# A Introduction

## 1 Plant surface

### 1.1 Cuticle and its function

Plant cuticles – as a continuous and extracellular membrane, synthesized by epidermal cells (Marga et al., 2001) with thickness between 0.1 to 20 µm (Schönherr and Baur, 1996) - represent the interface between plant and biotic and abiotic environment (Bargel et al., 2003) (Fig. 1). Primary aerial parts of plants like stems, leaves, fruits and petals are covered by the cuticle.

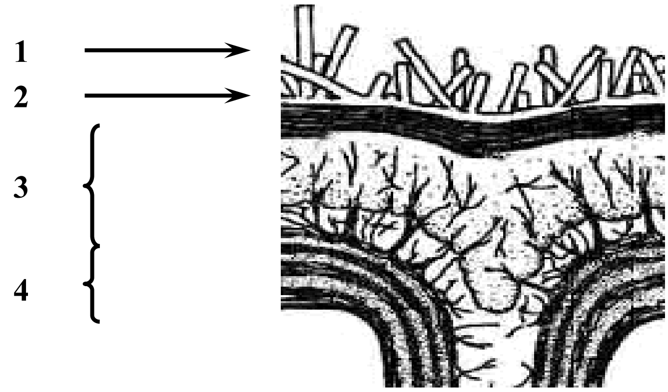


Figure 1: Schematic profile of plant cuticle (adapted from Jeffree, 1996); **1** epicuticular wax crystals, **2** epicuticular wax film, **3** intracuticular waxes and cutin, **4** cell wall.

The predominant structural model is a bilayer cuticular membrane, two layers distinguishable by their ontogeny, ultrastructure and chemical composition (Jeffree, 1996). The outermost part, which forms a layer outside the epidermal cell wall (Lee and Priestley, 1924), is composed predominantly of soluble and polymerized aliphatic lipids, while the inner layer, which is formed by impregnation of the cell wall, also contains substantial amounts of various embedded cell wall polysaccharides (Jeffree, 1996). During the early stages of its ontogeny, the cuticle is subtended by a layer of cell wall polysaccharides, which are characterised by a high pectin content. Therefore it is called pectin lamella. Later the cuticular membrane becomes more strong and cross-linked to the cell wall by embedment of cellulose microfibrils (Jeffree, 1996). The dominant structural polymer in the plant cuticle is cutin. Its detailed chemical composition has been comprehensively reviewed elsewhere (Baker, 1982; Holloway, 1982). It appears possible, since after saponification of cuticular membrane, insoluble residues cutan may present as well (Jeffree, 1996). The two polymers may occur in any ratio and differ in their abundance at different ontogenetic stages (Tegelaar, 1990). However, the structure has not yet been confirmed by conventional chemical analysis, and its biosynthetic origin is unknown. The cuticular membrane covering cells is often structured with papillae or accomplished by folding. An expansion of the cuticular membrane can be an influencing factor for folding-appearance.

The functions attributed to the cuticle are the protection and waterproofing of the plant surface (Holloway, 1994). Almost all kind of interactions between plant and environment are depending on the chemical and physical structure of the cuticle. The cuticle has to accomplish multiple physiological and ecological functions. Thereby, for transport of chemical substances cuticle forms a barrier, whereas barrier properties can be influenced by many abiotic and biotic environmental factors, as well as by the existence of cuticular waxes (see 1.3). Figure 2 displays the influencing factors.

