume element has a high sorption capacitance and the mobility of sorbed molecules may be up to 50 times larger than in the outer skin. If a sink exists in a leaf or fruit, 2,4-D molecules will move out of the inner volume element at a much faster rate, than the outer skin can be penetrated. There will be no accumulation of 2,4-D in cuticles as long as there is a sink (sorption in membrane and storage lipids, metabolization, conjugation etc.), and permeances of the outer volume elements are lower than the rates of removal into sinks.

The range of permeances measured for cuticular membranes of various plant species is 1 to 270 $\times$ 10^{-10} m/s (Riederer and Schönherr 1985). The time needed for 5% of a solute to penetrate from a droplet (the donor) through the outer volume element into a semi-infinite receiver (the inner volume element of the cuticle and the adjacent cells) can be calculated from eq. (1). According to Hartley and Graham-Bryce (1980)

$$-PAt/V_{donor} = \ln C_{donor}/C_o. \quad (1)$$

Given a hemispherical droplet of 10 μl (V_{donor}) having a contact area (A) with the cuticle, the time needed for a 5% decrease in the donor concentration (that is $C_{donor}/C_o = 0.95$) ranges from 160 hr to 35.5 min for the range of permeances given above. This example shows, that penetration through plant cuticles is a slow process when compared to transport across cellular membranes and penetration across the outer layer of cuticles will likely be the rate limiting step with most compounds, rather than uptake into cells and organelles.

Accumulation in cuticles lacking epoxy groups will occur only, if the compound is not removed from the inner volume element because there is no sink. Thus, attempts to monitor the history of environmental pollution by analyzing the amounts of pollutants sorbed in the cuticles (Buckley 1982) can only be successful if compounds are selected that are not metabolized. All others will not be found (or at least only in very low concentrations), because the rate of entry into the inner volume element through the transport limiting skin is much lower than the rate of efflux into a sink. The cuticle will appear empty, even though the atmosphere might have been heavily polluted.

For a pollutant to be phytotoxic it must be present in cells or organelles or participate in metabolism somehow. This means, there will be a sink for it and accumulation in the cuticles is therefore unlikely. Thus, compounds found in cuticles in high concentrations are probably not phytotoxic.

Compounds that accumulate in cuticles may not be phytotoxic but they may be toxic to other organisms, including man. The sorption compartment of cuticles is the inner volume element. The transport limiting layer is the outer volume element. Washing a leaf or a fruit will remove little or nothing of a compound sorbed in the inner volume element, if the permeance of the outer skin is low.

The permeability of cuticular membranes increases with increasing partition coefficient (Kerler and Schönherr 1987b). The dependence on partition coefficients of asymmetry is currently being investigated.

References

Buckley EH (1982) Accumulation of airborne polychlorinated biphenyls in foliage. Science 216:520–522

Crank J (1975) The mathematics of diffusion. Oxford University Press, London

Hartley GS, Graham-Bryce IJ (1980) Physical principles of pesticide behaviour. Academic Press, London

Holloway PJ (1982) Structure and histochemistry of plant cuticular membranes: an overview. In: Cutler DF, Alvin KL, Price CE (eds) The plant cuticle. Academic Press, London, pp 1–32

Kerler F, Riederer M, Schönherr J (1984) Non-electrolyte permeability of plant cuticles: A critical evaluation of experimental methods. Physiol Plant 62:599–606

Kerler F, Schönherr J (1987a) Accumulation of lipophilic chemicals in plant cuticles: Prediction from octanol/water partition coefficients. Arch Environ Contam Toxicol 17:1–6

——— (1987b) Permeation of lipophilic chemicals across plant cuticles: Prediction from octanol/water partition coefficients and molar volumes. Arch Environ Contam Toxicol 17:7–12

Riederer M, Schönherr J (1984) Accumulation and transport of (2,4-dichlorophenoxy)acetic acid in plant cuticles: I. Sorption in the cuticular membrane and its components. Ecotoxicol Environ Safety 8:236–247

——— (1985) Accumulation and transport of (2,4-dichlorophenoxy)acetic acid in plant cuticles: II. Permeability of the cuticular membrane. Ecotoxicol Environ Safety 9:196–208

——— (1986a) Covalent binding of chlorophenoxyacetic acids to plant cuticles. Arch Environ Contam Toxicol 15:97–105

——— (1986b) Thermodynamic analysis of nonelectrolyte sorption in plant cuticles: The effects of concentration and temperature on sorption of 4-nitrophenol. Planta 169:69–80

Rippen G (1984) Handbuch der Umweltchemikalien. Ecomed Verlagsgesellschaft, Landsberg/Lech

Schönherr J, Riederer M (1986) Plant cuticles sorb lipophilic compounds during enzymatic isolation. Plant Cell Environ 9:459–466

Schönherr J, Kerler F, Riederer M (1984) Cuticular lipids as interface between plant and environment. Developments in Plant Biology 9:491–498

Shafer WE, Schönherr J (1985) Accumulation and transport of phenol, 2-nitrophenol and 4-nitrophenol in plant cuticles. Ecotoxicol Environ Safety 10:239–252

Manuscript received February 17, 1987 and in revised form May 29, 1987.