Chapter 5
Epicuticular Wax Formation and Regeneration—A Remarkable Diffusion Phenomenon for Maintaining Surface Integrity and Functionality in Plant Surfaces

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5.1 Introduction

Diffusion processes are ubiquitous in organisms, varying from being essential short-distance transport phenomena to posing threats, such as uncontrolled leakages of substances. A membrane, consisting of a phospholipid double layer with integrated proteins and other additional functional molecules envelops all cells, the smallest units of life. This membrane represents the device to reconcile the need of protecting the cell interior from its environment while maintaining intracellular conditions with the necessary exchange of substances with the surroundings (see Fig. 5.1). This exchange occurs—apart from processes such as endo-/exocytosis where whole membrane patches are used as transport vehicles—often via "controlled diffusion", involving pores or channels formed by proteins. In this manner, the membrane is semipermeable allowing diffusion of water and uncharged small molecules, whereas other substances are hindered from passing through.

Controlled diffusion processes are thus central for managing cell metabolism and—in the end—the metabolism of multicellular plants and animals. Originally, water was the only immediate surroundings of unicellular and multicellular organisms, since there is general agreement that life evolved within the oceans, and therefore, within an aquatic environment. During evolution, however, life moved on land

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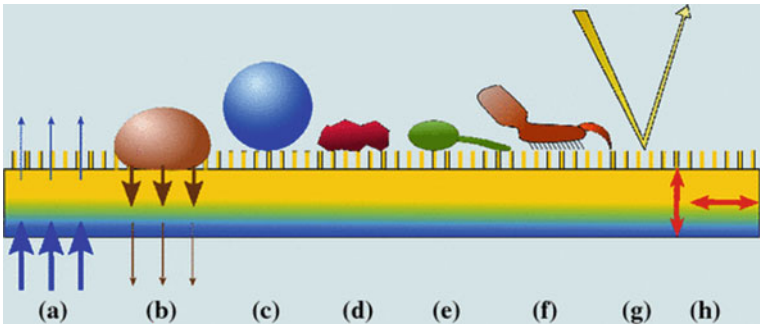

Fig. 5.1 Schematic summary of the most prominent functions of the cuticle as represented by a hydrophobic microstructured plant surface. **a** Transport barrier: limitation of uncontrolled water loss or leaching from the interior and **b** against foliar uptake. **c** Water repellency: control of surface water status. Anti-adhesive, self-cleaning properties: **d** reduction of contamination, **e** pathogen attack and **f** control of attachment and locomotion of insects. **g** Spectral properties: protection against harmful radiation. **h** Mechanical properties: resistance against mechanical stress and maintenance of physiological integrity (modified after [1])

and was confronted with the problem of a strong humidity gradient, namely the difference between water-saturated cells and tissues and the much drier air [2]. The water vapor deficit even at high relative humidity is huge and results in desiccation within a short time, lest there are any means to prevent this from happening. In fact, plants and animals are enveloped by desiccation barriers, which hinder water from rapid and uncontrolled loss into the atmosphere. There are, however, some exceptions, notably desiccation-tolerant organisms, such as mosses, some ferns, and a few seed plants, which can dry out and recover upon wetting without damage. All other organisms are necessarily equipped with a kind of "skin" preventing rapid desiccation.

To conserve water by suppressing water vapor diffusion into the atmosphere, the envelope has to be hydrophobic. Terrestrial plants developed a hydrophobic layer covering the outermost cells called epidermis [3]. This hydrophobic layer consists of two main components, the polymer cutin and soluble waxes, described further below, and is termed "cuticle" [4, 5] (see Figs. 5.1 and 5.2).

Due to its key importance for maintaining the hydrated state, the cuticle evolved during early stages of land plant evolution [7]. Cuticles are already present in 400 million year old plant fossils from the Lower Devonian, a time during which the vegetation consisted of quite small and leafless axes (Fig. 5.3). In fact, cuticle-like remains can be found in much older fossil material, dating back to the earliest times of land plant evolution from which only microfossil fragments are preserved [8].

Also, terrestrial animals need a protective cover against uncontrolled evaporation. In this respect, arthropods are interesting since they show a hydrophobic cover similar in many aspects to the plant cuticle. This is particularly the case for insects, since both plants and insects exchange gases with the atmosphere via their body surface. Land plants cannot be completely isolated from the atmosphere since they have to absorb CO_2 for photosynthesis and to evaporate water to maintain internal transport

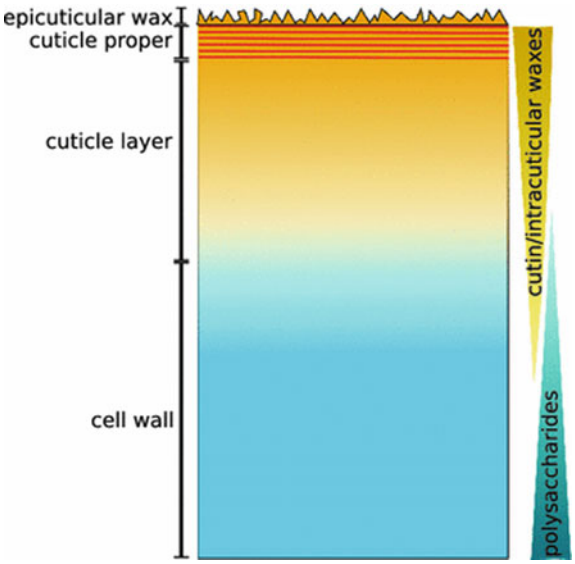

Fig. 5.2 Simplified scheme of the structural features of the plant cuticle and their major components (modified after [6])

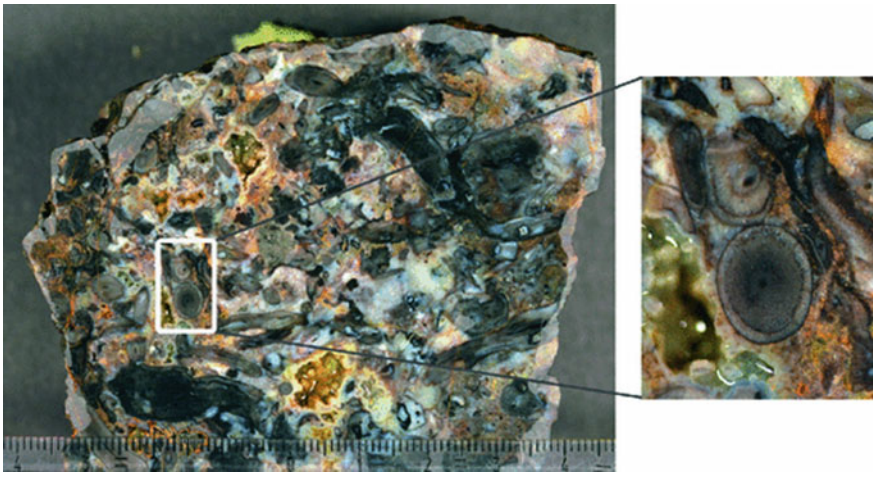

Fig. 5.3 A section through a piece of Rhynie Chert sediment (Scotland, near Aberdeen), containing axes of early land plants from the Lower Devonian, approximately 400 million years old. On the right, cross-sections of two plant axes are shown in detail. They belong to the so-called "rhyniophytic" plants thriving during that time on land. These plants were up to 20 cm high and consisted of leafless axes already covered by a cuticle

