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Alexei Solovchenko

Photoprotection in Plants

Optical Screening-based Mechanisms

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Dedicated to the memory of Prof. Mark N. Merzlyak (1946–2009)

Preface

The ability of certain plant pigments absorbing in the UV and/or photosynthetically active regions of the spectrum to act as internal light filters has been discussed for quite a time. However, the participation of these compounds in photoprotection of plants has received only occasional attention and is much less studied in comparison with "classic" photoprotective mechanisms such as elimination of reactive oxygen species, thermal dissipation of the excessive excitation energy of chlorophyll, and repair of photooxidative damage.

Until recently, the photoprotective function of different pigments received little attention. However, during the last two decades, the interest of the scientific community in these pigments (generally named "screening" or "sunscreen" pigments) has grown dramatically. According to major citation databases, the number of publications dedicated to various aspects of plant screening pigments increased more than 3 times and there were 5 times more citations of such works. Still, the coverage of the subject is far from uniform: the overwhelming majority of the works in the field were (and so far are) dedicated to UV-screening compounds, their natural occurrence, and physiology, and the number of studies on compounds attenuating visible radiation remains modest in comparison with the number of studies on UV-screening compounds. This situation seemingly stems from an explosion of interest in ozone holes and their consequences for terrestrial and aquatic ecosystems mediated by elevated UV levels. At the same time, potential photoprotective effects exerted by anthocyanins, carotenoids, and flavonols in the visible region were often overlooked.

Recently obtained experimental evidence fostered a rethinking of the physiological significance of a considerable number of well-known compounds, mainly secondary metabolites of plants. This is especially true for secondary carotenoids and many phenolics. Consequently, the photoprotective role of these compounds has been acknowledged in a considerable number of cases. Different mechanisms of photoprotection were discussed and optical screening turned out to be plausible in many situations. The marked achievements in research into screening-based photoprotection in plants became possible owing to recent progress in the development of

methods and equipment for the analysis of pigments and changes in plant optical properties induced by accumulation of these pigments. Particularly fruitful was the application of nondestructive optical reflectance-based approaches for quantification of screening pigments in situ.

To date, screening pigments have been discovered almost in all plant species investigated; in many cases, their chemical nature as well as their spectral properties have been documented and, most important, their photoprotective function was experimentally confirmed. The increasing number of works dedicated to anthocyanins and secondary carotenoids together with a large body of data on UV-screening compounds suggests that optical screening is an important defense mechanism of plants integral to the system of mechanisms protecting plants against photooxidative damage.

In spite of the breakthrough in the investigation of the diversity and biochemistry of plant screening pigments, a number of problems related to the spectra in planta, subcellular localization, and the physiological significance of screening pigments remain to be solved. There are also significant gaps in our knowledge about the buildup and relative efficiency of different groups of screening pigments. In particular, information on the in planta spectra of pigments which is crucial for characterization of their photoprotective functions is often lacking at present, especially for pigments absorbing in the visible part of the spectrum.

This monograph represents an attempt to develop an integral (but by no means comprehensive) view of plant photoprotective mechanisms based on optical screening of harmful radiation by extrathylakoid pigments. The first two chapters are dedicated to general questions related to optical screening and its place within the system of photoprotective mechanisms of plants, chemical diversity, and the natural occurrence of the key screening pigments. Chapter 3 addresses the induction and the dynamics of plant pigment composition in the case of accumulation of screening compounds. Chapter 4 discusses the general patterns of localization of screening pigments in cell compartments and their distribution in plant tissues. In Chap.5, the profound effects exerted by the buildup of screening pigments on the optical properties of plants are considered, and Chap.6 elucidates the employment of these effects for nondestructive estimation in situ of the screening pigment content and the efficiency of photoprotection provided by such pigments. The book concludes with a chapter dedicated to the relationships between the accumulation of screening pigments and the resistance of microalgae and higher plants to photoinhibition and photodestruction by high fluxes of UV radiation and photosynthetically active radiation.

I hope this book will be of use for lecturers, students, and specialists in the fields of plant physiology, ecological biophysics, and plant ecology.

Lomonosov State University, Moscow, Russia Alexei E. Solovchenko
April 2010

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Contents

Chapter 1
Optical Screening as a Photoprotective Mechanism

Abstract In this introductory chapter, the concept of photoprotection via "passive" screening of solar radiation by different extrathylakoid pigments is briefly outlined. The key differences between optical screening and other photoprotective mechanisms, such as enzymatic and nonenzymatic elimination of reactive oxygen species, thermal dissipation of the excessive chlorophyll excitation energy, and repair of oxidative damage, are discussed. The importance of screening and screen pigments for long-term photoacclimation is underlined together with specific advantages and drawbacks of this photoprotective mechanism.

The existence of plants as photoautotrophic organisms is characterized by uttermost dependence on the absorption and utilization of solar radiation energy in photosynthetic reactions. The photosynthetic pigments localized in the thylakoid membranes of chloroplasts efficiently capture light quanta and transfer their energy to other components of the photosynthetic apparatus, driving the ATP and NADPH syntheses, CO_2 fixation, etc. On the other hand, photosynthesis proceeds with an optimal rate only within a narrow irradiance range (Fig. 1.1), which is often lower than the fluxes of solar radiation reaching plants under natural conditions (Li et al. 2009; Ort 2001). Therefore, the light energy absorbed by the photosynthetic apparatus cannot be utilized completely in the course of photochemical reactions in many situations (Ensminger et al. 2006). The imbalance between the amount of light energy absorbed and the capacity of the plant to utilize it occurs under high fluxes of solar radiation and/or even under moderate irradiance combined with stresses of different nature, such as extreme temperatures (Ensminger et al. 2006), drought (Georgieva et al. 2010; Yordanov et al. 2000), and mineral nutrition deficiencies (Abadía and Abadía 1993). There are also other situations when plants are rendered sensitive to damage by excessive fluxes of solar radiation. Thus, in juvenile and senescing plants, the regulation of the functioning of the photosynthetic apparatus is not so perfect in comparison with that in mature leaves, making it less efficient in utilization of the absorbed light and therefore prone to photodamage by radiation

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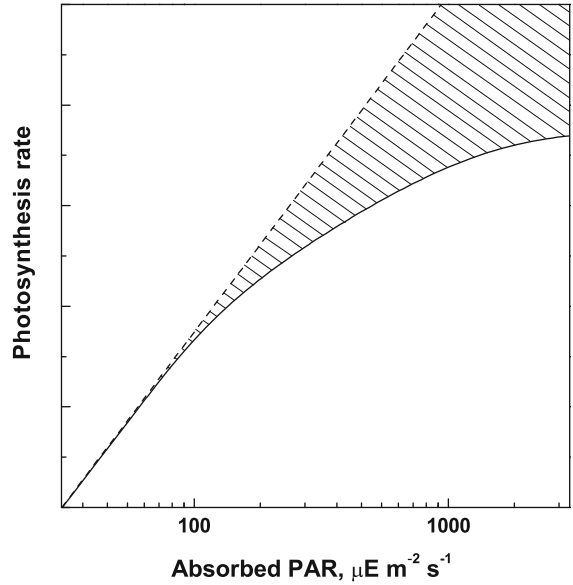

Fig. 1.1 The saturation of photosynthesis at high irradiances leads to the situation where a considerable part of the absorbed photosynthetically active radiation (PAR) cannot be utilized in photochemical reactions (*hatched area*) and imposes the threat of photooxidative damage unless it is removed via a photoprotective mechanism such as thermal dissipation

fluxes which usually do not harm mature plants (Abreu and Munne-Bosch 2007; Hughes et al. 2007; Lu et al. 2003; Munné-Bosch et al. 2001; Woodall and Stewart 1998).

Photodamage to photoautotrophic organisms under unfavorable environmental conditions proceeds primarily via increased generation of reactive oxygen species (ROS) photosensitized in the cells by chlorophylls (Asada 2006; Foyer and Noctor 2000) and a number of endogenous photosensitizers, such as porphyrins, flavins, and pterins (Kreitner et al. 1996; Massey 1994). Apart from the excessive photosynthetically active radiation (PAR), photodamage to plants could be induced by UV radiation, comprising 7–9% of the total energy of solar radiation reaching Earth's surface (Bjorn and Murphy 1985). Short-wavelength UV (UV-C, wavelengths below 280 nm) radiation is absorbed almost completely by the ozone layer of atmosphere. UV-B (280–315 nm) and UV-A (315–400 nm) radiation constitute approximately 5 and 90% of the total solar UV radiation, respectively (Rozema et al. 2002). High-energy UV-B quanta are able to damage plant cells directly, whereas the effects of less energetic UV-A radiation are usually ROS-mediated (Bornman et al. 1997; Rozema et al. 2002).

The essential need for plant survival under variable and often excessive fluxes of solar radiation brought about the development of certain adaptive systems including both regulatory and photoprotective mechanisms (Fig. 1.2) (Asada 2006; Demmig-Adams and Adams 2006; Li et al. 2009). Since the first photoautotrophic organisms on Earth were probably exposed to higher fluxes of harder UV radiation as compared with contemporary species, the enzymatic systems for repair of the UV-induced damage to nucleic acids and important proteins of the

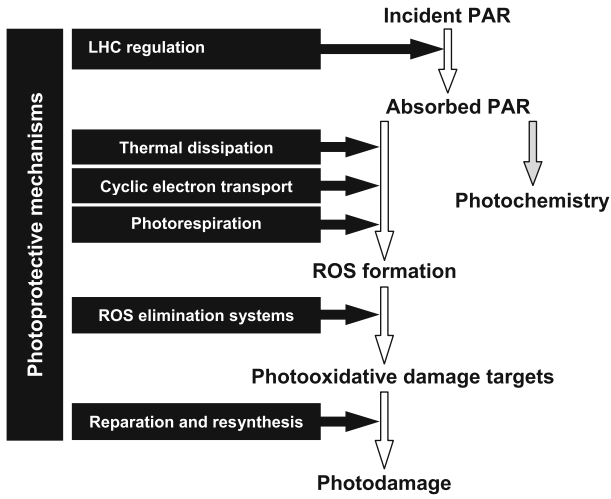

Fig. 1.2 Alternative flows of the energy of absorbed PAR and a multilevel system of "active" photoprotective mechanisms operating in chloroplasts

photosynthetic apparatus are thought to be among the first photoprotective mechanisms that evolved (Bornman et al. 1997; Cockell 1998; Cockell and Knowland 1999). Furthermore, the ROS-detoxifying systems, both enzymatic and nonenzymatic, are ubiquitous in and crucial for the prevention or amelioration of oxidative damage to plants (Asada 2006). Obviously, other mechanisms responsible for the maintenance of efficient photosynthesis in the wide range of radiation wavelengths and fluxes emerged at later stages of evolution (Demmig-Adams and Adams 2006).

It is important to realize that the aforementioned photoprotective mechanisms have certain aspects in common. All of them predominantly cope with the *consequences* of photodamage by UV radiation and PAR, i.e., repair damaged macromolecules, eliminate ROS and products of their reactions *already formed* in the cell (Fig. 1.2). Then, the efficient operation of these mechanisms requires sufficient levels of energy-rich and/or reducing compounds which are necessary for repair of DNA, resynthesis of the membrane lipids and proteins, as well as for regeneration of important low molecular mass antioxidants such as reduced glutathione and ascorbate (Foyer and Noctor 2005).

Over the last two decades, the concept of photoprotective mechanisms based on attenuation or "passive" optical screening of harmful radiation by extrathylakoid pigments has evolved and become widespread (Bilger et al. 2007; Burchard et al. 2000; Cockell and Knowland 1999; Merzlyak et al. 2008b; Morgan-Kiss et al. 2006; Sinha et al. 1998; Solovchenko and Merzlyak 2003, 2008; Steyn et al. 2009). The ability of plants to respond to strong irradiation by the synthesis and accumulation, within different cell compartments and tissue structures, of the compounds selectively absorbing in the UV or the visible part of the spectrum is the foundation of

these mechanisms. In higher plants, these compounds are concentrated in superficial structures such as the cuticle and epidermis and/or are distributed within cells and tissues (Lenk and Buschmann 2006; Lenk et al. 2007; Merzlyak et al. 2008a; Solovchenko and Merzlyak 2003). These mechanisms are distinct from the "classic" or "active" photoprotective systems (Fig. 1.2) in a number of ways. Primarily, they prevent photodamage by alleviating its *cause* – the excessive absorption of radiation by the photosynthetic apparatus and other photosensitive cell components (Fig. 1.3).

