



Tansley review

The effects of stress on plant cuticular waxes

Author for correspondence:
Tom Shepherd
Tel: +44 1382562731
Fax: +44 1382562426
Email: tsheph@scri.sari.ac.uk

Tom Shepherd and D. Wynne Griffiths

Quality Health & Nutrition, Scottish Crop Research Institute, Mynfield, Invergowrie, Dundee DD2 5DA

Received: 16 February 2006
Accepted: 5 May 2006

Contents

Summary	469	VIII Water, salinity and cold stress	482
I Introduction	470	IX Mechanical stress	485
II Biosynthesis of cuticular wax	470	X Altitude	486
III Deposition and crystalline morphology of cuticular wax	474	XI Pollution	486
IV Cuticular wax as a photoprotective layer	475	XII Genetic and environmental control of cuticular wax production	488
V Effects of irradiation and temperature on cuticular wax composition	478	XIII Conclusions	493
VI Contact angles and wettability	481	Acknowledgements	493
VII Humidity effects	482	References	493

Summary

Key words: abiotic stress, biosynthesis, composition, cuticular wax, morphology.

Plants are subject to a wide range of abiotic stresses, and their cuticular wax layer provides a protective barrier, which consists predominantly of long-chain hydrocarbon compounds, including alkanes, primary alcohols, aldehydes, secondary alcohols, ketones, esters and other derived compounds. This article discusses current knowledge relating to the effects of stress on cuticular waxes and the ways in which the wax provides protection against the deleterious effects of light, temperature, osmotic stress, physical damage, altitude and pollution. Topics covered here include biosynthesis, morphology, composition and function of cuticular waxes in relation to the effects of stress, and some recent findings concerning the effects of stress on regulation of wax biosynthesis are described.

New Phytologist (2006) **171**: 469–499

© The Authors (2006). Journal compilation © *New Phytologist* (2006)

doi: 10.1111/j.1469-8137.2006.01826.x

I. Introduction

Plants have evolved to exist in conditions which are rarely ideal for maintenance of normal physiology and may be at the limit for survival. Abiotic stress arises from exposure to climatic extremes such as drought, heat, cold and frost, and the effects of radiation levels, shade, altitude, soil nutrient and water status and pollution are also significant. In response, plants can adapt to, avoid and overcome the stress by means of various physiological and biochemical mechanisms, including evolution of a resistance-conferring genotype, or by development of genes which can produce ecologically adapted phenotypes. Stress resistance mechanisms fall broadly into two categories – avoidance and tolerance – which may occur together. Avoidance involves establishment of internal conditions where the plant's cells are unstressed although external conditions may be stressful, for example, control of high leaf temperatures by transpiration and prevention of drought by water conservation. Tolerance involves endurance of the stress such that plants can function under extremes of both internal and external stress. Examples include plants endurance of desiccation during drought and revival on hydration. The development of specialised physiological mechanisms is more a characteristic of tolerance, whereas avoidance more often utilises the capability of general physiological processes to provide mechanistic and morphological devices to shield plants from the effects of extreme conditions. Stress resistance may be induced following exposure to sublethal levels of stress, and hardiness towards one form of stress may confer some resistance to other stresses. This illustrates the interrelationship between different stress factors; for example, resistance to drought and resistance to high temperatures are often linked, as are resistance to freezing and resistance to cellular dehydration.

Development of a water-resistant cuticle was fundamental to the successful colonisation of land by plants (Edwards *et al.*, 1996) and such structures existed by approx. 400 million years ago. As the primary interface between the plant and its environment the cuticle plays a key role in maintaining the plant's integrity within an inherently hostile environment. Its outermost surface is covered in a hydrophobic layer of predominantly long chain aliphatic molecules, collectively referred to as cuticular wax, embedded in an underlying layer of the polymer cutin (Fig. 1). Before considering the functional significance of cuticular wax to plant stress, it is useful to summarise its origin and physicochemical nature.

II. Biosynthesis of cuticular wax

Synthesis of the major wax components occurs via sequential elongation of a C_2 primer derived from acetyl-CoA with C_2 units derived from malonyl-CoA. In this condensation-elongation process, acyl chains of up to C_{16} and C_{18} formed in the synthesis *de novo* are further extended to C_{30} or higher by a second elongation system. Modification of the acyl chain gives products including alkanes, aldehydes, primary alcohols, alkyl esters, secondary alcohols, ketones and various polyoxygenated compounds. The chemistry, biochemistry and molecular biology of cuticular wax biosynthesis have been extensively reviewed (Kolattukudy *et al.*, 1976; Bianchi, 1995; von Wettstein-Knowles, 1995; Kolattukudy, 1996; Post-Beittenmiller, 1996; Kunst & Samuels, 2003; Shepherd, 2003).

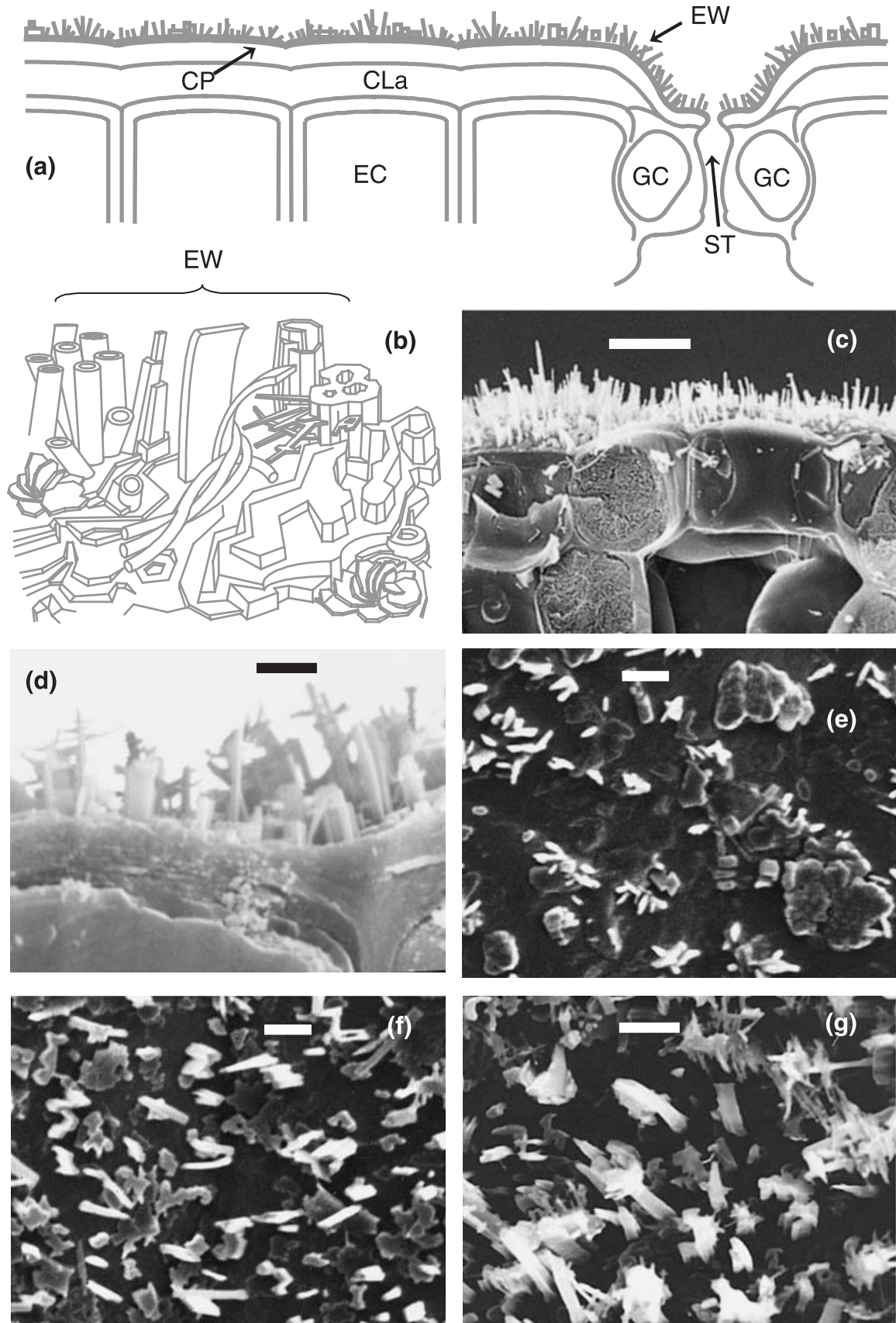
1. Formation of malonyl-CoA

The initial step in wax synthesis is formation of malonyl-CoA from acetyl-CoA, catalysed by the multifunctional enzyme system acetyl-CoA carboxylase (ACCase; refer to Appendix A, Table A1 for full list of abbreviations). A molecule of CO_2 derived from bicarbonate is added to the biotin moiety of biotin carboxylate carrier protein (BCCP) to form N-1' carboxybiotin-BCCP, a reaction catalysed by biotin carboxylase (BC). Subsequently the CO_2 is transferred to acetyl-CoA, forming malonyl-CoA, catalysed by carboxyltransferase (CT) (Fig. 2). Two types of ACCase occur: the plastidial form supplies malonyl-CoA for the synthesis *de novo*, whereas the cytosolic form supplies malonyl-CoA for further acyl chain elongation and synthesis of polketides and flavonoids (Schultz & Ohlrogge, 2002).

2. Synthesis *de novo* of acyl chains

De novo acyl chain synthesis catalysed by fatty acid synthase (FAS) occurs in plastids (reviewed by von Wettstein-Knowles, 1995; Schultz & Ohlrogge, 2002). The enzyme complex includes acyl carrier protein (ACP), to which acyl groups are linked via a pantetheine-4'-phosphate prosthetic group. Chain formation starts with transfer of an acetyl group from acetyl-CoA to a cysteine thiol of 3-ketoacyl-ACP synthase (KAS), another key component of FAS, to serve as the C_2 primer for elongation (Fig. 2, step a). The C_2 elongating substrate consists of a malonyl group which is transferred from malonyl-CoA to the ACP pantetheine thiol (step b). Malonyl-ACP is decarboxylated by KAS to form a carbanion,

Fig. 1 (a) Plant leaf cross-section showing epicuticular wax (EW); cuticle proper (CP) consisting of epicuticular wax, intracuticular wax and cutin; cuticular layer (CLa) consisting of intracuticular waxes, cutin and polysaccharides; epidermal cells (EC); guard cells (GC) and stomata (ST). (b–g) Some of the different crystal forms observed for epicuticular wax. Scanning electron micrographs showing leaf freeze-fracture cross-sections of kale, *Brassica oleracea*, cv. Fribor (c) and broccoli, *B. napus* (d) and crystalline epicuticular wax from young (e), older (f) and mature (g) leaves of glasshouse-grown swede, *Brassica napus* cv. Doon Major.



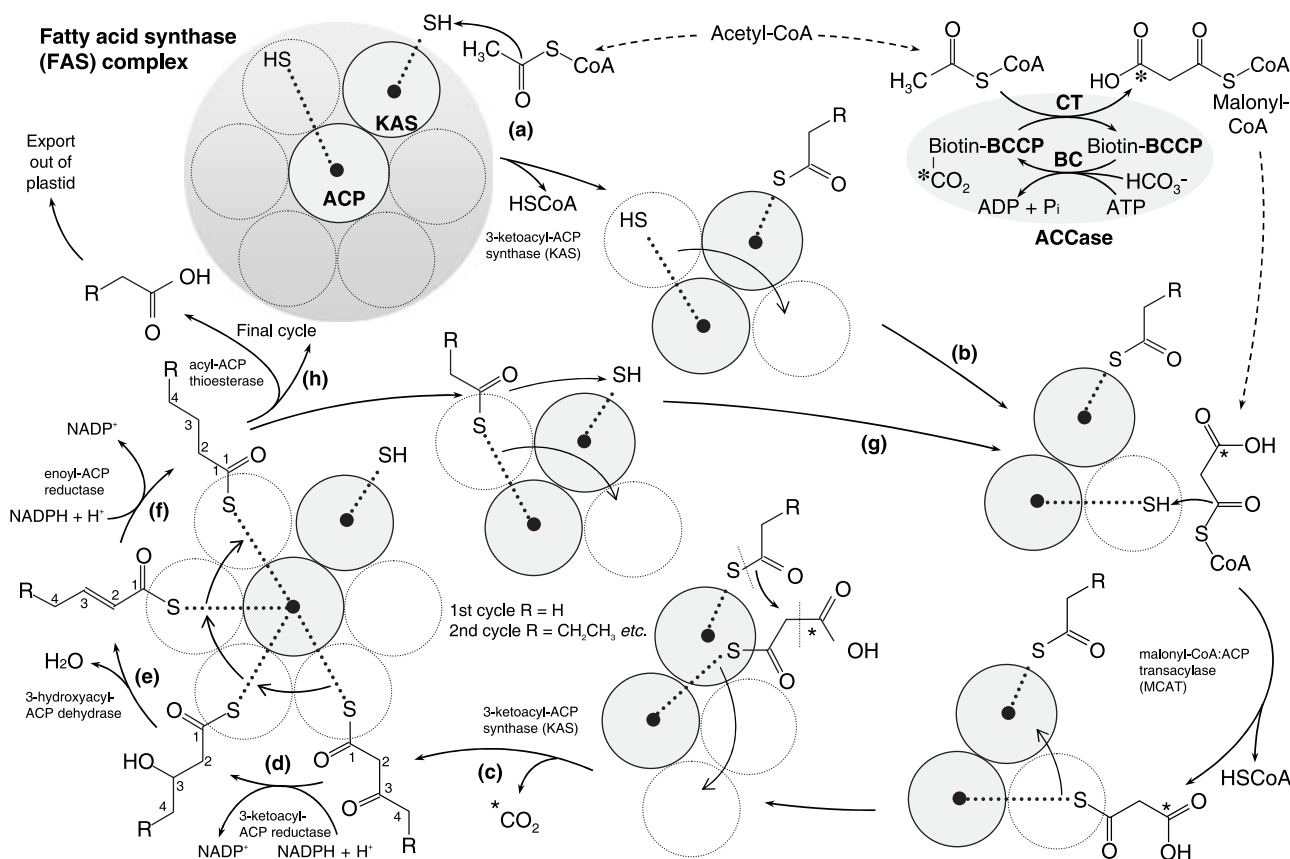


Fig. 2 Synthesis of malonyl-CoA by acetyl-CoA carboxylase (ACCase) and the synthesis *de novo* of acyl chains by fatty acid synthase (FAS) in plastids. BCCP, biotin carboxylate carrier protein; BC, biotin carboxylase; CT, carboxyltransferase; ACP, acyl carrier protein; KAS, 3-ketoacyl-ACP synthase. The spatial arrangement shown for the different enzyme components of FAS is representational and does not indicate a definitive structure. (Adapted from Shepherd (2003) with permission from Academic Press.)

which undergoes a Claisen condensation reaction with the acyl carbonyl group of the KAS-bound acetyl primer (step c). Overall, the acyl (acetyl) group is transferred to C-2 of malonyl-ACP, forming acetoacetyl-ACP and CO_2 , and the KAS cysteine thiol is regenerated. A three-reaction sequence follows, involving stereospecific reduction of 3-ketoacyl-ACP (step d), dehydration of D-3(R)-hydroxyacyl-ACP (step e) and reduction of $\Delta^2(E)$ -2,3-enoyl acyl-ACP to the corresponding C_4 acyl-ACP (step f), which completes the first elongation cycle. Transfer of the C_4 acyl group from ACP to the KAS cysteine thiol starts the next cycle, freeing ACP to accept another malonyl group from malonyl-CoA (step g). Repetition of the cycle a further six times gives palmitoyl-ACP (16:0-ACP), then one further time to produce stearoyl-ACP (18:0-ACP). When the acyl chain is 16 or 18 carbons long, a double bond may be inserted between C-9 and C-10 by the action of acyl-ACP desaturase.

Acyl chains to be incorporated into cellular complex lipids, cutin, suberin and wax components, are hydrolysed by thioesterases and the resultant fatty acids are transported across the plastidial membrane (Fig. 2, step h). An acyl-CoA

synthase at the outer plastidial membrane may then convert free acids to acyl-CoA derivatives.

3. Elongation and acyl chain modification

Further elongation of acyl chains derived from the synthesis *de novo* occurs in epidermal cells and involves a series of extra-plastidial elongation systems, collectively referred to as fatty acyl elongases (FAEs). Each FAE includes the condensing enzyme 3-ketoacyl-CoA synthase (KCS), 3-ketoacyl-CoA reductase, 3(R)-hydroxyacyl-CoA dehydrase and (E)-2,3-enoyl-CoA reductase (Millar & Kunst, 1997). Like FAS they catalyse a sequence of condensation, reduction, dehydration and reduction reactions, in which acyl chains are extended by C_2 units derived from malonyl-CoA. The enzymes catalysing the last three reactions are believed to be expressed constitutively throughout the plant and are associated with the various cellular condensing enzymes (Millar & Kunst, 1997). FAE differs from FAS in that the extending acyl chain is linked to CoA rather than ACP and the C_2 donor is malonyl-CoA, rather than malonyl-ACP. The major wax components arise

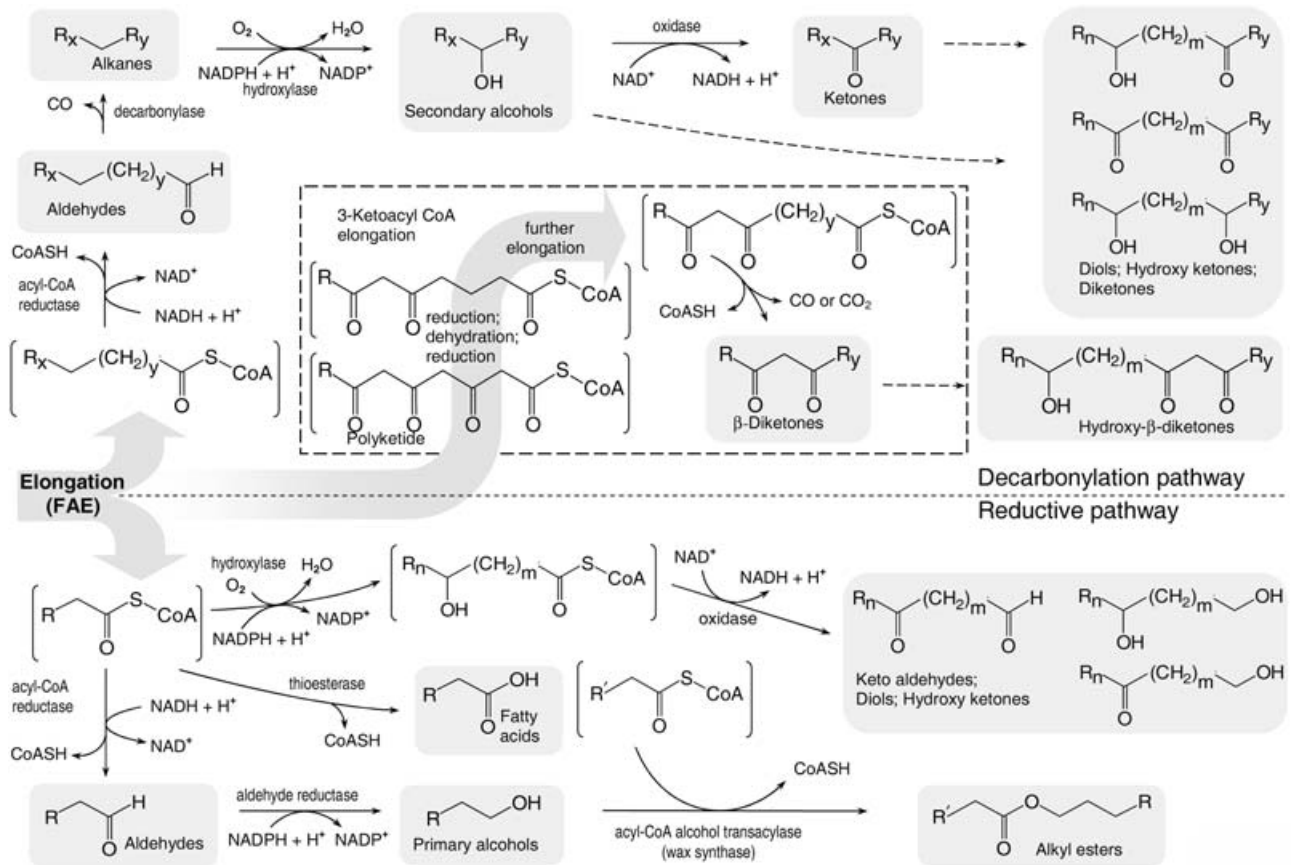


Fig. 3 Reactions and products of the decarbonylation and reductive pathways. Most products are derived directly from the products of elongation by fatty acid elongases (FAE). In some species, 3-ketoacyl-CoA compounds formed by the condensing enzyme 3-ketoacyl-CoA elongase are intermediates in the formation of β -diketones. (Adapted from Shepherd (2003) with permission from Academic Press.)

via further modification of the elongation products by enzyme complexes associated with the elongation systems. Two distinct pathways occur and, based on their key reactions, these have been named the reductive and decarbonylative pathways, respectively (Fig. 3).