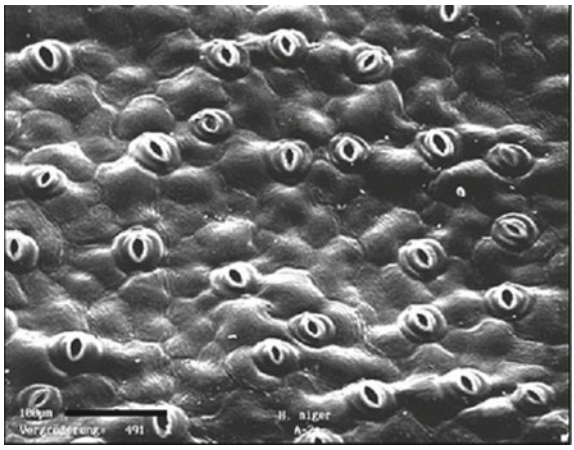

Fig. 5.4 A scanning electron microscope image of the lower leaf surface of *Helleborus niger*, showing stomata (bar: 100 μm). These micropores can be closed to control the exchange of CO_2 and water vapor between atmosphere and plant interior

processes. Plants are, therefore, forced to allow for a limited and controlled gas exchange and transpiration, facilitated by micropores, called stomata, which can be closed, according to the environmental conditions (Fig. 5.4). How micropore-based technologies in chemical engineering have, quite generally, been inspired by nature is referred to in great detail in Chap. 11.

The cuticle thus reconciles two conflicting tasks, namely suppression of outward diffusion of water vapor and uptake of CO_2. The solution is to pierce the isolating cover, the cuticle, with pores whose aperture can be regulated.

Terrestrial insects are very much under the same constraint, and consequently also developed a hydrophobic cuticle, which simultaneously serves as a gateway for respiration by being equipped with openings, the spiracles, leading to an internal tubing system, the tracheae. In fact, plants and terrestrial arthropods share many similarities with respect to evolutionary solutions against desiccation [9].

Conspicuous for both groups is the occurrence of wax blooms deposited upon the cuticles [1, 10, 11] (see Fig. 5.5). For both, essential functional roles are indicated.

For different plant species, cuticles can show quite different thicknesses, with plants from arid environments often showing considerably thicker cuticles than plants from humid habitats. The reason for that is not fully understood since the suppression of water vapor loss appears to be not dependent on cuticle thickness but on its chemical composition [12]. A thick cuticle can also contribute to mechanical stabilization [13] whereas wax crystals on plant cuticles are often associated with the famous Lotus effect, forming structured hydrophobic surfaces resulting in vigorous water repellency (contact angle $\gtrapprox 150°$) [14].

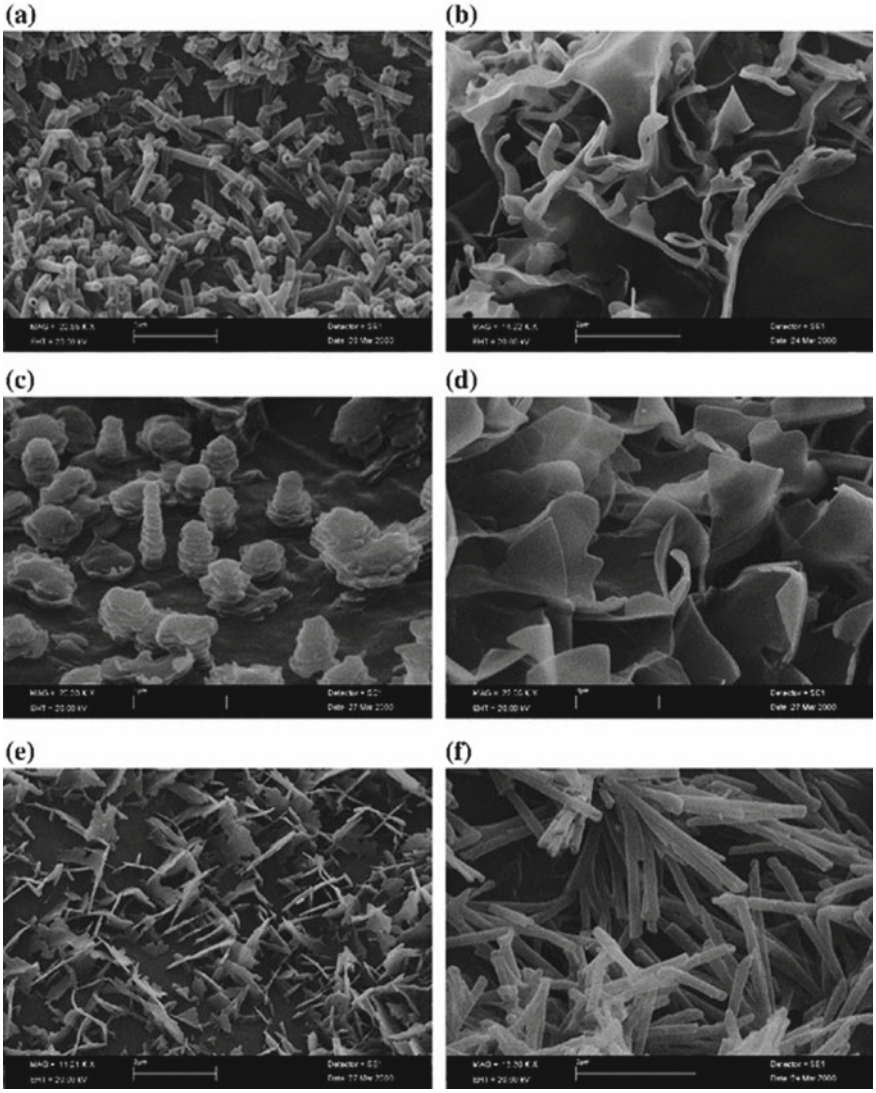

Fig. 5.5 Scanning electron microscopic images of epicuticular waxes from plants. **a** Nonacosanol-based tubules (bar: 1 μm). **b** Irregularly shaped wax crystals (bar: 2 μm). **c** Transversely rigid rodlets based on palmitone (bar: 1 μm). **d** Membraneous platelets (bar: 1 μm). **e** Irregularly shaped platelets (bar: 2 μm). **f** Tubules based on β-diketones (bar: 2 μm). Photographs: Institut für Botanik, TU Dresden