Plant evolution was accompanied by a continuous expansion of the diversity and an increase of structural complexity of molecules suitable for the photoprotective

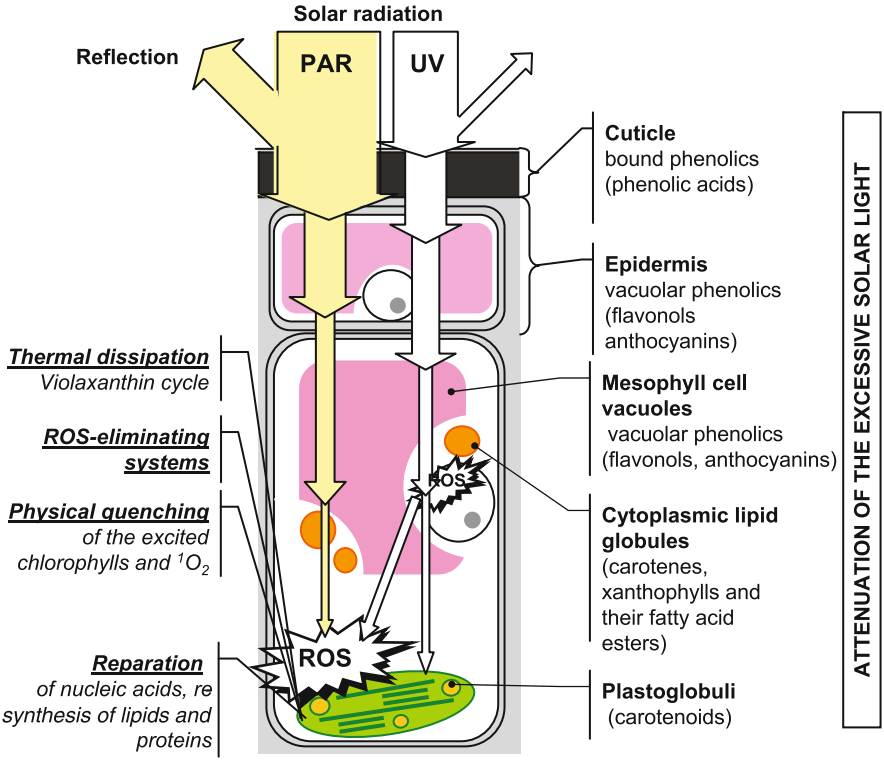

Fig. 1.3 Optical screening – an integral part of a system of photoprotective mechanisms in plants. Under unfavorable environmental conditions and in situations when the regulation of photosynthesis is impaired, high fluxes of solar radiation induce direct or indirect reactive oxygen species (ROS)-mediated damage to plants. Certain mechanisms are responsible for a decrease in ROS levels in the cell and cope with the consequences of photodamage (see also Fig. 1.2). The screening pigments attenuate the incident radiation, thereby removing, to a considerable extent, the cause of photodamage (harmful UV and the excessively absorbed visible quanta). (Reprinted from Solovchenko and Merzlyak (2008) with kind permission from Springer Science + Business Media), Fig. 1

function via radiation screening (which will be covered in detail in the next chapter) (Cockell and Knowland 1999). The vast majority of screening pigments discovered to the date in plants belong to four key groups of compounds differing in chemical structure and the biosynthetic pathways. Among others, they include mycosporine-like amino acids (Sinha et al. 1998) and extrathylakoid (also known as the secondary) carotenoids which do not transfer the absorbed light energy to chlorophylls (Ben-Amotz et al. 1989; Han et al. 2003). Together with carotenoids, the photoprotective function in plants is served by a large number of phenolic compounds (Hoch et al. 2003; Williams and Grayer 2004) and nitrogen-containing heterocyclic betalains (Strack et al. 2003). Different but complementary classes of photoprotective pigments disparate in chemical structure, spectral properties, and localization, e.g., phenolic compounds and carotenoids or phenolics and betalains, are present in many plant species simultaneously (Tanaka et al. 2008). Certain classes of screening pigments such as phenolics are ubiquitous and have been discovered in all plant species studied so far. However, the proposed photoprotective function of a screening compound should be rigorously proved in each case.

Recently obtained evidence suggests that plant screening pigments possess high photostability both in vitro and in planta (Merzlyak and Solovchenko 2002; Merzlyak and Chivkunova 2000). Therefore, a photoprotective screen, once formed, could be maintained with minimal expenditure of energy and valuable metabolites providing a reliable long-term protection against photodamage. It is important, therefore, that the efficiency of "passive" screening of radiation is far less affected by environmental stresses (such as extreme temperatures or drought; Munné-Bosch et al. 2001) which suppress photosynthesis and could impair the ability of the enzymatic systems to provide an adequate level of photoprotection (Asada 2006).

At the same time, the initial buildup of photoprotective compounds demands a considerable amount of photoassimilates and energy to be invested in biosynthesis of screening pigments. The induction of synthesis and accumulation of the pigments in amounts sufficient to accomplish their photoprotective function (as well as decomposition of earlier accumulated screening compounds) is a relatively slow process, which occurs on the timescale of hours and days. Owing to these circumstances, the screening-based mechanisms are warrantable mostly under the prolonged action of a stressor; hence, they are of high importance for long-term adaptation of plants.

To conclude, one can think of radiation screening by extrathylakoid pigments as a photoprotective mechanism relying on principles totally different from those of "classic" photoprotective mechanisms but integral to the whole system of protection of plants against photooxidative stress. Screening-based photoprotection is a first-line defense of plants against potentially harmful solar radiation, which takes a considerable time to deploy as well as to withdraw and is therefore effective for long-term photoacclimation of plants. In the following chapters, the components, the operation, and several approaches for assessment of the efficiency of screening-based photoprotection will be considered.

References

Abadía J, Abadía A (1993) Iron and plant pigments. In: Barton L, Hemming B (eds) Iron chelation in plants and soil microorganisms, vol 15. Academic, San Diego, pp 327–344

Abreu M, Munne-Bosch S (2007) Photo-and antioxidant protection and salicylic acid accumulation during post-anthesis leaf senescence in *Salvia lanigera* grown under Mediterranean climate. Physiol Plant 131:590–598

Asada K (2006) Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiol 141:391–396

Ben-Amotz A, Shaish A, Avron M (1989) Mode of action of the massively accumulated β-carotene of *Dunaliella bardawil* in protecting the alga against damage by excess irradiation. Plant Physiol 86:1286–1291

Bilger W, Rolland M, Nybakken L (2007) UV screening in higher plants induced by low temperature in the absence of UV-B radiation. Photochem Photobiol Sci 6:190–195

Bjorn L, Murphy T (1985) Computer calculation of solar ultraviolet radiation at ground level. Physiol Veg 23:555–561

Bornman J, Reuber S, Cen Y, Weissenböck G (1997) Ultraviolet radiation as a stress factor and the role of protective pigments. In: Lumsden P (ed) Plants and UV-B: responses to environmental change. Cambridge University Press, Cambridge, pp 157–168

Burchard P, Bilger W, Weissenbock G (2000) Contribution of hydroxycinnamates and flavonoids to epidermal shielding of UV-A and UV-B radiation in developing rye primary leaves as assessed by ultraviolet-induced chlorophyll fluorescence measurements. Plant Cell Environ 23:1373–1380

Cockell C (1998) Biological effects of high ultraviolet radiation on early earth – a theoretical evaluation. J Theor Biol 193:717–729

Cockell C, Knowland J (1999) Ultraviolet radiation screening compounds. Biol Rev 74:311–345

Demmig-Adams B, Adams W (2006) Photoprotection in an ecological context: the remarkable complexity of thermal energy dissipation. New Phytol 172:11–21

Ensminger I, Busch F, Huner N (2006) Photostasis and cold acclimation: sensing low temperature through photosynthesis. Physiol Planta 126:28–44

Foyer C, Noctor G (2000) Oxygen processing in photosynthesis: regulation and signalling. New Phytol 146:359–388

Foyer C, Noctor G (2005) Oxidant and antioxidant signalling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. Plant Cell Environ 28:1056–1071

Georgieva K, Sarvari E, Keresztes A (2010) Protection of thylakoids against combined light and drought by a lumenal substance in the resurrection plant *Haberlea rhodopensis*. Ann Bot 105:117–126

Han Q, Shinohara K, Kakubari Y, Mukai Y (2003) Photoprotective role of rhodoxanthin during cold acclimation in *Cryptomeria japonica*. Plant Cell Environ 26:715–723

Hoch W, Singsaas E, McCown B (2003) Resorption protection. Anthocyanins facilitate nutrient recovery in autumn by shielding leaves from potentially damaging light levels. Plant Physiol 133:1296–1305

Hughes N, Morley C, Smith W (2007) Coordination of anthocyanin decline and photosynthetic maturation in juvenile leaves of three deciduous tree species. New Phytol 175:675–685

Kreitner M, Ebermann R, Alth G (1996) Quantitative determination of singlet oxygen. Production by porphyrins. J Photochem Photobiol B Biol 36:109–111

Lenk S, Buschmann C (2006) Distribution of UV-shielding of the epidermis of sun and shade leaves of the beech (*Fagus sylvatica* L.) as monitored by multi-colour fluorescence imaging. J Plant Physiol 163:1273–1283

Lenk S, Buschmann C, Pfundel E (2007) In vivo assessing flavonols in white grape berries (*Vitis vinifera* L. cv. Pinot Blanc) of different degrees of ripeness using chlorophyll fluorescence imaging. Funct Plant Biol 34:1092

Li Z, Wakao S, Fischer BB, Niyogi KK (2009) Sensing and responding to excess light. Annu Rev Plant Biol 60:239–260

Lu Q, Wen X, Lu C, Zhang Q, Kuang T (2003) Photoinhibition and photoprotection in senescent leaves of field-grown wheat plants. Plant Physiol Biochem 41:749–754

Massey V (1994) Activation of molecular oxygen by flavins and flavoproteins. J Biol Chem 269:22459–22462

Merzlyak MN, Chivkunova OB (2000) Light-stress-induced pigment changes and evidence for anthocyanin photoprotection in apples. J Photochem Photobiol B Biol 55:155–163

Merzlyak M, Solovchenko A (2002) Photostability of pigments in ripening apple fruit: a possible photoprotective role of carotenoids during plant senescence. Plant Sci 163:881–888

Merzlyak MN, Chivkunova OB, Solovchenko AE, Naqvi KR (2008a) Light absorption by anthocyanins in juvenile, stressed, and senescing leaves. J Exp Bot 59:3903–3911

Merzlyak MN, Melo TB, Naqvi KR (2008b) Effect of anthocyanins, carotenoids, and flavonols on chlorophyll fluorescence excitation spectra in apple fruit: signature analysis, assessment, modelling, and relevance to photoprotection. J Exp Bot 59:349–359

Morgan-Kiss R, Priscu J, Pocock T, Gudynaite-Savitch L, Huner N (2006) Adaptation and acclimation of photosynthetic microorganisms to permanently cold environments. Microbiol Mol Biol Rev 70:222–252

Munné-Bosch S, Jubany-Mari T, Alegre L (2001) Drought-induced senescence is characterized by a loss of antioxidant defences in chloroplasts. Plant Cell Environ 24:1319–1327

Ort D (2001) When there is too much light. Plant Physiol 125:29–32

Rozema J et al (2002) The role of UV-B radiation in aquatic and terrestrial ecosystems – an experimental and functional analysis of the evolution of UV-absorbing compounds. J Photochem Photobiol B Biol 66:2–12

Sinha R, Klisch M, Gröniger A, Häder D (1998) Ultraviolet-absorbing/screening substances in cyanobacteria, phytoplankton and macroalgae. J Photochem Photobiol B Biol 47:83–94

Solovchenko A, Merzlyak M (2003) Optical properties and contribution of cuticle to UV protection in plants: experiments with apple fruit. Photochem Photobiol Sci 2:861–866

Solovchenko A, Merzlyak M (2008) Screening of visible and UV radiation as a photoprotective mechanism in plants. Russ J Plant Physiol 55:719–737

Steyn WJ, Wand SJE, Jacobs G, Rosecrance RC, Roberts SC (2009) Evidence for a photoprotective function of low-temperature-induced anthocyanin accumulation in apple and pear peel. Physiol Plant 136:461–472

Strack D, Vogt T, Schliemann W (2003) Recent advances in betalain research. Phytochemistry 62:247–269

Tanaka Y, Sasaki N, Ohmiya A (2008) Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids. Plant J 54:733

Williams C, Grayer R (2004) Anthocyanins and other flavonoids. Nat Prod Rep 21:539–573

Woodall G, Stewart G (1998) Do anthocyanins play a role in UV protection of the red juvenile leaves of *Syzygium*? J Exp Bot 49:1447–1450

Yordanov I, Velikova V, Tsonev T (2000) Plant responses to drought, acclimation, and stress tolerance. Photosynthetica 38:171–186

Chapter 2
Screening Pigments: General Questions

Abstract The chapter begins with the issue of the specificity of the photoprotective function served by different compounds absorbing in visible and UV parts of the spectrum and important reservations that should be made before associating the function of radiation screening with a plant pigment. Current hypotheses of the evolution of optical screening mechanisms and screening compounds in microalgae and higher plants are discussed. The chapter concludes with an overview of the most important classes of plant screening pigments, a brief account of their diversity, natural occurrence, and spectral properties in vitro.

2.1 The Specificity of the Screening Pigments' Function

The question of specificity is of crucial importance for the discussion of the potential photoprotective function of a plant pigment. This is especially true in the case of compounds which play multifaceted roles in plant organism, such as phenolics (Close and Beadle 2003a; Close and McArthur 2002; Harborne 1976) and carotenoids. The latter, for example, participate in different photoprotective mechanisms, including elimination of reactive oxygen species (ROS) and dissipation of excessive energy absorbed by chlorophylls (Demmig-Adams and Adams 2006; Young 1991; Young and Britton 1990). Indeed, a change in metabolism and pigment composition indirectly increasing the resistance of plants to high fluxes of radiation does not necessarily represent a specific high-light response. In particular, numerous responses of plants to the spectral quality of the radiation are mediated by phytochrome and cryptochrome photoreceptors and induce various biochemical and photomorphogenic effects, including biosynthesis of certain phenolic compounds (Hahlbrock and Scheel 1989; Mohr and Drumm-Herrel 1983) which are not necessarily screening-related. Generally, obtaining solid evidence for the

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participation of certain substances in photoprotection via screening of radiation is complicated because many constituents of plant cells, apart from mycosporin-like amino acids (MAA) and phenolics, absorb in the UV and visible parts of the spectrum but serve no specific photoprotective function. For example, the structural phenylpropanoids and lignins comprising the cell wall as well as the condensed aromatic compounds of the plant cuticle strongly absorb in the UV region, especially in the UV-B range (Krauss et al. 1997; Markstädter et al. 2001; Solovchenko and Merzlyak 2003).

Certain organisms (such as cyanobacteria from the genera *Gloeocapsa* and *Oscillatoria*) possess highly efficient constitutive systems of nucleic acid repair and elimination of ROS which allow the microorganisms to withstand naturally elevated fluxes of UV radiation without the induction of additional protective mechanisms such as accumulation of screening compounds (Cockell and Knowland 1999). In some diatoms (e.g., in certain representatives of the genera *Thalassiosira* and *Chaetoceros*) complex morphology of their silica cell shells can introduce a gross (several orders of magnitude) variation in the intensity of the actual UV fluxes reaching sensitive cell structures. This makes it difficult to assess the expression of the UV-induced effects and the role of the UV-screening pigments in such organisms (Cockell and Knowland 1999; Moisan and Mitchell 2001; Shick and Dunlap 2002). Furthermore, constitutive components of the cell wall of Antarctic diatoms from the genera *Proboscia* and *Nitzschia* considerably attenuate UV radiation, diminishing the UV dose absorbed by the protoplast (Cockell and Knowland 1999; Moisan and Mitchell 2001).