4. Decarbonylation pathways

Acyl-CoAs are reduced to intermediate aldehydes from which alkanes are formed by decarbonylation (Fig. 3). Secondary alcohols are formed via stereospecific hydroxylation of alkanes, and oxidation of the alcohol gives the corresponding ketone. Further reactions can occur, including esterification of secondary alcohols with fatty acids and formation of diols, hydroxy ketones and diketones via additional hydroxylation and oxidation. In some species, such as cereals and grasses, a variation of the decarbonylation pathway is thought to be the source of β -diketones (von Wettstein-Knowles, 1995; Kolattukudy, 1996). During acyl chain elongation, 3-ketoacyl-CoA intermediates are formed by 3-ketoacyl-CoA elongase in a series of cycles where the usual reduction, dehydration and reduction steps are omitted following the condensation

reaction. After three such steps, a polyketide is formed, from which a β -diketone is produced via a sequence of reduction, dehydration and reduction, more full cycles of elongation and finally decarbonylation or decarboxylation. Further reactions may include insertion of additional oxygenated substituents.

5. Reductive pathways

Acyl-CoA is reduced to an intermediate aldehyde and then to a primary alcohol (Fig. 3). A single enzyme carries out both reductions in some plants, and the aldehyde intermediate remains bound. Fatty acyl-CoA reductase has been purified from leaves of pea, *Pisum sativum* (Vioque & Kolattukudy, 1997), and jojoba, *Simmondsia chinensis*, embryo (Metz *et al.*, 2000). Free fatty acids are formed by hydrolysis of acyl-CoA. Several mechanisms are possible for synthesis of long-chain esters, including direct esterification of acids with alcohols and transfer of acyl groups from phospholipids, glycerolipids or acyl-CoA to alcohols. Wax synthase (acyl-CoA alcohol transacylase) has been identified from several plants, and purified from jojoba (Lardizabal *et al.*, 2000; Metz *et al.*, 2000). Ketoaldehydes, ketoalcohols,

diols and hydroxy fatty acids may be formed by introduction of oxygenated substituents into the acyl chain. Some of these compounds are found in the free form and as esters, while others only occur as components of polymers such as estolides, cutin and suberin.

III. Deposition and crystalline morphology of cuticular wax

The mechanism of wax deposition on the leaf surface is poorly understood (see review by Kunst & Samuels, 2003). Following synthesis at sites such as the endoplasmic reticulum (ER), Golgi apparatus and plasma membrane (PM), individual components must move to the PM, pass through the cell wall and cuticle and on to the cuticle surface. Direct movement between ER and PM where they are in very close proximity is one possibility and movement to the PM within intracellular vesicles is another, perhaps derived from the ER, Golgi and PM by exocytosis. Jenks *et al.* (1994) reported an increase in the local density of vesicles adjacent to the site of wax excretion when wax production in sorghum was induced by light. However, the nature and origin of the vesicles could not be determined. Passage of wax components through the cell wall and cuticle probably occurs via diffusion, possibly in a solvated form, through molecular-scale spaces and channels consisting mostly of temporary openings between polymer and wax chains, since there is little evidence for macroscopic pits, pores or excretory structures. Small lipid transport proteins (LTPs) associated with the cell wall may facilitate diffusion (Yamada, 1992; see also reviews by Post-Beittenmiller, 1996; Kunst & Samuels, 2003). Being hydrophobic, LTPs can probably move from the outer layer of the PM, through the cell wall and on to the cuticle surface, and such a protein has been found within broccoli cuticular wax (Pyee *et al.*, 1994). However, there is currently no additional experimental evidence supporting this idea.

The existence of layers within cuticular waxes was verified using differential removal-extraction procedures. In earlier studies, for example with blackberry, *Rubus fruticosus* (Haas & Rentschler, 1984), the outer layer designated as 'epicuticular wax' was removed using materials like collodion, whereas more recently Jetter *et al.* (2000) used a cryoadhesive with *Prunus laurocerasus* for this purpose. Inner layers designated as 'intracuticular wax', which could not be removed mechanically, were extracted with solvent. The inter-layer boundary was considered to delineate the outer limit of the cutin matrix. The chemical basis and extent of such differentiation vary between species. Epicuticular wax from *R. fruticosus* consisted primarily of free alcohols, alcohol acetates and esters, with lesser amounts of free fatty acids and alkanes. Intracuticular wax had similar amounts of the alcohols, fewer esters and more free fatty acids, and triterpenoid acids were exclusive to this fraction (Haas & Rentschler, 1984). In the case of *P. laurocerasus*, epicuticular wax consisted entirely of aliphatic compounds, whereas intracuticular wax consisted primarily of triterpenoids (Jetter *et al.*, 2000).

Seen under the electron microscope, epicuticular waxes usually have a microcrystalline structure, sometimes arising from an underlying amorphous layer (Jeffree, 1996). Numerous morphological forms classified by Barthlott *et al.* (1998) may be present, including rods, ribbons, filaments, tubes and plates. Some of these can be related to the presence of specific wax components (Table 1). Compounds with mid-chain oxy-substituents, such as β -diketones, hydroxy- β -diketones, diols and secondary alcohols, are associated with tubes, whereas primary alcohols with a terminal oxy-substituent are associated with platelets. Changes in the homolog distribution within a class of compound, for example the primary alcohols, are also associated with crystalline modifications (Table 1).

When Jeffree (1974) and co-workers (Jeffree *et al.*, 1975) recrystallised leaf waxes from various solvents using a porous surface and wick-fed delivery system, the waxes formed similar

Table 1 Association of cuticular wax crystalline forms with specific classes of chemical component in the wax

Plant species	Crystal morphology	Composition associated with morphology	Reference
Various	Tubules	β -Diketones or secondary alcohol nonacosan-10-ol, also diols	Baker (1982); Gulz (1994); Jetter & Riederer (1994, 1995)
<i>Eucalyptus gunnii</i>	Branched rodlets (clusters)	β -Diketones	Koch <i>et al.</i> (2006)
Barley (<i>Hordeum vulgare</i>)	Tubes	β -Diketones and hydroxy- β -diketones	von Wettstein-Knowles (1974)
Norway spruce (<i>Pinus abies</i>); stone pine (<i>P. cembra</i>);	Fine rod-like tubes	Secondary alcohol (S)-nonacosan-10-ol	Anfodillo <i>et al.</i> (2002); Prügler <i>et al.</i> (1994); Koch <i>et al.</i> (2006)
<i>Tropaeolum majus</i>	Branched tubules		Avato (1987)
Maize (<i>Zea mays</i>) (juvenile waxes of wild type)	Platelets and attached rods (star-like structures)	Primary alcohols, particularly the C ₃₂ homologue	
Maize			Beattie & Marcell (2002)
Normal plants	Crenellated platelets	Primary alcohols, predominantly C ₃₂	
gl mutants	Semicircular platelets	Primary alcohols, predominantly C ₂₈ , C ₃₀	
gl mutants	Globules	Primary alcohols, predominantly C ₂₆	
Maize	Reduced density of crystal forms	Increased levels of alkyl esters	Beattie & Marcell (2002)

structures to those found on leaves. They concluded that wax morphology was influenced more by the physicochemical properties of the constituents rather than by the underlying cuticular membrane or means of delivery to the surface. More recently, Kirsch *et al.* (1997) found that the transport characteristics (permeability) of recrystallised waxes from several species mirror the barrier properties of isolated cuticular membranes and intact leaves with wax *in situ*. They argued similarly that properly reconstituted wax layers have similar crystalline morphologies to those of intact waxes.

Temperature, light intensity and humidity influence wax morphology, and these parameters tend to act together so it can be difficult to distinguish between their respective effects. In general, higher temperatures favour structures parallel to the cuticle surface such as plates and flakes, while lower temperatures favour more vertical structures such as rods and tubes (Table 2). Complex structures may form at higher temperatures, for example the dendritic lattices observed in *Brassica* waxes which can develop layered structures reminiscent of rain forest canopies (Fig. 1c,d,g). Greater illumination often leads to shorter, less elaborate structures, although this is not always the case. Some examples of the effect of temperature and light on wax morphology are listed in Table 2, principally for members of the *Brassicaceae*. Established structures may also transform into other forms if conditions change. Tubular crystal forms are thermodynamically unstable because of their high surface area/volume ratio, and with an input of energy, for example heat, they transform into more compact planar and thermodynamically stable forms. This accounts for the observation of Baker (1974) that tube waxes on normal brussels sprouts turned into dendrites within 48 h of raising the temperature from 15 to 35°C, with little compositional change. Baker (1974) suggested that local temperature and crystallisation rate may be more significant than solute concentration and chemical composition in determining wax crystal structure. Slow crystallisation favours linear structures, whereas rapid cooling favours dendrite formation.

The ease of interconversion between crystalline forms is also more readily explained if waxes are carried to the cuticle surface in a solvent. Transportation of wax precursors through the cuticle where they are subject to final modification (von Wettstein-Knowles, 1974), or extrusion of waxes through the cuticle or via specialised pores, notwithstanding the lack of evidence for such structures, could not account for the crystallisation of different structures such as dendrites on the upper reaches of existing crystals. Plants produce a wide range of volatile organic compounds, including terpenes, short-chain aldehydes, ketones, alcohols and esters, etc., all of which could serve as a wax delivery solvent. Although the quantities involved are low, a steady rate of production would be sufficient to deliver the wax to the cuticle surface and volatiles may persist within the boundary layer long enough to aid in crystallisation and interconversion of wax structures under appropriate conditions.

IV. Cuticular wax as a photoprotective layer

Electromagnetic radiation incident on plant cuticles covers a wide range of energies and wavelengths, from high-energy, short-wavelength ultraviolet (UV) through visible and near-infrared (IR) to lower-energy, longer-wavelength middle-far IR (Fig. 4a). In recent years quantities of UV-B radiation reaching the earth's surface have increased due to stratospheric ozone (O₃) depletion caused by atmospheric pollutants such as chlorofluorocarbons (Farman *et al.*, 1985; Kerr & McElroy, 1993; Webb, 1997; Holmes & Keiller, 2002). This is likely to continue due to the long-term persistence of chlorofluorocarbons within the upper atmosphere (Molina & Rowland, 1974). Both UV-B and UV-A are harmful to plant growth, UV-B being most damaging (Barnes & Cardoso-Vilhena, 1996; Holmes & Keiller, 2002).

Leaves may reflect the radiation via classical specular reflection and light scattering or they may absorb the energy. The key absorption event occurs in the photosynthetically active region (PAR) of the visible spectrum (400–700 nm), through the action of photosynthetic pigments, with further absorptions by water at longer wavelengths (Fig. 4a). The presence within the epidermis of UV-B-absorbing pigments, primarily flavonoids, with minimal PAR absorption, provides a major protective barrier to damage (Robberecht *et al.*, 1980; Caldwell *et al.*, 1983; Rozema *et al.*, 1997; Cockell & Knowland, 1999; Liakoura *et al.*, 2001). Where absorption is high, reflectance is generally low and vice versa (Barnes & Cardoso-Vilhena, 1996), and UV reflectance can range from < 10% in most plant species studied to date, to 70% in a few others (Caldwell *et al.*, 1983; Barnes *et al.*, 1996). In a study with four species, Robberecht *et al.* (1980) found that, whereas the amounts of UV-B reflected (5–40%) and absorbed (60–95%) varied between species, less than 1% was transmitted into the mesophyll.

Reflectivity is strongly influenced by the surface topography, which is primarily determined by leaf hairs and the cuticular wax layer, particularly at visible wavelengths. The importance of cuticular wax has been shown in various studies by removal of wax from waxy-leaved species. Reflectance was reduced and photosynthesis increased in glaucous *Eucalyptus* species, but had little effect in non-waxy species (Cameron, 1970). Similarly, reflectance from *Kalanchoe pumila* was greatly reduced and absorbance increased at visible and near-IR wavelengths (Eller, 1979). In a recent study using 45 different *Eucalyptus* and *Kalanchoe* species, Holmes and Keiller (2002) found that waxy leaves were more reflective at UV (330 nm) and photosynthetic (680 nm) wavelengths than various types of hairy leaves. Removal of wax generally reduced reflectance at both wavelengths, and the reduction was proportionally greater at 330 nm; however, waxless varieties showed little change in reflectivity. Reflectance was reduced over the range 270–500 nm in *E. cinerea* and *E. gunnii*, and mainly over 400–500 nm (PAR) in *K. pumilla*, showing that reflectance characteristics can vary widely. Light dispersion is particularly associated with

Table 2 Effect of temperature and irradiation on leaf wax crystal morphology

Plant species	Variable parameter ^a	Effect on wax quantity	Effect on wax morphology or composition	Reference
Cauliflower (<i>Brassica napus</i>), glaucous and glossy (glabrous) forms; Field rape (<i>B. napus</i>)	T	Lower T > higher T	Dendrites, crusts, plates (crystal growth parallel to surface rather than upwards at higher T)	Whitecross (1963); Whitecross & Armstrong (1972)
	T		Lower T (15/10°C): rodlets up to 3.4 µm long (av. 1.8 µm), base diameter 0.2–0.6 µm, no platelets Mid-T (24/19°): complex branched platelets with fused primary branches forming an elevated platform 0.3 µm above leaf surface Fewer rods, transition at 15/10–21/16°C Higher T (27/22°): platelets with increased complexity of branch fusion	Armstrong and Whitecross (1976)
Brussels sprout (<i>Brassica oleracea</i>)	T	Lower T > higher T	Lower T (15°C): tubes, cylindrical rods Higher T (35°C): dendrites, crusts, plates (crystal growth parallel to surface rather than upwards). Dendrites rather than tubes form within 48 h of raising T from 15 to 35°C	Baker (1974)
<i>B. oleracea</i>	E T E	Higher E > lower E 15°C > 25°C 440 µ E m ⁻² > 145 µ E m ⁻²	Lower E: reduces size and distribution of crystalline forms Higher T (25°C): large parallel dendrites (high and low E) Higher E (15°C): smaller less elaborate dendrites Lower E (15°C): rods perpendicular to leaf surface	Reed and Tukey (1982)
<i>B. oleracea</i>	Night T		Higher T: heat-resistant cv. Sousyu, larger plates (crystal growth parallel to surface rather than upwards) Higher T: heat-susceptible cv. Kinsyun, plate density lower	Welker and Furuya (1994, 1995)
Swede (<i>B. napus</i>); Kale (<i>B. oleracea</i>)	T, E		Higher T/lower E: increased dendrite formation Higher E/lower T: shorter crystalline structures	Shepherd <i>et al.</i> (1995b)
Carnation (<i>Dianthus caryophyllus</i>)	T, E	15°C < 25°C 440 µ E m ⁻² > 145 µ E m ⁻²	Higher T, E: length of rods increased, density decreased	Reed and Tukey (1982)

^aTemperature (day/night); E, illumination (light) intensity.

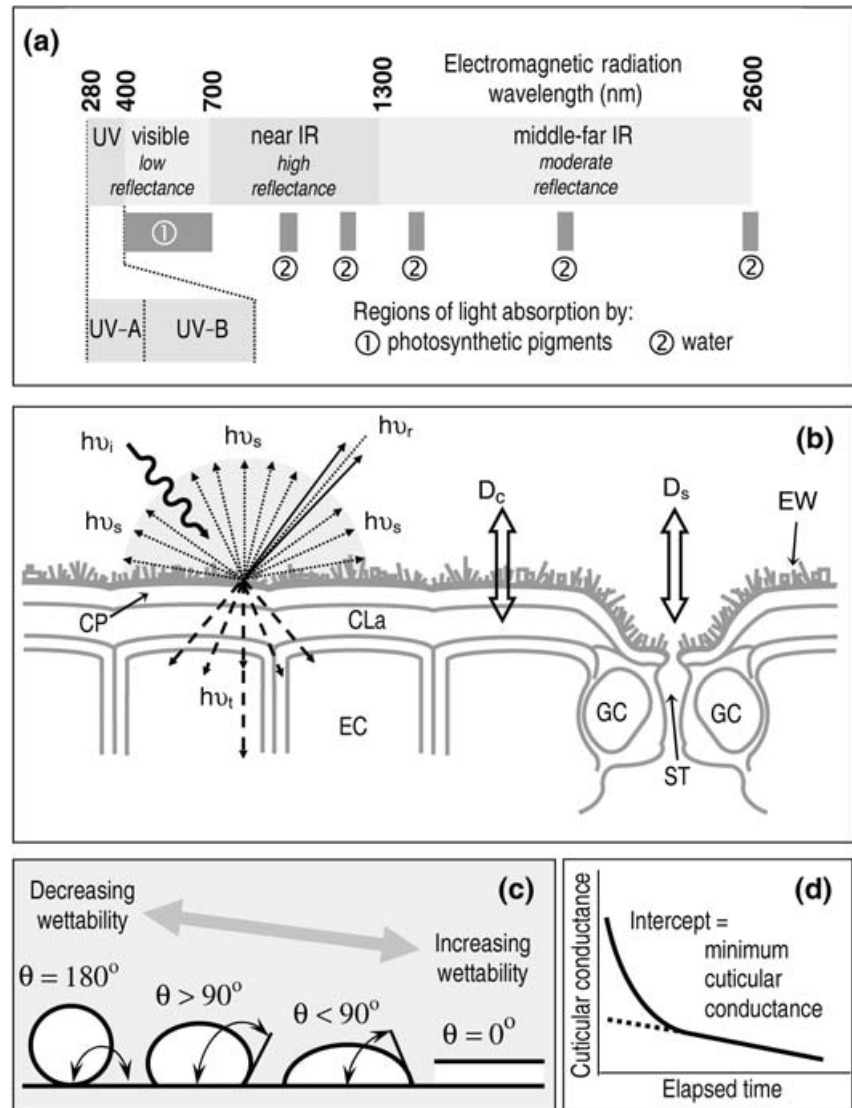


Fig. 4 Leaf surface properties. (a) Wavelength distributions for radiation incident on plant leaves, absorption events and surface reflectivity. (b) Leaf cross-section showing incident ($h\nu_i$), reflected ($h\nu_r$), scattered ($h\nu_s$) and transmitted ($h\nu_t$) electromagnetic radiation, paths of stomatal (D_s) and cuticular (D_c) diffusion or transpiration. EW, epicuticular wax; CP, cuticle proper consisting of epicuticular wax, intracuticular wax and cutin; CLa, cuticular layer, consisting of intracuticular waxes, cutin and polysaccharides; EC, epidermal cells; GC, guard cells; ST, stomata. (c) Variation in leaf surface contact angle (θ) and wettability from spherical droplet to water film. (d) Variation of cuticular conductance with time following onset of water stress.

glaucousness (Johnson *et al.*, 1983; Juniper & Jeffree, 1983; Blum, 1986; Barnes & Cardoso-Vilhena, 1996; Febrero *et al.*, 1998), and wax morphology (Table 3). Filamentous structures (tubes, rods, ribbons) common to glaucous lines lower incident radiation by increasing reflectance more than plates (Cameron, 1970; Sanchez-Diaz *et al.*, 1972; Blum, 1975a,b; Juniper & Jeffree, 1983; Febrero *et al.*, 1998). Reflectance can vary with temperature, for example, reflectance from *Eucalyptus* increased at higher day/night temperatures over the range 15/10–27/22°C, with a maximum at intermediate temperatures (21/16°C) (Cameron, 1970).

Reflectance (PAR) by glaucous and non-glaucous lines of wheat was proportional to the amount of wax present, and elevated reflectance reduced light transmission to the underlying mesophyll cells in glaucous durum wheat by 12% (Johnson *et al.*, 1983). Normally this is insufficient to affect photosynthesis, but might be a factor under non-saturating light

conditions. Eller (1979) suggested that light reflected from glaucous surfaces may help to illuminate leaves in shaded locations lower in the canopy. Such an effect would benefit plants such as wheat with large leaf area indices, and increased photosynthesis at lower levels may balance reduced photosynthesis at higher locations. Reduced absorption at visible and near-IR wavelengths due to enhanced reflectance can lower tissue temperatures, thus reducing the vapour pressure difference between the tissue and the air, which helps to reduce transpirational water loss. Usually the reduction in transpiration is greater relative to any reduction in photosynthesis, causing an increase in transpiration efficiency (TE), net photosynthesis/transpiration. For example, in comparison with non-glaucous plants, photosynthetic tissue in glaucous durum wheat was up to 0.7°C cooler for droughted field-grown plants or 0.3°C cooler for well watered glasshouse-grown plants, photosynthesis and stomatal conductance were reduced, day and night

Table 3 Effect of water stress on leaf surface reflectivity and associated cuticular wax morphology

Plant species	Stress	Leaf appearance	Reflection (wavelength, nm)	Associated crystal/surface morphology	Reference
Lehmann lovegrass (<i>Eragrostis lehmanniana</i>)	W (RES)			Large wax plates	Hull <i>et al.</i> (1978)
Western Red Cedar (<i>Thuja plicata</i>)	W (SUS)			Large plates absent	Krasowski and Owens (1990)
Durum wheat (<i>Triticum turgidum</i>)	W (RES)			Thick crusts and clumps of wax	Johnson <i>et al.</i> (1983)
Bartley (<i>Hordeum vulgare</i>)	W	GLC vs NG	↑ Ears, sheaths, flag leaves ^a (flag _{abax} > flag _{adax}) (400–700) ↑ × 30–50% (400–700)	Fine tubes rods and ribbons even covering stomatal pores (GLC flag leaf abaxial surface)	Febrero <i>et al.</i> (1998)
	W vs. NS	GLC, NG	≈ (700–2200)		
	W, NS	GLC vs. NG	↑ × 20% (400–700) ≈ (700–2200)		
<i>Eucalyptus</i> sp.		GLC vs. NG	↑	Long tubes and rods (GLC); small platelets (NG)	Cameron (1970)
Sorghum (<i>Sorghum bicolor</i>)		GLC (abax) Normal (GLC)	↑	Dense filaments (abax) Thick amorphous layer covered in flakes	Sanchez-Diaz <i>et al.</i> (1972) Blum (1975a,b)

W, water stress; S, salinity stress; NS, non-stressed; RES, resistant; SUS, susceptible or least resistant; GLC, glaucous; NG, non-glaucous (glabrous); adax, adaxial leaf surface; abax, abaxial leaf surface; ↓ reduction or ↑ increase in value; ≈, relatively unchanged.