5.2 Qualitative Explanation of Self-repairing Wax Layers

5.2.1 Chemical Properties of the Cuticle

The cuticle may be regarded as a natural composite comprising two major hydrophobic components: an insoluble polymer fraction composed of cutin and, in some species, cutan as well as soluble lipids of diverse chemistry, collectively called waxes. In addition, a certain amount of polysaccharides is present (overview in [15, 16]). The outer very thin region (usually less than 100 nm), called cuticle proper, contributes 99% of the barrier efficiency [17], while the region determining the thickness of up to 20 μm, is called the cuticle layer [18, 19]. The chemical composition and internal structure of the cuticle seem to show a high degree of variability during ontogeny and among different plant species and organs. Whereas intra-cuticular waxes may be either amorphous or crystalline, epicuticular waxes (Fig. 5.5) are assumed to be of crystalline nature [20–22].

5.2.2 Wax Transport and Cuticle Self-repair

The crystal nature of epicuticular waxes imply self-assembly as the driving force for the formation of such structures. This has been proven after extraction and recrystallization of waxes from organic solvents revealing morphologically similar structures as compared to the plant surface [20, 21, 23–29]. To allow self-assembly of complex three-dimensional structures, the individual molecules must be mobile within a suitable matrix or solvent in which they are free to find an energetically favorable position, which also includes phase separation of different components or component classes found in wax mixtures. Recrystallisation of extracted waxes from a solution is considerably influenced by temperature, chemical nature of the solvent and the underlying substrate resulting in a large structural variability [20, 22].

The most intriguing problem, however, was the process of wax deposition onto the surface, as the molecules have to move from inside the cell through a hydrophilic cell wall and the hydrophobic cuticle and finally onto the ridges and edges of the growing crystals. Several hypotheses have been published from ectodesmata to the involvement of transport proteins [30–32]. One obvious hypothesis is the existence of some kind of channels or pathways, but no evidence of trans-cuticular structures that could serve as pathways for wax molecules has yet been found in the plant cuticle by Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM) or Atomic Force Microscopy (AFM) investigations [28, 33].

While studying the various phenomena related to water repellency and self-cleaning of natural and artificial surfaces, one particular interest was the ability of plants to reestablish these properties after damaging the surface. Wax crystals are weak structures very susceptible to mechanical influences, and therefore, easily altered or completely removed. Since plants are able to maintain the functionality of

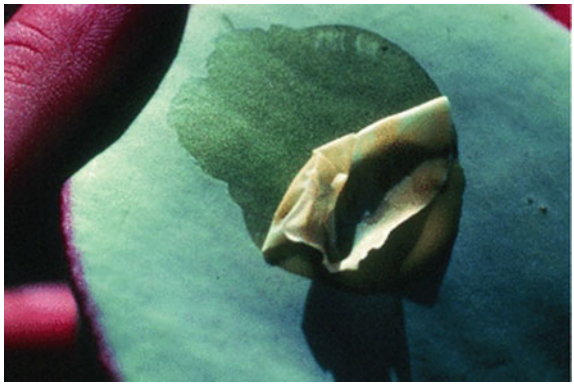

Fig. 5.6 Exposed cuticular surface after applying and peeling off the glue prior to regeneration of wax layers

the surface over quite a considerable period of time, the question turned up whether at all and if, how quick and to which extent wax layers and structures are re-established. To address this question, we performed a number of experiments with different plant species, from bud break to senescence, i.e., when the leaves are shed. The experimental setup was rather simple: during one vegetation period we applied glue, not containing organic solvents to the leaves, let it dry, and peeled it off (Fig. 5.6). Subsequently samples were taken on a regular basis to check for regeneration of wax layers and crystals.

Generally, a wide range of species was able to regenerate waxes after removal within a few days or up to 2 weeks. Many species could achieve that only in the young stages of leaf development, while others maintained this capability during the whole lifespan. Only very few species showed wax regeneration confined to later stages of development. Interestingly, the regeneration was confined to the area from which the wax layer has been removed, independently of cell borders, meaning that the reestablishment of a wax cover happened within the area of one cell (Fig. 5.7).

During these experiments, we faced the problem that very young and delicate leaves were destroyed during the attempt to peel off the glue. So we waited for the leaves to expand expecting that the glue would fall off by itself. However, since the material was highly elastic, the polymer film expanded together with the expanding leaf without being dropped. So we waited even longer expecting that the glue would be separated by the emerging wax cover that should act like a separating agent.

However, to our biggest surprise, this did not happen as well but now the wax cover emerged on the surface of the glue (Fig. 5.8).

This accidental and unexpected result of our experiment allowed only one conclusion: the transport of wax molecules must be independent of the living cell, since no transporters, channels or other cellular components can be involved in the movement through the polymer (i.e. the glue).

As a consequence, we isolated the cuticle enzymatically to remove every component of the living protoplast, covered it with a pure polyurethane film, and span the resulting specimen over a diffusion chamber filled with water (Fig. 5.9).

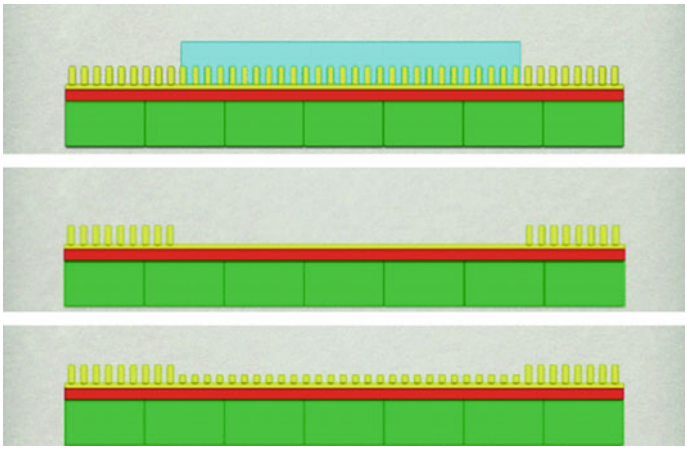

Fig. 5.7 Upper: Glue applied to the leaf surface. Center: Leaf surface after removing wax by peeling off the glue. Lower: Wax regeneration occurring in the areas only where the wax has been removed, independently of cell borders

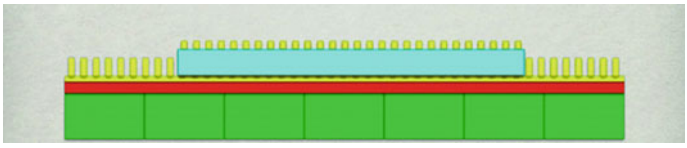

Fig. 5.8 Wax regeneration—the observation on young leaves. Waxes move through the glue and crystallize similar in size and distribution as on the leaf surface