Taking into account the diversity of functions of UV- and visible-absorbing compounds in plants as well as cross-resistance induced by other stressors is a prerequisite for studying the effects of photoprotective compounds. The concentration of certain MAA participating, apart from photoprotection, in the maintenance of osmotic homeostasis in halophile species, even at the background UV levels, can reach as high as 100 mM (Oren and Gunde-Cimerman 2007). Then, the diversity of phenolic functions in higher plants could complicate the revealing of their specific photoprotective function (Harborne 1976, 1980; Harborne and Williams 2000). Consequently, the knowledge of the role of a compound in the physiology of the species under question is important to ascertain its participation in photoprotection. Detailed investigation of the irradiation-induced accumulation of the tentative photoprotective compound as well as careful interpretation of the data on the subcellular localization, tissue distribution, and function are also essential.

It is generally accepted that compliance with several criteria is necessary to consider a certain compound as a photoprotective (screening) pigment (Cockell and Knowland 1999):

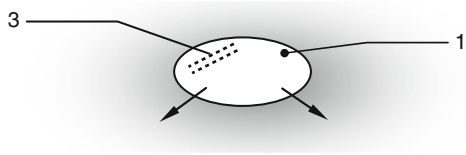
1. The compound should strongly absorb radiation in the spectral band(s) overlapping with the absorption band(s) of the photosynthetic pigments, endogenous photosensitizers (Jung and Kim 1990), and/or photosensitive components (such as nucleic acids and/or proteins) of the cell.

2. The irradiation in the corresponding spectral range should trigger the synthesis of the pigment in the natural and model systems (e.g., cell or tissue cultures).
3. The accumulation of the compound in question should induce resistance to the radiation in the spectral range of the pigment absorption.

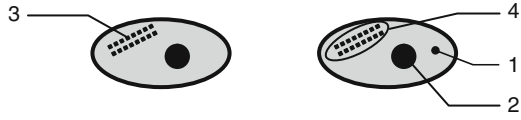
Only compliance with all of the criteria listed above might be considered as solid evidence of the participation of certain compound in the protection against photodamage via radiation screening. In addition, the similarity of the action spectrum of the induction of synthesis of a compound and its absorption spectrum could also represent evidence for participation of this compound in photoprotection via radiation screening; i.e., irradiation in the band of the absorption maximum of a screening compound should induce its biosynthesis most efficiently (Garcia-Pichel and Castenholz 1993). The mutants deficient in the synthesis of different photoprotective pigments are another useful tool for elucidation and proving the specificity of screening pigment function in plants.

2.2 The Evolution of Screening Pigments in Plants

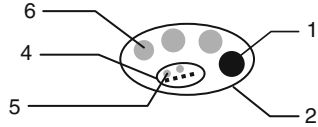
According to the results of a number of theoretical reconstructions and simulations, photoprotection via screening of radiation was very important for ancient photoautotrophic organisms. It is generally accepted that the spectrum of solar radiation before oxygenic photosynthesis emerged and became widespread differed from that measured near Earth's surface at present in having a higher proportion of short-wavelength UV radiation that penetrated the atmosphere in the absence of oxygen and the ozone layer (Cockell 1998; Cockell and Knowland 1999). Under such conditions, an evolutionary advantage was probably gained by ancient photochemotrophic organisms excreting inorganic products of their metabolism (such as elementary sulfur and iron ions) in the form of suspensions or solutions nonselectively attenuating the incident radiation (Cockell 1998); see also Fig. 2.1 (label 1). However, the suspensions of fine inorganic particles possess a strong, spectrum-independent scattering, which strongly attenuates, together with harmful UV radiation, the photosynthetically active radiation necessary for photosynthesis. Then, the phototrophic microorganisms, especially their plankton forms, rarely possessed a supply of mineral substrates sufficient for the creation of such primitive inorganic "screens." Apparently, this was the primary driver of the evolutionary transition to the synthesis of organic screening pigments selectively absorbing radiation in certain spectral ranges (Fig. 2.1, labels 2, 3). The phototrophs capable of building such screens appeared to be more competitive. Owing to their improved ability to withstand high fluxes of solar radiation, they succeeded in opening previously inaccessible niches such as upper layers of ocean and terrestrial landscapes, and eventually dominated the biosphere (Cockell 1998; Cockell and Knowland 1999; Fig. 2.1, label 4).



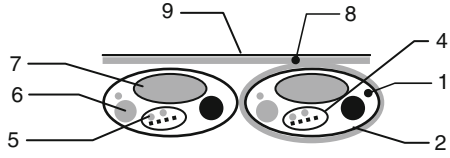
1. **Predecessors of contemporary photoautorophs (prokaryotic)** accumulated photoprotective compounds, presumably similar to scytonemin, in the sheath or extracelluarly.



2. **Microalgae** accumulate UV-protective compounds (*e.g.* MAA), mainly in the cytoplasm



3. **Green eukaryotic microalgae** accumulate UV-protective MAA and phenolics in the cytoplasm and the extrathylakoid carotenoids, absorbing in the visible range, within oil bodies



4. **Terrestrial plants** accumulate more complex UV-protective phenolics (mainly phenolic acid and flavonol derivatives) within cuticle and vacuoles of epidermal and mesophyll cells and extrathylakoid carotenoids in plastoglobuli within plastids

Fig. 2.1 The hypothetical evolution of the diversity and localization of screening compounds in plants. The main trends include (**a**) the increase in the diversity, (**b**) and the complexity of chemical structures of the screening pigments, and (**c**) their compartmentalization within certain cell organelles and structures. *1* cytoplasm, *2* nucleus, *3* photosynthetic membranes, *4* chloroplast, *5* plastoglobuli, *6* cytoplasmic lipid globules ("oil bodies"), *7* vacuole, *8* cell wall, *9* cuticle. (Solovchenko, unpublished)

According to the data from comparative biochemistry, the first screening compounds could have evolved from common constitutive metabolites possessing an aromatic ring or rings within their structure, such as phenylalanine derivatives. These compounds probably gradually took on the function of photoprotection. This hypothesis is supported by a wide diversity of functions fulfilled in contemporary plants by screening compounds such as phenolics (Hahlbrock and Grisebach 1979; Hahlbrock and Scheel 1989; Harborne 1976) and carotenoids (Demmig-

Adams et al. 1996). There is a ground to believe that primitive ancient phototrophs synthesized a limited number of UV-absorbing compounds with a relatively simple structure resembling that of the MAA backbone (Cockell 1998). However, the versatile metabolic pathways of phototrophic microorganisms could well serve as a rich source of compounds absorbing in the UV and visible parts of the spectrum. In particular, organic substances possessing a linear and/or cyclic system of conjugated double bonds and π electrons characteristic of all natural screening pigments are the most efficient UV absorbers (Bandaranayake 1998; Cockell and Knowland 1999; Kolb et al. 2003; Mazza et al. 2000; Morgan-Kiss et al. 2006; Solovchenko and Merzlyak 2008). These circumstances are thought to facilitate, with time, the appearance of the contemporary diversity of screening compounds (see the next section). Indeed, contemporary microalgae and higher plants are characterized by much more diverse photoprotective substances with a wide variety of chemical structures and spectral properties (Bandaranayake 1998; Cockell and Knowland 1999; Kolb et al. 2003; Solovchenko and Merzlyak 2008).

Expansion of plants to terrestrial habitats with more severe (in comparison with aquatic) environmental conditions, including higher fluxes of solar radiation, was accompanied by dramatic changes in the composition and localization of screening pigments. In present-day plant species, they are represented mainly by MAA and phenolic compounds and, less frequently, betalains and secondary carotenoids (Close and Beadle 2003b; Close and McArthur 2002; Gould 2004; Hoch et al. 2003; Hughes 2009; Hughes et al. 2005; Karageorgou and Manetas 2006; Merzlyak et al. 2008a, b; Pietrini et al. 2002; Solovchenko and Schmitz-Eiberger 2003; Steyn et al. 2002, 2009; Zeng et al. 2010).

From the view of the evolution of higher-plant photoprotective pigments, the loss or acquisition of certain classes of photoprotective pigments by entire families (e.g., Caryophyllaceae, lacking anthocyanins; Mabry and Dreiding 1980; Stafford 1994; Strack et al. 2003; Tanaka et al. 2008) is of special interest. In these cases, the function of protection from strong visible radiation is, as a rule, taken over by other compounds disparate in terms of chemical structure but featuring similar spectral properties, such as ketocarotenoids in some species of *Aloe* and *Cryptomeria* and betalains, e.g., in Caryophyllales (Strack et al. 2003). The reason for these substitutions remains unknown.

2.3 The Diversity of Screening Pigments

Screening pigments discovered in photoautotrophs including microalgae and higher plants can be roughly divided into four principal groups: MAA (Oren and Gunde-Cimerman 2007; Shick and Dunlap 2002; Sinha et al. 2007), phenolic compounds with key subgroups of phenylpropanoids, flavonols, and anthocyanins (Agati et al. 2009; Buer et al. 2010; Burchard et al. 2000; Giordano et al. 2005; Merzlyak et al. 2008b; Meyer et al. 2009; Treutter 2006; Vogt 2010), betalains (Ibdah et al. 2002;

Strack et al. 2003), and carotenoids (Hagen et al. 1994; Hormaetxe et al. 2005; Merzlyak et al. 2005).

MAA and most of phenolic compounds (phenolic acids and flavonols) play a crucial role in UV screening. Certain flavonols when present in high amounts (Havaux and Kloppstech 2001; Smith and Markham 1998) and anthocyanins play an important role in protection against photodamage by visible radiation (Hughes 2009; Merzlyak and Chivkunova 2000; Steyn et al. 2009; Zeng et al. 2010). The participation of betalains (Ibdah et al. 2002; Vogt et al. 1999; Wang and Liu 2007) and certain carotenoids in screening of light in the blue-green part of the spectrum has been reported (Diaz et al. 1990; Han et al. 2003; Hormaetxe et al. 2005, 2007; Merzlyak and Solovchenko 2002; Merzlyak et al. 2005; Weger et al. 1993). A brief account of the natural occurrence, chemical structure, and spectral properties of the key screening pigments will be presented in the following sections.

2.3.1 Mycosporin-Like Amino Acids

Many more primitive photoautotrophs, including cyanobacteria, red and green microalgae, as well as dinoflagellates (Gómez et al. 1998; Karsten et al. 2005; Sinha and Häder 2007), accumulate MAA, the compounds resembling water-soluble mycosporines initially discovered in fungi. The first reports on a photoprotective role of MAA were published about 35 years ago (Shick and Dunlap 2002). Since that time, a considerable number of works (Karsten et al. 2005; Korbee et al. 2005; Kräbs et al. 2004; Oren and Gunde-Cimerman 2007; Singh et al. 2008), including comprehensive reviews (Oren and Gunde-Cimerman 2007; Shick and Dunlap 2002; Sinha et al. 2007), dedicated mainly to the UV protection of marine organisms and the biochemistry of these compounds, have been published.

The structure of the MAA chromophore (Fig. 2.2) is represented by cyclohexene or cycloheximine groups formed at the early stages of the shikimate pathway. The addition of various substituents at later stages provides for the vast diversity of MAA molecules encountered in nature. MAA have molar extinction coefficients in the range 24–50 mM^{-1} cm^{-1} (Gröniger et al. 2000; Sinha and Häder 2007). These compounds emit no measurable fluorescence and do not form free-radical products upon irradiation. Owing to a low quantum yield of triplet formation, it is unlikely than MAA could exert a noticeable photodynamic effect via singlet oxygen generation. By contrast, MAA were reported to be efficient quenchers of ROS (He and Häder 2002a, b). Thus, mycosporine glycine in its ground state prevented photodamage of some bacteria by photosensitizer-generated singlet oxygen (Suh et al. 2003). Meanwhile, MAA showed only a moderate antioxidative activity (Shick and Dunlap 2002). The above-mentioned properties together with high photostability both in vitro and in vivo make MAA efficient UV-screening compounds.

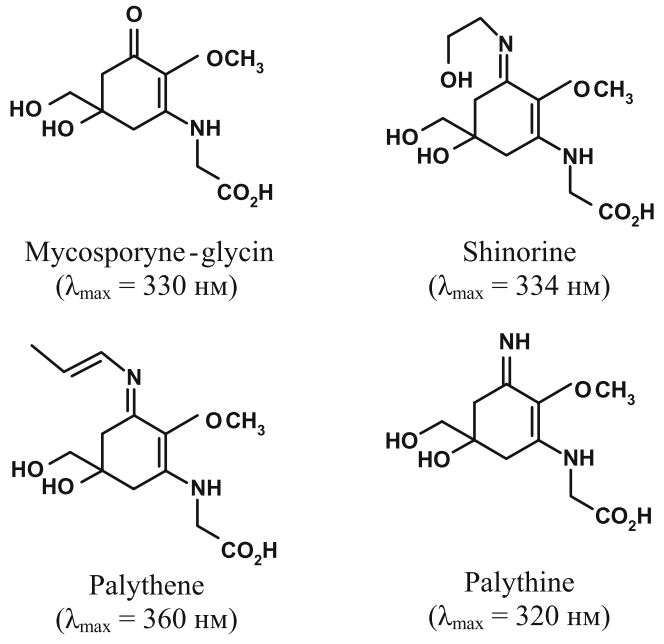


Fig. 2.2 Selected mycosporin-like amino acids and their absorption maxima (Cockell and Knowland 1999)

Additional details on MAA biosynthesis, natural occurrence, and functions can be found in Karsten et al. (2005) and Shick and Dunlap (2002).