^aGeneral order ears > sheaths > flag leaves.

transpiration by the ear were up to 50% less and overall TE was reduced. This can be beneficial at critical stages of development; for example, a 0.5°C fall in tissue temperature was calculated to equate with a 30 g per plant saving in water, sufficient to extend grain filling by at least 3 d (Richard *et al.*, 1986).

Reflectance and photosynthesis are dependent on the angle of incidence of radiation. At angles between 90° and 60°, reflectance from non-glaucous *E. regnans* was constant and then increased rapidly at shallower angles (Howard, 1967). Photosynthetic rates fall at angles below 72°, to 70% of normal values at 45°, and approach zero at 5° (Kriedemann *et al.*, 1964).

V. Effects of irradiation and temperature on cuticular wax composition

An increase in wax thickness is a response of many plants including various *Brassica* sp. (Baker, 1974; Reed & Tukey, 1982; Shepherd *et al.*, 1995b), carnation (Reed & Tukey, 1982) and barley (Giese, 1975) to higher irradiation levels (Tables 2, 4). In her study using barley, Giese (1975) found that deposition rates and leaf-surface wax density in 4-d-old plants were 2.5 times greater in the light than in the dark. When 4-d-old dark-grown plants were exposed to light, rates of wax deposition increased 7.5-fold in the first 24 h after exposure, thereafter settling down to the same rate and wax density as for light-grown plants. While demonstrating the stimulatory effect of light, these findings also indicated that rates of wax deposition were density-limited. Temperature also affects wax production and in numerous plants, particularly *Brassica* sp., more waxes are produced at lower temperatures (Table 2), although this is not always the case.

In addition to affecting wax quantity, light and temperature influence composition, and, in some instances, such compositional changes have been studied in detail (Table 4). It is usually possible to relate compositional changes to the channelling of acyl precursors into free fatty acids and the products of the reductive and decarbonylation pathways, to changes in their chain-length (CL) distributions, or in some cases to both. In studies with brussels sprouts, *B. oleracea*, Baker (1974) found that normal (glaucous) plants and the *gl4* mutant generally had more fatty acids and products of the reductive pathways at lower irradiation levels and higher temperatures, and more of the decarbonylation products at higher irradiation and lower temperatures. Patterns were more complex among several glossy (*gl*) mutants. In comparison, the effects were reversed for temperature (*gl2* and *gl3*) or showed intermediate product distributions for temperature (*gl1*) and light (*gl1*, *gl2* and *gl3*). In another study (Shepherd *et al.*, 1995b, 1997) with the brassicas kale, *B. oleracea*, and swede, *B. napus*, glasshouse-grown plants (lower irradiation, higher temperature) were compared with plants grown outdoors (higher irradiation, lower temperature). Both species responded similarly under the different conditions, and there was also

Table 4 Effect of temperature and irradiation on leaf wax composition

	Variable parameter	Effect on wax quantity	Reductive pathways ^a				Decarbonylation pathways ^a			Reference
			Fatty acids	Aldehydes	Primary alcohols	Esters	Alkanes	Secondary alcohols	Ketones <i>Ketols</i>	
Brussels sprout (<i>Brassica oleracea</i>)										
Normal	T (15 vs 35°C) ^b		< (× 6.0)	< (× 7.0)	< (× 3.0)	< (× 2.0)	> (× 1.5)	> (× 1.3)	> (× 1.4)	Baker (1974)
<i>gl4</i>			< (× 2.0)	> (× 1.1)	< (× 2.0)	< (× 4.0)	> (× 1.4)	> (× 1.4)	< (× 1.2)	
<i>gl1</i>			< (× 1.2)	≈	> (× 1.2)	> (× 1.8)	< (× 1.6)	> (× 24)	< (× 2.0)	
<i>gl2</i>			≈	> (× 1.7)	> (× 1.1)	=	< (× 3.3)	≈	> (× 1.5)	
<i>gl3</i>			≈	> (× 1.2)	> (× 1.9)	< (× 1.1)	< (× 2.5)	≈	< (× 4.0)	
Normal	E (80 vs 38 W m ⁻²) ^c		< (× 2.0)	< (× 1.6)	< (× 1.6)	≈	≈	< (× 1.2)	> (× 1.6)	
<i>gl4</i>			< (× 1.5)	≈	≈	≈	≈	> (× 1.2)	< (× 1.3)	
<i>gl1</i>			< (× 1.4)	< (× 1.3)	> (× 1.4)	> (× 2.3)	≈	< (× 2.5)	> (× 2.0)	
<i>gl2</i>			< (× 1.6)	≈	> (× 1.3)	> (× 2.0)	≈	> (× 6.0)	> (× 1.5)	
<i>gl3</i>			< (× 1.2)	≈	≈	≈	≈	≈	≈	
Kale (<i>B. oleracea</i>); Swede (<i>B. napus</i>)	E, T	High E/low T > low E/high T (× 3.9–6.3)	> (× 1.2)	< (× 1.3)	> (× 1.3)	> (× 1.6)	> (× 1.1)	> (× 1.2)	< (× 1.2)	Shepherd <i>et al.</i> (1995b)
			< (× 1.2)	< (× 3.2)	> (× 2.3)	> (× 1.5)	> (× 1.1)	< (× 1.8)	< (× 1.8)	
									< (× 1.1)	
									< (× 1.3)	
<i>Citrus aurantium</i>	T (day 25 vs 30°C) ^d		> (× 1.8)		> (× 1.6)	> (× 1.2)	> (× 1.8)			Riederer & Schneider (1990)
	T (night 15° vs 20°) ^d		< (× 1.8)		< (× 1.8)	≈	< (× 1.6)			Hass (1977)
<i>Hedera helix</i>	T (18 vs 28°C) ^e			>>	<					Gonzalez <i>et al.</i> (1996)
Pea (<i>Pisum sativum</i>)	E (UV-B) 6.5 ^f , 0 kJ m ⁻² d ⁻¹	High E > low E (× 1.2–1.3)	> (× 2.5)		< (× 7)	> (× 4.8)	> (× 2)			
Tobacco (<i>Nicotiana tabacum</i>)	E (UV-B) 5.66, 0 kJ m ⁻² d ⁻¹	High E ≤ low E	> (× 1.8) ^g					< (n-) (× 1.1) ^g > (br-) (× 1.2) ^g		Barnes <i>et al.</i> (1996)
Barley (<i>Hordeum vulgare</i>)	E (light vs dark)	High E > low E (15–10)°C	≈	> (× 5.7)	≈	< (× 1.3)	≈			Giese (1975)

^aValues (× *n*) represent the relative change in the percentage composition for each compound class.

^bValues shown are based on data for lower illumination levels (38 W m⁻²) at 70% humidity; values for higher illumination levels (80 W m⁻²) are similar.

^cValues are for 21°C and 70% humidity.

^dNight-time temperature was 20°C for the daytime comparison, daytime temperature was 25° for the night-time comparison.

^eAldehydes comprised 40% of the wax at 18°C, but were not detected at 28°C.

^fEquivalent to 20% ozone depletion.

^gEffect seen for adaxial leaf surface only.

some similarity with the earlier findings of Baker (1974), decarbonylation products being relatively more abundant under higher irradiation/lower temperatures. The distribution of different compound classes within the major pathways can also change, suggesting that individual steps and transformations within the pathways (Fig. 3) may be differentially sensitive to the environment. However, this does not always happen. For example, Giese (1975) found with barley that the proportions of wax decarbonylation (1%) and reduction (89%) products or free fatty acids formed only by elongation (10%) were the same in the light and the dark. In their studies with *Citrus aurantium*, Riederer & Schneider (1990) showed that changes in daytime and night-time temperatures may differentially effect wax composition. Higher daytime temperature during leaf development reduced the quantities per unit area of alkanes, primary alcohols, fatty acids and alkyl esters, whereas, except for the esters, the amounts of these components increased with higher night-time temperatures.

The studies of Giese (1975) and Shepherd *et al.* (1995b, 1997) also showed the influence of environment on CL, with a shift towards shorter components under higher illumination (Figs 5, 6a). A shift to lower CL is seen for every class of component in barley wax, which is dominated by the products of the reductive pathways. In the light, one CL of a single distribution dominates a given wax class, whereas in the dark, two

prominent CLs or groups are found (Fig. 5). The *Brassica* wax is dominated by decarbonylation products, which occur as a single distribution under the different conditions (Fig. 6a).

Environmental factors can also influence the distribution of isomers within a class of compound. Some waxes contain branched-chain (*br*-) components, formed by utilisation of a *br*-primer unit in the synthesis *de novo* (Baker & Holloway, 1975; Shepherd *et al.*, 1995a, 1997; Shepherd, 2003). Higher levels of UV-B were associated with increased abundance of *br*-relative to *n*-alkanes in *Nicotiana tabacum* and an increase in the proportion of shorter ($C_{<30}$) to longer ($C_{>30}$) homologues (Barnes *et al.*, 1996). Similarly, both free and esterified *iso*- (*i*-) and *anteiso*- (*a*-) *br*-acids and alcohols in waxes from kale and swede were generally more abundant than the *n*-components under greater illumination and lower temperature (Fig. 6b) (Shepherd *et al.*, 1997). Environmental effects on CL were seen; at higher illumination *n*-acids, *n*-alcohols and *i*-acids showed a shift to shorter CL for kale but to longer CL for swede. One pair of kale and swede genotypes showed a shift to shorter *i*-alcohols, while the other pair showed a shift to longer CL. There were no apparent relationships between CL and environment for *a*-components. These effects may also have been attributable to differences in UV-B since plants with the fewest *br*-components were grown in a glasshouse while those with higher levels were grown outdoors.

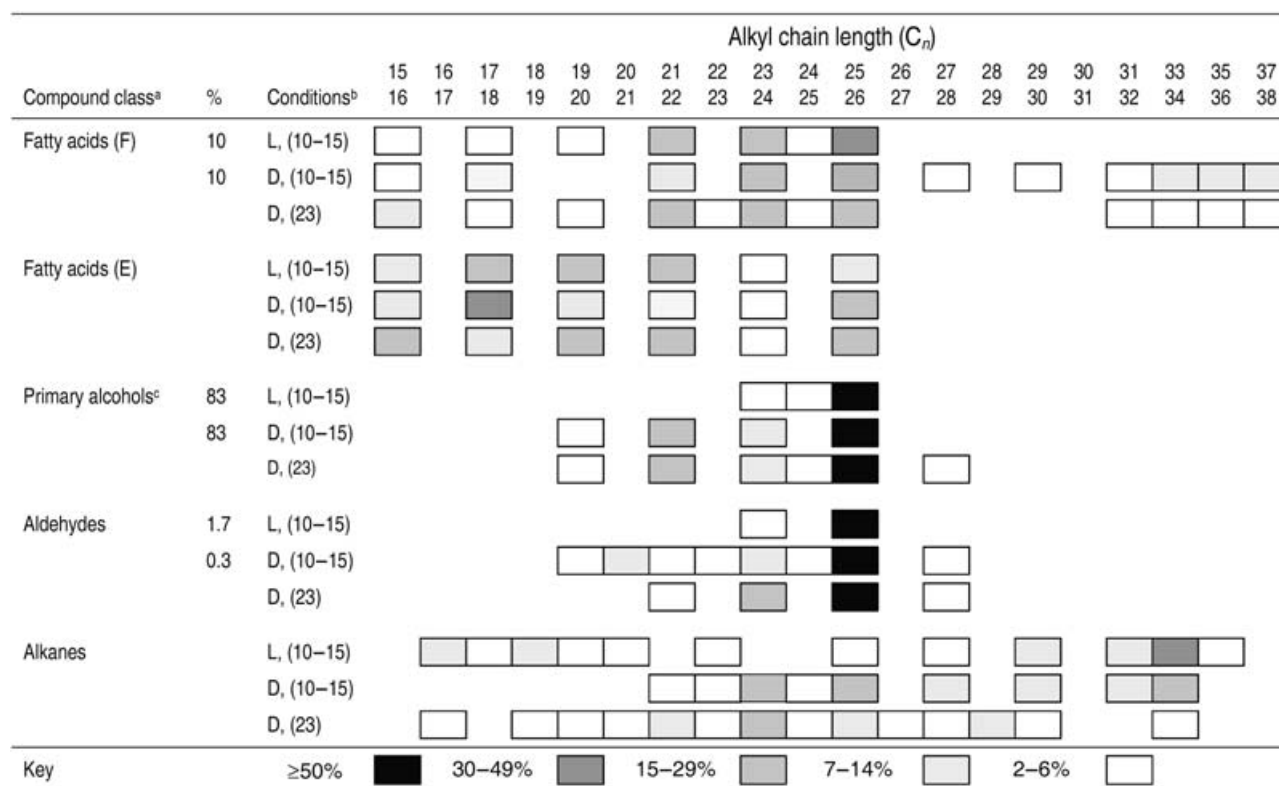


Fig. 5 Distribution of wax components from wax of barley (*Hordeum vulgare*) under different growth conditions. ^aEsters were also detected (4.7 and 6.1%) in the light and dark (10–15°C), respectively; ^bIllumination levels, growth temperature (°C); ^cFree and esterified; L, grown under illumination; D, grown in the dark; F, free; E, esterified. (Based on data tabulated in Giese (1975).)

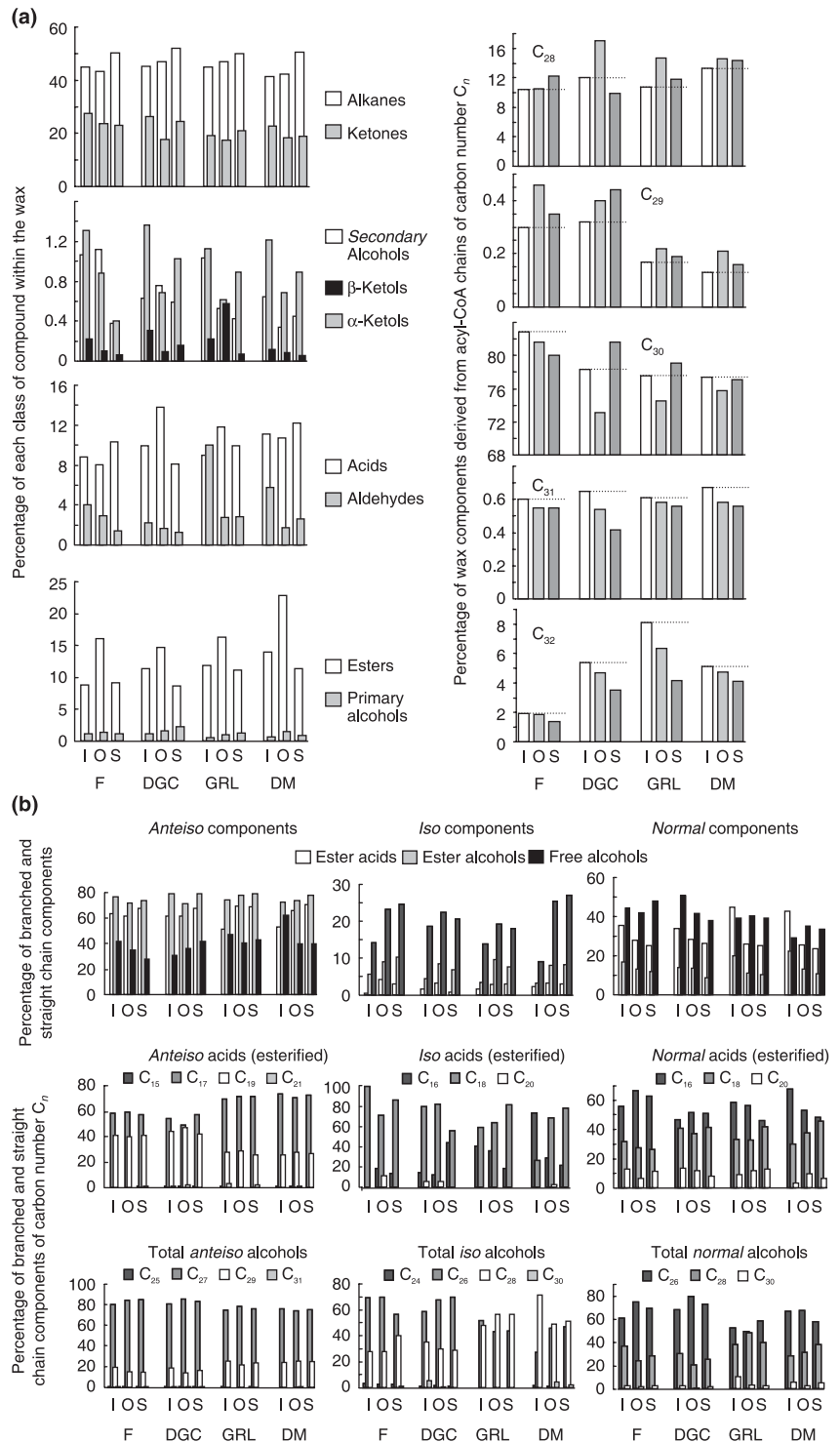


Fig. 6 Distribution of wax components from kale (*Brassica oleracea* var. *acephola*) genotypes Fribor and DGC and swede (*Brassica napus* var. *rapifera*) genotypes GRL and Doon Major under different growth conditions. Plants were grown indoors (I) in a glasshouse and outdoors (O) in a semi-shaded location in Scotland, and outdoors (S) in Switzerland. (a) Products of the associated pathways and overall chain length (CL) distribution. (b) Total and CL distributions for *anteiso*- and *iso*-branched and *normal*-esterified acids, alcohols and free alcohols in wax esters components. (Based on data tabulated in Shepherd *et al.* (1995b, 1997).)

VI. Contact angles and wettability

Leaf surface wettability is influenced by the physicochemistry of the cuticular wax and, to a lesser extent, leaf turgor (Butler, 1996; Cape, 1996). Wettability is measured in terms of the contact angle (CA), the angle subtended between the leaf

surface and the plane of a tangent to the surface of a water droplet originating at the contact point for air, leaf and water (Fig. 4c). Values of CA vary widely from 120° for very hydrophobic *Eucalyptus globulus* (Hietala *et al.*, 1997) to 29° for very hydrophilic *Vicia faba*. At angles < 90° water droplets tend to spread, ultimately forming water films, particularly

when leaves are shaken by wind. Conversely, formation of isolated drops is a characteristic of angles $> 90^\circ$ (Cape, 1996). Contact angles are therefore indicative of water retention capacity, which is significant for surface-colonising insects and microorganisms (Butler, 1996; Huttunen, 1996) and for foliar deposition and uptake of nutrients, pollutants and agrochemicals (Cape, 1996). Droplets on wettable leaves can focus solar radiation up to 20 times (Brewer *et al.*, 1991), possibly by acting as planoconvex lenses. This may increase the transmission of damaging UV-B to the epidermis and upper mesophyll. Some stress-induced changes in CA and wettability are associated with altered wax composition and morphology (Table 5). Reductions in CA of up to 30° were correlated with temperature-induced recrystallisation of conifer needle wax, largely composed of (S)-nonacosan-10-ol, from tubular to planar forms (Anfodillo *et al.*, 2002), and increased abundance of *br*-alkanes in the wax from *N. tabacum* following exposure to elevated UV-B (Barnes *et al.*, 1996).

VII. Humidity effects

Suppression of wax production by high humidity and low light intensity is a particular problem associated with tissue culture (Sutter & Langhans, 1979, 1982). Plants grown *in vitro* are susceptible to desiccation due to the lack of waxes and require protective environments (humidity tents, intermittent mist and shade) for 8–10 d for hardening and survival before transfer to a glasshouse. Wax deposition on *B. oleracea*, *E. gunnii* and *Tropaeolum majus* was increased by reducing relative humidity from 100 to 35% or from 98% to 20–30% (Baker, 1974; Sutter & Langhans, 1982; Koch *et al.*, 2006). Higher humidity may affect wax morphology (Table 5); for example, formation of larger crystalline tubules in *B. oleracea* is restricted, leaving dendrites as the major morphological type (Baker, 1974; Koch *et al.*, 2006), and the diameter and degree of clustering of branched rodlets in *E. gunnii* are reduced. High humidity does not always affect wax coverage and composition, for example, as found by Riederer & Schneider (1990) for *Citrus* leaf cuticles, or structure, as found for *T. majus*, where the formation of branched tubules is unaffected (Koch *et al.*, 2006).