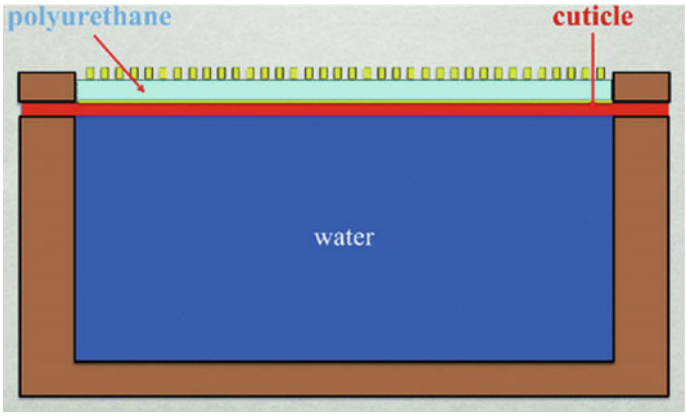

Fig. 5.9 In vitro experiments with isolated cuticles in a diffusion chamber

The result was basically the same as was observed on leaves. The wax moved through the polymer membrane following the transpiration gradient built up by evaporating water. The structures formed on the polymer surfaces again were virtually identical to those found on leaves in situ. In a final approach, we also removed the cuticle and replaced the latter with a polymer membrane alone that was applied to a filter paper, replacing the cell wall. The latter was loaded with wax and the sandwich was again placed on top of a diffusion chamber (Fig. 5.10).

The experiment again revealed the same result. Waxes moved through the polymer and crystallized on the surface. These results were independent of the type of polymer (e.g., PU, PP, PE, PC) or the used wax (e.g., plant waxes, montan waxes, artificial waxes). In case of plant waxes, the size, individual morphology, and distribution was virtually the same as on the leaf surface (Fig. 5.11).

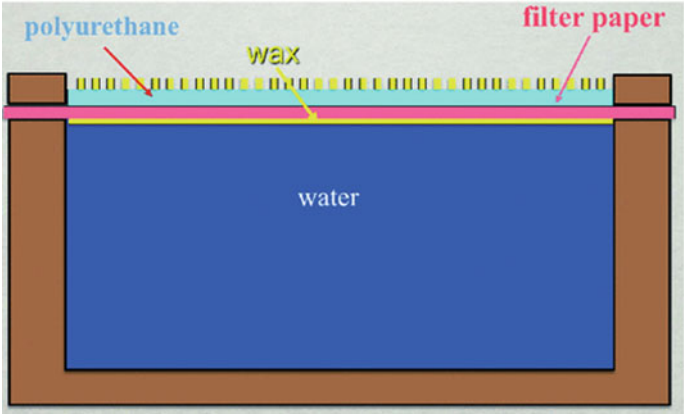

Fig. 5.10 Completely artificial approach in which a filter paper was loaded with wax of different origin that moved through a polyurethane membrane together with water

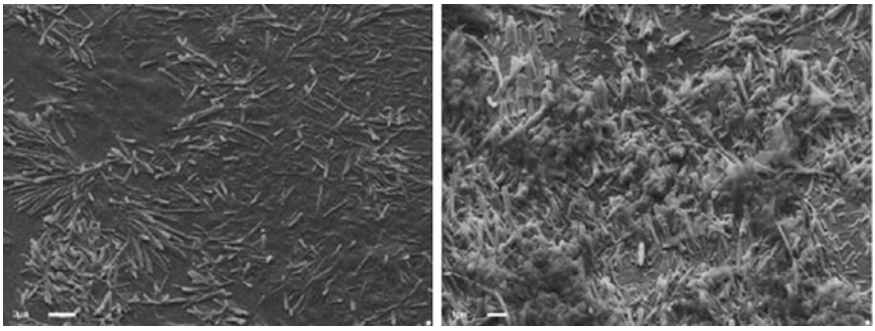

Fig. 5.11 Wax tubules, based on β-diketones recrystallized after movement through artificial polyurethane membranes. Size and distribution are not distinguishable from plant surfaces (left), while a higher density is achieved by a longer diffusion time (right)

Neinhuis et al. [34] consequently proposed a co-transport of wax components with water that constantly is lost via the cuticle, although in very small amounts. Assuming such a process is appealing since no pathways, carrier molecules or sensors are needed. Since cuticular waxes are the main permeability barriers, the transport to the outside slows down while more wax is deposited on the surface, so it is self-regulating. In addition, it easily explains the intriguing phenomenon of wax regeneration. Since removal of epicuticular wax also partly removes the water barrier, more wax is able to move through the cuticle in this particular spot and builds up a new layer without affecting neighboring area. AFM in situ demonstrated the rather quick reassembly of new wax layers after their removal under environmental conditions in vivo. AFM time-series pictured the formation of mono- and bi-molecular wax films and the growth of three-dimensional platelets, either directly on the cuticle or on already existing wax layers within minutes [28, 35].

5.2.3 *Summary of Sect. 5.2*

The qualitative explanation of cuticle self-repair and wax transport to the plant surface can be summarized as follows:

- Intact cuticles are very efficient barriers against evaporation of water from the plant interior. Hence, if the wax layer is degraded, evaporation from this zone increases, generating a current of liquid water from the plant interior.
- This water current transports the wax molecules from the epidermal cells (where they are presumably produced) toward the outer fringe of the cuticle. There the water evaporates. Being much heavier than the water molecules, the wax molecules do not evaporate, they rather form wax crystals rebuilding hereby the damaged cuticle layer by layer.
- As this repair process proceeds, both evaporation and the evaporation-driven water current decrease and smaller amounts of wax molecules are transported to the damaged cuticle. Finally, the cuticle attains its original thickness and the repair process comes to a halt.

The advantages of this self-regulating model over other hypotheses are:

- Neither distinct pathways (such as micro-channels or ectodesmata) nor the existence of lipid transfer proteins have to be postulated. The waxes move through the cuticle due to the presence of the water flow, hence neither organic solvents nor special receptors are necessary.
- It explains almost all phenomena we have observed, including some which were hitherto hard to explain, such as the wax regeneration and the appearance and the distribution patterns of epicuticular wax in distinct leaf areas.

5.3 Quantitive Explanation of Self-repairing Wax Layers

In this section, we present a condensed version of a quantitive model of cuticle repair, i.e., notably of the movement of the wax molecules deployed for this purpose. It emerged from the qualitative scenario outlined in the previous section. A detailed account can be found in [36].