2.3.2 Phenolic Compounds

Phenolic compounds are amazingly ubiquitous in nature: they have been found in every plant species studied so far; more than 20,000 phenolic species are known to date, and most of them were discovered in plants (Harborne 1980, 2001). These compounds are characterized by an extreme diversity of chemical structure (Fig. 2.3; Harborne 1980, 2001; Harborne and Williams 1998, 2000).

The basic structure of a phenolic compound is formed by one or more aromatic rings with a hydroxyl group(s) as a substituent(s) (Harborne 1980). Phenolics are synthesized in chloroplasts or cytoplasm and, after glycosylation, they are transported to and accumulated within the vacuoles (Lancaster et al. 1994; Moskowitz and Hrazdina 1981) or are excreted into the apoplast, where they remain within the cell wall or are incorporated in the cuticle (Baur et al. 1998; Krauss et al. 1997). Phenolics serve a plethora of protective functions in plants. For a long time, the main phenolic-dependent protective mechanism in plants was thought to be the defense

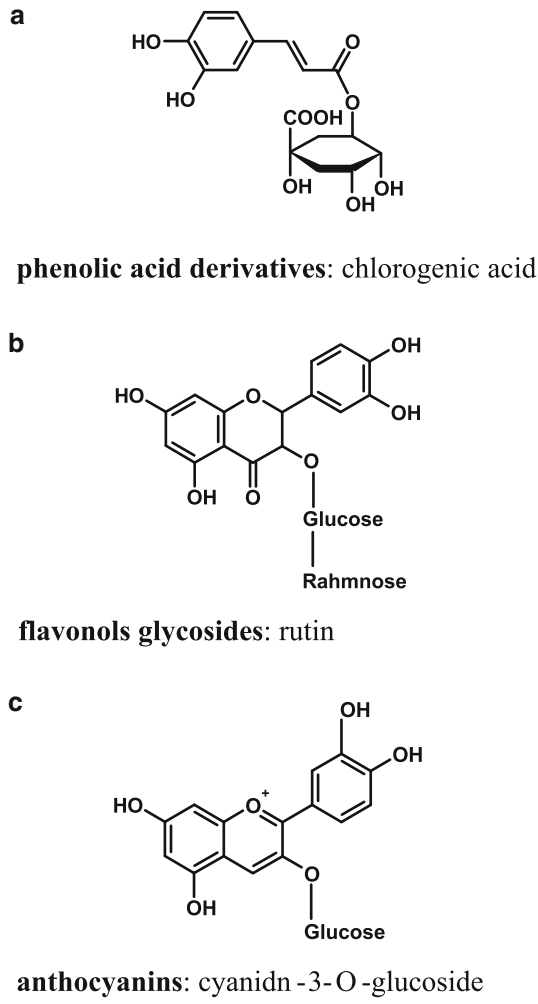


Fig. 2.3 Typical representatives of phenolic compound groups important for radiation screening in plants (Markham 1989; Strack and Wray 1989)

against phytopathogens and herbivores (Close and McArthur 2002; Harborne 1976, 2001). This paradigm has changed recently to accommodate the important photoprotective function of phenolics in plants which was supported by a large body of experimental evidence (Bidel et al. 2007; Caldwell et al. 1983; Close and McArthur 2002; Day et al. 1993, 1994; DeLucia et al. 1992; Georgieva et al. 2010; Ibdah et al. 2002; Kolb et al. 2003; Merzlyak et al. 2004; Meyer et al. 2009; Solovchenko and Merzlyak 2003; Solovchenko and Schmitz-Eiberger 2003; Vogt et al. 1999).

The most important (in the context of radiation screening) group of phenolic compounds includes hydroxycinnamates and other phenylpropanoid derivatives (compounds with a C_6–C_3 backbone), flavonols ,and anthocyanins (flavonoids possessing a C_6–C_3–C_6 backbone). Simple phenols and phenolic acids (C_6–C_1)

appear to be relatively uninvolved in radiation screening, probably because of their high toxicity preventing their accumulation in the amounts necessary for a screening function.

Many phenolic compounds exert a strong antiradical activity in vitro (Afanas'ev et al. 1998; Korkina and Afanas'ev 1997; Kostyuk et al. 2004; Rice-Evans et al. 1997; Russo et al. 2000; Saija et al. 1995). In particular, flavonoids are good chelators of transient metal ions (such as $Fe^{2+/3+}$), efficient radical scavengers (Deng et al. 1997), and singlet oxygen quenchers (Tournaire et al. 1993). Much less evidence of ROS-eliminating activity of phenolics and in planta is available. As a result, the function of phenolics as free-radical scavengers in vivo for the protection of the photosynthetic apparatus is much debated. Still, there are reports on the antioxidative activity of anthocyanins, chlorogenic acid, and quercetin glycosides in planta. Flavonols can eliminate ROS in illuminated chloroplasts and plant tissues (Agati et al. 2007; Takahama 1983). It is supposed that the peroxidase reaction with flavonols (Yamasaki et al. 1997) or anthocyanins (Gould et al. 2002) could be important for scavenging of hydrogen peroxide. However, unambiguous evidence supporting the predominance of the antioxidant activity of phenolic compounds for the accomplishment of their photoprotective function in vivo remains to be obtained.

The characteristic absorption spectrum of screening-relevant phenolic compounds in the UV region usually contains two bands (Fig. 2.4). The first band, peaking around 280 nm, appears due to the presence of an aromatic ring(s); it is detected in the spectra of all phenolics. The second, long-wavelength band is situated in the 300–360-nm range; the exact position of its maximum varies for different classes of phenolics. In anthocyanidins and their glycosylated forms known as anthocyanins, the maximum of the second absorption band is located in the blue-green part of the visible spectrum (Markham 1989; Markham et al. 2001; Smith and Markham 1998). Particularly, the long-wavelength absorption band of cyanidin, the predominant aglycone of anthocyanins responsible for reddish coloration of leaves and fruit in many species, is centered at 525 nm (Strack and Wray 1989).

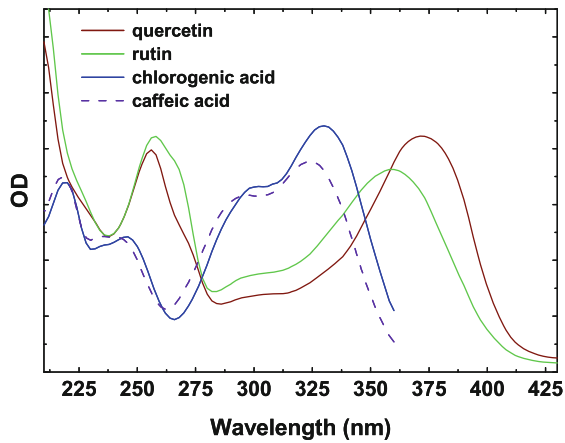


Fig. 2.4 Absorption spectra of certain flavonols and phenolic acids in methanol. (Solovchenko, unpublished)

The molar absorption coefficients of most of the phenolic compounds relevant to screening are within the range from 10 to 35 mM^{-1} cm^{-1} (Markham 1989; Moskowitz and Hrazdina 1981; Strack and Wray 1989). In solutions, flavonols and anthocyanins often undergo inter- and intramolecular copigmentation (Figueiredo et al. 1999; Gonnet 1999; Lancaster et al. 1994). As a result, the increase of the absorption coefficients, bathochromic shifts of the maxima, and peak flattening are observed, significantly affecting the efficiency of absorption of light by these compounds localized within the cells and tissues. In the case of flavonols (such as quercetin and kaempferol glycosides), their in planta tautomerization induces more profound bathochromic shifts of the long-wavelength absorption maxima (Markham et al. 2001; Smith and Markham 1998), which could be particularly significant for visible-radiation screening. Discussion of the consequences of concentration- and copigmentation-dependent effects on the in planta spectroscopy of screening pigments will follow in Chap. 5.

2.3.3 Betalains

This is an interesting group of water-soluble nitrogen-containing compounds of limited occurrence within flowering plants. Specifically, they are encountered mostly in the nine families of the order Caryophyllales (Mabry and Dreiding 1980; Stafford 1994). Two main classes of betalains are distinguished: purple-to-rose betacyanins and yellowish betaxanthins (Fig. 2.5). These classes of betalains are formed by conjugation of the betalamic acid chromophore with cyclodioxiphenylalanine or other amino acids, respectively. Betalains also occur in plants as glycosides, acylglycosides, or more complex species: ferulic acid esters and flavonol conjugates synthesized as a result of UV irradiation.

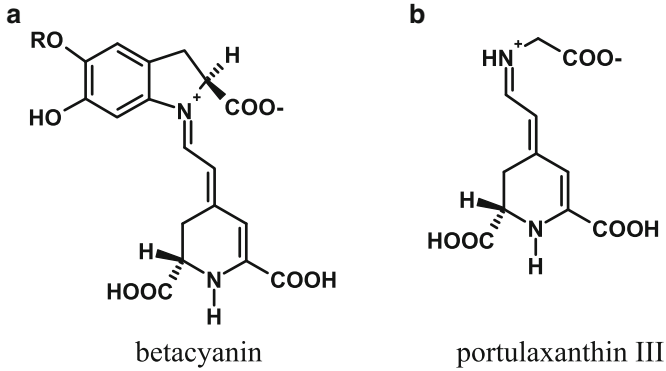


Fig. 2.5 Typical representatives of two important betalain groups: (**a**) betacyanins and (**b**) betaxanthins (Strack et al. 2003)

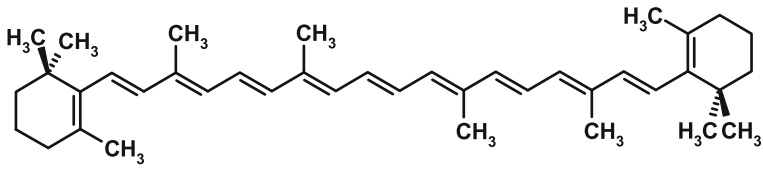
The absorption spectra of betacyanins are characterized by a broad band with a maximum near 593–543 nm; a bathochromic shift to 550 nm is possible as a result of intramolecular copigmentation. The spectra of betaxanthins feature three main bands with maxima near 217, 262, and 546–471 nm (Stafford 1994). Betalains are free-radical scavengers, more efficient at alkaline and neutral pH (Cai et al. 2003; Escribano et al. 1998). The similarity of the spectral properties and subcellular localization of betalains and anthocyanins suggests that the former fulfill the function of anthocyanins in species lacking these pigments (Strack et al. 2003).

2.3.4 Carotenoids

Carotenoids are accessory pigments that are ubiquitous in photoautotrophs. These pigments participate in light harvesting, fulfill photoprotective function, and stabilize the pigment–protein complexes of the photosynthetic apparatus (Green and Durnford 1996; Pascal et al. 2005; Pérez-Bueno et al. 2008; Ruban et al. 2007). More than 800 carotenoid species with linear or cyclic structures have been discovered in plants thus far (Britton 1985).

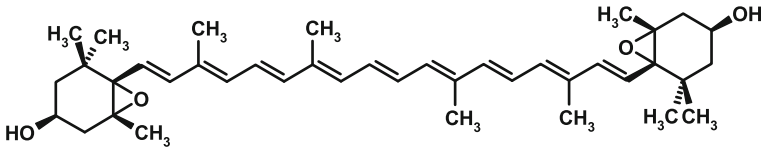
Carotenoids are terpenoid compounds formed via condensation of eight isoprenoid monomers. Yellow-to-orange carotenoids are formed as a result of the stepwise desaturation of their colorless precursors. Upon attaining certain levels of unsaturation, cyclization of the end groups takes place, yielding one or two ionone rings. In higher plants, carotenoids can be synthesized in the dark, but their quantity and composition are controlled by blue-light and UV receptors (Hirschberg 2001; Römer and Fraser 2005; Tanaka et al. 2008; Ye et al. 2009). Carotenoids are divided, according to their substituent composition, into two groups: carotenes, simple hydrocarbon compounds, and xanthophylls containing oxygen atoms within hydroxy, epoxy, or keto groups (Figs. 2.6 and 2.7). The carotenoids of most plant species are represented by carotenes and xanthophylls with characteristic three-headed absorption maxima in the blue part of the spectrum, 400–480 nm (Britton 1995b; Goodwin 1961; Young 1993). The composition of "photosynthetic" or primary carotenoids is highly conserved (Green and Durnford 1996; Young 1993), but under stressful conditions certain species accumulate unusual red secondary carotenoids such as rhodoxanthin (4′,5′-didehydro-4,5′-*retro*-β,β-carotene-3,3′-dione). The presence of conjugated keto groups in the molecules of ketocarotenoids causes the considerable bathochromic shift of the main absorption maximum (cf. Figs. 2.8 and 2.9) in comparison with the carotenoids native to the photosynthetic apparatus (Britton 1985, 1995a). To the best of our knowledge, no evidence has been obtained on the involvement of rhodoxanthin or other red carotenoids in photoprotection within thylakoid membranes. It was reported that the light-harvesting chlorophyll–protein complex of *Cryptomeria japonica* does not retain rhodoxanthin (Han et al. 2003). In contrast to some other xanthophylls nonnative to the photosynthetic apparatus, rhodoxanthin did not facilitate the reassembly of the monomeric recombinant LHCIIb complex (Phillip et al. 2002).

a



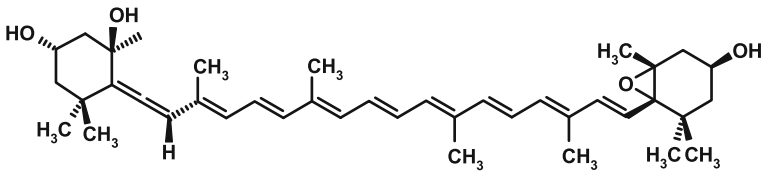
carotenes: β-carotene (*Dunaliella salina*)

b



xanthophylls: violaxanthin (*Malus* × *domestica*)

c



xanthophylls: neoxanthin (*M.* × *domestica*)

Fig. 2.6 Carotenoids native to the photosynthetic apparatus, which could be accumulated as secondary (extrathylakoid) carotenoids (Britton 1995b; Knee 1988; Rabbani et al. 1998)

The form and intensity of the carotenoid absorption peaks in solution (Fig. 2.8) are determined by the number of conjugated double bonds in the carbon skeleton, the number and the kind of the substituents, as well as the kind and polarity of the solvent. The carotenoid molar absorption coefficient of the maximum located in the blue-green region of the spectrum can be as high as 180 mM^{-1} cm^{-1} (Britton 1995b).