VIII. Water, salinity and cold stress

1. Water stress

Leaf transpiration has stomatal (TR^{st}) and cuticular (TR^{cu}) components. TR^{st} is controlled by stomatal conductance, largely determined by tissue water status at a given vapour pressure difference between the leaf surface and the air. TR^{cu} is affected by the physicochemical characteristics of the leaf surface, such as wax thickness and, to a greater extent, wax microstructure, which largely determine the leaf surface's hydraulic permeability and transport characteristics (Svenningsson, 1988; Xu *et al.*, 1995, reviewed by Riederer &

Table 5 Association of contact angles with wax composition and crystalline morphology

Plant species	Contact angle (CA)	Associated wax crystal morphology and composition	Reference
Norway spruce (<i>Pinus abies</i>); Stone pine (<i>P. cembra</i>)	$> 113^\circ$, in February ^a ($CA_{abax} 30^\circ > CA_{adax}$) 83° , in August ^a ($CA_{abax} 30^\circ > CA_{adax}$)	Tubular, mainly (S)-nonacosan-10-ol Planar, mainly (S)-nonacosan-10-ol	Anfodillo <i>et al.</i> (2002)
Tobacco (<i>Nicotiana tabacum</i>)	$< 75^\circ$, elevated UV-B ($CA_{abax} 15^\circ > CA_{adax}$) $90\text{--}106^\circ$, zero or ambient UV-B ($CA_{abax} \geq CA_{adax}$)	Increase in the ratio of <i>br</i> - to <i>n</i> -alkanes Increase in the ratio of <i>n</i> - to <i>br</i> -alkanes	Barnes <i>et al.</i> (1996)
<i>Brassica oleracea</i>	160° , low RH ^b 110° , high RH ^c	Tubules with dendritic branches Dendrites, increased ratio of primary alcohols to aldehydes and ketones to secondary alcohols	Koch <i>et al.</i> (2006)
<i>Eucalyptus gunnii</i>	160° , low RH ^b 150° , high RH ^c	Branched rodlets (dense clusters) Both unbranched and branched rodlets (non-clustered)	Koch <i>et al.</i> (2006)
<i>Tropaeolum majus</i>	155° , low RH ^b 135° , high RH ^c	Both mainly triacontane-14,16-dione and β -amyryn Branched tubules, unaffected by RH, mainly (S)-nonacosan-10-ol	Koch <i>et al.</i> (2006)

n-, normal (straight) chain; *br*-, branched chain; *adax*, adaxial leaf surface; *abax*, abaxial leaf surface.

^aAt treeline.

^b20–30% relative humidity (RH).

^c98% RH.

Schreiber, 1996; Schreiber *et al.*, 1996). Under extreme water deficit, stomata close and stomatal conductance falls, and water loss by the cuticular route becomes significant (Fig. 4b,d). TR^{cu} is technically difficult to measure accurately (see reviews by Kerstiens, 1996a,b), incomplete stomatal closure being the most significant problem, even under conditions promoting maximal stomatal closure. Therefore the term minimum conductance (g_{min}) is used to allow for any contribution from (TR^{cu}), and unless stomata-free preparations are used, this is the value determined experimentally.

Wax deposition is often a response to water stress, and this can occur rapidly within a few days (Bengston *et al.*, 1978; Premachandra *et al.*, 1991). Stress-resistant plants, for example those adapted to arid conditions, often have thicker waxes than those from more temperate locations or that are otherwise susceptible to stress. However, this is not always the case, Tischler & Burson (1995) found no relationship between geographical location and wax coverage or heat-tolerance in their study of several heat-tolerant bahiagrass (*Paspalum notatum*) cytotypes. Several studies have attempted to show correlations between g_{min} , reflectivity, morphology and specific wax components (Tables 3, 6); however, their findings have to be interpreted with caution due to uncertainty about stomatal contributions to the measured values. Although there is no simple relationship between g_{min} and the amount or composition of wax (Kerstiens, 1996a,b), there does appear to be a relationship with CL. Hauke & Schreiber (1998) reported that an increase in the mean CL of the constituents in ivy, *Hedera helix* wax from C_{27} to C_{33} over the first 60 d of growth was in greater accordance with the observed reduction in g_{min} than the actual wax composition.

2. The barrier membrane model of cuticular waxes

The ability of hydrocarbon chains to coalign and form a hydrophobic barrier is well known, for example the suppression of evaporation by monolayers of long-chain alcohols (Langmuir & Schaefer, 1943; Mansfield, 1955). The model for the molecular organisation of wax molecules outlined by Riederer & Schneider (1990) and Riederer & Schreiber (1996) involves coalignment of the hydrocarbon backbone of wax constituents normal to the cuticle surface, forming monolayers extending laterally parallel to the cuticle (Sitte & RENNIER, 1963), which constitute a solid crystalline zone. Many such sheets may be stacked, and between them are regions populated by the ends of alkyl chains, which could include the polar functional groups of acids, alcohols and aldehydes in addition to terminal methyl groups. If the constituents were of uniform CL, then this intermediate region would be indistinguishable from the crystalline zone. However, waxes usually have a skewed or often bimodal CL distribution, and consequently the intermediate region has a distinct volume fraction into which chain ends may protrude and is considered to be in a solid amorphous state. Adjacent

layers may be bridged by intercalation of longer chains such as alkyl esters, and, in principle, secondary alcohols and ketones could also link the layers. The layered sheets are probably discontinuous, surrounded by further solid amorphous regions including components not present in the crystalline regions. Since the crystalline regions are considered impermeable in the barrier membrane model, water and solutes diffuse through the cuticular wax via the amorphous zones, and molecules travel many times further than the simple thickness of the wax, due to the large aspect ratios (width : thickness) of the crystalline regions. Using published crystallographic (Small, 1984) and density data for alkanes (Le Roux, 1969), Jetter *et al.* (2000) estimated that the cuticular wax of *Prunus laurocerasus* consisted of an alkane layer 130–160 nm thick, similar to a value of < 200 nm based on scanning electron microscopy (SEM) measurements of detached wax. Assuming molecular packing in *cis* configuration perpendicular to the cuticle (Sitte & RENNIER, 1963), the wax was estimated to be 35–45 molecules thick.

These layered sheets, which may not appear crystalline in SEM images, constitute the basic structure out of which the microcrystalline forms protrude. In some cases, microcrystals may be vertical extensions of the basic structure, or partially detached regions. However, given the association between morphology and composition, it is likely that some microcrystals are outgrowths with a different chemical composition to the underlying sheets. Formation of tubular structures, characteristic of secondary alcohols and ketones, etc., implies a different mode of molecular packing, perhaps imposed by the stereochemistry in the region of the oxo-substituents. Alternatively, different crystal forms may arise from different crystallisation rates as suggested by Baker (1974). Such structures are likely to have amorphous zones, and the inner surface of tubes may be a channel for movement and deposition of wax constituents, perhaps in association with a solvent. This may aid morphological transformations such as dendrite formation on existing structures. If alkyl esters tend to bridge amorphous regions in the underlying layers rather than form ester-specific structures, it may be significant that increased abundance of esters in maize wax appears to reduce the efficiency and amount of visible crystal formation, as reported by Beattie & Marcell (2002).

The significance of alkane CL to the barrier properties of cuticular wax has been elegantly demonstrated by Dodd and co-workers in their studies of the geographic distribution of several tree species (Dodd *et al.*, 1998; Dodd & Rafii, 2000; Dodd & Poveda, 2003). In a study of 27 populations of *Austrocedrus chilensis* from Chile and Argentina, mediterranean populations adapted to warmer and more arid conditions had higher proportions of longer (C_{34} – C_{37}) alkanes in their waxes than those of mesic populations adapted to cooler and more humid conditions at the rainforest margin. However, within the overall range (C_{21} – C_{37}), both populations had similar CL distributions for the shorter homologs (Dodd *et al.*, 1998).

Table 6 Effect of water or salinity stress compared with non-stressed plants on abundance and composition of cuticular wax

Plant species	Stress	Effect on wax quantity	Cuticular transpiration	Composition or characteristic associated with adaptation	Reference
Sorghum (<i>Sorghum bicolor</i>) ^a	W (RES)	↑ (× 1.15)	↓ (RES > SUS)	Bloom (glaucous)	Jordan <i>et al.</i> (1983, 1984)
	W (SUS)	↑ (RES > SUS)	↓	Bloomless	
Durum wheat (<i>Triticum turgidum</i>); common wheat (<i>Triticum aestivum</i>)	W (RES)	↑ Ears (× 1.73) ↑ Sheaths (× 1.2) ↑ Flag leaves (× 1.51)			Johnson <i>et al.</i> (1983)
Maize (<i>Zea mays</i>) ^b	W	↑ (× 1.35) after 10 d ↑ (× 1.43) after 20 d			Premachandra <i>et al.</i> (1991)
Tomato (<i>Lycopersicon esculentum</i>)	W, S	↑			Xu <i>et al.</i> (1995)
Oats (<i>Avena sativa</i>)	W (RES)	↑ (RES > SUS)	↓ (RES > SUS)	Primary alcohols ↑ (mainly C ₂₆) Alkanes ↓ (possible switch to formation of β-diketones) Total fatty acids ↑ (free, esterified) Primary alcohols ↑ (mainly C ₂₆) Alkanes ↑ Total fatty acids ≈ (free, esterified)	Bengston <i>et al.</i> (1978); von Wettstein-Knowles (1972)
	W (SUS)	↑	↓	Alkanes ↑	
Cotton (<i>Gossypium hirsutum</i>)	W (RES)	↑ Leaves (× 1.69) ↑ Bracts (× 1.47) ↑ Bolls (× 1.04)		Alkanes ↑ (× 10) (C ₂₄ → C ₃₄) ^c Alkanes ↑ (× 2.5) (C ₂₄ → C ₃₀) ^c Alkanes ↓ (× 0.3) (C ₂₄ /C ₂₆ → C ₂₈ /C ₃₀) ^c	Bondada <i>et al.</i> (1996)
Austrocedrus chilensis	W (RES)			Higher proportion of longer (C ₃₄ –C ₃₇) alkanes	Dodd <i>et al.</i> (1998)
Peanut (<i>Arachis hypogaea</i>)	S (RES)	↑	↓	Primary and sec-alcohols; β-diketones ↑ Alkanes ↓ (possible conversion to s-alcohols and β-diketones)	Rao <i>et al.</i> (1981)
56 weed species (mostly dicotyledenous)	W	No effect	↑	More fatty acids, absence of alcohols	Rama Das <i>et al.</i> (1979)
15 angiospermous weed species	W	No effect	↓	More aldehydes	Rao <i>et al.</i> (1980)
Grape (comparison of intact wax with wax fractions and individual compounds on artificial membranes)			↓ (Equally effective) ↓ (Less effective) ≈ (Ineffective)	Intact wax or paraffin wax; aldehyde and hydrocarbon fractions; alcohol fraction (mainly C ₂₄ , C ₂₆ , C ₂₈) Fatty acid fraction (mainly C ₂₄ , C ₂₆); C ₁₈ primary alcohol	Grncarevic & Radler (1967)
Caatinga (<i>Capparis yco</i> , <i>Ziziphus joazeiro</i>) (a) Caatinga (<i>Aspidosperma pyrifolium</i>); Cerrado (<i>Tocoyena formosa</i>), (b) Cerrado (<i>Aristolochia esperanzae</i>)	W	No effect	↓ (Most effective) ↓ (Least effective)	Oleanic acid; C ₁₈ fatty acid <i>n</i> -Alkanes, triterpene alcohols (a) Ursolic acid (b) hentriacontan-16-one	Oliveira <i>et al.</i> (2003)

W, water stress; S, salinity stress; RES, resistant; SUS, susceptible or least resistant; ↓ reduction or ↑ increase in value; ≈ relatively unchanged.

^aDryland conditions compared with well watered.

^b40 d old at start of stress treatment.

^cShift in homolog distribution from shorter to longer chain lengths.

These differences are thought to reflect increased bridging or protrusion of alkanes into the amorphous regions within the wax microstructure of mediterranean populations, impeding cuticular water loss. The annual rainfall related most closely to lower carbon CLs, and annual mean temperature to the longest CLs. It was concluded that the effects were primarily due to ecogenic adaptation.

In a second study, Dodd & Rafii (2000) compared three similar species, *A. chilensis*, *Fitzroya cupressoides* and *Pilgerodendron uviferum*. They span > 2500 km in range and are adapted to different conditions, but with overlapping range. The northernmost, *A. chilensis*, ranges from northerly mediterranean dry rock sites to the humid temperate rainforest margin and cool dry Patagonian steppe in the south. *F. cupressoides* occupies a narrow belt of moist coastal and Andean sites and *P. uviferum* extends to the southern tip of South America, in conditions ranging from warm humid and cool humid to continental. Alkane distributions in cuticular waxes can be analysed statistically to give the weighted mean (N) of CL C_n and dispersion (d) of C_n about the mean, and a lower value of d corresponds to a narrower CL distribution. Crystallinity is inversely proportional to d/N and mixtures with a narrow CL range have a narrower lipid phase transition temperature (T_m) range, retaining their crystalline structure for longer. Consequently, mixtures with higher mean CLs and narrower CL ranges have higher melting points and maintain crystallinity at higher ambient temperatures (Riederer & Schneider, 1990). *A. chilensis* and *F. cupressoides* had the highest average values of N (33.2, 32.2) and lowest values of d (1.9, 1.8), respectively, whereas *P. uviferum* had the lowest value of N (27.2) and highest value of d (3.0). This corresponds to higher melting points for *A. chilensis* than *P. uviferum*, where the difference in T_m is c. 12°C. Therefore, an elevated amount of the longest CL alkanes, with a reduced CL range in mediterranean populations of *A. chilensis*, helps to stabilize the wax's internal structure in the hottest and most arid conditions, and is another facet of an adaptation where reduced transpiration is the driving force. The parameters N and d for alkane CL distribution can also exhibit altitudinal variation. Populations of *Juniperus communis* from the Pyrenees growing at low and high altitudes had the highest values of N , while d increased with altitude (Dodd & Poveda, 2003). This was considered as indicative of possible adaptations to minimise g_{min} under hot summer conditions at low altitude and physiological drought caused by freezing at high altitude.

3. Salinity and cold stress

Increased wax deposition on plants such as peanut, *Arachis hypogaea* and salt-sensitive jojoba on exposure to salinity stress (Rao *et al.*, 1981; Mills *et al.*, 1997, 2001) appears to be primarily a response to water deficit. Wax deposition on leaves of salt-sensitive jojoba is also induced by exogenous abscisic acid (ABA) (Mills *et al.*, 2001). The increase in wax loading

caused by exposure of cabbage seedlings to moderate NaCl-induced water stress before transplantation was used to improve drought resistance in transplanted plants and is a useful hardening technique (Fujiwara *et al.*, 2002). Increased leaf wax production by ornamental trees irrigated with treated sewage effluent (reuse water) has been cited by Jordan *et al.* (2001) as an indicator of levels of foliar damage and stress equivalent to use of saline solution. These findings were used to recommend limitations on spray irrigation with reuse water, which is common practice in municipal areas of the southwestern United States.

Cold stress varies widely in severity and effect. Exposure of maize to a 7-d-long cold spell reduced quantities of cuticular wax on the third leaf of four-leaf plants by 29%, resulting in increased wettability and increased herbicide retention (Gauvrit & Gaillardon, 1991). Under more severe conditions, frozen soil causes frost drought which lowers water potential and inhibits transpiration and CO₂ uptake due to stomatal closure (Esch & Mengel, 1998b). Poor frost tolerance in willow, *Salix* sp., was correlated with increases in wax load and n -alkane content. Leaf surfaces were covered by more wax spheres and the increased hydrophobicity of the wax on a frost-susceptible high-producer clone was reflected in a CA value of 93°, approx. 20° higher than the other clones (Hietala *et al.*, 1997).

IX. Mechanical stress

Under windy conditions, wax can be removed by fracturing of crystals and abrasion due to aerodynamic loading, impact of raindrops, dust and snow, etc., and by leaf-to-leaf contact. In wind tunnel experiments, wax crystals on the leaves of *Picea stichensis* became flattened and smeared after exposure to airflow at 11 ms⁻¹ for 1 wk, and structures within and around the stomatal antechambers of *P. stichensis* and *Pinus sylvestris* were particularly vulnerable (Van Gardingen *et al.*, 1991). Waxes can reform rapidly after mechanical removal, for example the fine structure of broccoli, *B. oleracea* wax regenerated within 3 d (Schwab *et al.*, 1993). However, rates of replacement may not be fast enough to maintain normal levels (Hall & Jones, 1961). Latimer & Severson (1997) investigated the relative effects of wind, brushing (thigmic stress) and dehydrating moisture stress conditioning (MSC) on *B. oleracea* as mechanisms for conditioning and hardening glasshouse-grown plants before field planting. After 9 d treatment, reductions in wax levels due to brushing and wind were similar (31–38%), but that due to MSC was 11%. After 15 d, wax was reduced by 15% due to brushing and 6% due to wind, but had increased by 17% due to MSC, indicative of wax redeposition although the rate of removal by brushing was greater than the rate of renewal. There were no changes in wax composition during the treatments and after transplanting to the field, the plants were indistinguishable. A study of the seasonal development of wax on three plantain sp., *Hosta plantaginea*, *H. lancifolia*

and the glaucous genotype *Hosta* 'Krossa Regal', carried out by Jenks *et al.* (2002) illustrates the typical effects of weathering during a normal growing season. Wax coverage increased initially in the period from the expanding leaf stage to spring, then fell from spring to summer, increasing again from summer to autumn. The mid-season reduction was attributed to weathering by rain, wind and possibly heat stress. Specific compounds, β -diketones (C_{29} , C_{31} 10,12-diones), were abundant on both leaf surfaces of 'Krossa Regal' during development up to spring, then remained abundant on the inner abaxial surface, but became minor components on the exposed outer adaxial surface, possibly due to differential weathering. The extent of wax erosion during a growing season can vary greatly between species. Neinhuis & Barthlott (1998) found that wax on ginkgo eroded at a much slower rate than that on oak, *Quercus robur*, which eroded rapidly a few weeks after leaf expansion ceased. The reason for such differences is unclear but might relate to differences in the susceptibility of different crystal structures to damage, or may reflect a greater ability of ginkgo to regenerate its wax.

X. Altitude

Plants growing at high altitude experience interacting stresses, including weathering, dehydration and low temperatures. For some conifers the altitude limit of the alpine treeline may be determined by the extent of winter needle desiccation, resulting in increased g_{\min} (reviewed by Kerstiens, 1996a; Körner, 1998). Lack of warmth in the short growing season may impair epidermal development, leaving the needles susceptible to water loss by TR^{cut} during the following winter. Damage may arise from strong winds and abrasion by driven snow, resulting in increased water loss (Tranquillini, 1979; DeLucia & Berlyn, 1983; Hadley *et al.*, 1991; Herrick & Frieland, 1991; Anfodillo *et al.*, 2002). Grace (1990) attributed the treeline limit for Scots pine, *Pinus sylvestris*, in the Cairngorms, Scotland, to stomatal dysfunction and cuticular damage rather than impaired cuticular development. Increased leaf or needle wax coverage is a characteristic of growth at higher elevation in some species such as Norway spruce, *Pinus abies* and stone pine, *Pinus cembra* in southern alpine environments (Günthardt, 1984; Riolo, 1999).

Surface characteristics determined for conifer needles, such as CA, reflectance and g_{\min} , can show altitudinal variation. Anfodillo *et al.* (2002) found for *P. abies* that CA both increased with altitude and exhibited a seasonal reduction from winter to summer, except at the lowest altitudes where the pattern was reversed. At high altitudes (treeline) CA ranged from $> 113^\circ$ in February to 83° in August (Table 4) as wax morphology changed from less wettable tubular forms to more wettable planar forms. Similar changes in CA were seen for *P. cembra*, for which variations were seen between adaxial and abaxial surfaces, the latter having CAs up to 30° greater than the former. Contact angles were also generally greater to windward

than leeward. At low (valley bottom) and mid-altitudes, g_{\min} rose from winter to summer, although g_{\min} was unaffected by altitude. This was believed to indicate defective stomatal closure rather than changes in cuticle permeability.

Reflectance (PAR) for the co-occurring Alaskan conifers black spruce, *Picea mariana* and white spruce, *P. glauca* increased with both latitude and altitude over the range 60–930 m (Richardson *et al.*, 2003). Both species had similar reflectance spectra, but reflectance was higher in black spruce than white spruce, probably due to differences in wax loading, which may represent a photoprotective adaptation in black spruce for colonisation of more stressful sites. Interpretation of the data suggested that a 1000 m increase in elevation was effectively similar to a 6° increase in latitude. Adaptation to different geographic locations can alter wax composition (see section VIII.2). In a study of the effects of UV-B on geographically adapted *Poa* sp., Pilon *et al.* (1999) found that differences in wax loading and composition, or production of UV-absorbing compounds such as triterpenes, were associated with location rather than exposure to UV-B. Wax loading on alpine plants was at the low end of the range found for all plants, although there was no clear altitudinal effect. Alkanes and aldehydes were far more abundant or were only found in alpine plant waxes, whereas primary alcohols were more abundant from lowland plants. Triterpenes (squalene, lupeol, β -amyrin) were found in waxes from lowland plants but were absent from the alpine varieties. Levels of esters were similar.

XI. Pollution

In high concentrations, and under prolonged exposure, acidic and oxidising gases and aerosols can help to degrade cuticular wax and impair stomatal function (Roberts & Cannon, 1989). This may impose chronic water stress, which can lead to defoliation and death. Various aspects of the interaction between atmospheric pollutants and the leaf cuticle have been reviewed by Heath (1980) and contributors in Percy *et al.* (1994a).

1. Acid rain

The wettability of cuticular wax influences the retention and spread of acidified droplets and potentially the extent of damage. Acidity is also a factor: greater damage is caused by rain or mist at pH 3 than at pH 5 (Knittel & Pell, 1991; Esch & Mengel, 1998a; Hoard *et al.*, 1998). Formation of necrotic lesions within the leaf is characteristic of acid damage, which may be accompanied by damaged or altered wax morphology, resulting in an increase in g_{\min} and water stress. Consequently, plants previously exposed to acid rain can become more sensitive to drought and desiccation under water deficit, causing more pronounced stomatal closure and loss of turgor than for drought alone, as reported by Mena-Petite *et al.* (1999) for *Pinus radiata*, and by Esch and Mengel (1998a,b) for spruce,

Picea abies, under frost drought conditions. However, this need not always apply; for example, the susceptibility of *Zea mays* to drought stress was unaffected by simulated acid rain, and the wax was morphologically unchanged (Knittel & Pell, 1991). The damaging effects of acid precipitation are enhanced when combined with wind action. Hoad *et al.* (1998) found that the greatest damage to birch occurred with a combination of direct or indirect wind action and acid mist at pH 3. By contrast, sheltered leaves were very resistant to the effects of the acid mist.