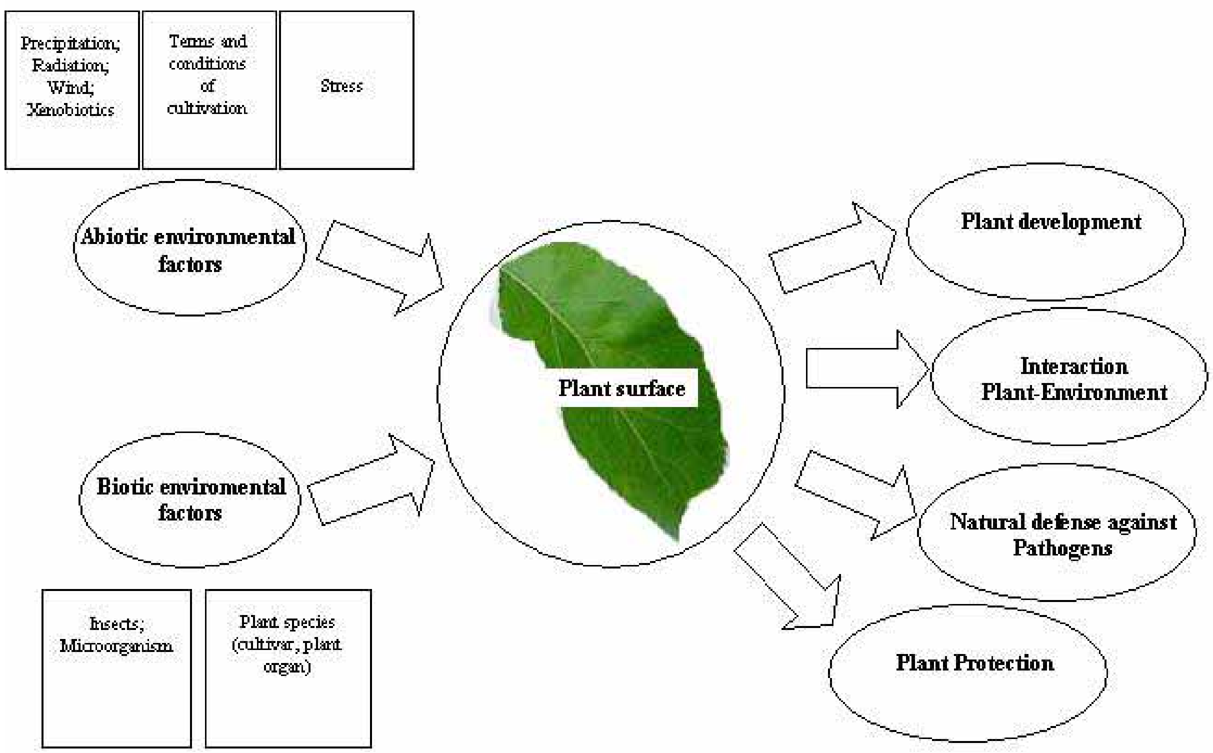


Figure 2: Influencing factors on chemical and physical properties of plant surface (left side) and effect on plants (right side).

The cuticle reduces leaching of ions and nutrients (Tyree at al., 1992; Niederl et al., 1998), and represents a major penetration barrier into leaf tissues of xenobiotics (Schönherr and Riederer, 1989; Schreiber and Schönherr, 1993). Bacterial and fungal attacks can be repelled by the existence of the cuticle and thus infection can be minimized (Barnes and Cardoso-

Wilhena 1996; Kerstiens 1996; Schreiber and Schönherr 1992). Besides, damage caused by harmful solar radiation (especially UV-B: 280-320 nm) can be impeded and alleviated, respectively (Kerstiens, 1996).

## 1.2 Cuticular waxes and their functions

Cuticular waxes are embedded within the cutin matrix of plant cuticular membranes and make up epicuticular films and aggregates (Riederer and Markstädter, 1996). Wax consists of various soluble lipids, being predominantly linear, long-chain and aliphatic molecules - particularly fatty acids and their derivates alcohols, esters, tritperenes, alkanes with addition of varying proportions of cyclic compounds including pentacyclic triterpenoids and hydroxycinnamic acid derivatives (Riederer and Markstädter, 1996). They are synthesized from $C_{16}$ and $C_{18}$-precursors, produced in the plastids (Bird and Gray, 2003). The proportion of the single chemical compounds differs among plant species, and even the cultivar influences the chemical composition (Post-Beittenmiller, 1996). Cuticular waxes constitute between 1 to 10 % of the total cuticle (Walton, 1990).

Extrusion of wax to the surface via pores was first proposed by de Bary (1871, 1884), however, electron microscopy has failed to reveal transcuticular pores in sense of open transcuticular channels via which wax might pass freely. Recent research dealing with permeation of substances (wax monomers, proteins, sugar, ions) and water through cuticles, revealed no generally accepted mechanism for their penetration (Neinhuis et al., 2001). Thus, Neinhuis et al. (2001) suggested a co-transport mechanism of wax compounds and water.