5.3.1 Equation of Mass Transfer Through the Cuticle

We make use of a few assumptions which keep the mathematics manageable, thereby providing insight into the model structure: We employ the porous medium approximation, allowing us to restrict the mathematics to one dimension (the z-direction in Fig. 5.12), thus largely following the introductory remarks on diffusive movement in Chap. 2, Eqs. (2.6)–(2.11); we assume that the properties of the biological structures along the z-axis are (approximately) constant within each of the four different layers depicted in Fig. 5.12; and we assume stationary conditions, that is, none of the transport processes involved depends explicitly on time.

In the framework described in Sect. 5.2, the wax molecules are transported from the places where they are formed (presumably the epidermal cells depicted in Fig. 5.12) to the epicuticular wax layer where they are deployed by "swimming" passively in the midst of (liquid) water molecules. These vaporize at the plant surface into the atmosphere, causing hereby the flow of the liquid water molecules which is ultimately fed by soil water ascending through the plant's vascular water system. In addition to "swimming" with the flux of water, wax molecules are also subject to the

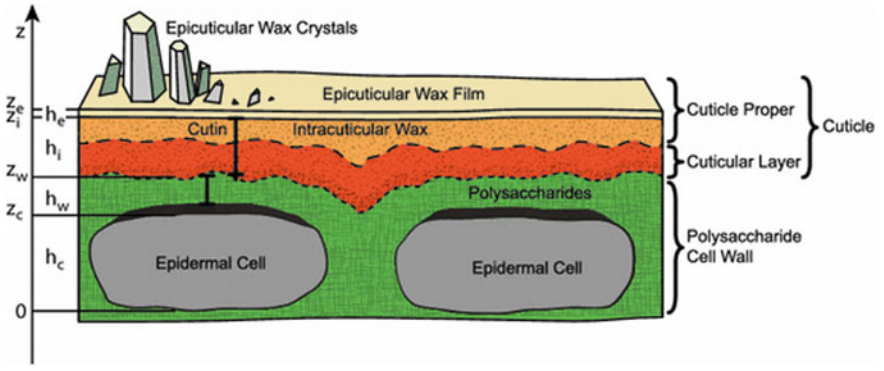

Fig. 5.12 Plant cuticle structure. Schematic diagram highlighting the major structural features of the cuticle and underlying epidermal cell layer. h_e, h_i, h_w and h_c denote the thicknesses of the various layers, z_e, z_i, z_w and z_c the z-coordinates of their outer fringes. (Typical) numerical values of these quantities are given in Table 5.1. (Not drawn to scale, modified after [3]). For photographs of epicuticular waxes, see Fig. 5.5

Table 5.1 List of variables and numerical values. Subscripts c, w, i, e refer to the different structural layers depicted in Fig. 5.12. Numerical data for diffusion coefficients and thicknesses of cutin layer and wax film layer are partly based on Tables 2 and 3 in [37] for cultivar "Elstar" and partly derived by educated guessing. Similarly, the value of K_c is based on [38]. The diffusion coefficient of the polysaccharide layer has been set arbitrarily to one tenth of the diffusion coefficient of the cutin layer

Quantity	Value	Quantity	Value
h_c	16 μm	R	8.314 J/mol/K
h_w	0.5 μm	T	20 °C
h_i	11.93 μm	g	9.81 m/s^2
h_e	4.14 μm	V_w	18.07×10^{-6} m^3/mol
S_c	4.33×10^{-12} m^2/s	V_{wax}	404×10^{-6} m^3/mol
S_w	7.16×10^{-11} m^2/s	ρ_w	18.07×10^{-6} m^3/mol
S_i	7.16×10^{-10} m^2/s	w_{rel}	0.6
S_e	3.03×10^{-10} m^2/s	ψ_{leaf}	−204 m
K_c	1×10^{-14} m/s	ξ	4 /s
K_w	1.69×10^{-15} m/s	c_s	10 mol/m^3
K_i	1.69×10^{-14} m/s	c_t	5.53 mol/m^3
K_e	7.18×10^{-15} m/s		

transport mechanism emerging from their Brownian motion, i.e., to a diffusive flux in the direction of decreasing wax concentrations (see Fig. 2.2a). With Fig. 5.12, wax concentration is easily understood to assume its maximum value, namely its saturation concentration c_s, at $z = z_e$, given the immediate vicinity of the epicuticular wax crystals. Wax concentration is thus expected to decrease from the leaf surface into its interior, giving rise to a diffusive flux just opposite to the flux of the water molecules. As it turned out, both transport mechanisms are equally important and indispensable in order to formulate a coherent mathematical model.

Considering both transport mechanisms, the flux $j(z)$ of wax molecules of concentration $c(z)$ is given by the expression (see, e.g., [39, 40])

$$j = -S\frac{dc}{dz} + cJ. \tag{5.1}$$

The first term on the right-hand side describes diffusion. $S = Dn/\tau$ denotes the (effective) diffusion coefficient in a porous medium, n and τ are porosity and tortuosity of that medium, respectively, and D is the diffusion coefficient in bulk liquid. The second term refers to the movement of the wax molecules within the flux $J(z)$ of (liquid) water, referred to (see also Sect. 2.2) as advection.

We assume that the wax molecules originate only within the epidermal cells, i.e., within the interval $0 < z < z_c$ (cf. Fig. 5.12). We further assume that the wax production rate can be described by the function

$$q = \xi[c_t - c(z)] \tag{5.2}$$

where c_t denotes a threshold concentration of the wax molecules and ξ is a rate constant. Depending on whether $c_t > c(z)$ or $c_t < c(z)$ is realized, q acts as source term (i.e., producing wax molecules, $q > 0$) in the first case and as sink term (removing wax molecules, $q < 0$) in the second case. Outside the interval $0 < z < z_c$ we set $q = 0$. Obviously, the maximum production rate of wax molecules amounts to $q_{max} = \xi c_t$.

Transport equation (5.1) and wax production rate (5.2) are connected by the continuity equation (see also Eq. (2.8)) which is simply the mathematical version of molecular bookkeeping: any change in the number of wax molecules within any (fictitious) volume of space is caused by inflowing molecules, outflowing molecules, and—possibly—generation (or destruction) of wax molecules within the volume. Due to the simplifying assumptions, stationarity and one-dimensionality, the continuity equation reads in our case

$$0 = -\frac{dj}{dz} + q. \tag{5.3}$$

Since we know already wax flux (5.1) and wax production rate (5.2), we can insert these expressions into (5.3) and obtain

$$0 = S\frac{d^2c}{dz^2} - \frac{d(cJ)}{dz} + \xi(c_t - c) \qquad \text{if} \quad 0 < z < z_c \tag{5.4a}$$

$$0 = S\frac{d^2c}{dz^2} - \frac{d(cJ)}{dz} \qquad \text{if} \quad z_c < z < z_e. \tag{5.4b}$$

The first line applies within the epidermal cells (where the wax is presumably produced), whereas the second line is valid outside the epidermal cells (no wax production, thus $q = 0$ in the continuity equation (5.3)). Both lines represent linear differential equations of second order for the wax concentration $c(z)$.