The major photosynthetic carotenoids of higher plants include β-carotene and a number of xanthophylls such as lutein, neoxanthin, violaxanthin, antheraxanthin, and zeaxanthin; the structures of xanthophylls of unicellular algae are much more diverse (Britton 1985, 1995a; Young 1993). Many microalgal species are able to accumulate secondary carotenoids which do not participate in photosynthesis and are represented by carotenoids both native (e.g., β-carotene; Rabbani et al. 1998; Ye et al. 2009) and nonnative – such as astaxanthin (Zhekisheva et al. 2002), canthaxanthin (León et al. 2007), and rhodoxanthin (Han et al. 2003; Merzlyak

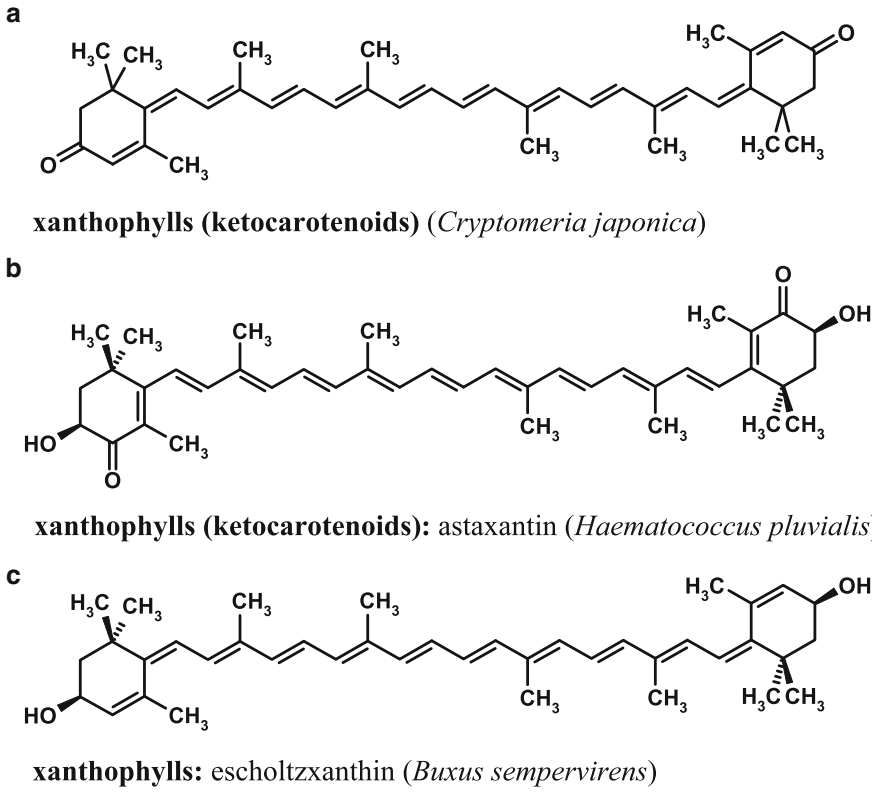


Fig. 2.7 Carotenoids nonnative to the photosynthetic apparatus involved in optical screening of visible radiation (Britton 1995b; Hormaetxe et al. 2005; Wang et al. 2003)

et al. 2005; Weger et al. 1993) – to the photosynthetic apparatus. The secondary xanthophylls are often accumulated in the form of fatty acid esters (Zhekisheva et al. 2002). Higher plants are also capable of extrathylakoid accumulation of carotenoids, mainly in the form of xanthophyll fatty acid esters, whose composition is species-dependent; for more details, see Chap. 3 and (Knee 1988).

The "classic" mechanisms of photoprotection with participation of carotenoids have been relatively well studied. Carotenoids are potent scavengers of free radicals, including free-radical forms of oxygen (Demmig-Adams et al. 1996; Frank and Cogdell 1996; Krinsky 1979). Carotenoids with a large number (10 or 11) of conjugated double bonds readily quench the excited states of chlorophyll, including chlorophyll triplets, as well as singlet oxygen physically (Krinsky 1979). Certain carotenoids undergo cyclic transformations known as xanthophyll cycles which yield carotenoid species capable of efficient thermal dissipation of the excitation energy of chlorophyll, preventing photodamage to the photosynthetic apparatus (Demmig-Adams and Adams 2006). The mechanism of photoprotection of algae and higher plants by extrathylakoid or extraplastidic carotenoids based on optical

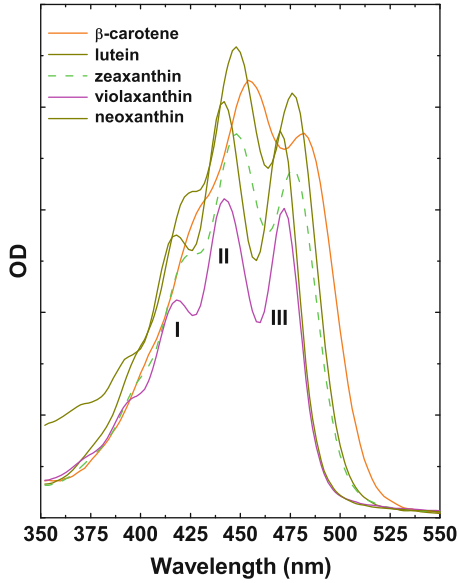


Fig. 2.8 Absorption spectra of the most important higher-plant carotenoids in acetone. The absorption peaks are numbered according to Britton (1995b). (Solovchenko, unpublished)

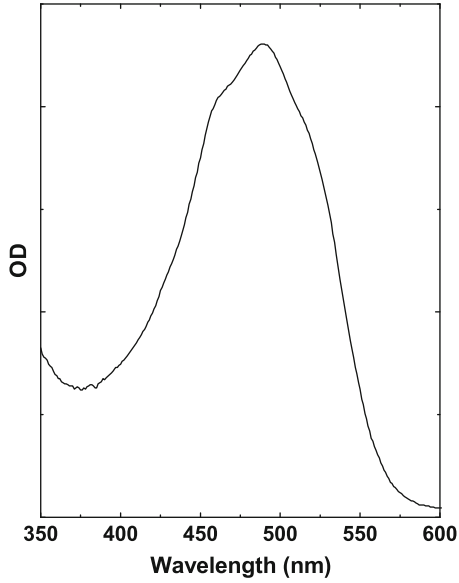


Fig. 2.9 Absorption spectra of rhodoxanthin in acetone. (Solovchenko, unpublished)

screening of the excessive radiation has been discussed for several decades and is generally acknowledged by now (Ben-Amotz et al. 1989; Bidigare et al. 1993; Hagen et al. 1993, 1994; Hanagata and Dubinsky 1999; Hormaetxe et al. 2005;

Hu et al. 2008; Merzlyak and Solovchenko 2002; Solovchenko and Merzlyak 2008) though not as generally as UV protection by phenolic compounds.

2.3.5 Other Screening Pigments

The overwhelming majority of screening compounds discovered to date belong to four principal groups as outlined already. There are also compounds involved in screening of solar radiation but resembling none of the major categories. This group is growing in size and will obviously continue to grow since the vast diversity of screening compounds, especially in phototrophic microorganisms, remain to a considerable extent unexplored and many such compounds are discovered every year. For example, the Antarctic microalga *Phaeocystis pouchetii* responds to elevated UV irradiation with accumulation of a compound with absorption maxima near 323, 271, and 211 nm, and does not fall into any of the currently known screening pigment classes (Marchant et al. 1991).

For example, lichen acids serve multiple roles in the protection of lichens from biotic and environmental stresses (Adams et al. 1993; Bachereau and Asta 1997; Hawksworth and Hill 1984; Solhaug and Gauslaa 1996). In particular, lichen acids are important for the protection of the photobiont against photooxidative damage by solar radiation, which imposes considerable risks under harsh conditions characteristic of lichen.

A special case is constituted by cyanolichens which accumulate scytonemin as a screening pigment (Budel et al. 1997). Scytonemin is one of the most studied cyanobacterial screening compounds often encountered in the sheath of mate-forming cyanobacteria (planktonic species mostly lack scytonemin). Scytonemin is a long-known (Nägeli and Schwenderer 1877) protective compound and whose participation in UV photoprotection via optical screening was rigorously confirmed (Garcia-Pichel et al. 1992). According to the NMR data, scytonemin is a dimer formed via polycondensation of tryptophan and phenylpropanoid precursors. The absorption maximum of scytonemin in vivo is situated near 400 nm (Proteau et al. 1993), suggesting that its tail absorption could be significant for protection against short-wavelength visible radiation. Importantly, scytonemin accumulated in high amounts provides reliable UV protection not only for dividing cells but also for desiccated cells, which possess no other photoprotective mechanisms (Garcia-Pichel et al. 1992).

2.4 Concluding Remarks

The larger part of the contemporary diversity of UV-radiation- and visible-radiation-screening compounds is represented by various phenolic compounds occurring in higher plants. MAA and related substances encountered mainly in cyanobacteria

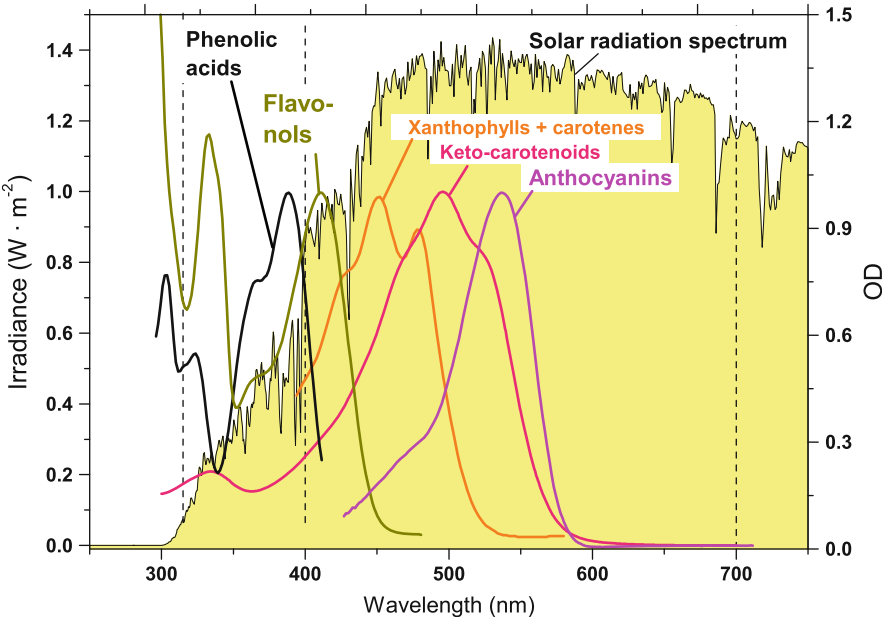


Fig. 2.10 Absorption spectra of the representatives of key groups of photoprotective pigments and the energy spectrum of solar radiation near Earth's surface. The absorption maxima of the most phenolics are located in the UV-B and UV-A regions; anthocyanins possess a long-wavelength maximum in the green region, the short-wavelength part of the spectrum is not shown). This is the band where the maximum of energy in the solar spectrum is located. The photoprotective carotenoids absorb in the blue-green range. The spectra are normalized to their absorption maxima. With kind permission from Springer Science+Business Media: Solovchenko and Merzlyak (2008), Fig. 3

and in certain microalgae and macrophytic algal species are less assorted, but this group is rapidly expanding because new compounds belonging to it are being discovered. There are also more exotic and less abundant groups of screening pigments such as betalains and lichen acids. The participation of secondary carotenoids of numerous microalgal and higher-plant species, especially those lacking anthocyanins, in photoprotection via screening became evident as a result of recent investigations. These compounds greatly differ in terms of their biosynthetic origin and chemical structure, but all of them possess pronounced absorption bands with high extinction coefficients in the UV and/or visible parts of the spectrum (Fig. 2.10). Different taxa of photoautotrophic organisms differ in their ability to synthesize various groups of photoprotective screening pigments (Fig. 2.11). Nevertheless, the combinations of screening compounds simultaneously present in the cells and tissues of many algae and plants could efficiently attenuate radiation in the very broad spectral band extending from the UV region to the blue-green and even to the yellow-orange region of the visible part of the spectrum (Fig. 2.11). The rest of this book focuses primarily on phenolics as the

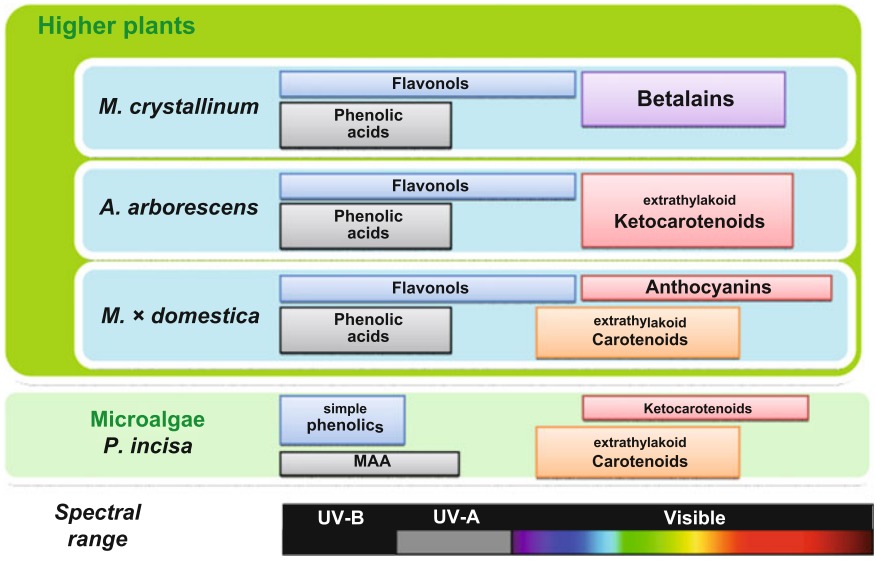


Fig. 2.11 Typical compositions of screening pigments in microalgae and higher plants. Most algae contain mycosporine-like amino acids as UV screening compounds and assorted secondary carotenoids for protection against damage by visible radiation. In higher plants, various phenolics are ubiquitous UV protectants. Anthocyanins, betalains, and red ketocarotenoids constitute there alternative groups of photosynthetically active radiation screening pigments with similar absorption properties but are disparate in other regards. (Solovchenko, unpublished)

most abundant and obviously ubiquitous screening compounds and carotenoids, the radiation-screening function is which is being vigorously investigated.