The micromorphology of epicuticular waxes can be described as very complex in form and structure, shaped sometimes like crystals, or like an amorphous film; all in all 23 types have been classified (Barthlott et al., 1998), whereas the first attempt in classification was made by de Bary (1871). Using a light microscope he identified four main structural forms: needles, rods, granular layers and films. On his part, Baker (1982) divided the form of epicuticular waxes in plates, tubular waxes, ribbons, rodlets, filaments and dendrites.

An influence of endogenous and exogenous factors on amount and chemical composition of cuticular waxes can be stated; a pronounced effect is ontogenesis. Markstädter (1994) reported an increase of wax coverage from 20 up to 700 µg per leaf during leaf expansion (*Fagus sylvatica* L.).

In addition to genetic factors (Wissemann, 2000), environmental factors, e.g. light (Von Wettstein-Knowles et al., 1980; Letchamo and Gosselin, 1996), relative humidity, water stress (Sutter, 1984; Prior et al., 1997) and, ontogenetic development are reported to affect the chemical composition of epicuticular waxes (Rhee et al., 1998).

In spite of the low mass of cuticular wax compared to the mass of the cuticle, cuticular waxes are responsible for up to 99 % of the resistance of the cuticular membrane to water loss (Riederer and Schreiber, 1995).

Limiting diffusional flow of water and solutes are main functions of plant cuticles (Riederer and Schreiber, 1995; Schönherr, 1982). The wettability of plant surfaces is influenced by the chemical composition and micromorphology of epicuticular waxes, thus controlling leaching, epiphyllic microflora and foliar uptake of pesticides (Brunskill, 1956; Challen, 1962). Moreover, the cuticle makes a contribution to the attenuation of photosynthetically active radiation in order to avoid light inhibition and of ultraviolet radiation (reviewed by Riederer and Markstädter, 1996). However, wettability and light reflection may be affected to a much larger extent by the presence of trichomes and epidermal topography than by epicuticular waxes (Kerstiens, 1996).

## 1.3 Influence of environmental factors

Environmental factors, like global irradiance, water supply, temperature, acid rain, do influence growth and development of earth living plants. UV-radiation < 290 nm is completely absorbed by the atmosphere, while relevant radiation has a wavelength longer than 290 nm. Figure 3 shows a general overview of effects of UV-B radiation reaching earth's surface. In a variably manner plants do respond to environmental influences, like modified growth, changed efficiency of photosynthesis rate, changed thickness of the cuticle, physicochemical characteristics of the plant surface.