As noted above, we assume that the properties of the plant tissue—represented by the variables D, n and τ, which are amalgamated to the effective diffusion coefficient $S = Dn/\tau$—are approximately constant within each of the four different layers of Fig. 5.12. They may, however, vary from one layer to the next. This implies that Eq. (5.4a) has to be solved with S equated with S_c while (5.4b) has to be solved separately for the three layers between $z = z_c$ and $z = z_e$, with S adopting the values S_w, S_i and S_e. Since the differential equation (5.4) is of second order, each of these piecewise solutions comes with the two so-called "integration constants", adding up to altogether $4 \times 2 = 8$ arbitrary constants, which can (and must) be fixed according to the boundary conditions which specify the definite system (for details see below).

Once Eq. (5.4) have been solved for $c(z)$ in this way, the wax flux $j(z)$ follows by inserting the result into (5.1).

However, before solving Eq. (5.4), the still unknown water flux $J(z)$ between the epidermal cell and the outer fringe of the cuticle must be determined.

5.3.2 Solution of the Water Flux Equation

Because the plant tissues we deal with can be treated as porous media and because the fluid velocities inside these are low it is reasonable to describe $J(z)$ by means of Darcy's Law (see Sects. 2.3 and 11.3.2 and, e.g., [39–41]). In one dimension, it reads

$$J = -K\frac{d\psi}{dz}. \tag{5.5}$$

$K(z)$, the hydraulic conductivity, contains information about the flowing liquid (which is in our case water loaded with wax crystals) and the conductivity of the structures through which the liquid flows. Similarly as before, we assume that $K(z)$ is constant within each of the four tissue layers of Fig. 5.12 but may vary from layer to layer.

$\psi(z)$ denotes the water potential whose gradient $d\psi/dz$ is the driving force of the water current. Water potential is closely related to the chemical potential of water (see also Sect. 10.4): it represents the work needed within a given system to move one mole of pure water at atmospheric pressure to some other point (at the same temperature and pressure). In the fields of hydrogeology and plant physiology, it is a very useful concept because it allows to treat certain aspects of liquid water and water vapor within the same formalism (see e.g. [41]).

Within epidermal cells (i.e., for liquid water), typical values of ψ are around $\psi_{leaf} \approx -2$ MPa. The water potential of atmospheric water vapor depends strongly on temperature T and relative humidity w_{rel}; for $T = 20\,^{\circ}\mathrm{C}$ and $w_{rel} = 0.5$ it amounts to $\psi_{wv} \approx -93.5$ MPa, for instance. In soil research and hydrological research, water potential is usually expressed in units of pressure head. In these units, the equivalent values are $\psi_{leaf} \approx -204$ m and $\psi_{wv} \approx -7028$ m. The latter value is obtained from the expression [41]

$$\psi_{wv} = \frac{RT}{V_w \rho_w g} \log w_{rel}, \tag{5.6}$$

where R, g, ρ_w and V_w denote the gas constant, the gravitational acceleration, and the density and molar volume of liquid(!) water, respectively. In what follows, we will express water potential in units of pressure head (Pressure units (Pa = kg/m/s^2) are obtained by multiplying pressure head (units m) by $\rho_w g \approx 9.81 \times 10^3\,\mathrm{kg/m^2/s^2}$, as can be seen from (5.6)).

The water flux equation is derived from the continuity equation for liquid water which reduces due to our assumptions to $0 = dJ/dz$. Insertion of (5.5)—while keeping in mind the assumption that $K(z)$ is constant within each layer—yields the differential equation

$$0 = \frac{d^2\psi}{dz^2}. \tag{5.7}$$

Similarly as in the case of (5.4), this equation has to be solved separately for each layer. Each of the four solutions of equation (5.7) contains two arbitrary constants. These are determined from the condition of continuity for the water potential $\psi(z)$ and the water flux $J(z)$ at the layer margins at z_c, z_w and z_i and from two boundary conditions for $\psi(z)$: We require $\psi(0) = \psi_{leaf}$ and $\psi(z_e) = \psi_{wv}$ with ψ_{wv} as given in (5.6).

Application of this procedure is straightforward. It results, however, in lengthy expressions for $\psi(z)$; since we do not need them in what follows we give here only what results if we insert $\psi(z)$ into expression (5.5) for $J(z)$:

$$J = \frac{\psi_{leaf} - \psi_{wv}}{\frac{h_c}{K_c} + \frac{h_w}{K_w} + \frac{h_i}{K_i} + \frac{h}{K_e}}. \tag{5.8}$$

h_e, h_i, h_w and h_c denote the thicknesses of the various layers, as indicated in Fig. 5.12, and K_e, K_i, K_w and K_c are the respective water conductivities. $J > 0$ indicates a water flux toward positive z-values, i.e., toward the plant surface. In what follows, h_e denotes the thickness of the intact epicuticular wax film, while h denotes its actual thickness during any stage of the repair process (thus, $0 \leq h \leq h_e$).

Several features of expression (5.8) are noteworthy:

- It shows a close analogy to Ohm's law in electrodynamics: if water flux J is identified with electric current and water potential difference $\psi_{leaf} - \psi_{wv}$ (the driving force of water flux) with voltage, then the four terms in the denominator of the right hand side of (5.8) represent four resistances connected in series.
- J is independent of z, simplifying the solution of the differential equation (5.4) for the wax flux j considerably. (This property was to be expected from the physics of the situation: no water sources or sinks are present).
- The water flux J depends roughly reciprocally on the thickness h of the epicuticular wax film. This corroborates the qualitative conception developed in Sect. 5.2: the water flux decreases while the repair process proceeds (i.e., $h \to h_e$) and the wax layer regains its original thickness h_e.

5.3.3 *Solution of the Transport Equation*

By inserting the expression (5.5) determined for the water flux J into Eq. (5.4), we are now able to determine the concentration $c(z)$ of the wax molecules and, subsequently, by inserting into Eq. (5.1), their flux j. Solving a differential equation of second order in four adjacent layers gives rise to eight integration constants which may be determined by taking account of the respective boundary conditions, ending up in a number of quite lengthy expressions. All these relations and how they have been determined may be found in [36]. Here we confine ourselves to the graphical

representation of the solution in three characteristic situations as resulting with the parameters summarized in Table 5.1

Figure 5.13 displays the wax concentration $c(z)$ and the wax flux $j(z)$ along the pathway of wax molecules between epidermal cell and epicuticular wax film (cf. Fig. 5.12) and the wax production rate $q = \xi[c_t - c(z)]$ within the epidermal cells as resulting as a solution of equations (5.4) and (5.1).