2. Wax erosion and stomatal damage

Airborne pollutants accelerate erosion of cuticular waxes; for example, aerosols containing antimony (Sb) eroded wax on Japanese cedar, *Cryptomeria japonica* 1.5 times faster than found for unpolluted trees (Sase *et al.*, 1998; Takamatsu *et al.*, 2001a,b). Erosion rates also depend on plant age and geographic location. In conifers, juvenile waxes are particularly vulnerable to damage; for example, wax degradation by sulphur dioxide (SO₂) and absorption of S by Scots pine, *Pinus sylvestris* were greatest on the two youngest needle age classes (Manninen & Huttunen, 1995). Pollution-induced changes to wax morphology in the stomatal antechambers of some conifer needles are similar to ageing effects, starting with fusion of wax rods at the tip, ultimately leading to loss of crystalline structure (Bermadinger-Stabentheiner, 1994; Huttunen, 1994). Resistance to damage can vary between species exposed to similar pollution loads. Waxes on young needles of Norway spruce were damaged within a year of exposure, whereas those of silver fir, *Abies alba* required 3 years' exposure before damage occurred (Bednarova, 2001a). Reduced pollution levels can lead to reduction in damage and ultimately to recovery (Bednarova, 2001b). Stomatal damage is common and may include collapse, depression, fusion or degradation of guard cells, and occlusion with wax clumps (Paoletti *et al.*, 1997). In addition, acid rain and drought can leach calcium, causing formation of CaSO₄ microcrystals in pine needles, particularly at the interface between stomatal antechambers and substomatal cavities (Huttunen *et al.*, 1989; Turunen *et al.*, 1994; Pritchard *et al.*, 2000). This process effectively neutralises the acid and may provide protection from further damage.

3. Ozone

Ozone is a common air pollutant and some of its effects may be related to membrane degradation via oxidation of double bonds in fatty acids and sulfhydryl groups in proteins. At 65 nl l⁻¹, ozone severely reduced formation of new cuticular wax via the synthesis *de novo*, although the existing wax was unaffected (Carlsson *et al.*, 1994; Hellgren *et al.*, 1995). Ozone reduced wax synthesis in red spruce needles (Percy *et al.*, 1992), degraded wax in Norway spruce (Barnes *et al.*, 1988) and decreased chloroplast size and photosynthesis in Norway

spruce (Sutinen *et al.*, 1990; Wallin *et al.*, 1990) and wheat (Ojanperä *et al.*, 1992). Impaired wax synthesis could arise from a reduced supply of photosynthetically fixed carbon to the synthesis *de novo* and by disruption of the membrane integrity of the plastids where the synthesis occurs.

4. Carbon dioxide

According to some reports, climate change may double atmospheric CO₂ levels by the end of the century (Long *et al.*, 2004), accompanied by increasing temperatures, changes in rainfall patterns and increased UV-B exposure due to ozone depletion (see also section IV). Consequently, there is much interest in interaction between these parameters, particularly whether the generally beneficial effects of elevated CO₂ may compensate for the deleterious effects of the other parameters, in particular increased UV-B. Effects of elevated CO₂ are variable. Vanhatalo *et al.* (2001) found that elevated CO₂ alone, and in combination with O₃, increased wax coverage on the abaxial leaf surface of an inland clone of pubescent birch, *Betula pubescens*, but not on that of a coastal clone. By contrast, wax coverage fell when longleaf pine, *Pinus palustris*, was exposed to elevated CO₂ in studies of the interactions between elevated CO₂, water status and nitrogen supply (Prior *et al.*, 1997), and carbon allocation under conditions of elevated CO₂ and water stress (Runion *et al.*, 1999). Similarly, wax coverage on the Crassulacean acid metabolism (CAM) species *Agave deserti* was reduced by 40% when CO₂ levels were doubled, reflectance was 14% lower and transmittance 6% higher (Graham & Nobel, 1996). Elevated CO₂ can partially ameliorate some of the adverse effects of UV-B radiation in canola, *Brassica napus* (Qaderi & Reid, 2005). Cuticular wax coverage similarly doubled under elevated levels of CO₂ and UV-B alone. Although in combination there was a further slight increase, elevated CO₂ increased wax deposition sufficiently to provide protection against UV-B. Percy *et al.* (2002) investigated the effects of elevated CO₂, and O₃ on Aspen, *Populus tremuloides*, singly and in combination. They found that both CO₂ and O₃ alone increased cuticular wax production (16 and 23%, respectively); however, in combination, these effects were negated.

5. Direct effects on wax composition

There is a growing body of evidence (Table 7) that pollutants can modify wax composition, and that they may therefore have a direct effect on the biosynthesis of wax constituents, affecting both the distribution of products from the associated pathways and CL distribution. Percy *et al.* (1994b) have also shown, using recrystallised whole wax, that most wax constituents, once crystallised, are stable to treatment with ozone at 70 and 150 p.p.b. and acid at pH 3.0 and 1.0, levels of acidity to which plants can be exposed under some pollution scenarios. In exception to this, alkyl esters are

Table 7 Effect of atmospheric pollutants on wax chemical composition

Plant species	Pollutant	Compositional change	Reference
Canola (<i>Brassica napus</i>)	AR (pH ≤ 4.2)	↓ Nonacosane, nonacosanol-15-ol, nonacosanone	Percy & Baker (1987)
Sitka spruce (<i>Picea sitchensis</i>)	AR (pH ↑)	↑ Nonacosan-10-ol (two clones), nonacosane diols and hydroxy acids (one clone), estolides (one clone) ↓ Estolides (one clone)	Percy & Baker (1990)
Norway spruce (<i>P. abies</i>)	AR + O ₃ O ₃	↑ Nonacosan-10-ol (three clones) ↑ Nonacosan-10-ol, secondary diols; ↓ Estolides, ω-hydroxy fatty acids	Lütz <i>et al.</i> (1990); Günthardt-Goerg (1994); Dixon <i>et al.</i> (1997)
Norway spruce (<i>P. abies</i>); Sitka spruce (<i>P. sitchensis</i>)	O ₃ CO ₂ (700 p.p.m. vs. ambient)	↑ Alkanes, ≈ alcohols ↑ Nonacosan-10-ol, fatty acids (C ₁₄ –C ₂₆) ≈ 4,10- and 5,10-nonacosandiol	Prügel & Lognay (1994)
Red spruce (<i>P. rubens</i>)	O ₃ (> 70 p.p.b.)	↓ Secondary alcohols, diols, esters, fatty acids	Percy <i>et al.</i> (1992)
Aspen (<i>Populus tremuloides</i>)	CO ₂ ; O ₃ CO ₂ + O ₃	↑ Hydrocarbons, fatty acids ≈ hydrocarbons, ↑ fatty acids	Percy <i>et al.</i> (2002)
Norway spruce (<i>P. abies</i>); ivy (<i>Hedera helix</i>)	AR, O ₃	Shortening of alkane CL	Kerfourn & Garrec (1992)
Rye grass (<i>Lolium perenne</i>)	SO ₂ (winter) SO ₂ (summer)	↓ C ₂₄ alkane; ↑ C ₃₃ alkane ↑ C ₂₅ alkane	Shelvey & Koziol (1986)

AR, simulated acid rain, mist or fog; CL, chain length; ↓ reduction or ↑ increase in value.

subject to hydrolysis with increasing acidity, being converted to totally free fatty acids at pH 1.0.

XII. Genetic and environmental control of cuticular wax production

Detection of, and response to, environmental stimuli, for example, osmotic stress induced by cold, drought and salinity, involve a sequence of events starting with detection of a stress-induced signal and leading via a cascade of signalling steps to the final response. Intermediate stages can involve release of secondary signalling molecules such as inositol phosphates, reactive oxygen species and ABA, modulation of intracellular Ca²⁺, initiation of protein phosphorylation cascades and induction of proteins involved in cellular protection and transcriptional control of stress-related genes (reviewed by Xiong *et al.*, 2002; Zhu, 2002). Although numerous forms of stress influence cuticular waxes, they commonly induce changes in the amount and composition of wax, effects which are closely associated with the control of biosynthesis. Current understanding of this process comes mainly from the use of chemical and photoperiod inhibition (von Wettstein-Knowles, 1979) and mutants such as *eceriferum* (*cer*) in *Arabidopsis* and barley and *gl* in maize and various brassicas to block biosynthesis at particular points, leading to measurable accumulation of intermediates (Table 8a,b). Chain elongation and the channelling of acyl intermediates into the products of the reductive and decarbonylation pathways are shown schematically in Fig. 7. ACCase has a key role in the regulation of acyl chain production by supplying malonyl-CoA (Fig. 2) for elongation. Activation of partly purified ACCase from pea *in vitro* involves light-stimulated reduction of the enzyme by

thioredoxin (Sasaki *et al.*, 1997), and the activity of the CT β-subunit may be regulated by phosphorylation and dephosphorylation (Savage & Ohlrogge, 1999). Plastidial chain extension by FAS involves multiple tissue-specific isoforms of ACP, and in *Arabidopsis*, expression of the leaf-specific ACP4 is light-stimulated (Bonaventure & Ohlrogge, 2002), possibly based on the presence of I-box like motifs found in light-inducible promoters (Terzaghi & Cashmore, 1995). Post-transcriptional control is also exerted due to the greater association of ACP transcripts with polyribosomes in the light than in the dark.

The Claisen reaction catalysed by KAS is another key event during chain elongation by FAS (Fig. 2, step c), and multiple KAS isoforms with different CL specificities were revealed through the effect of inhibitors such as cerulenin and arsenite on fatty acid synthesis (Fig. 7). Formation of C₄ chains involves KAS III, further elongation to C₁₄ and C₁₆ involves KAS I, and KAS II catalyses the final step to C₁₈ (von Wettstein-Knowles, 1995; von Wettstein-Knowles *et al.*, 2000; Carlsson *et al.*, 2002). Genes encoding the major KAS isoforms have been cloned and expressed sequence tag (EST) clones for each KAS have been identified from various species. Extensive homology exists between KAS I and II, whereas KAS III is similar to another condensing enzyme, polyketide chalcone synthase (CHS). Transfer of acyl groups from acyl-ACP to KAS I (Fig. 2 step g) is inhibited by free ACP formed during the process and the inhibition is reversed by acylation of ACP, whereas ACP has little effect on KAS II or KAS III. However, C₁₆-ACP is a feedback inhibitor of KAS III, and therefore overproduction of acyl chains by KAS I inhibits initiation of new chains by KAS III. Reduced KAS II activity in the *Arabidopsis* mutant *fab1* results in accumulation of

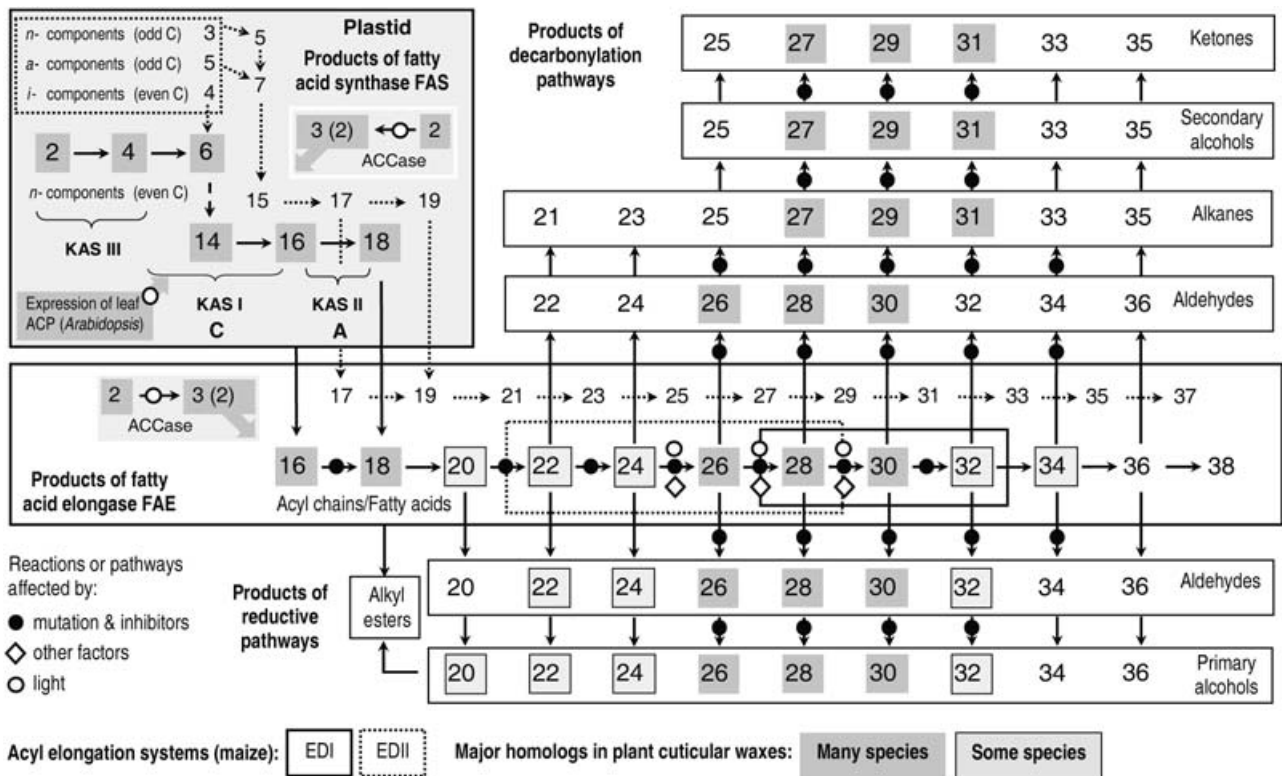


Fig. 7 Schematic representations of acyl chain elongation by fatty acid synthase (FAS) in plastids and extra-plastidial fatty acid elongase (FAE). The subsequent channelling of acyl intermediates into the associated decarbonylative and reductive pathways is shown. Numbers shown indicate the length of the acyl chains. The major products of elongation are *normal*- (*n*-) straight chains with even carbon numbers derived from a C₂ primer unit (acetyl-CoA). Odd carbon number chains and chains with methyl branches are formed by the use of alternative primers. Propionyl-CoA (C₃); isobutyryl-CoA (C₄), 3-methylbutyryl-CoA (C₅) and 2-methylbutyryl-CoA (C₅) are primers used for formation of odd carbon *n*-chains, even carbon *iso*- (*i*-) branched chains, odd carbon *i*-branched chains and odd carbon *anteiso* (*a*-) branched chains, respectively. Several of the elongation steps or sequences have chain length-specific isoforms of the condensing enzyme 3-ketoacyl-ACP synthase (KAS) in FAS. (Adapted from Shepherd (2003) with permission from Academic Press.)

palmitic acid (C₁₆), rendering the mutant susceptible to low temperature stress (Carlsson *et al.*, 2002).

Defects at specific points in elongation by FAE are features of many of the *cer*, *gl* and other mutants, resulting in accumulation of shorter products than found for wildtype plants. Chemical inhibition and variation in photoperiod can also give similar effects, and, overall, impaired elongation can occur between C₁₆ and C₁₈, and at each step between C₂₀ and C₃₂ (Fig. 7, Table 8). These findings support the presence of sequential elongation systems with multiple isoforms of elongase, or subunits such as the condensing enzyme KCS, with specificity for a particular step or sequence of steps in elongation. The associated pathways may also be defective, changing the distribution of the different classes of compound (Fig. 7, Table 8b). Such alterations are often responsible for the phenotype of the mutation (von Wettstein-Knowles, 1995; Kolattukudy, 1996; Post-Beittenmiller, 1996; Kunst & Samuels, 2003), which bears a certain similarity with some of the stress-induced phenotypic modifications.

Of the many genes involved in wax biosynthesis, relatively few have known identities or function. Some involved with

elongation and the associated pathways have been cloned, and their gene products studied. Genes encoding KCS have been identified from seeds of *Arabidopsis*, brassica and jojoba. Several *Arabidopsis* genes, *KCS1* (Todd *et al.*, 1999), *CER6* (*CUT1*) (Millar *et al.*, 1999; Fiebig *et al.*, 2000; Hooker *et al.*, 2002), *FDH* (fiddlehead) (Yephremov *et al.*, 1999; Pruitt *et al.*, 2000) and *HIC* (high carbon dioxide) specific to stomatal guard cells (Holroyd *et al.*, 2002; Hetherington & Woodward, 2003), encode KCS in leaves and stems. Up to 20 members of the *Arabidopsis* KCS gene family were recently found in epidermal tissues of stem tops during gene expression studies, with *KCS1*, *CER6* and *FDH* having the greatest expression levels (Suh *et al.*, 2005). The *kcs1-1* and *cer6* mutations affect each elongation step between C₂₄ and C₃₀, with reduced levels of the corresponding C₂₆–C₃₀ aldehydes and primary alcohols (*kcs1-1*, *cer6*) and C₂₉ alkanes, secondary alcohols and ketones (*cer6*). The main effect of *cer6* occurs between C₂₄ and C₂₆; however, for *kcs1-1* no complete blockages in CL extension are evident, suggesting that there is considerable redundancy in elongase activity and CL specificity. Of the other enzymes associated with FAE, maize *gl8* encodes a 3-ketoacyl reductase

Table 8 Effects of inhibitors, photoperiod and mutation (a) On elongation and (b) elongation and compound distribution within the associated pathways (Adapted from Shepherd (2003) with permission from Academic Press.)

Step	Inhibitors/mutation	Plant	Suggested function	References ^a
(a) Effects on elongation				
C ₁₆ → C ₁₈	Cerulenin Arsenite	Barley (<i>Hordeum vulgare</i>)		Mikkelsen (1978)
C ₂₀ → C ₂₂	Arsenite, 2-ME Cerulenin	Barley Leek		Mikkelsen (1978)
C ₂₂ → C ₂₄	Photoperiod	Barley		Giese (1975)
C ₂₄ → C ₂₆	Photoperiod <i>cer6^c</i> ; <i>kcs-1^c</i>	Barley <i>Arabidopsis thaliana</i>	3-ketoacyl-CoA synthase (KCS)	Giese (1975) Jenks <i>et al.</i> (1995); Todd <i>et al.</i> (1999); Hooker <i>et al.</i> (2002)
C ₂₆ → C ₂₈	Cyanide; photoperiod <i>gl3</i> <i>cer6^c</i> ; <i>kcs-1^c</i> <i>cer2^{cs}</i> , <i>cer2-133 884^s</i> , <i>cer9</i>	Barley Marrow stem kale (<i>Brassica oleracea</i>) <i>A. thaliana</i>	3-ketoacyl-CoA synthase (KCS); regulator (<i>cer2</i>); lipid transfer protein (<i>cer9</i>)	Mikkelsen (1978); Giese (1975) Jenks <i>et al.</i> (1995); Todd <i>et al.</i> (1999); Hooker <i>et al.</i> (2002); Rashotte <i>et al.</i> (2004)
C ₂₈ → C ₃₀	TCA <i>gl1</i> , <i>gl2</i> , <i>gl3</i> , <i>gl5</i> <i>gl3^c</i> <i>cer2^{cs}</i> , <i>cer6^c</i> , <i>kcs-1^c</i>	Maize (<i>Zea mays</i>) (EDI); <i>B. oleracea</i> , Barley Brussels sprout (<i>B. oleracea</i>); Cauliflower (<i>B. oleracea</i>); <i>B. napus</i> Maize <i>A. thaliana</i>		Avato <i>et al.</i> (1984); Macey (1974); Mikkelsen (1978) Baker (1974); Netting <i>et al.</i> (1972); Holloway <i>et al.</i> (1977a) Avato <i>et al.</i> (1985)
C ₃₀ → C ₃₂	TCA <i>gl2^c</i> , <i>gl4^c</i> , <i>gl16</i> <i>wa</i>	Maize (EDI); barley Maize Pea (<i>Pisum sativum</i>)	3-ketoacyl-CoA synthase (KCS)	Hannoufa <i>et al.</i> (1993); McNevin <i>et al.</i> (1993); Jenks <i>et al.</i> (1995); Todd <i>et al.</i> (1999); Hooker <i>et al.</i> (2002) Avato <i>et al.</i> (1984); Mikkelsen (1978) Avato <i>et al.</i> (1985) Holloway <i>et al.</i> (1977b)

Table 8b Effects of inhibitors, photoperiod and mutation on elongation and compound distribution within the associated pathways