References

Adams W, Demmig-Adams B, Lange O (1993) Carotenoid composition and metabolism in green and blue-green algal lichens in the field. Oecologia 94:576–584

Afanas'ev I, Ostrachovich E, Korkina L (1998) Effect of rutin and its copper complex on superoxide formation and lipid peroxidation in rat liver microsomes. FEBS Lett 425:256–258

Agati G, Matteini P, Goti A, Tattini M (2007) Chloroplast-located flavonoids can scavenge singlet oxygen. New Phytol 174:77–89

Agati G, Stefano G, Biricolti S, Tattini M (2009) Mesophyll distribution of 'antioxidant' flavonoid glycosides in *Ligustrum vulgare* leaves under contrasting sunlight irradiance. Ann Bot 104:853–861

Bachereau F, Asta J (1997) Effects of solar ultraviolet radiation at high altitude on the physiology and the biochemistry of a terricolous lichen (*Cetraria islandica* (L.) ach.). Symbiosis 23:197–217

Bandaranayake W (1998) Mycosporines: are they nature's sunscreens? Nat Prod Rep 15:159–172

Baur P, Stulle K, Uhlig B, Schönherr J (1998) Absorption von Strahlung im UV-B und Blaulichtbereich von Blattkutikeln ausgewählter Nutzpflanzen. Gartenbauwissenschaft 63:145–152

Ben-Amotz A, Shaish A, Avron M (1989) Mode of action of the massively accumulated β-carotene of *Dunaliella bardawil* in protecting the alga against damage by excess irradiation. Plant Physiol 86:1286–1291

Bidel L, Meyer S, Goulas Y, Cadot Y, Cerovic Z (2007) Responses of epidermal phenolic compounds to light acclimation: in vivo qualitative and quantitative assessment using chlorophyll fluorescence excitation spectra in leaves of three woody species. J Photochem Photobiol B Biol 88:163–179

Bidigare R et al (1993) Evidence a photoprotective for secondary carotenoids of snow algae. J Phycol 29:427–434

Britton G (1985) General carotenoid methods. Methods Enzymol 111:113

Britton G (1995a) Structure and properties of carotenoids in relation to function. FASEB J 9:1551–1558

Britton G (1995b) UV/visible spectroscopy. In: Britton G, Liaaen-Jensen S, Pfander H (eds) Carotenoids, vol 1B. Birkhauser, Basel, pp 13–62

Budel B, Karsten U, Garcia-Pichel F (1997) Ultraviolet-absorbing scytonemin and mycosporine-like amino acid derivatives in exposed, rock-inhabiting cyanobacterial lichens. Oecologia 112:165–172

Buer CS, Imin N, Djordjevic MA (2010) Flavonoids: new roles for old molecules. J Integr Plant Biol 52:98–111

Burchard P, Bilger W, Weissenbock G (2000) Contribution of hydroxycinnamates and flavonoids to epidermal shielding of UV-A and UV-B radiation in developing rye primary leaves as assessed by ultraviolet-induced chlorophyll fluorescence measurements. Plant Cell Environ 23:1373–1380

Cai Y, Sun M, Corke H (2003) Antioxidant activity of betalains from plants of the Amaranthaceae. J Agric Food Chem 51:2288–2294

Caldwell M, Robberecht R, Flint S (1983) Internal filters: prospects for UV-acclimation in higher plants. Physiol Plant 58:445–450

Close D, Beadle C (2003a) Alternate energy dissipation? Phenolic metabolites and the xanthophyll cycle. J Plant Physiol 160:431–434

Close D, Beadle C (2003b) The ecophysiology of foliar anthocyanin. Bot Rev 69:149–161

Close D, McArthur C (2002) Rethinking the role of many plant phenolics-protection from photodamage not herbivores? Oikos 99:166

Cockell C (1998) Biological effects of high ultraviolet radiation on early earth – a theoretical evaluation. J Theor Biol 193:717–729

Cockell C, Knowland J (1999) Ultraviolet radiation screening compounds. Biol Rev 74:311–345

Day T, Martin G, Vogelmann T (1993) Penetration of UV-B radiation in foliage: evidence that the epidermis behaves as a non-uniform filter. Plant Cell Environ 16:735–741

Day T, Howells B, Rice W (1994) Ultraviolet absorption and epidermal-transmittance spectra in foliage. Physiol Plant 92:207–218

DeLucia E, Day T, Vogelman T (1992) Ultraviolet-B and visible light penetration into needles of two species of subalpine conifers during foliar development. Plant Cell Environ 15:921–929

Demmig-Adams B, Adams W (2006) Photoprotection in an ecological context: the remarkable complexity of thermal energy dissipation. New Phytol 172:11–21

Demmig-Adams B, Gilmore A, Adams W (1996) In vivo function of carotenoids in higher plants. FASEB J 10:403–412

Deng W, Fang X, Wu J (1997) Flavonoids function as antioxidants: by scavenging reactive oxygen species or by chelating iron? Radiat Phys Chem 50:271–276

Diaz M, Ball E, Luttge U (1990) Stress-induced accumulation of the xanthophyll rhodoxanthin in leaves of *Aloe vera*. Plant Physiol Biochem 28:679–682

Escribano J, Pedreño M, Garcia-Carmona F, Muñoz R (1998) Characterization of the antiradical activity of betalains from *Beta vulgaris* L. roots. Phytochem Anal 9:124–127

Figueiredo P, George F, Tatsuzawa F, Toki K, Saito N, Brouillard R (1999) New features of intramolecular copigmentation byacylated anthocyanins. Phytochemistry 51:125–132

Frank H, Cogdell R (1996) Carotenoids in photosynthesis. Photochem Photobiol 63:257–264
Garcia-Pichel F, Castenholz R (1993) Occurrence of UV-absorbing, mycosporine-like compounds among cyanobacterial isolates and an estimate of their screening capacity. Appl Environ Microbiol 59:163–169
Garcia-Pichel F, Sherry N, Castenholz R (1992) Evidence for an ultraviolet sunscreen role of the extracellular pigment scytonemin in the terrestrial cyanobacterium *Chiorogloeopsis* sp. Photochem Photobiol 56:17–23
Georgieva K, Sarvari E, Keresztes A (2010) Protection of thylakoids against combined light and drought by a lumenal substance in the resurrection plant *Haberlea rhodopensis*. Ann Bot 105:117–126
Giordano C, Massai R, Agati G (2005) On the role of flavonoids in the integrated mechanisms of response of *Ligustrum vulgare* and *Phillyrea latifolia* to high solar radiation. New Phytol 167:457–470
Gómez I, Pérez-Rodríguez E, Vicegla B, Figueroa F, Karsten U (1998) Effects of solar radiation on photosynthesis. UV-absorbing compounds and enzyme activities of the green alga *Dasycladus vermicularis* from southern Spain. J Photochem Photobiol B Biol 47:46–57
Gonnet J (1999) Colour effects of co-pigmentation of anthocyanins revisited-2. A colorimetric look at the solutions of cyanin co-pigmented by rutin using the CIELAB scale. Food Chem 66:387–394
Goodwin TW (1961) Biosynthesis and function of carotenoids. Annu Rev Plant Physiol 12:219–244
Gould K (2004) Nature's Swiss army knife: the diverse protective roles of anthocyanins in leaves. J Biomed Biotechnol 5:314–320
Gould K, McKelvie J, Markham K (2002) Do anthocyanins function as antioxidants in leaves? Imaging of H_2O_2 in red and green leaves after mechanical injury. Plant Cell Environ 25:1261–1269
Green B, Durnford D (1996) The chlorophyll-carotenoid proteins of oxygenic photosynthesis. Annu Rev Plant Biol 47:685–714
Gröniger A, Sinha R, Klisch M, Häder D (2000) Photoprotective compounds in cyanobacteria, phytoplankton and macroalgae – a database. J Photochem Photobiol B Biol 58:115–122
Hagen C, Braune W, Greulich F (1993) Functional aspects of secondary carotenoids in *Haematococcus lacustris* [Girod] Rostafinski (Volvocales). IV: protection from photodynamic damage. J Photochem Photobiol B Biol 20:153–160
Hagen C, Braune W, Björn L (1994) Functional aspects of secondary carotenoids in *Haematococcus lacustris* (Volvocales). III. Action as a sunshade. J Phycol 30:241–248
Hahlbrock K, Grisebach H (1979) Enzymic controls in the biosynthesis of lignin and flavonoids. Annu Rev Plant Physiol 30:105–130
Hahlbrock K, Scheel D (1989) Physiology and molecular biology of phenylpropanoid metabolism. Annu Rev Plant Biol 40:347–369
Han Q, Shinohara K, Kakubari Y, Mukai Y (2003) Photoprotective role of rhodoxanthin during cold acclimation in *Cryptomeria japonica*. Plant Cell Environ 26:715–723
Hanagata N, Dubinsky Z (1999) Secondary carotenoid accumulation in *Scenedesmus komarekii* (Chlorophyceae, Chlorophyta). J Phycol 35:960–966
Harborne J (1976) Functions of flavonoids in plants. In: Goodwin T (ed) Chemistry and biochemistry of plant pigments. Academic, New York, pp 736–778
Harborne J (1980) Plant phenolics. In: Bell E, Charlwood B (eds) Secondary plant products. Springer, Berlin, pp 329–402
Harborne J (2001) Twenty-five years of chemical ecology. Nat Prod Rep 18:361–379
Harborne J, Williams C (1998) Anthocyanins and other flavonoids. Nat Prod Rep 15:631–652
Harborne J, Williams C (2000) Advances in flavonoid research since 1992. Phytochemistry 55:481–504
Havaux M, Kloppstech K (2001) The protective functions of carotenoid and flavonoid pigments against excess visible radiation at chilling temperature investigated in *Arabidopsis npq* and *tt* mutants. Planta 213:953–966
Hawksworth D, Hill D (1984) The lichen-forming fungi. Blackie, Glasgow

He Y, Häder D (2002a) Reactive oxygen species and UV-B: effect on cyanobacteria. Photochem Photobiol Sci 1:729–736

He Y, Häder D (2002b) UV-B-induced formation of reactive oxygen species and oxidative damage of the cyanobacterium *Anabaena* sp.: protective effects of ascorbic acid and N-acetyl-L-cysteine. J Photochem Photobiol B Biol 66:115–124

Hirschberg J (2001) Carotenoid biosynthesis in flowering plants. Curr Opin Plant Biol 4:210–218

Hoch W, Singsaas E, McCown B (2003) Resorption protection. Anthocyanins facilitate nutrient recovery in autumn by shielding leaves from potentially damaging light levels. Plant Physiol 133:1296–1305

Hormaetxe K, Becerril J, Fleck I, Pintó M, Garcia-Plazaola J (2005) Functional role of red (retro)-carotenoids as passive light filters in the leaves of *Buxus sempervirens* L.: increased protection of photosynthetic tissues? J Exp Bot 56:2629–2636

Hormaetxe K, Becerril J, Hernández A, Esteban R, Garcia-Plazaola J (2007) Plasticity of photoprotective mechanisms of *Buxus sempervirens* L. leaves in response to extreme temperatures. Plant Biol 9:59–68

Hu Z, Li Y, Sommerfeld M, Chen F, Hu Q (2008) Enhanced protection against oxidative stress in an astaxanthin-overproduction *Haematococcus* mutant (Chlorophyceae). Eur J Phycol 43:365–376

Hughes N (2009) The photoprotective role of anthocyanin pigments in leaf tissues. In: vol. PhD. Wake Forest University, Winston-Salem, p 169

Hughes N, Neufeld H, Burkey K (2005) Functional role of anthocyanins in high-light winter leaves of the evergreen herb *Galax urceolata*. New Phytol 168:575–587

Ibdah M, Krins A, Seidlitz H, Heller W, Strack D, Vogt T (2002) Spectral dependence of flavonol and betacyanin accumulation in *Mesembryanthemum crystallinum* under enhanced ultraviolet radiation. Plant Cell Environ 25:1145–1154

Jung J, Kim H (1990) The chromophores as endogenous sensitizers involved in the photogeneration of singlet oxygen in spinach thylakoids. Photochem Photobiol 52:1003–1009

Karageorgou P, Manetas Y (2006) The importance of being red when young: anthocyanins and the protection of young leaves of *Quercus coccifera* from insect herbivory and excess light. Tree Physiol 26:613

Karsten U, Friedl T, Schumann R, Hoyer K, Lembcke S (2005) Mycosporine-like amino acids and phylogenies in green algae: *Prasiola* and its relatives from the Trebouxiophyceae (Chlorophyta). J Phycol 41:557–566

Knee M (1988) Carotenol esters in developing apple fruits. Phytochemistry 27:1005–1009

Kolb C, Kopecky J, Riederer M, Pfundel E (2003) UV screening by phenolics in berries of grapevine (*Vitis vinifera*). Funct Plant Biol 30:1177–1186

Korbee N, Figueroa F, Aguilera J (2005) Effect of light quality on the accumulation of photosynthetic pigments, proteins and mycosporine-like amino acids in the red alga *Porphyra leucosticta* (Bangiales, Rhodophyta). J Photochem Photobiol B Biol 80:71–78