Subfigure (c) shows the (net) wax flux $j(z)$. It is the sum of the diffusive component (represented by the first term in expression (5.1)) and of the advective component (the second term in (5.1)). These two are displayed in subfigure (d); the upper three curves represent advective components, cJ, the lower three curves depict the diffusional parts, $-S\,dc/dz$. Positive fluxes are directed toward the cuticle, negative fluxes point to the leaf interior. Blue curves are related to a damaged cuticle (the outer fringe is located at $z = z_i$), green curves represent an intact cuticle (the outer fringe is at $z = z_e$), and red curves represent the fictitious case of an epicuticular wax film which is twice as thick as it ought to be (the outer fringe is at $z = z_e + h_e$).

Comparison between the blue and green curves allows to visualize the repair scenario:

- As long as the cuticle is undamaged, the green curves in subfigures (a), (c), and (d) terminate at $z = z_e$, and the green curves representing advection and diffusion (subfigure (d)) have for all points with $z > z_w$ the same distance to the z-axis, thus adding up to a vanishing net wax flux (green curve in subfigure (c)).
- When the cuticle is damaged the repair process begins. This is illustrated by the blue curves which terminate at $z = z_i$: the absolute values of both advection and diffusion flux have increased, compared to the intact cuticle (see subfigure (d)), but now results a net flux toward the cuticle (see subfigure (c)).
- During cuticle regrowth, all blue curves "migrate" toward the green curves, that is, the absolute values of advection and diffusion flux decrease and converge slowly until they have merged with the green curves; then the net flux ceases and the repair process is completed.

Notice, that the model predicts also what happens to (fictitious) protrusions of height $h > h_e$, extending from the epicuticular wax film: This case is represented by the red curves. The one representing the net flux (subfigure (c)) runs for $z > z_w$ below the z-axis, indicating a negative net flux directed toward the plant interior; this means that the protrusions are dissolved and transported to the leaf interior. This process stops when the cuticle has been eroded to thickness h_e and the red curve has migrated to and merged with the green curve.

Comparison of subfigures (b) and (c) of Fig. 5.13 illustrates the continuity equation (5.3) which states that the gradient of the net wax flux equals the injection (or removal) of wax molecules: The region $0 < z \lessapprox 8\,\mu\text{m}$ acts as a wax source (indicated by $q > 0$). Wax molecules that are generated in the region $z \lessapprox 2\,\mu\text{m}$ flow toward the plant interior (indicated by $j < 0$, subfigure (c)), those produced in the interval $2\,\mu\text{m} \lessapprox z \lessapprox 8\,\mu\text{m}$ flow a short distance toward the cuticle (indicated by $j > 0$). In the case of an intact cuticle (green curves), all of them are removed from the cell liquid in the region $8\,\mu\text{m} \lessapprox z < z_c$ which acts as wax sink ($q < 0$). If the cuticle

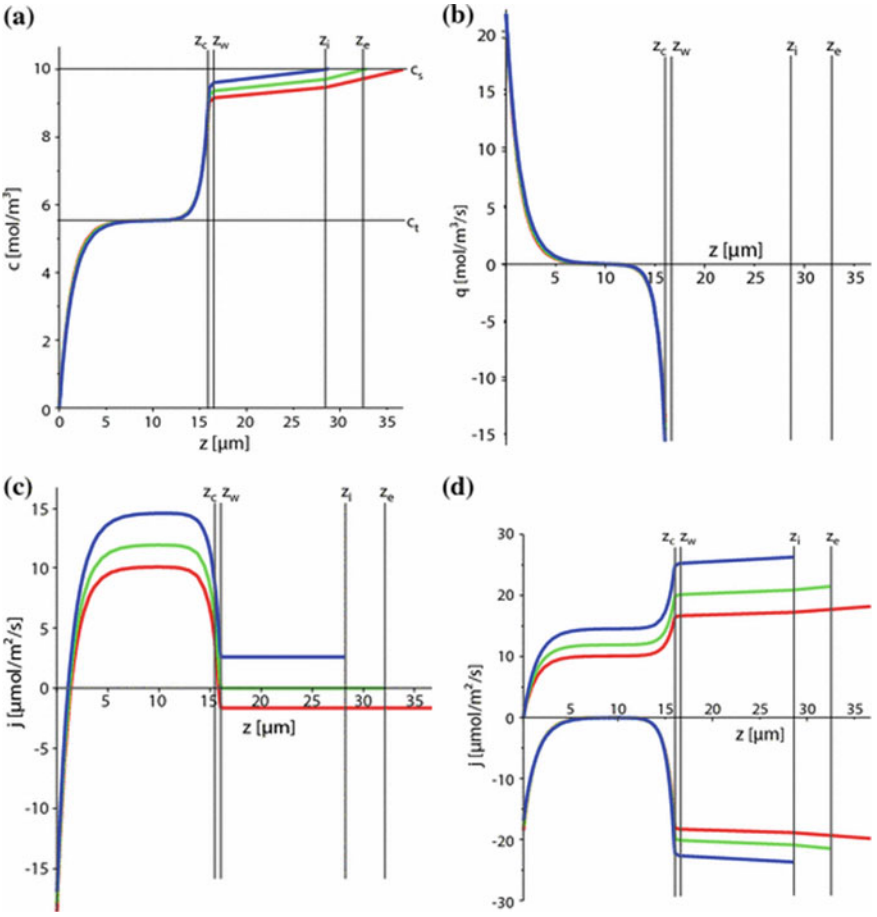

Fig. 5.13 Wax concentration (**a**) and wax fluxes (**c**, **d**) along the pathway of wax molecules between epidermal cell and epicuticular wax film (cf. Fig. 5.12). The (net) wax flux $j(z)$ in subfigure (**c**) is the sum of the diffusive component (lower three curves in subfigure (**d**)) and of the advective component (upper three curves in subfigure (**d**)). Positive fluxes are directed toward the cuticle, negative fluxes point to the leaf interior. (For detailed explanation, see text.) Vertical lines delineate the tissue layers defined in Fig. 5.12; the horizontal lines in subfigure (**a**) denoted c_s and c_t mark the saturation and the threshold wax concentrations. Subfigure (**b**) depicts the wax insertion (or removal) rate $q = \xi[c_t - c(z)]$ within the epidermal cells. Positive values indicate insertion, negative values indicate the removal of wax molecules. Notice that the graph depicts three nearly identical curves. Blue curves are related to a damaged cuticle (the outer fringe is located at $z = z_i$), green curves represent an intact cuticle (the outer fringe is at $z = z_e$), and red curves represent the fictitious case of an epicuticular wax film which is twice as thick as it ought to be. Numerical values are as in Table 5.1

is damaged (blue curves), however, a certain fraction of the injected wax molecules reaches and repairs the cuticle.