Step	Inhibitors/mutation	Plant	Suggested function	References ^a
(b) Effects on elongation and compound distribution within the associated pathways				
<i>Acyl</i> → <i>aldehyde</i>				
C_{26} , C_{28} , C_{30} , C_{32}	<i>gl1</i> , <i>gl11</i> ^c	Maize	<i>gl11</i> (EDI) component, possible Acyl-CoA reductase	Avato <i>et al.</i> (1985); Maddaloni <i>et al.</i> (1991); Sturaro <i>et al.</i> (2005) Wang <i>et al.</i> (2002)
	<i>TAA1a</i>	Wheat (<i>Triticum aestivum</i>) (tapetum tissue pollen)	Acyl-CoA reductase	
C_{26} – C_{34} C_{30}	<i>cer8</i> , <i>cer3</i> ; <i>cer7</i> ^s <i>cer23</i> ^s ; <i>wax2/yre</i> ; <i>rst1</i>	<i>A. thaliana</i>	FA release from Acyl-CoA (reduced); Acyl-CoA reductase (<i>wax2/yre</i>)	Jenks <i>et al.</i> (1995) Rashotte <i>et al.</i> (2004); Chen <i>et al.</i> (2003); Kurata <i>et al.</i> (2003); Chen <i>et al.</i> (2005)
<i>Aldehyde</i> → <i>alcohol</i>				
C_{26} , C_{28} , C_{30}	<i>gl2</i> <i>gl1</i> ; <i>gl8</i> ; <i>gl5</i> ^c	Brussels sprout Maize	Reductase (<i>gl5</i> possibly <i>gl1</i>)	Baker (1974) Maddaloni <i>et al.</i> (1991); Xu <i>et al.</i> (1997, 2002); Sturaro <i>et al.</i> (2005)
C_{26} – C_{32}	<i>cer4</i> ^c	<i>A. thaliana</i>		Hannoufa <i>et al.</i> (1993); Jenks <i>et al.</i> (1995)
C_{30} – C_{32}	<i>cer1</i> , 16 <i>TAA1a</i>	Wheat (tapetum tissue pollen)	Lipid transfer protein Acyl-CoA reductase	Jenks <i>et al.</i> (1995) Wang <i>et al.</i> (2002)
<i>Acyl</i> → <i>alcohol</i>				
C_{28} C_{28} , C_{30}	<i>cer23</i> ^s <i>cer24</i> ^s	<i>A. thaliana</i>	FA release from Acyl-CoA (enhanced) Acyl-CoA reductase	Rashotte <i>et al.</i> (2004)
<i>Aldehyde</i> → <i>alkane</i>				
C_{30}	<i>gl1</i> ; <i>gl8</i> ^c	Maize		Maddaloni <i>et al.</i> (1990, 1991); Sturaro <i>et al.</i> (2005)
	<i>cer22</i> ^s <i>EPI23</i>	<i>A. thaliana</i> <i>Kleinia odora</i>	Aldehyde decarbonylase or regulator Aldehyde decarbonylase	Rashotte <i>et al.</i> (2004) Kolattukudy (1996)
C_{26} – C_{34}	<i>cer1</i> ^c , <i>cer16</i>	<i>A. thaliana</i>	Lipid transfer protein	Hannoufa <i>et al.</i> (1993); McNevin <i>et al.</i> (1993); Jenks <i>et al.</i> (1995); Aarts <i>et al.</i> (1995)
<i>Acyl</i> → <i>alkane</i>				
C_{28} , C_{30} , C_{32}	<i>gl1</i> , <i>gl2</i> , <i>gl3</i> <i>cer2</i> ^c	Brussels sprout <i>A. thaliana</i>	Acyl-CoA transferase	Baker (1974) Hannoufa <i>et al.</i> (1993)
<i>Alkane</i> → <i>secondary alcohol</i> → <i>ketone</i>				
C_{27} , C_{29} , C_{31}	<i>gl1</i> , <i>gl2</i> , <i>gl3</i>	Brussels sprout		Baker (1974)
<i>Acyl</i> → <i>fatty acid</i>				
	<i>cer3</i> ^c	<i>A. thaliana</i>		Hannoufa <i>et al.</i> (1993); Jenks <i>et al.</i> (1995)

^aFor reviews see: von Wettstein-Knowles (1995); Post-Beittenmiller (1996); Kunst & Samuels (2003). ^c, genes have been cloned; ^s, stem only; 2-ME, 2-Mercaptoethanol; TCA, trichloroacetic acid. Adapted from Shepherd (2003) with permission from Academic Press.

localised to the ER (Xu *et al.*, 1997, 2002; Dietrich *et al.*, 2005) and similar sequences have been found in *Arabidopsis*, barley and leek. Zheng *et al.* (2005) have identified from *Arabidopsis* the first enoyl-CoA reductase associated with FAE. Although the biochemical function of *Arabidopsis* CER1 is unknown, it has been suggested that it encodes a fatty acid decarboxylase (Hannoufa *et al.*, 1993; McNevin *et al.*, 1993; Aarts *et al.*, 1995; Jenks *et al.*, 1995). TAA1a from bread wheat encodes a fatty acyl-CoA reductase which forms the alcohol moieties of wax esters present in the tapetum tissues of pollen (Wang *et al.*, 2002). *Arabidopsis* CER24 and WAX2/YRE may be acyl-CoA reductases involved in alcohol (Rashotte *et al.*, 2004), and alkane (Chen *et al.*, 2003; Kurata *et al.*, 2003) formation, respectively, and RST-1 (resurrection 1) also appears to be involved in the latter reaction (Chen *et al.*, 2005), although its function is unknown. Maize GL1 has a high amino acid sequence homology with *Arabidopsis* WAX2, and is probably the maize WAX2 ortholog (Sturaro *et al.*, 2005).

In some species, separate but parallel elongation systems appear to be linked with the different associated pathways, which may not share a common pool of precursors. This may also be characteristic of different developmental stages. In maize and barley, an elongation system, ED-I, active in seedlings, is responsible for formation of very long-chain alcohols, aldehydes, acids and alkanes (Fig. 7). A second system, ED-II, active over all stages of growth, produces shorter acyl chains primarily for ester formation, although some ED-I products may also be made (Mikkelsen, 1978; Avato *et al.*, 1984, 1990). ED-I is defective in some maize *gl* mutants or through the action of trichloroacetic acid (TCA), and a shift occurs in the acyl CL distribution away from C₃₂ to shorter chains, particularly in primary alcohols (Avato *et al.*, 1984; Bianchi *et al.*, 1985; Avato *et al.*, 1990; Beattie & Marcell, 2002). This has a strong parallel with the light-induced changes in CL found by Giese (1975) for the same components in barley wax (Fig. 5). In barley and wheat, ED-I is paralleled by another system which elongates 3-ketoacyl chains for production of β -diketones (Fig. 3). A mutation in barley *CER-CQU*, which encodes a multifunction enzyme with 3-ketoacyl elongase activity, impairs formation of β -diketones, while alkane and alcohol formation is unaffected (von Wettstein-Knowles, 1995). A similar system to ED-I functions in wheat seedlings, whereas the 3-ketoacyl elongation system becomes active in mature plants. Such a switch between parallel pathways for formation of alkanes and β -diketones appears to occur in some species under water or salinity stress (von Wettstein-Knowles, 1972; Bengtson *et al.*, 1978; Rao *et al.*, 1981).

Branched constituents common to some species are thought to come from elongation systems with a specificity for *br*-precursors. In brassicas, *br*- (*i*-, *a*-) constituents are abundant in free and esterified fatty acids and alcohols; however, *br*-components are not usually found in the other products of the associated pathways. Defects in this specificity for *n*-compounds

occur in some brassica mutants (*gl4*, *gl6* and *glNilla*), resulting in the formation of *br*-alkanes and *br*-secondary alcohols (Netting *et al.*, 1972; Baker & Holloway, 1975). Changes in the distribution of *br*- and *n*-components under different lighting regimes are features of tobacco and brassica waxes (Barnes *et al.*, 1996; Shepherd *et al.*, 1997), again suggesting that the stress, in this case exposure to UV-B, changes the activity of parallel elongation systems.

Developmental factors may have a key role in control of biosynthesis, since provision of a waterproof protective coat is essential at the earliest stages of growth. There is evidence from studies with leek, *Allium porrum*, that induction of microsomal elongation by FAE leads that of plastidial *de novo* synthesis by FAS, suggesting that the processes are under different developmental regulation (Rhee *et al.*, 1998). Developmental patterns can vary between species, for example, onset of wax synthesis in leek follows cell elongation, whereas expression of *Arabidopsis* CER2 and CER3 is associated with elongating tissues (Xia *et al.*, 1996) and cell division in the meristem (Hannoufa *et al.*, 1996), respectively. Therefore, environmental factors are more likely to modulate normal developmental signals. How this occurs is not well understood. Stimulation by light is a key process in the synthesis *de novo* and probably constitutes the single greatest factor determining the light sensitivity of wax production. Some details have emerged about the influence of environmental factors on components of FAE and other enzymes involved in wax biosynthesis. Hooker *et al.* (2002) found that light is essential for expression of *Arabidopsis* CER6, accumulation of the CER6 transcript is also enhanced by osmotic stress and ABA, and overexpression of CER6 increases wax accumulation. The CER6 promoter region contains elements similar to I-box and GT1-binding sites found in light-inducible promoters (Terzaghi & Cashmore, 1995), and ABA-responsive *cis*-acting elements (ABRE) involved in ABA-regulated gene expression by drought and cold (Guiltinan *et al.*, 1990). If this is more general of the other KCS condensing enzymes, it may contribute to the CL sensitivity of FAE and may form part of a signalling mechanism for regulating wax production under osmotic stress. By contrast, expression of the regulatory gene CER2 is not induced by light, temperature, drought or other stress conditions, but may be induced by cytokinins in young leaves (Xia *et al.*, 1997) or by specific transcription factors.

Genes encoding three transcription factors, designated SHN (shine), including SHN1/WIN1 (wax inducer 1) (Aharoni *et al.*, 2004; Broun *et al.*, 2004), have recently been identified in *Arabidopsis*. From the observed phenotypes of the *shn* mutant and WIN1 overexpressors in leaves, they may influence early chain elongation, particularly between C₁₈ and C₂₂, which affects the overall quantities of all biosynthetic end-products. They may differentially regulate channelling of precursors towards decarboxylation rather than the reductive pathways (SHN/WIN1), or more specifically competing reactions utilising C₃₀ acyl-CoA, that is, decarboxylation to

form C₂₉ products or further elongation to give C₃₂ products (SHN). Within the decarbonylation pathway they appear to exert control on the hydroxylation and oxidation reactions that form C₂₉ secondary alcohols and ketones (SHN). Overexpression of *WIN1* in transgenic *Arabidopsis* induced some genes involved in wax biosynthesis (*KCSI*, *CER1*, *CER2*), whereas others (*CER6/CUT1*, *FDH*, *CER3*) were unaffected (Broun *et al.*, 2004). This is consistent with the observed phenotype based on the known functions of the respective gene products (Fig. 7, Table 8a,b). However, a loss-of-function *shn* mutant has not yet been found, and therefore the regulation of wax biosynthesis by the three SHN transcription factors in epidermal cells remains to be confirmed. Another transcription factor possibly involved in regulating wax biosynthesis, *WXP1* (wax production), was recently characterised from the model legume *Medicago truncatula* (Zhang *et al.*, 2005). When overexpressed in transgenic alfalfa, *Medicago sativa*, *WXP1* activated wax production, reduced water loss and enhanced drought tolerance. Expression of *WXP1* in *M. truncatula* shoots was reversible, being induced by drought and ABA, but on removal of the stimuli, transcription levels returned to normal. Alcohols derived from the reductive pathway are the major components in alfalfa leaf waxes, whereas alkanes formed by decarbonylation predominate in stems. The main effect of *WXP1* was to increase production of the C₃₀ alcohol in leaves, whereas there was little change in stem wax composition, which is consistent with the specific involvement of *WXP1* in the reductive pathway, in contrast with *Arabidopsis SHN1/WIN1*, which is mainly involved in the decarbonylation pathway. However, *WXP1* also up-regulated three *FAE* genes, orthologs to *Arabidopsis CER2*, pointing to its possible involvement in regulation of acyl chain elongation as found for *SHN1/WIN1*. Greater resistance to cuticular transpirational water loss with an increase in the abundance of the longer chain C₃₀ alcohol is consistent with mechanistic effects similar to those described previously for alkanes in cuticular waxes (section VIII.2).

The SHN/WIN proteins belong to the plant-specific family of APETALA (AP2) (DNA binding-domain)/ethylene-responsive element binding factor (EREBP or ERF) transcription factors associated with plant development and response to environmental stress (Singh *et al.*, 2002). Different members of this family regulate genes involved in response to drought, cold, jasmonates and ethylene as part of the signalling cascade associated with plant sensing and response to environmental stimuli (Xiong *et al.*, 2002; Zhu, 2002). *WXP1* also belongs to the APT2 domain-containing group of transcription factors, but is distinct from most of the known examples on the basis of phylogenetic protein sequence analysis (Zhang *et al.*, 2005).

XIII. Conclusions

Cuticular waxes have protected plants from the vigours of their environment for a very long time and it is only now

becoming clear how this is achieved. There is an elegance about the ways in which plants have come to assemble a few simple molecules into the battery of protective structures and mechanisms that are deployed as their outermost line of defence. Our understanding of the interrelationship between wax composition and function and the basic mechanisms of their production is growing, but there is still much that needs clarification. Some of the major outstanding issues are the mechanisms of wax deposition on to the cuticle surface, control of composition at genetic, molecular and morphological levels, and how environmental pressures elicit an adaptive response. It is becoming apparent that in *Arabidopsis*, the *CER6* gene may play a fundamental role in the plant's response to all types of environmental challenge, but it remains to be seen how general this may be. As of yet relatively little is known about what regulates the conversion of elongated acyl chains into the various products of the associated pathways, or indeed the mechanisms of such transformations. However, the identification of stress-inducible transcription factors in *M. truncatula* and *A. thaliana*, which appear to differentially regulate the utilization of acyl chains by the reductive and decarbonylation pathways, respectively, offers an insight as to how this might occur more generally. It has been suggested that overexpression of genes encoding transcription factors might be an effective general method for manipulation of wax biosynthesis in leaves (Zhang *et al.*, 2005). This could be very useful both for mechanistic investigations and for transgenic production of stress-resistant clones of economically important crop species.

Acknowledgements

The authors would like to acknowledge the support of the Scottish Executive Environment and Rural Affairs Department.

References

- Aarts MG, Keijzer CJ, Stiekema WJ, Pereira A. 1995. Molecular characterisation of the *CER1* gene of *Arabidopsis* involved in epicuticular wax biosynthesis and pollen fertility. *Plant Cell* 7: 2115–2127.
- Aharoni A, Dixit S, Jetter R, Thoenes E, van Arkel G, Pereira A. 2004. The SHINE clade of AP2 domain transcription factors activates wax biosynthesis, alters cuticle properties, and confers drought tolerance when overexpressed in *Arabidopsis*. *Plant Cell* 16: 2463–2480.
- Anfodillo T, Pasqua Di Bisceglie D, Urso T. 2002. Minimum cuticular conductance and cuticle features of *Picea abies* and *Pinus cembra* needles along an altitudinal gradient in the dolomites. *Tree Physiology* 22: 479–487.
- Armstrong DJ, Whitecross MI. 1976. Temperature effects on formation and fine structure of *Brassica napus* leaf waxes. *Australian Journal of Botany* 24: 309–318.
- Avato P. 1987. Chemical genetics of epicuticular wax formation in maize. *Plant Physiology and Biochemistry* 25: 179–190.
- Avato P, Bianchi G, Gentinetta E, Salamini F. 1984. Effect of trichloroacetic acid on wax composition of normal and mutant maize (*Zea mays* L.). *Journal of Experimental Botany* 35: 245–251.
- Avato P, Bianchi G, Pogna N. 1990. Chemosystematics of surface lipids from maize and some related species. *Phytochemistry* 29: 1571–1576.

- Avato P, Bianchi G, Salamini F. 1985. Absence of long chain aldehydes in wax of the *glossyII* mutant of maize. *Phytochemistry* 24: 1995–1997.
- Baker EA. 1974. The Influence of environment on leaf wax development in *Brassica oleracea* var. *gemmifera*. *New Phytologist* 73: 955–966.
- Baker EA. 1982. Chemistry and morphology of plant epicuticular waxes. In: Cutler DF, Alvin KL, Price CE, eds. *The plant cuticle*. London, UK: Academic Press, 139–165.
- Baker EA, Holloway PJ. 1975. Branched-chain constituents of brussels sprout wax. *Phytochemistry* 14: 2463–2467.
- Barnes JD, Cardoso-Vilhena J. 1996. Interactions between electromagnetic radiation and the plant cuticle. In: Kerstiens G, ed. *Plant cuticles an integrated functional approach*. Oxford, UK: BIOS Scientific Publishers Ltd., 157–174.
- Barnes JD, Davison AW, Booth TA. 1988. Ozone accelerates structural degradation of epicuticular wax on Norway spruce needles. *New Phytologist* 110: 309–318.
- Barnes JD, Percy KE, Paul ND, Jones P, McLaughlin CK, Mullineaux PM, Creissen G, Wellburn AR. 1996. The influence of UV-B radiation on the physicochemical nature of tobacco (*Nicotiana tabacum* L.) leaf surfaces. *Journal of Experimental Botany* 47: 99–109.
- Barthlott W, Neinhuis C, Cutler D, Ditsch F, Meusel I, Theisen I, Wilhelmi H. 1998. Classification and terminology of plant epicuticular waxes. *Botanical Journal of the Linnean Society* 126: 237–260.
- Beattie GA, Marcell LM. 2002. Effect of alterations in cuticular wax biosynthesis on the physicochemical properties and topography of maize leaf surfaces. *Plant, Cell & Environment* 25: 1–16.
- Bednarova E. 2001a. Reaction of the assimilatory tissues of Norway spruce (*Picea abies* [L.] karst.) and silver fir (*Abies alba* Mill.) to immission stress in regions under a long-term pollution load. *Ekologia-Bratislava* 20: 36–45.
- Bednarova E. 2001b. Situation in epicuticular waxes of birch (*Betula pendula*) leaves in air-polluted regions. *Ekologia-Bratislava* 20: 284–291.
- Bengston C, Larsson S, Liljengberg C. 1978. Effect of water stress on cuticular transpiration rate and amount and composition of epicuticular wax in seedlings of six oat varieties. *Physiologia Plantarum* 44: 319–324.
- Bermadinger-Stabentheiner E. 1994. Problems in interpreting effects of air pollutants on spruce epicuticular waxes. In: Percy KE, Cape JN, Jagels R, Simpson CJ, eds. *Air pollutants and the leaf cuticle*. Heidelberg, Germany: Springer-Verlag, 322–327.
- Bianchi G. 1995. Plant waxes. In: Hamilton RJ, ed. *Waxes: chemistry, molecular biology and functions*. Dundee, UK: The Oily Press, 175–222.
- Bianchi A, Bianchi G, Avato P, Salamini F. 1985. Biosynthetic pathways of epicuticular wax of maize as assessed by mutation, light, plant age and inhibitor studies. *Maydica* 30: 179–198.
- Blum A. 1975a. Effect of the *bm* gene on epicuticular wax on the water relations of *Sorghum bicolor*. *Israel Journal of Botany* 24: 50.
- Blum A. 1975b. Effect of the *bm* gene on epicuticular wax deposition and the spectral characteristics of sorghum leaves. *SABRAO Journal of Breeding and Genetics* 7: 45–52.
- Blum A. 1986. The effect of heat stress on wheat leaf and ear photosynthesis. *Journal of Experimental Botany* 37: 111–118.
- Bonaventure G, Ohlrogge JB. 2002. Differential regulation of mRNA levels of acyl carrier protein isoforms in *Arabidopsis*. *Plant Physiology* 128: 223–235.
- Bondada BR, Oosterhuis BM, Murphy JB, Kim KS. 1996. Effect of water stress on the epicuticular wax composition and ultrastructure of cotton (*Gossypium hirsutum* L.) leaf, bract and boll. *Environmental and Experimental Botany* 36: 61–69.
- Brewer CA, Smith WK, Vogelmann TC. 1991. Functional interaction between leaf trichomes, leaf wettability and the optical properties of water droplets. *Plant, Cell & Environment* 14: 955–962.
- Broun P, Poindexter P, Osborne E, Jiang C-ZJ, Riechmann JL. 2004. WIN1, a transcriptional activator of epidermal wax accumulation in *Arabidopsis*. *Proceedings of the National Academy of Sciences of the USA* 101: 4706–4711.
- Butler DR. 1996. The presence of water on leaf surfaces and its importance for microbes and insects. In: Kerstiens G, ed. *Plant cuticles an integrated functional approach*. Oxford, UK: BIOS Scientific Publishers Ltd., 267–282.
- Caldwell MM, Robberecht R, Flint SD. 1983. Internal filters: prospects for UV-acclimation in higher plants. *Physiologia Plantarum* 58: 445–450.
- Cameron RJ. 1970. Light intensity and the growth of *Eucalyptus* seedlings II. The effect of cuticular waxes on light absorption in leaves of *Eucalyptus* species. *Australian Journal of Botany* 18: 275–284.
- Cape JN. 1996. Surface wetness and pollutant deposition. In: Kerstiens G, ed. *Plant cuticles an integrated functional approach*. Oxford, UK: BIOS Scientific Publishers Ltd., 283–300.
- Carlsson AS, Hellgren LI, Selldén G, Sandelius AS. 1994. Effects of moderately enhanced levels of ozone on the acyl lipid composition of leaf lipids of garden pea (*Pisum sativum*). *Physiologia Plantarum* 91: 754–762.
- Carlsson AS, LaBrie ST, Kinney AJ, von Wettstein-Knowles P, Browse J. 2002. A *KAS2*cDNA complements the phenotypes of the *Arabidopsis fabI* mutant that differs in a single residue bordering the substrate binding pocket. *Plant Journal* 29: 761–770.
- Chen X, Goodwin SM, Boroff VL, Liu X, Jenks MA. 2003. Cloning and characterisation of the *WAX2* gene of *Arabidopsis* involved in cuticle membrane and wax production. *Plant Cell* 15: 1170–1185.
- Chen X, Goodwin MS, Liu X, Chen X, Bressan RA, Jenks MA. 2005. Mutation of the *RESURRECTION1* locus of *Arabidopsis* reveals an association of cuticular wax with embryo development. *Plant Physiology* 139: 909–919.
- Cockell CS, Knowland J. 1999. Ultraviolet radiation screening compounds. *Biological Reviews* 74: 311–345.
- DeLucia EH, Berlyn GP. 1983. The effect of increasing elevation on leaf cuticle thickness and transpiration in balsam fir. *Canadian Journal of Botany* 62: 2423–2431.
- Dietrich CR, Perera MADN, Yandeu-Nelson MD, Meeley RB, Nikolau BJ, Schnable PS. 2005. Characterisation of two *GL8* paralogs reveals that the 3-ketoacyl reductase component of fatty acid elongase is essential for maize (*Zea mays* L.) development. *Plant Journal* 42: 844–861.
- Dixon M, LeThiec C, Garrec JP. 1997. An investigation into the effects of ozone and drought, applied singly and in combination, on the quantity and quality of the epicuticular wax of Norway spruce. *Plant Physiology and Biochemistry* 35: 447–454.
- Dodd RS, Poveda MM. 2003. Environmental gradients and population divergence contribute to variation in cuticular wax composition of *Juniperus communis*. *Biochemical Systematics and Ecology* 31: 1257–1270.
- Dodd RS, Rafii ZA. 2000. Habitat-related adaptive properties of plant cuticular lipids. *Evolution* 54: 1438–1444.
- Dodd RS, Rafii ZA, Power AB. 1998. Ecotypic adaptation in *Austrocedrus chilensis* in cuticular hydrocarbon composition. *New Phytologist* 138: 699–708.
- Edwards D, Abbott GD, Raven JA. 1996. Cuticles of early land plants: a palaeoecophysiological evaluation. In: Kerstiens G, ed. *Plant cuticles an integrated functional approach*. Oxford, UK: BIOS Scientific Publishers Ltd., 1–31.
- Eller BM. 1979. Die Strahlungsökologische bedeutung von epidermisaufgaben (The significance of layers superimposed to the epidermis for leaf ecology and leaf radiation budget). *Flora* 168: 146–192.
- Esch A, Mengel K. 1998a. Combined effects of acid mist and frost on the water status of young spruce trees (*Picea abies*). *Chemosphere* 36: 645–650.
- Esch A, Mengel K. 1998b. Combined effects of acid mist and frost drought on the water status of young spruce trees (*Picea abies*). *Environmental and Experimental Botany* 39: 57–65.
- Farman JC, Gardiner BG, Shanklin JD. 1985. Large losses of total ozone in Antarctica reveal seasonal ClO_x–NO_x interaction. *Nature* 315: 207–210.
- Febrero A, Fernández S, Molina-Cano JL, Araus JL. 1998. Yield, carbon isotope discrimination, canopy reflectance and cuticular conductance of barley isolines of differing glaucousness. *Journal of Experimental Botany* 49: 1575–1581.
- Fiebig A, Mayfield JA, Miley NL, Chau S, Fischer RL, Preuss D. 2000. Alterations in *CER6*, a gene identical to *CUT1*, differentially affects long-chain lipid content on the surface of pollen and stems. *Plant Cell* 12: 2001–2008.