Korkina L, Afanas'ev I (1997) Antioxidant and chelating properties of flavonoids. In: Sies H (ed) Antioxidants in disease mechanisms and therapy. Academic, New York, pp 151–163

Kostyuk V, Potapovich A, Strigunova E, Kostyuk T, Afanas'ev I (2004) Experimental evidence that flavonoid metal complexes may act as mimics of superoxide dismutase. Arch Biochem Biophys 428:204–208

Kräbs G, Watanabe M, Wiencke C (2004) A monochromatic action spectrum for the photoinduction of the UV-absorbing mycosporine-like amino acid shinorine in the red alga *Chondrus crispus*. Photochem Photobiol 79:515–519

Krauss P, Markstadter C, Riederer M (1997) Attenuation of UV radiation by plant cuticles from woody species. Plant Cell Environ 20:1079–1085

Krinsky N (1979) Carotenoid protection against oxidation. Pure Appl Chem 51:649–660

Lancaster J, Grant J, Lister C, Taylor M (1994) Skin color in apples: influence of copigmentation and plastid pigments on shade and darkness of red color in five genotypes. J Am Soc Hortic Sci 119:63–69

León R, Couso I, Fernández E (2007) Metabolic engineering of ketocarotenoids biosynthesis in the unicelullar microalga *Chlamydomonas reinhardtii*. J Biotechnol 130:143–152

Mabry T, Dreiding A (1980) Betalains. In: Bell EA, Charwood BV (eds) Encyclopedia of plant physiology, vol 8. Springer, Berlin, pp 513–533

Marchant H, Davidson A, Kelly G (1991) UV-B protecting compounds in the marine alga *Phaeocystis pouchetii* from Antarctica. Mar Biol 109:391–395

Markham K (1989) Flavones, flavonols and their glycosides. In: Harborne J, Dey P (eds) Methods in plant biochemistry, vol 1. Academic, New York, pp 197–235

Markham K, Gould K, Ryan K (2001) Cytoplasmic accumulation of flavonoids in flower petals and its relevance to yellow flower colouration. Phytochemistry 58:403–413

Markstädter C, Queck I, Baumeister J, Riederer M, Schreiber U, Bilger W (2001) Epidermal transmittance of leaves of *Vicia faba* for UV radiation as determined by two different methods. Photosynth Res 67:17–25

Mazza C, Boccalandro H, Giordano C, Battista D, Scopel A, Ballare C (2000) Functional significance and induction by solar radiation of ultraviolet-absorbing sunscreens in field-grown soybean crops. Plant Physiol 122:117–126

Merzlyak MN, Chivkunova OB (2000) Light-stress-induced pigment changes and evidence for anthocyanin photoprotection in apples. J Photochem Photobiol B Biol 55:155–163

Merzlyak M, Solovchenko A (2002) Photostability of pigments in ripening apple fruit: a possible photoprotective role of carotenoids during plant senescence. Plant Sci 163:881–888

Merzlyak MN, Melo TB, Razi Naqvi K (2004) Estimation of leaf transmittance in the near infrared region through reflectance measurements. J Photochem Photobiol B Biol 74:145–150

Merzlyak M, Solovchenko A, Pogosyan S (2005) Optical properties of rhodoxanthin accumulated in *Aloe arborescens* Mill. leaves under high-light stress with special reference to its photoprotective function. Photochem Photobiol Sci 4:333–340

Merzlyak MN, Chivkunova OB, Solovchenko AE, Naqvi KR (2008a) Light absorption by anthocyanins in juvenile, stressed, and senescing leaves. J Exp Bot 59:3903–3911

Merzlyak MN, Melo TB, Naqvi KR (2008b) Effect of anthocyanins, carotenoids, and flavonols on chlorophyll fluorescence excitation spectra in apple fruit: signature analysis, assessment, modelling, and relevance to photoprotection. J Exp Bot 59:349–359

Meyer S, Louis J, Moise N, Piolot T, Baudin X, Cerovic ZG (2009) Developmental changes in spatial distribution of in vivo fluorescence and epidermal UV absorbance over *Quercus petraea* leaves. Ann Bot 104:621–633

Mohr H, Drumm-Herrel H (1983) Coaction between phytochrome and blue/UV light in anthocyanin synthesis in seedlings. Physiol Plant 58:408–414

Moisan T, Mitchell B (2001) UV absorption by mycosporine-like amino acids in *Phaeocystis antarctica* Karsten induced by photosynthetically available radiation. Mar Biol 138:217–227

Morgan-Kiss R, Priscu J, Pocock T, Gudynaite-Savitch L, Huner N (2006) Adaptation and acclimation of photosynthetic microorganisms to permanently cold environments. Microbiol Mol Biol Rev 70:222–252

Moskowitz A, Hrazdina G (1981) Vacuolar contents of fruit subepidermal cells from *Vitis* species 1. Plant Physiol 68:686–692

Nägeli C, Schwenderer S (1877) Das mikroskop. Engelmanne, Leipzig

Oren A, Gunde-Cimerman N (2007) Mycosporines and mycosporine-like amino acids: UV protectants or multipurpose secondary metabolites? FEMS Microbiol Lett 269:1–10

Pascal A et al (2005) Molecular basis of photoprotection and control of photosynthetic light-harvesting. Nature 436:134–137

Pérez-Bueno M, Johnson M, Zia A, Ruban A, Horton P (2008) The Lhcb protein and xanthophyll composition of the light harvesting antenna controls the pH-dependency of non-photochemical quenching in *Arabidopsis thaliana*. FEBS Lett 582:1477–1482

Phillip D, Hobe S, Paulsen H, Molnar P, Hashimoto H, Young A (2002) The binding of xanthophylls to the bulk light-harvesting complex of photosystem II of higher plants: a specific requirement for carotenoids with a 3-hydroxy-β-end group. J Biol Chem 277:25160–25169

Pietrini F, Iannelli M, Massacci A (2002) Anthocyanin accumulation in the illuminated surface of maize leaves enhances protection from photo-inhibitory risks at low temperature, without further limitation to photosynthesis. Plant Cell Environ 25:1251–1259

Proteau P, Gerwick W, Garcia-Pichel F, Castenholz R (1993) The structure of scytonemin, an ultraviolet sunscreen pigment from the sheaths of cyanobacteria. Cell Mol Life Sci 49:825–829

Rabbani S, Beyer P, Lintig J, Hugueney P, Kleinig H (1998) Induced β-carotene synthesis driven by triacylglycerol deposition in the unicellular alga *Dunaliella bardawil*. Plant Physiol 116:1239–1248

Rice-Evans C, Miller N, Paganga G (1997) Antioxidant properties of phenolic compounds. Trends Plant Sci 2:152–159

Römer S, Fraser P (2005) Recent advances in carotenoid biosynthesis, regulation and manipulation. Planta 221:305–308

Ruban A et al (2007) Identification of a mechanism of photoprotective energy dissipation in higher plants. Nature 450:575–578

Russo A et al (2000) Bioflavonoids as antiradicals, antioxidants and DNA cleavage protectors. Cell Biol Toxicol 16:91–98

Saija A, Scalese M, Lanza M, Marzullo D, Bonina F, Castelli F (1995) Flavonoids as antioxidant agents: importance of their interaction with biomembranes. Free Radic Biol Med 19:481–486

Shick J, Dunlap W (2002) Mycosporine-like amino acids and related gadusols: biosynthesis, accumulation, and UV-protective functions in aquatic organisms. Annu Rev Physiol 64:223–262

Singh S, Klisch M, Sinha R, Hader D (2008) Effects of abiotic stressors on synthesis of the mycosporine-like amino acid shinorine in the cyanobacterium *Anabaena variabilis* PCC 7937. Photochem Photobiol 84:1500–1505

Sinha R, Häder D (2007) UV-protectants in cyanobacteria. Plant Sci 174:278–289

Sinha R, Singh S, Häder D (2007) Database on mycosporines and mycosporine-like amino acids (MAAs) in fungi, cyanobacteria, macroalgae, phytoplankton and animals. J Photochem Photobiol B Biol 89:29–35

Smith G, Markham K (1998) Tautomerism of flavonol glucosides: relevance to plant UV protection and flower colour. J Photochem Photobiol A Chem 118:99–105

Solhaug K, Gauslaa Y (1996) Parietin, a photoprotective secondary product of the lichen *Xanthoria parietina*. Oecologia 108:412–418

Solovchenko A, Merzlyak M (2003) Optical properties and contribution of cuticle to UV protection in plants: experiments with apple fruit. Photochem Photobiol Sci 2:861–866

Solovchenko A, Merzlyak M (2008) Screening of visible and UV radiation as a photoprotective mechanism in plants. Russ J Plant Physiol 55:719–737

Solovchenko A, Schmitz-Eiberger M (2003) Significance of skin flavonoids for UV-B-protection in apple fruits. J Exp Bot 54:1977–1984

Stafford H (1994) Anthocyanins and betalains: evolution of the mutually exclusive pathways. Plant Sci 101:91–98

Steyn W, Wand S, Holcroft D, Jacobs G (2002) Anthocyanins in vegetative tissues: a proposed unified function in photoprotection. New Phytol 155:349–361

Steyn WJ, Wand SJE, Jacobs G, Rosecrance RC, Roberts SC (2009) Evidence for a photoprotective function of low-temperature-induced anthocyanin accumulation in apple and pear peel. Physiol Plant 136:461–472

Strack D, Wray V (1989) Anthocyanins. In: Harborne J, Dey P (eds) Methods in plant biochemistry, vol 1. Academic, New York, pp 325–356

Strack D, Vogt T, Schliemann W (2003) Recent advances in betalain research. Phytochemistry 62:247–269

Suh H, Lee H, Jung J (2003) Mycosporine glycine protects biological systems against photodynamic damage by quenching singlet oxygen with a high efficiency. Photochem Photobiol 78:109–113

Takahama U (1983) Redox reactions between kaempferol and illuminated chloroplasts. Plant Physiol 71:598–601

Tanaka Y, Sasaki N, Ohmiya A (2008) Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids. Plant J 54:733

Tournaire C et al (1993) Antioxidant activity of flavonoids: efficiency of singlet oxygen ($^1\Delta_g$) quenching. J Photochem Photobiol B Biol 19:205

Treutter D (2006) Significance of flavonoids in plant resistance: a review. Environ Chem Lett 4:147–157

Vogt T (2010) Phenylpropanoid biosynthesis. Mol Plant 3:2–20

Vogt T, Ibdah M, Schmidt J, Wray V, Nimtz M, Strack D (1999) Light-induced betacyanin and flavonol accumulation in bladder cells of *Mesembryanthemum crystallinum*. Phytochemistry 52:583–592

Wang C, Liu T (2007) Involvement of betacyanin in chilling-induced photoinhibition in leaves of *Suaeda salsa*. Photosynthetica 45:182–188

Wang B, Zarka A, Trebst A, Boussiba S (2003) Astaxanthin accumulation in *Haematococcus pluvialis* (Chlorophyceae) as an active photoprotective process under high irradiance. J Phycol 39:1116–1124

Weger H, Silim S, Guy R (1993) Photosynthetic acclimation to low temperature by western red cedar seedlings. Plant Cell Environ 16:711–717

Yamasaki H, Sakihama Y, Ikehara N (1997) Flavonoid-peroxidase reaction as a detoxification mechanism of plant cells against H_2O_2. Plant Physiol 115:1405–1412

Ye Z-W, Jiang J-G, Wu G-H (2009) Biosynthesis and regulation of carotenoids in Dunaliella: progresses and prospects. Biotechnol Adv 26:352–360

Young A (1991) The photoprotective role of carotenoids in higher plants. Physiol Plant 83:702–708

Young A (1993) Occurrence and distribution of carotenoids in photosynthetic systems. In: Young A, Britton G (eds) Carotenoids in photosynthesis. Chapman and Hall, London, pp 16–71

Young A, Britton G (1990) Carotenoids and stress. In: Alscher R, Cumming J (eds) Stress responses in plants: adaptation and acclimation mechanisms. Wiley-Liss, New York, pp 87–112

Zeng X-Q, Chow WS, Su L-J, Peng X-X, Peng C-L (2010) Protective effect of supplemental anthocyanins on *Arabidopsis* leaves under high light. Physiol Plant 138:215–225

Zhekisheva M, Boussiba S, Khozin-Goldberg I, Zarka A, Cohen Z (2002) Accumulation of oleic acid in *Haematococcus pluvialis* (Chlorophyceae) under nitrogen starvation or high light is correlated with that of astaxanthin esters. J Phycol 38:325–331

Chapter 3
Stress-Induced Buildup of Screening Pigments

Abstract This chapter extends the discussion of screening pigments with an outline of possible mechanisms for the induction and regulation of their biosynthesis under stresses. Typical patterns of changes in pigment content and composition during the accumulation of screening pigments in plants are presented. Special attention is paid to the role of solar UV radiation in the induction of phenolics (which are admittedly the most ubiquitous and probably most studied screening pigment group) and to photostability of extrathylakoid carotenoids (the screening function of which is being vigorously investigated).

As stated in the previous chapter, the pronounced buildup of screening pigment content observed in response to elevated levels of radiation of the corresponding spectral band is a prerequisite for the function of the screening pigment. The patterns of accumulation of different groups of screening pigments in microalgae and higher plants during their acclimation to high fluxes of solar radiation are considered below. However, before proceeding to the discussion, several reservations have to be made. Firstly, there are many works on accumulation of phenolics in leaves and other plant organs induced by natural and artificial UV radiation and the ecological and physiological significance of this phenomenon which are given full credit but could not be cited here in full number owing to space constraints. Therefore, only important trends will be outlined with a minimum of references; for detailed accounts of these experimental works, one may refer to recent reviews in the field (see, e.g., Beggs et al. (1986), Caldwell et al. (2007), Rozema et al. (1997)). Secondly, considerable attention has been paid to accumulation of secondary carotenoids which could serve as screening compounds since much less is known about this phenomenon in comparison with accumulation of phenolics.