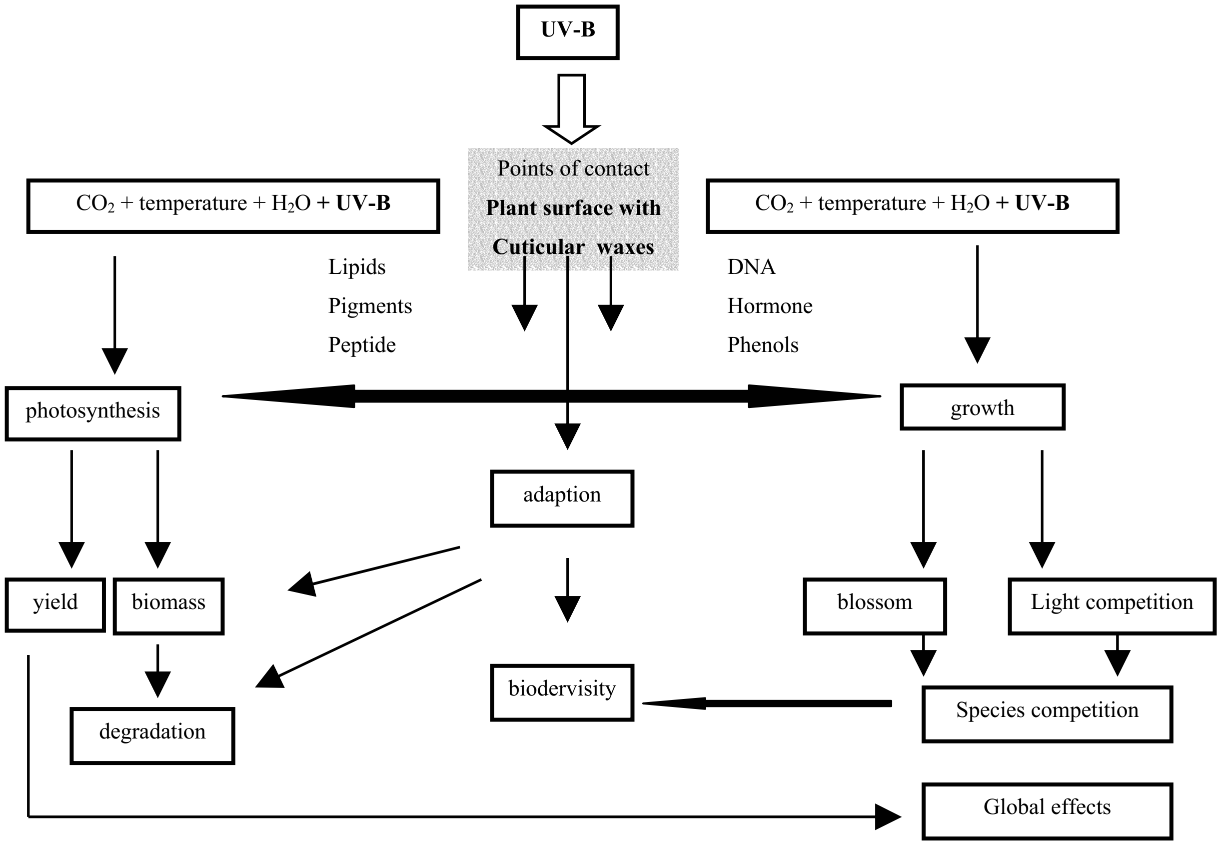


Figure 3: Overview on effects of UV-B radiation on earth living plants.

In the past decades few authors studied the influence of enhanced UV-B radiation inter alia as a consequence of an increasing global UV- radiation. Steinmüller and Tevini (1985) studied the adaxial and abaxial surface of the monokotyledon species barley (*Hordeum vulgare* L. cv. Villa) besides the two dikotyledones cucumber (*Cucumis sativus* L. cv. Delikatess) and bean (*Phaseolus vulgaris* L. cv. Favorit) in their reaction to enhanced UV-B radiation. Between the species there were differences in wax mass e.g. barley leaves had about five times more wax than from bean leaves. The irradiance caused an increase in total wax mass in all plants and a shift in chemical wax composition in different way depending on the studied plant. A significant change in the micromorphology of the studied leaves could not be stated.

The reaction in changing the physicochemical characteristics is depending on e.g. plant species and organ, age of the plant, UV-B dose. Gordon et al. (1998) studied the effects of UV-B radiation on epicuticular wax production and chemical composition of four *Picea* species. The authors stated that, the wax mass recovered from the needle surface did not vary with increasing UV-B dose after 35 d, whereas it changed between the species. No observations were reported concerning the micromorphology of the surface.

Kakani et al. (2003) observed the effects of UV-B (8 (ambient) and 16 kJ $m^{-2}$ $d^{-1}$ (high)) on cotton (*Gossypium hirsutum* L.) and found a reduction in plant height by 53 % over control shorter than plants not exposed to enhanced UV-B radiation. A changed density of stomata after UV-B treatment was another observation of Kakani et al. (2003). An increase in UV-B irradiance can cause a change in chemical composition of surface waxes, as e.g. in *Pisum sativum* L., where a shift from alcohol to esters and hydrocarbons was analysed (Gonzalez et al., 1996) micromorphology was investigated as well, and for leaves no change after induction of water deficit was stated.

What are the causal reactions leading to changes of chemical composition of surface waxes? Steinmüller and Tevini (1985) stated that the changed wax mass can be a function of modified leaf area (irradiated leaves show a reduced leaf area), but distribution pattern within some wax classes is an indication that UV-B radiation affects wax biosynthesis. There is a dose-dependence, as effects on the adaxial side were much more pronounced than on the abaxial side.

Barnes et al. (1996) studied surface leaf wax of UV-B radiated tobacco (*Nicotiana tabacum* L.). They reasoned that there must be an impact on wax biosynthesis as branching of wax molecules upon UV-B irradiation. The mechanism involves effects on the microsomal-based elongases which are responsible for the addition of $C_2$ units within the elongase-decarboxylase pathway. The reaction to water deficit is besides others a function of species and organ.