- Fujiwara T, Nakayama M, Kikuchi S, Yoshioka H, Sato F. 2002. Applying NaCl to suppress succulent growth and acclimatize cabbage plug seedlings. *Journal of the Japanese Society for Horticultural Science* 71: 796–804.
- Gauvrit C, Gaillardon P. 1991. Effect of low-temperatures on 2,4-D behaviour in maize plants. *Weed Research* 31: 135–142.
- Giese BN. 1975. Effects of light and temperature on the composition of epicuticular wax of barley leaves. *Phytochemistry* 14: 921–929.
- Gonzalez R, Paul ND, Percy K, Ambrose M, McLaughlin CK, Barnes JD, Areses M, Wellburn AR. 1996. Responses to ultraviolet-B radiation (280–315 nm) of pea (*Pisum sativum*) lines differing in leaf surface wax. *Physiologia Plantarum* 98: 852–860.
- Grace J. 1990. Cuticular water loss unlikely to explain tree-line in Scotland. *Oecologia* 84: 64–68.
- Graham EA, Nobel PS. 1996. Long-term effects of a doubled atmospheric CO₂ concentration on the CAM species *Agave deserti*. *Journal of Experimental Botany* 47: 61–69.
- Grcarevic M, Radler F. 1967. The effect of wax components on cuticular transpiration – model experiments. *Planta* 75: 23–27.
- Guiltinan MJ, Marcotte WR Jr, Quatrano RS. 1990. A plant leucine zipper protein that recognises an abscisic acid response element. *Science* 250: 267–270.
- Gulz PG. 1994. Epicuticular leaf waxes in the evolution of the plant kingdom. *Journal of Plant Physiology* 143: 453–464.
- Günthardt MS. 1984. Epicuticular wax of *Picea abies* needles. In: Sigenthaler P-A, Eichenberger W. *Structure, function and metabolism of plant lipids*. Amsterdam, Netherlands: Elsevier, 499–502.
- Günthardt-Goerg MS. 1994. The effect of the environment on the structure, quantity, composition of spruce needle wax. In: Percy KE, Cape JN, Jagels R, Simpson CJ, eds. *Air pollutants and the leaf cuticle*. Heidelberg, Germany: Springer-Verlag, 165–166.
- Hadley JH, Frieland AJ, Herrick GT, Amundson RG. 1991. Winter desiccation and solar radiation in relation to spruce decline in the Northern Appalachians. *Canadian Journal of Forest Research* 21: 269–272.
- Hall DM, Jones RL. 1961. Physiological significance of surface waxes on leaves. *Nature* 191: 95–99.
- Hannoufa A, McNevin J, Lemieux B. 1993. Epicuticular waxes of *eceriferum* mutants of *Arabidopsis thaliana*. *Phytochemistry* 33: 851–855.
- Hannoufa A, Negru V, Eisner G, Lemieux B. 1996. The *CER3* gene of *Arabidopsis thaliana* is expressed in leaves, stems, root, flowers and apical meristems. *Plant Journal* 10: 459–467.
- Hass K. 1977. Einfluss von temperature und blattalter auf das cuticularwachs von *Hedera helix*. *Biochemie und Physiologie der Pflanzen* 171: 25–31.
- Hass K, Rentschler I. 1984. Discrimination between epicuticular and intracuticular wax in blackberry leaves: ultrastructural and chemical evidence. *Plant Science Letters* 36: 143–147.
- Hauke V, Schreiber L. 1998. Ontogenetic and seasonal development of wax composition and cuticular transpiration of ivy (*Hedera helix* L.) sun and shade leaves. *Planta* 207: 67–75.
- Heath RL. 1980. Initial events in injury to plants by air pollutants. *Annual Reviews of Plant Physiology* 31: 395–431.
- Hellgren LI, Carlsson AS, Sellén G, Sandelius AS. 1995. In situ leaf metabolism in garden pea (*Pisum sativum* L.) exposed to moderately enhanced levels of ozone. *Journal of Experimental Botany* 46: 221–230.
- Herrick GT, Frieland AJ. 1991. Winter desiccation and injury of subalpine red spruce. *Tree Physiology* 8: 23–36.
- Hetherington AM, Woodward FI. 2003. The role of stomata in sensing and driving environmental change. *Nature* 424: 901–907.
- Hietala T, Mozes N, Genet MJ, Rosenqvist H, Laakso S. 1997. Surface lipids and their distribution on willow (*Salix*) leaves: a combined chemical, morphological and physiochemical study. *Colloids and Surfaces B: Biointerfaces* 8: 205–215.
- Hoad SP, Marzoli A, Grace J, Jeffree CE. 1998. Response of leaf surfaces and gas exchange to wind stress and acid mist in birch (*Betula pubescens*). *Trees-Structure and Function* 13: 1–12.
- Holloway PJ, Brown GA, Baker EA, Macey MJK. 1977a. Chemical composition and ultrastructure in epicuticular wax in three lines of *Brassica napus* L. *Chemistry and Physics of Lipids* 19: 114–127.
- Holloway PJ, Hunt GM, Baker EA, Macey MJK. 1977b. Chemical composition and ultrastructure of epicuticular wax in four mutants of *Pisum sativum* L. *Chemistry and Physics of Lipids* 20: 141–155.
- Holmes MG, Keiller DR. 2002. Effects of pubescence and waxes on the reflectance of leaves in the ultraviolet and photosynthetic wavebands: a comparison of a range of species. *Plant, Cell & Environment* 25: 85–93.
- Holroyd GH, Hetherington AM, Gray JE. 2002. A role for the cuticular waxes in the environmental control of stomatal development. *New Phytologist* 153: 433–439.
- Hooker TS, Millar AA, Kunst L. 2002. Significance of the expression of the CER6 condensing enzyme for cuticular wax production in *Arabidopsis*. *Plant Physiology* 129: 1568–1580.
- Howard JA. 1967. Spectral energy relations of isobilateral leaves. *Australian Journal of Biological Science* 19: 757–766.
- Hull HM, Wright LN, Bleckman CA. 1978. Epicuticular wax ultrastructure among lines of *Eragrostis lehmanniana* Nees, developed for seedling drought tolerance. *Crop Science* 18: 699–704.
- Huttunen S. 1994. Effects of air pollutants on epicuticular wax structure. In: Percy KE, Cape JN, Jagels R, Simpson CJ, eds. *Air pollutants and the leaf cuticle*. Heidelberg, Germany: Springer-Verlag, 81–96.
- Huttunen S. 1996. Interactions between epiphytic microbes and deposited compounds. In: Kerstiens G, ed. *Plant cuticles an integrated functional approach*. Oxford, UK: BIOS Scientific Publishers Ltd., 303–317.
- Huttunen S, Turunen M, Reinikainen J. 1989. Studies on scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* L. Karst) needle cuticles. *Annals of the Science of Forestry* 46: 553–556.
- Jeffree CE. 1974. Method for recrystallising selected components of plant epicuticular waxes as surfaces for the growth of micro-organisms. *Transactions of the British Mycological Society* 63: 626–629.
- Jeffree CE. 1996. Structure and ontogeny of plant cuticles. In: Kerstiens G, ed. *Plant cuticles an integrated functional approach*. Oxford, UK: BIOS Scientific Publishers Ltd., 33–82.
- Jeffree CE, Baker EA, Holloway PJ. 1975. Ultrastructure and recrystallisation of plant epicuticular waxes. *New Phytologist* 75: 539–549.
- Jenks MA, Gaston CH, Goodwin MS, Keith JA, Teusink RS, Wood KV. 2002. Seasonal variation in cuticular waxes of *Hosta* genotypes differing in leaf surface glaucousness. *Horticultural Science* 37: 673–677.
- Jenks MA, Rich PJ, Ashworth EN. 1994. Involvement of cork cells in the secretion of epicuticular wax filaments on *Sorghum bicolor* (L.) Moench. *International Journal of Plant Science* 155: 506–518.
- Jenks MA, Tuttle HA, Eigenbrode SD, Feldmann KA. 1995. Leaf epicuticular waxes of the *eceriferum* mutants in *Arabidopsis*. *Plant Physiology* 108: 369–377.
- Jetter R, Riederer M. 1994. Epicuticular crystals of nonacosan-10-ol: in-vitro reconstitution and factors influencing crystal habits. *Planta* 195: 257–270.
- Jetter R, Riederer M. 1995. In-vitro reconstitution of epicuticular wax crystals: formation of tubular aggregates by long-chain secondary alkanediols. *Botanica Acta* 108: 111–120.
- Jetter R, Schäffers, Riederer M. 2000. Leaf cuticular waxes are arranged in chemically and mechanically distinct layers: evidence from *Prunus laurocerasus* L. *Plant, Cell & Environment* 23: 619–628.
- Johnson DA, Richards RA, Turner NC. 1983. Yield, water relations, gas exchange, and surface reflectance of near-isogenic wheat lines differing in glaucousness. *Crop Science* 24: 318–325.
- Jordan LA, Davitt DA, Morris RL, Neuman DS. 2001. Foliar damage to ornamental trees sprinkler-irrigated with reuse water. *Irrigation Science* 21: 17–25.
- Jordan WR, Monk RL, Miller FR, Rosenow DT, Clark LE, Shouse PJ. 1983. Environmental physiology of sorghum. I. Environmental and genetic control of epicuticular wax load. *Crop Science* 23: 552–558.
- Jordan WR, Shouse PJ, Blum A, Miller FR, Monk RL. 1984. Environmental physiology of sorghum. II. Epicuticular wax load and cuticular transpiration. *Crop Science* 24: 1168–1173.
- Juniper BE, Jeffree CE. 1983. *Plant surfaces*. London, UK: Edward Arnold.

- Kerfourn C, Garrec JP. 1992. Modifications in the alkane composition of cuticular waxes from spruce needles (*Picea abies*) and ivy leaves (*Hedera helix*) exposed to ozone fumigation and acid fog: comparison with needles from declining spruce trees. *Canadian Journal of Botany* 70: 861–869.
- Kerr JB, McElroy CT. 1993. Evidence for large upward trends in ultraviolet-B radiation linked to ozone depletion. *Science* 262: 1032–1034.
- Kerstiens G. 1996a. Cuticular water permeability and its physiological significance. *Journal of Experimental Botany* 47: 1813–1832.
- Kerstiens G. 1996b. Diffusion of water vapour and gases across cuticles and through stomatal pore presumed closed. In: Kerstiens G, ed. *Plant cuticles an integrated functional approach*. Oxford, UK: BIOS Scientific Publishers Ltd., 121–134.
- Kirsch T, Kaffarnik F, Riederer M, Schreiber L. 1997. Cuticular permeability of the three tree species *Prunus laurocerasus* L., *Ginkgo biloba* L. & *Juglans regia* L. comparative investigation of the transport properties of intact leaves, isolated cuticles and reconstituted cuticular waxes. *Journal of Experimental Botany* 48: 1035–1045.
- Knittel R, Pell EL. 1991. Effects of simulated acidic rain on upper leaf surfaces of *Zea mays* foliage. *Canadian Journal of Botany* 69: 2637–2642.
- Koch K, Hartmann KD, Schreiber L, Barthlott W, Neinhuis C. 2006. Influences of air humidity during the cultivation of plants on wax chemical composition, morphology and leaf surface wettability. *Environmental and Experimental Botany* 56: 1–9.
- Kolattukudy PE. 1996. Biosynthetic pathways of cutin and waxes, and their sensitivity to environmental stress. In: Kerstiens G, ed. *Plant cuticles an integrated functional approach*. Oxford, UK: BIOS Scientific Publishers Ltd., 83–108.
- Kolattukudy PE, Croteau R, Buckner JS. 1976. Biochemistry of plant waxes. In: Kolattukudy PE, ed. *Chemistry and biochemistry of natural waxes*. Amsterdam, the Netherlands: Elsevier, 289–347.
- Körner C. 1998. A re-assessment of high elevation treeline positions and their explanation. *Oecologia* 115: 445–459.
- Krasowski MJ, Owens JN. 1990. Growth and morphology of western cedar seedlings as affected by photoperiod and moisture stress. *Canadian Journal of Forestry Research* 21: 340–352.
- Kriedemann PE, Neales TF, Ashton DH. 1964. Photosynthesis in relation to leaf orientation and light interception. *Australian Journal of Biological Science* 17: 591–600.
- Kunst L, Samuels AL. 2003. Biosynthesis and secretion of plant cuticular wax. *Progress in Lipid Research* 42: 51–80.
- Kurata T, Kawabata-Awai C, Sakuradani E, Shimizu S, Okada K, Wada T. 2003. The *YORE-YORE* gene regulates multiple aspects of epidermal cell differentiation in *Arabidopsis*. *Plant Journal* 36: 55–66.
- Langmuir L, Schaefer JV. 1943. Rates of evaporation of water through compressed monolayers on water. *Journal of the Franklin Institute* 235: 119–162.
- Lardizabal KD, Metz JG, Sakamoto T, Hutton WC, Pollard MR, Lassner MW. 2000. Purification of a jojoba embryo wax synthase, cloning of its cDNA and production of high levels of wax in seeds of transgenic *Arabidopsis*. *Plant Physiology* 122: 645–655.
- Latimer JG, Severson RF. 1997. Effect of mechanical and moisture-stress conditioning on growth and cuticle composition of broccoli transplants. *Journal of the American Society of Horticultural Science* 122: 788–791.
- Le Roux JH. 1969. Fischer-Tropsch waxes II. Crystallinity and physical properties. *Journal of Applied Chemistry* 19: 86–88.
- Liakoura V, Manetas Y, Karabourniotis G. 2001. Seasonal fluctuations in the concentration of U.V.-absorbing compounds in the leaves of some Mediterranean plants under field conditions. *Physiologia Plantarum* 111: 491–500.
- Long SP, Ainsworth EA, Rogers A, Ort DR. 2004. Rising atmospheric carbon dioxide: plants FACE the future. *Annual Reviews of Plant Biology* 55: 591–628.
- Lütz C, Heinzmann U, Gulz P-G. 1990. Surface structures and epicuticular wax composition of spruce needles after long-term treatment with ozone and acid mist. *Environmental Pollution* 64: 313–322.
- Macey MJK. 1974. Wax synthesis in *Brassica oleracea* as modified by trichloroacetic acid and glossy mutations. *Phytochemistry* 13: 1353–1358.
- Maddaloni M, Albano M, Motto M, Salamini F. 1991. Unstable alleles generated at various glossy loci. *Maize Genetics Cooperative Newsletter* 65: 25–26.
- Maddaloni M, Bossinger G, DiFonze N, Motto M, Salamini F, Bianchi A. 1990. Unstable alleles of the *GLOSSY-1* locus show a light-dependent variation in the pattern of somatic reversion. *Maydica* 35: 409–420.
- Manninen S, Huttunen S. 1995. Scots pine needles as bioindicators of sulfur deposition. *Canadian Journal of Forest Research* 25: 1559–1569.
- Mansfield WW. 1955. Influence of monolayers on the natural rate of evaporation of water. *Nature* 175: 247–249.
- McNevin JP, Woodward W, Hannoufa A, Feldmann KA, Lemieux B. 1993. Isolation and characterisation of *Eceriferum* (cer) mutants induced by T-DNA insertions in *Arabidopsis thaliana*. *Genome* 36: 610–618.
- Mena-Petite A, Duñabeitia MK, González-Moro B, Muñoz-Rueda A, Lacuesta M. 1999. Sequential effects of acidic precipitation and drought on water relations of *Pinus radiata* seedlings. *Journal of Plant Physiology* 155: 93–100.
- Metz JG, Pollard MR, Anderson L, Hayes TR, Lassner MW. 2000. Purification of a jojoba embryo fatty acyl-coenzyme A reductase and expression of its cDNA in high erucic acid rapeseed. *Plant Physiology* 122: 635–644.
- Mikkelsen JD. 1978. The effects of inhibitors on the biosynthesis of long chain lipids with even carbon-numbers in barley spike epicuticular wax. *Carlsberg Research Communications* 43: 15–35.
- Millar AA, Clemens S, Zachgo S, Giblin EM, Taylor DC, Kunst L. 1999. *CUT1*, an *Arabidopsis* gene required for cuticular wax biosynthesis and pollen fertility, encodes a very-long-chain fatty acid condensing enzyme. *Plant Cell* 11: 825–838.
- Millar AA, Kunst L. 1997. Very-long-chain fatty acid biosynthesis is controlled through the expression and specificity of the condensing enzyme. *Plant Journal* 12: 121–131.
- Mills D, Wenkart S, Benzioni A. 1997. Micropropagation of jojoba. In: Bajaj YPS, ed. *Biotechnology in agriculture and forestry*, Vol. 40. Berlin, Germany: Springer-Verlag, 370–393.
- Mills D, Zhabg G, Benzioni A. 2001. Effect of different salts and of ABA on growth and mineral uptake in jojoba shoots grown *in vitro*. *Journal of Plant Physiology* 158: 1031–1039.
- Molina MJ, Rowland FS. 1974. Stratospheric sink for chlorofluoromethanes: chlorine atom-catalysed destruction of ozone. *Nature* 249: 810–812.
- Neinhuis C, Barthlott W. 1998. Seasonal changes of leaf surface contamination in beech, oak, and ginkgo in relation to leaf micromorphology and wettability. *New Phytologist* 138: 91–98.
- Netting AG, Macey MJK, Barber HN. 1972. Chemical genetics of a subglauous mutant of *Brassica oleracea*. *Phytochemistry* 11: 579–585.
- Ojanperä K, Sutenin S, Pleijel H, Selldén G. 1992. Exposure of spring wheat, *Triticum aestivum* L. cv. Drabant, to different concentrations of ozone in open-top chambers: effects on the ultrastructure of the flag leaf. *New Phytologist* 120: 39–48.
- Oliveira AFM, Meirelles ST, Salatino A. 2003. Epicuticular waxes from caatinga and cerrado species and their efficiency against water loss. *Anais de Academia Brasileira de Ciências* 75: 431–439.
- Paoletti E, LaScala S, Raddi P. 1997. Leaf surface response to abiotic stress factors in a beech stand in central Italy. *Ekologia-Bratislava* 16: 281–293.
- Percy KE, Awmack CS, Lindroth RL, Kubiske ME, Kopper BJ, Isebrands JG, Pregitzer KS, Hendrey GR, Dickson RE, Zak DR, Oksanen E, Sober J, Harrington R, Karnosky DF. 2002. Altered performance of forest pests under atmospheres enriched by CO₂ and O₃. *Nature* 420: 403–407.
- Percy KE, Cape JN, Jagels R, Simpson CJ. 1994a. *Air pollutants and the leaf cuticle*. Heidelberg, Germany: Springer-Verlag.
- Percy KE, Jenen KF, McQuattie CJ. 1992. Effects of ozone and acidic fog on red spruce needle epicuticular wax production, chemical composition, cuticular membrane ultrastructure and needle wettability. *New Phytologist* 122: 71–80.