5.3.4 Restoration of the Wax Layer as a Function of Time

Provided the restoration proceeds slowly, compared to the travel time τ of a wax molecule between epidermal cell and epicuticular wax layer, the solution of the wax transport equation (5.1) can be exploited to derive the temporal development of the wax layer repair, although it has been derived under the assumption of stationarity. The values given in Table 5.1 imply for the velocity of the water current $J \approx 2.17\,\mu\mathrm{m/s}$ and thus $\tau = z_e/J \approx 15\,\mathrm{s}$ for the travel time of a wax particle between the epidermal cell and the cuticle. Hence, if the repair process lasts perhaps 1 h, this approach is certainly justified.

In order to calculate the temporal development of the epicuticular wax layer restoration, we assume that it has been eroded completely before the restoration process begins. That is, at the starting point $t = 0$ of the restoration process the outer fringe of the cuticle is located at $z = z_i$, equivalent to $h = 0$ (h denotes the actual thickness of the wax layer, h_e its thickness when it is intact, cf. Fig. 5.12).

The water brought there by the water flux J evaporates from the eroded area, leaving behind the much heavier wax molecules that came by the wax flux j. The wax molecules organize themselves as crystals, thus restoring the wax layer until it reaches its original thickness h_e whereupon the wax flux j ceases.

If V_{wax} denotes the molar volume of the wax molecules, the thickness h of the wax layer regrows with the velocity $dh/dt = V_{wax}\, j(h)$. In view of the structure of the expressions for $j(z)$ and $J(z)$ (cf. (5.8)), this is an ordinary but non-linear differential equation for $h(t)$.

Its non-linearity precludes a straightforward solution but an approximation approach (for details, see [36]) allows to calculate the thickness of the wax layer h as a function of time, resulting in

$$h(t) = h_e\Big[1 - \mathrm{e}^{(j_1 V_{wax}\, t)}\Big], \tag{5.9}$$

with $j_1 := (\partial j/\partial h)|_{h=h_e}$. Notice the implication $h(0) = 0$, that is, the cuticle layer started to (re-)grow at time $t = 0$. Its original thickness h_e approaches the wax crystal layer asymptotically, as $t \to \infty$. Thus, the repair process lasts—in principle—infinitely long; the time which is necessary to rebuild for instance 95% of the layer is, however, finite and amounts to the value $t_{95} := \ln(20)/(-j_1 V_{wax})$.

Figure 5.14 illustrates the result (5.9) for two different cases:

- In subfigure (a), temperature is kept constant and the relative atmospheric humidity w_{rel} adopts three different values. The time spans t_{95} increase with increasing w_{rel}: this is to be expected because the water potential difference $\left|\psi_{leaf} - \psi_{wv}\right|$ which is

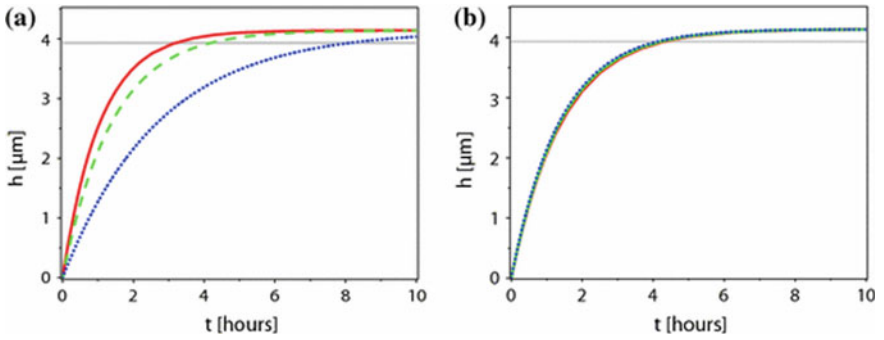

Fig. 5.14 Growth of the wax layer with time according to expression (5.9). The intersections with the gray, horizontal line indicate the time it takes to rebuild the wax layer to 95% of its original thickness of $h_e = 4.14\,\mu\text{m}$. **a** Temperature is kept constant at $T = 293\,\text{K} = 20\,^\circ\text{C}$ while the relative atmospheric humidity w_{rel} and the threshold concentration c_t of wax molecules in epidermal cell assume the values $(w_{rel}, c_t) = (0.8, 7.78\,\text{mol/m}^3)$ (blue, dotted line), $(w_{rel}, c_t) = (0.6, 5.53\,\text{mol/m}^3)$ (green, broken line) and $(w_{rel}, c_t) = (0.4, 3.48\,\text{mol/m}^3)$ (red, continuous line). The related time spans are $t_{95} = 8.13\,\text{h}$ (blue line), $t_{95} = 4.20\,\text{h}$ (green line) and $t_{95} = 3.19\,\text{h}$ (red line). **b** $w_{rel} = 0.6$ is kept constant, T and c_t assume the values $(T, c_t) = (30\,^\circ\text{C}, 5.65\,\text{mol/m}^3)$ (blue line), $(T, c_t) = (20\,^\circ\text{C}, 5.53\,\text{mol/m}^3)$ (green line) and $(T, c_t) = (10\,\text{C}, 5.42\,\text{mol/m}^3)$ (red line). The three curves are nearly indistinguishable; their common t_{95} time amounts to $t_{95} = 4.20\,\text{h}$. Other numerical values are as in Table 5.1. t_{95} is defined in the text

the driving force of evaporation decreases if w_{rel} is increased, according to (5.6). Accordingly, the wax supply for restoration decelerates.

- In subfigure (b), relative atmospheric humidity is kept constant and temperature is varied ($T = 10$, 20 and 30 °C). The related curves are nearly indistinguishable.

5.4 Conclusions

The model presented here corroborates, extends and quantifies the conjecture of Neinhuis et al. [34] which explains almost all phenomena observed in connection with cuticle repair.

They proposed the co-transport of wax components with water which relies on comparatively simple physics instead of postulating sophisticated living structures such as carrier molecules or specialized pathways for wax molecules; they were also able to confirm their hypothesis qualitatively by carrying out experiments with isolated cuticles and artificial membranes.

Adding diffusion as a transport mechanism which counteracts water transport of the wax components leads to a further clarification of the observations: the presence of two antagonistic transport mechanisms allows for the scenario that the two driving forces are balanced in the case of intact cuticles and that a damaged cuticle causes an imbalance resulting in net wax transport and cuticle self-repair which lasts until the balance is readjusted.

The model explains these findings in detail: its mathematical structure allows, for instance, to conclude that the thickness of the epicuticular wax layer and the typical restoration time after degradation (which are the result of two physical processes that are independent of living structures) are nonetheless controlled by living structures, namely the epidermal cells which generate the wax molecules. In the framework of the model, the cells have two degrees of freedom at their disposal to regulate the wax production: they can predefine both the thickness h_e and the restoration time t_{95} of the epicuticular wax layer by fine-tuning the parameters c_t and ξ of expression (5.2).

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