Drought induces a large decrease in photosynthetic activity (Angelopoulos et al., 1996; Munné-Bosch et al., 1999). However, mechanistic bases of this inhibition of photosynthesis are not well understood. Inhibition of photosynthesis could be based on changes of electron transport as affected by oxidative processes in chloroplast after exposure of plants to drought. An insufficient water supply can cause a higher content of epicuticular wax compared to sufficient water supply (Letchamo and Gosselin, 1996). As well as the mass of epicuticular waxes as the chemical composition were affected. Water stressed organs of cotton (*Gossypium hirsutum* L.) had higher levels of long-chained alkanes in comparison with well watered plants (Bondada et al., 1996).

Leaf surface wax has been considered as an important component of drought tolerance (Premachandra et al., 1991), besides several other morphological and physiological adaptions are known to impart drought tolerance, like root structure, accumulation of osmotica, leaf folding, reduction in leaf area, regulation of transpiration rate are some of these mechanisms (Joshi et al., 1998; Subbarao et al., 1995; Blum, 1998).

Environmental factors involved in plant growth and development might not be separated from each other.

## 1.4 Implications of modified cuticula and surface waxes

Why there is importance for studying the possible modifications of plant surface caused by environmental factors?

A modified surface can influence the interaction plant:microorganism. The dense biofilm, which covers the cuticular surface, consists of epiphyllic microorganism, under changed environmental conditions the composition of microorganism is modified (Blakeman, 1982; Andrews and Harries, 2000; Herrera-Campos et al., 2004). These factors can impact the environmental behaviour of the plant. Changes in wettability caused by degradation of epicuticular wax crystals (Riederer, 1989) offer, in general, a more suitable microhabitat for most phyllosphere organism (Knoll and Schreiber, 1998), whereas surface wetness may hinder sporulation of others (Butler, 1996). Besides these aspects, pest management aspects are important. Deposition, retention and distribution of spray droplets are affected by amount and chemical composition of epicuticular wax and by leaf surface micro-roughness (McWorther, 1993). The surface exposure of wax chemical groups determine the adequate wetting of the leaf, which is an essential basis for pesticide efficiency (Challen, 1962; Holloway, 1970; Fogg, 1947; Furmidge, 1962). Changes in wax chemistry and physical properties may affect wettability and markedly alter spray retention and penetration, as documented for expanding peach leaves (Bukovac et al., 1979).

Besides the surface wax properties, density of trichomes are influenced as well (Barnes et al., 1996), which do impact the behaviour of water droplets on leaf surfaces (Brewer et al., 1991) and leaf optical properties (Ehleringer, 1984).

The change in physicochemical characteristics of the surface, evoked by abiotic and biotic environmental factors, can impact behaviour of the plant towards environment.

## 1.5 The objective target of the present study

Clearly the surface of plants – as the first point of contact between earth living plants and it’s surrounding area - is subjected to impact of it`s surrounding environment.

The object of our study was *M. domestica* Borkh., particularly the leaves and fruits; whereas both are characterised by a different physical properties of the surface wax. The leaves do have amorphous wax film, whereupon the fruits do have crystals. The chemical composition is comparable. By means of these two different organs, the impact of environmental factors, like enhanced UV-B radiation and deficit in water supply on physicochemical characteristics should be studied, because of possible variation of surface characteristics, which do impact plant-environmental-behaviour.

Moreover ontogenesis as a crucial factor impairing surface wax morphology and chemical composition was assayed. Former studies showed a definite content of tocopherols in surface wax layer of e.g. Rubus (Robertson et al., 1991). Because of it`s prevention of the plant from damage caused by a grat number of abiotic and biotic stressores, we tested the surface wax layer of apple leaves for tocopherol content.

A change in surface chemical and physical characteristics can have implications on agricultural aspects i.e. plant protection, therefore retention and rainfastness of a well established fungicide mancozeb on UV-B influenced leaf surfaces were studied. To what extent there is an interaction between two environmental factors concerning their influence on adaxial apple leaf surface, is to be examined as well.

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