- Percy KE, McQuattie CJ, Rebbeck JA. 1994b. Effects of air pollutants on epicuticular wax chemical composition. In: Percy KE, Cape JN, Jagels R, Simpson CJ, eds. *Air pollutants and the leaf cuticle*. Heidelberg, Germany: Springer-Verlag, 67–79.
- Percy KE, Baker EA. 1987. Effects of simulated acid rain on production, morphology and composition of epicuticular wax and on cuticular membrane development. *New Phytologist* 107: 577–589.
- Percy KE, Baker EA. 1990. Effects of simulated acid rain on epicuticular wax production, morphology, chemical composition and on cuticular membrane thickness in two clones of Sitka spruce (*Picea sitchensis* (Bong.) Carr). *New Phytologist* 116: 79–87.
- Pilon JJ, Lambers H, Bass W, Tosserams M, Rozema J, Atkin OK. 1999. Leaf waxes of slow-growing alpine and fast-growing lowland *Poa* species: inherent differences and responses to UV-B radiation. *Phytochemistry* 50: 571–580.
- Post-Beittenmiller D. 1996. Biochemistry and molecular biology of wax production in plants. *Annual Review of Plant Physiology and Plant Molecular Biology* 47: 405–430.
- Premachandra GS, Saneoka H, Kanaya M, Ogata S. 1991. Cell membrane stability and leaf surface wax content as affected by increasing water deficits in maize. *Journal of Experimental Botany* 42: 167–171.
- Prior SA, Pritchard SG, Runion GB, Rogers HH, Mitchell RJ. 1997. Influence of atmospheric CO₂ enrichment, soil N and water stress on needle surface wax formation in *Pinus palustris* (Pinaceae). *American Journal of Botany* 84: 1070–1077.
- Pritchard SG, Prior SA, Rogers HH, Peterson CM. 2000. Calcium sulfate deposits associated with needle substomatal cavities of container grown longleaf pine (*Pinus palustris*) seedlings. *International Journal of Plant Sciences* 161: 917–923.
- Prügel B, Lognay G. 1994. Preliminary observations on the influence of increasing atmospheric CO₂ levels on cuticular waxes of spruce needles. In: Percy KE, Cape JN, Jagels R, Simpson CJ, eds. *Air pollutants and the leaf cuticle*. Heidelberg, Germany: Springer-Verlag, 295–304.
- Prügel B, Loosveldt P, Garrec JP. 1994. Changes in the content and constitution of the cuticular wax of *Picea abies* (L.) Karst. in relation to needle ageing and tree decline in five European forest areas. *Trees* 9: 80–87.
- Pruitt RE, Vielle-Calzada J-P, Ploense SE, Grossniklaus U, Lolle SJ. 2000. FIDDLEHEAD, a gene required to suppress epidermal cell interactions in *Arabidopsis*, encodes a putative lipid biosynthetic enzyme. *Proceedings of the National Academy of Sciences of the USA* 97: 1311–1316.
- Pyee J, Yu H, Kolattukudy PE. 1994. Identification of a lipid transfer protein as the major protein in the surface wax of broccoli (*Brassica oleracea*) leaves. *Archives of Biochemistry and Biophysics* 311: 460–468.
- Qaderi MM, Reid DM. 2005. Growth and physiological responses of canola (*Brassica napus*) to UV-B and CO₂ under controlled environment conditions. *Physiologia Plantarum* 125: 247–259.
- Rama Das VS, Raja Reddy K, Krishna ChM, Sambamurthy S, Rao JVS. 1979. Transpirational rates in relation to quantity of leaf epicuticular waxes. *Indian Journal of Experimental Biology* 17: 158–163.
- Rao GG, Basha SKM, Rao GR. 1981. Effect of NaCl salinity on amount and composition of cuticular wax and cuticular transpiration rate in peanut *Arachis hypogaea* (L.). *Indian Journal of Experimental Biology* 19: 880–881.
- Rao JVS, Sambamurthy S, Raja Reddy K. 1980. The quantity of leaf epicuticular wax in relation to the rates of transpiration in some angiospermous weed species. *Comparative Physiology and Ecology* 5: 175–179.
- Rashotte AM, Jenks MA, Ross AS, Feldmann KA. 2004. Novel *eceriferum* mutants in *Arabidopsis thaliana*. *Planta* 219: 5–13.
- Reed DW, Tukey HB Jr. 1982. Light intensity and temperature effects on epicuticular wax morphology and internal cuticle ultrastructure of carnation and brussels sprouts leaf cuticles. *Journal of American Horticultural Science* 107: 417–420.
- Rhee Y, Hlousek-Radojic A, Ponsamuel J, Liu D, Post-Beittenmiller D. 1998. Epicuticular wax accumulation and fatty acid elongation activities are induced during leaf development of leeks. *Plant Physiology* 116: 901–911.
- Richards RA, Rawson HM, Johnson DA. 1986. Glauousness in wheat: its development and effect on water-use efficiency, gas exchange and photosynthetic tissue temperatures. *Australian Journal of Plant Physiology* 13: 465–473.
- Richardson AD, Berlyn GP, Duigan SP. 2003. Reflectance of Alaskan black spruce and white spruce foliage in relation to elevation and latitude. *Tree Physiology* 28: 537–544.
- Riederer M, Schneider G. 1990. The effect of environment on the permeability and composition of citrus leaf cuticles. *Planta* 180: 154–165.
- Riederer M, Schreiber L. 1996. Waxes—the transport barriers of plant cuticles. In: Hamilton RJ, ed. *Waxes: chemistry, molecular biology and functions*. Dundee: UK: The Oily Press, 131–156.
- Riolo A. 1999. Variazioni stagionali di alcuni componenti fogliari in *Picea abies* (L.) Karst, durante l'acclimatazione al freddo. Italy: University of Padova. *M.Sc. Thesis*.
- Robberecht R, Caldwell MM, Bullings WD. 1980. Leaf ultraviolet optical properties along a latitude gradient in the arctic-alpine life zone. *Ecology* 61: 612–619.
- Roberts BR, Cannon WN. 1989. Changes in xylem pressure potential of red spruce seedlings treated with ozone and simulated acid rain. *Canadian Journal of Forest Research* 19: 1200–1203.
- Rozema J, van de Staaij J, Björn LO. 1997. UV-B as an environmental factor in plant life: stress and regulation. *Trends in Ecology and Evolution* 12: 22–28.
- Runion GB, Entry JA, Prior SA, Mitchell RJ, Rogers HH. 1999. Tissue chemistry and carbon allocation in seedlings of *Pinus palustris* subjected to elevated atmospheric CO₂ and water stress. *Tree Physiology* 19: 329–335.
- Sanchez-Diaz MF, Hesketh JD, Kramer PJ. 1972. Wax filaments on sorghum leaves as seen with a scanning electron microscope. *Journal of the Arizona Academy of Sciences* 7: 6–7.
- Sasaki Y, Kozaki A, Hatano M. 1997. Link between light and fatty acid synthesis: Thioredoxin-linked reductive activation of plastidic acetyl-CoA carboxylase. *Proceedings of the National Academy of Sciences of the USA* 94: 11096–11101.
- Sase H, Takamatsu T, Yoshida T, Inubushi K. 1998. Changes in properties of epicuticular wax and the related water loss in Japanese cedar (*Cryptomeria japonica*) affected by anthropogenic environmental factors. *Canadian Journal of Forest Research* 28: 546–556.
- Savage LJ, Ohlrogge JB. 1999. Phosphorylation of pea chloroplast acetyl-CoA carboxylase. *Plant Journal* 18: 521–527.
- Schreiber L, Kirsch T, Riederer M. 1996. Diffusion through cuticles: principles and models. In: Kerstiens G, ed. *Plant cuticles an integrated functional approach*. Oxford, UK: BIOS. Scientific Publishers Ltd., 109–119.
- Schultz DJ, Ohlrogge JB. 2002. Metabolic engineering of fatty acid biosynthesis. In: Kuo TM, Gardner HW, eds. *Lipid biotechnology*. New York, NY, USA: Marcel Dekker, pp. 1–25.
- Schwab M, Noga G, Barthlott W. 1993. Einfluß eines Mg- und Ca-mangels auf synthese, chemische zusammensetzung und mikromorphologische ausbildung von epicuticulären wachsen bei kohlrabiblätern. *Angewandte Botanik* 67: 172–179.
- Shelvey JD, Koziol MJ. 1986. Seasonal and SO₂ induced changes in epicuticular wax of ryegrass. *Phytochemistry* 25: 415–420.
- Shepherd T. 2003. Wax pathways. In: Thomas B, Murphy DJ, Murray BG, eds. *Encyclopedia of applied plant sciences*. Oxford, UK: Elsevier, 1204–1225.
- Shepherd T, Robertson GW, Griffiths DW. 1995a. Compositional analysis of intact alkyl esters in leaf epicuticular wax of swede by capillary gas chromatography and electron-impact mass spectrometry. *Phytochemical Analysis* 6: 65–73.
- Shepherd T, Robertson GW, Griffiths DW, Birch ANE. 1997. Effects of environment on the composition of epicuticular wax esters from kale and swede. *Phytochemistry* 46: 83–96.
- Shepherd T, Robertson GW, Griffiths DW, Birch ANE, Duncan G. 1995b. Effects of environment on the composition of epicuticular wax from kale and swede. *Phytochemistry* 40: 407–417.

- Singh K, Foley RC, Onate-Sanchez L. 2002. Transcription factors in plant defense and stress responses. *Current Opinions in Plant Biology* 5: 430–436.
- Sitte P, Rennier R. 1963. Untersuchungen an cuticularen zellwandschichten. *Planta* 60: 19–40.
- Small DM. 1984. Lateral chain packing in lipids and membranes. *Journal of Lipid Research* 25: 1490–1500.
- Sturaro M, Hartings H, Schmelzer E, Velasco R, Salamini F, Motto M. 2005. Cloning and characterisation of *GLOSSY1*, a maize gene involved in cuticle membrane and wax production. *Plant Physiology* 138: 478–489.
- Suh MC, Samuels AL, Jetter R, Kunst L, Pollard M, Ohlrogge J, Beisson F. 2005. Cuticular lipid composition, surface structure, and gene expression in *Arabidopsis* stem epidermis. *Plant Physiology* 139: 1649–1665.
- Sutinen S, Skärby L, Wallin G, Sellén G. 1990. Long-term exposure of Norway spruce, *Picea abies* (L.) Karst., to ozone in open-top chambers. II. Effects on the ultrastructure of needles. *New Phytologist* 115: 345–355.
- Sutter E, Langhans RW. 1979. Epicuticular wax formation on carnation plantlets regenerated from shoot tip culture. *Journal of the American Society for Horticultural Science* 104: 493–496.
- Sutter E, Langhans RW. 1982. Formation of epicuticular wax and its effect on water loss in cabbage plants regenerated from shoot-tip culture. *Canadian Journal of Botany* 60: 2896–2902.
- Svenningsson M. 1988. Epi- and intracuticular lipids and cuticular transpiration rates of primary leaves of eight barley (*Hordeum vulgare*) cultivars. *Physiologia Plantarum* 73: 512–517.
- Takamatsu T, Sase H, Takada J. 2001a. Some physiological properties of *Cryptomeria japonica* leaves from Kanto, Japan: potential factors causing tree decline. *Canadian Journal of Forest Research* 31: 663–672.
- Takamatsu T, Sase H, Takada J, Matsushita R. 2001b. Annual changes in some physiological properties of *Cryptomeria japonica* leaves from Kanto, Japan. *Water, Air and Soil Pollution* 130: 941–946.
- Terzaghi WB, Cashmore AR. 1995. Light-regulated transcription. *Annual Review of Plant Physiology and Plant Molecular Biology* 46: 445–474.
- Tischler CR, Burson BL. 1995. Evaluating different bahiagrass cytotypes for heat tolerance and leaf epicuticular wax content. *Euphytica* 84: 229–235.
- Todd J, Post-Beittenmiller D, Jaworski JG. 1999. *KCSI* encodes a fatty acid elongase 3-ketoacyl-CoA synthase affecting wax biosynthesis in *Arabidopsis thaliana*. *Plant Journal* 17: 119–130.
- Tranquillini W. 1979. *Physiological ecology of the alpine timberline*. Heidelberg, Germany: Springer-Verlag. (Ecological Studies 31.)
- Turunen M, Huttunen S, Back J. 1994. Observations on the effects of acid rain treatment on needle surfaces of Scots pine and Norway spruce seedlings. In: Percy KE, Cape JN, Jagels R, Simpson CJ, eds. *Air pollutants and the leaf cuticle*. Heidelberg, Germany: Springer-Verlag, 315–319.
- Van Gardingen PR, Grace J, Jeffree CE. 1991. Abrasive damage by wind to the needle surfaces of *Picea stichensis* (Bong) Carr and *Pinus sylvestris* L. *Plant Cell and Environment* 14: 185–193.
- Vanhatalo R, Huttunen S, Back J. 2001. Effects of elevated [CO₂] and O₃ on stomatal and surface wax characteristics on leaves of pubescent birch grown under field conditions. *Trees-Structure and Function* 15: 304–313.
- Vioque J, Kolattukudy PE. 1997. Resolution and purification of an aldehyde-generating and an alcohol-generating fatty acyl-CoA reductase from pea leaves (*Pisum sativum* L.). *Archives of Biochemistry and Biophysics* 340: 64–72.
- Wallin G, Skärby L, Sellén G. 1990. Long-term exposure of Norway spruce, *Picea abies* (L.) Karst., to ozone in open-top chambers. I. Effects on the capacity of net photosynthesis, dark respiration and leaf conductance of shoots of different ages. *New Phytologist* 115: 335–344.
- Wang A, Xia G, Xie W, Dumonceaux T, Zou J, Datla R, Selvaraj G. 2002. Male gametophyte development in bread wheat (*Triticum aestivum* L.): molecular, cellular, and biochemical analyses of a sporophytic contribution to pollen wall ontogeny. *Plant Journal* 30: 613–623.
- Webb AR. 1997. Monitoring changes in UV-B radiation. In: Lumsden PJ, ed. *Plants and UV-B; responses to environmental change*. Cambridge, UK: Cambridge University Press, 13–30.
- Welker OA, Furuya S. 1994. Surface structures of leaves in heat tolerant plants. *Journal of Agronomy and Crop Sciences* 173: 279–288.
- Welker OA, Furuya S. 1995. Influence of heat stress on growth and leaf epicuticular structure of cabbages. *Journal of Agronomy and Crop Sciences* 174: 53–62.
- von Wettstein-Knowles PM. 1972. Genetic control of β -diketone and hydroxy- β -diketone synthesis in epicuticular waxes of barley. *Planta* 106: 113–130.
- von Wettstein-Knowles PM. 1974. Ultrastructure and origin of epicuticular wax tubes. *Journal of Ultrastructure Research* 46: 483–498.
- von Wettstein-Knowles PM. 1979. Genetics and biosynthesis of plant epicuticular waxes. In: Appleqvist L, Liljenberg C, eds. *Advances in the biochemistry and physiology of plant lipids*. Amsterdam, Holland: Elsevier/North Holland Biomedical Press, 1–26.
- von Wettstein-Knowles PM. 1995. Biosynthesis and genetics of waxes. In: Hamilton RJ, ed. *Waxes: chemistry, molecular biology and functions*. Dundee, UK: The Oily Press, 91–129.
- von Wettstein-Knowles PM, Olsen JG, McGuire KA, Larsen S. 2000. Molecular aspects of β -ketoacyl synthase (KAS) catalysis. *Biochemical Society Transactions* 28: 601–607.
- Whitecross MI. 1963. Studies on the plant cuticle. Australia: University of Sydney. *PhD thesis*.
- Whitecross MI, Armstrong DJ. 1972. Environmental effects on epicuticular waxes of *Brassica napus* L. *Australian Journal of Botany* 20: 87–95.
- Xia Y, Nikolau BJ, Schnable PS. 1996. Cloning and characterisation of *CER2*, an *Arabidopsis* gene that affects cuticular wax accumulation. *Plant Cell* 8: 1291–1304.
- Xia Y, Nikolau BJ, Schnable PS. 1997. Developmental and hormonal regulation of the *Arabidopsis CER2* gene that codes for a nuclear-localised protein required for the normal accumulation of cuticular waxes. *Plant Physiology* 115: 925–937.
- Xiong L, Schumaker KS, Zhu J-K. 2002. Cell signaling during cold, drought and salt stress. *Plant Cell Supplement* 2002: S165–S183.
- Xu H, Gauthier L, Gosselin A. 1995. Stomatal and cuticular transpiration of greenhouse tomato plants in response to high solution electrical conductivity and low soil water content. *Journal of the American Society of Horticultural Science* 120: 417–422.
- Xu X, Dietrich CR, Delledonne M, Xia Y, Wen T-J, Robertson DS, Nikolau BJ, Schnable PS. 1997. Sequence analysis of the cloned *glossy8* gene of maize suggests that it may code for a β -ketoacyl reductase required for the synthesis of cuticular waxes. *Plant Physiology* 115: 501–510.
- Xu X, Dietrich CR, Lessire R, Nikolau BJ, Schnable PS. 2002. The endoplasmic reticulum-associated maize GL8 protein is a component of the acyl-coenzyme A elongase involved in the production of cuticular waxes. *Plant Physiology* 128: 924–934.
- Yamada M. 1992. Lipid transfer proteins in plants and microorganisms. *Plant and Cell Physiology* 33: 1–6.
- Yephremov A, Wisman E, Huijser P, Huijser C, Wellesen K, Saedler H. 1999. Characterization of the *FIDDLEHEAD* gene of *Arabidopsis* reveals a link between adhesion response and cell differentiation in the epidermis. *Plant Cell* 11: 2187–2201.
- Zhang J-Y, Broeckling CD, Blancaflor EB, Sledge MK, Sumner LW, Wang Z-Y. 2005. Overexpression of *WXPI* a putative *Medicago truncatula* AP2 domain-containing transcription factor gene, increases cuticular wax accumulation and enhances drought tolerance in transgenic alfalfa (*Medicago sativa*). *Plant Journal* 42: 689–707.
- Zheng H, Rowland O, Kunst L. 2005. Disruptions of the *Arabidopsis* enoyl-CoA reductase gene reveal an essential role for very-long-chain fatty acid synthesis in cell expansion during plant morphogenesis. *Plant Cell* 17: 1467–1481.
- Zhu J-K. 2002. Salt and drought stress signal transduction in plants. *Annual Review of Plant Biology* 53: 247–273.

Appendix A

Table A1 Abbreviations

<i>a</i> -	<i>anteiso</i> -branched chain	IR	infrared
ABA	abscisic acid	<i>i</i> -	<i>iso</i> -branched chain
ACCase	acetyl-CoA carboxylase	KAS	3-ketoacyl-ACP synthase
ACP	acyl carrier protein	KCS	3-ketoacyl-CoA synthase
BC	biotin carboxylase	LTPs	lipid transfer proteins
BCCP	biotin carboxylate carrier protein	MCAT	malonyl-CoA : ACP transacylase
<i>br</i> -	branched chain	MSC	moisture stress conditioning
CA	contact angle	N	weighted mean of CL
CaSO ₄	calcium sulfate	<i>n</i> -	<i>normal</i> straight chain
CER	eceriferum	O ₃	ozone
CL	chain length	PAR	photosynthetically active region
C _{<i>n</i>}	carbon number	PM	plasma membrane
CT	carboxyltransferase	Sb	antimony
CUT	cuticular wax production	SO ₂	sulfur dioxide
D	dispersion of C _{<i>n</i>} about the mean CL	SHN	shine
EC	epidermal cells	SEM	scanning electron microscopy
ER	endoplasmic reticulum	TCA	trichloroacetic acid
EST	expressed sequence tag	TE	transpiration efficiency
FAE	fatty acid elongase	T _{<i>m</i>}	lipid phase transition temperature
FAS	fatty acid synthase	TR ^{cu}	cuticular transpiration
FDH	fiddlehead	TR st	stomatal transpiration
GL	glossy	UV	ultraviolet
σ _{min}	minimum cuticular conductivity	WIN1	wax inducer 1
HIC	high carbon dioxide	WXP1	wax production 1
H ₂ S	hydrogen sulfide		



About New Phytologist

- *New Phytologist* is owned by a non-profit-making **charitable trust** dedicated to the promotion of plant science, facilitating projects from symposia to open access for our Tansley reviews. Complete information is available at www.newphytologist.org.
- Regular papers, Letters, Research reviews, Rapid reports and both Modelling/Theory and Methods papers are encouraged. We are committed to rapid processing, from online submission through to publication 'as-ready' via *OnlineEarly* – the 2004 average submission to decision time was just 30 days. Online-only colour is **free**, and essential print colour costs will be met if necessary. We also provide 25 offprints as well as a PDF for each article.
- For online summaries and ToC alerts, go to the website and click on 'Journal online'. You can take out a **personal subscription** to the journal for a fraction of the institutional price. Rates start at £109 in Europe/\$202 in the USA & Canada for the online edition (click on 'Subscribe' at the website).
- If you have any questions, do get in touch with Central Office (newphytol@lancaster.ac.uk; tel +44 1524 594691) or, for a local contact in North America, the US Office (newphytol@ornl.gov; tel +1 865 576 5261).