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Nevertheless, it is appears that screening of visible radiation by extrathylakoid carotenoids plays an important role in photoprotection of certain microalgae and higher-plant species devoid of both anthocyanins and betalains. Thirdly, for the purposes of this and the following chapters, it is convenient to use apple fruits as an example since they represent a useful natural system for studies of photoprotection-related processes in plants (Merzlyak and Solovchenko 2002; Merzlyak and Chivkunova 2000; Merzlyak et al. 2005b; Solovchenko et al. 2006) and possess a fully functional photosynthetic apparatus operating at rates commensurate with those recorded in leaves (Blanke and Lenz 1989). In fruit growing on the periphery of a canopy, one of the surfaces (referred to as sunlit) is affected by strong direct sunlight, whereas the opposite (shaded) surface of the *same* fruit is predominantly illuminated by lower fluxes of diffuse light, allowing their paired comparison. Therefore, one could obtain, within a single fruit, samples of photosynthesizing tissue acclimated to high or low light intensity. A number of studies devoted to the elucidation of screening pigments have been carried out in our laboratories using apples as a model (Merzlyak and Solovchenko 2002; Merzlyak et al. 2002, 2003, 2005b; Solovchenko et al. 2005, 2006; Solovchenko and Merzlyak 2003; Solovchenko and Schmitz-Eiberger 2003); the results of these will be used as illustrations where appropriate.

3.1 Buildup of Mycosporine-Like Amino Acid and Phenolic Sunscreens

The induction of the synthesis of screening compounds in plants by elevated fluxes of natural solar radiation as well as by artificial photosynthetically active radiation (PAR) and/or UV radiation is one of the most documented and extensively reviewed stress response (see, e.g., Caldwell et al. (2007), Cockell and Knowland (1999), Vogt et al. (1999)). At the same time, the buildup of screening compounds is often induced by other abiotic (or environmental) stresses such as extreme temperatures (Bilger et al. 2007; Lancaster et al. 2000; Morgan-Kiss et al. 2006) and phosphorus and nitrogen deficiencies (Olsen et al. 2009) as well as biotic stresses such as phytopathogen and herbivore attacks (Close and McArthur 2002; Harborne 2001).

It is generally accepted that the pathways of biosynthesis of various pigments can be upregulated by signals perceived by different photoreceptors, transmitted or relayed by hormones (such as gibberellins and ethylene; Cecchi et al. 2005) and nonspecific inductors such as reactive oxygen species (ROS) as well as by redox signals originating from the photosynthetic electron transport chain under diverse stresses (Foyer and Noctor 2005; Steinbrenner and Linden 2003). The following sections contain a brief outline of the regulatory mechanisms relevant to the buildup of screening pigments.

3.1.1 Induction and Regulation of the Synthesis of Mycosporine-Like Amino Acids

Current information about the induction and regulation of the biosynthesis of mycosporine-like amino acids (MAA) in algae is rather scarce. According to the experimental evidence reviewed in Shick and Dunlap (2002) and Sinha et al. (2001), the content of MAA in photoautotrophic organisms is positively related to the total solar irradiance they experience under natural conditions. However, covariation of solar PAR and UV radiation fluxes imposes certain difficulties in determining the action spectrum of the induction of MAA synthesis. Exclusion of different ranges from solar radiation incident on diverse microalgae and macroalgae revealed stimulating effects of UV-B radiation, UV-A radiation, white light lacking UV radiation, and blue light, but no effect of red or green light, suggesting the involvement of photoreceptors with specific spectral sensitivity, presumably blue-light/UV-A receptors, perhaps the flavoprotein cryptochromes (Shick and Dunlap 2002). According to recent findings, the maximum of the action spectrum for MAA induction in sea microalgae is located in the UV-B region near 310 nm (Klisch and Häder 2002). It also appears that the induction of MAA synthesis by solar radiation is a high-irradiance, dose-dependent response (Shick and Dunlap 2002).

3.1.2 Induction of Biosynthesis of Phenolic Compounds

Naturally elevated fluxes of UV radiation and PAR (i.e., experienced by plants in high-altitude habitats and equatorial regions) as well as artificially supplemented UV radiation induce a number of responses in plants (Jansen et al. 1998). One of the most common among them is a differential induction of synthesis and accumulation of screening compounds, predominantly those of a phenolic nature. The content and composition of the phenolics (see Chap. 2) accumulated in plants under high fluxes of solar radiation vary widely depending on the species and the environmental conditions of growth – this phenomenon is extensively documented in the literature (for recent reviews, see Bornman (1999), Bornman et al. (1997), Caldwell et al. (2007)).

Phenolic acids, their conjugates, and, more often, flavonol glycosides are generally induced in plants by strong sunlight and artificial UV radiation. For instance, supplementation with 8.9 kJ m^{-2} day^{-1} UV-B radiation induced a distinct increase in UV-absorbing compounds in rape (*Brassica napus* L.) epidermis in comparison with plants grown under background UV-B fluxes. Notably, the flavonol glycoside content increased most prominently: for kaempferol glycosides, a 23–36 times increase was recorded (Olsson et al. 1998). Another common example is given by conifer plants such as Scots pine (*Pinus sylvestris* L.) and Engelmann spruce (*Picea engelmannii* Parry ex Engelm.), in which UV-B irradiation also induces

a considerable increase in flavonol glycoside content (DeLucia et al. 1992; Schnitzler et al. 1996; Turunen et al. 1999). Irradiation with elevated levels of UV radiation also brings about cuticle and epidermis thickening (Jansen et al. 1998); these processes facilitate the accumulation of the screening phenolics in cuticle and epidermal cells (see Chap. 4).

Anthocyanin accumulation is also readily induced in diverse plants species by stresses, especially by strong solar irradiation on the background of low temperature (Steyn et al. 2002, 2009; Zeliou et al. 2009); therefore, anthocyanins are sometimes referred to as "stress pigments" (Chalker-Scott 1999). Biosynthesis of these pigments is regulated mainly by phytochrome and cryptochrome systems; accumulation of anthocyanins represents another well-known high-irradiation response (Beggs and Wellmann 1994; Saure 1990). Interestingly, the transient buildup of anthocyanins or "flush" is often observed in young (so-called juvenile pigmentation) and senescing plants (especially on the background of low temperatures) and plant organs but is lacking in mature plants (Chalker-Scott 1999; Karageorgou and Manetas 2006). In other words, the anthocyanin screen in these cases is deployed when the photosynthetic apparatus is especially vulnerable, i.e., when it is not yet mature, undergoes ordered dismantling during senescence (Hoch et al. 2001a, b, 2003), or is acclimating to a stressor. After maturation of the photosynthetic apparatus or upon removal of the stressor, the anthocyanin screen, which is no longer necessary, disappears (Hughes et al. 2007; Karageorgou and Manetas 2006). However, the processes of degradation of phenolic compounds involved in withdrawal of phenolic sunscreens are much less understood in comparison with the pathways of their biosynthesis. Most of the studies in the field have been dedicated to phenolic decomposition/catabolism in vitro, in food, or in animals or humans (Cheynier 2006). According to current knowledge, flavonoids, including anthocyanins, usually undergo enzymatic degradation via a reaction catalyzed by vacuolar flavonol hydroxylases. Still, the mechanism of sequestration of phenolics or their degradation products from the compartments of their accumulation remains to a considerable extent unclear.

The molecular mechanisms of the induction of phenolic compound biosynthesis in response to stressors such as elevated levels of UV radiation or PAR appear to be quite complicated (for a detailed description of the receptor and signal transduction network controlling phenolic metabolism in plants, see Hahlbrock and Scheel (1989), Jenkins (2009), Logemann et al. (2000), Mol et al. (1996), Tanaka et al. (2008), Wade et al. (2001), Weisshaar and Jenkins (1998)). In particular, biosynthesis of phenolic compounds can be induced by changes in the spectral quality of illumination by specific photoreceptors (phytochromes, cryptochromes, UV-A receptors) (Beggs and Wellmann 1994; Ensminger and Schäfer 1992; Jenkins 2009); ROS could be involved in those processes as well (Mackerness 2000).

Signals originating from photoreceptors control key stages of phenolic biosynthesis pathways such as the phenylpropanoid pathway, which synthesizes important precursors of screening pigments of a phenolic nature (Dixon and Paiva 1995). In most of the systems studied, the biosynthesis of phenolics is accomplished by differential upregulation of the transcription of genes encoding key enzymes of

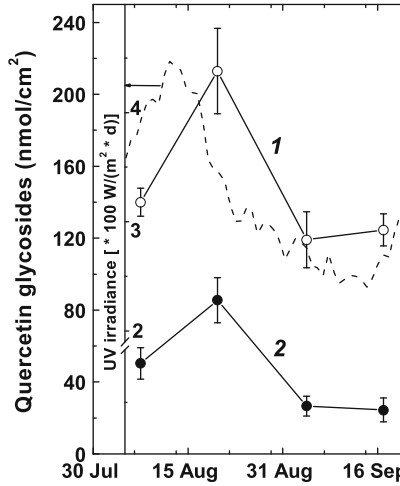

Fig. 3.1 The time course of skin quercetin glycoside content on sunlit (*open symbols*) and shaded (*closed symbols*) surfaces of Braeburn apple fruit and daily UV irradiance (*dashed line*). (Reproduced from Solovchenko et al. (2005) with kind permission)

the phenylpropanoid pathway (phenylalanine ammonia lyase, *pal*) and the enzyme (s) synthesizing precursors of flavonols (e.g., chalcone synthase, *chs*) (Beggs and Wellmann 1994; Dixon and Paiva 1995; Jenkins 2009).

The time of the induction of phenolic screening pigment synthesis in response to elevated irradiation varies, depending on the species under consideration, from several hours (in young grasses) to several days or even weeks (in woody species) (Tevini et al. 1991). For example, the UV-irradiation-dependent increase in quercetin glycoside content in apple fruit skin occurs with a time lag of about 10 days (Fig. 3.1). Experiments with radiolabeled phenolic precursors showed that flavonols accumulated after UV irradiation (mainly quercetin and kaempferol) were predominantly synthesized de novo. Then, UV stress often induces the formation of more complex, conjugated (e.g., with betalains) flavonol species (Ibdah et al. 2002; Strack et al. 2003).

3.1.3 Accumulation of Different Phenolic Compounds in Response to Strong Solar Irradiation

The massive accumulation of screening phenolics appears to be an irradiance-dependent response, that is, the amount of screening compound(s) is proportional to the dose of solar radiation (or, more precisely, its UV component, see, e.g., Fig. 3.1). The irradiance-dependent buildup of phenolics tends to occur locally, i.e., in the cells affected by elevated levels of radiation. This response can be modulated by signals from receptor systems sensing the radiation in the visible (presumably, blue) part of the spectrum. For example, the exclusion of the UV component from solar radiation reduces considerably the magnitude of buildup of quercetin glycosides on the sunlit surface of apple fruit (Fig. 3.2). Similar observations exist for

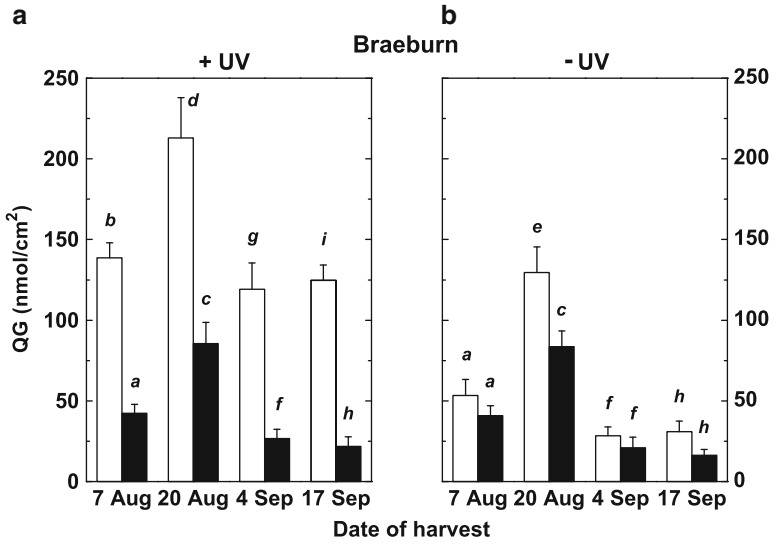

Fig. 3.2 Changes in quercetin glycoside content of sunlit (*open bars*) and shaded (*closed bars*) skin of Braeburn fruits grown under unaltered sunlight (**a**) and with UV radiation filtered out (**b**) Significantly different values are labeled with *different letters*. (Reproduced from Solovchenko et al. (2005) with kind permission)

other species, such as *P. sylvestris* (Turunen et al. 1999) and *Vigna unguiculata* L. (Lingakumar et al. 1999). Interestingly, in fruit grown without UV irradiation, the amount of these compounds remained at the levels characteristic of shaded (adapted to low fluxes of solar radiation) tissues but never dropped to zero (cf. Fig. 3.2a, closed bars, b). In addition, the quercetin glycoside content in fruit tissues developing under a solar spectrum containing no UV radiation, though 2–3 times lower in comparison with fruit grown under a full solar spectrum follow the same trend. One could speculate that the phenolic contents recorded in the absence of UV irradiation represent changes in the constitutive level of these compounds which is regulated in an irradiance-independent manner, probably with participation of blue-light photoreceptors. However, the similarity of the phenolic content trends under a full solar spectrum and in the absence of UV irradiation does not necessarily suggest cross talk between the UV- and blue-light-dependent regulatory mechanisms of phenolic biosynthesis. Instead, this could simply arise from tight correlation between the proportions of radiation in the UV and blue parts of solar spectrum. An additional physiological significance of phenolics contained in tissues adapted to low fluxes of sunlight could be related to protection from diffuse UV radiation, the proportion of which in solar radiation scattered by clouds and the canopy could be even higher than in direct solar beams (Parisi and Downs 2004).

Interestingly, the induction of a UV-protective phenolic screen often occurs to a considerable extent independently from adaptation of the photosynthetic apparatus to PAR irradiance levels. Thus, exclusion of the UV component from the solar

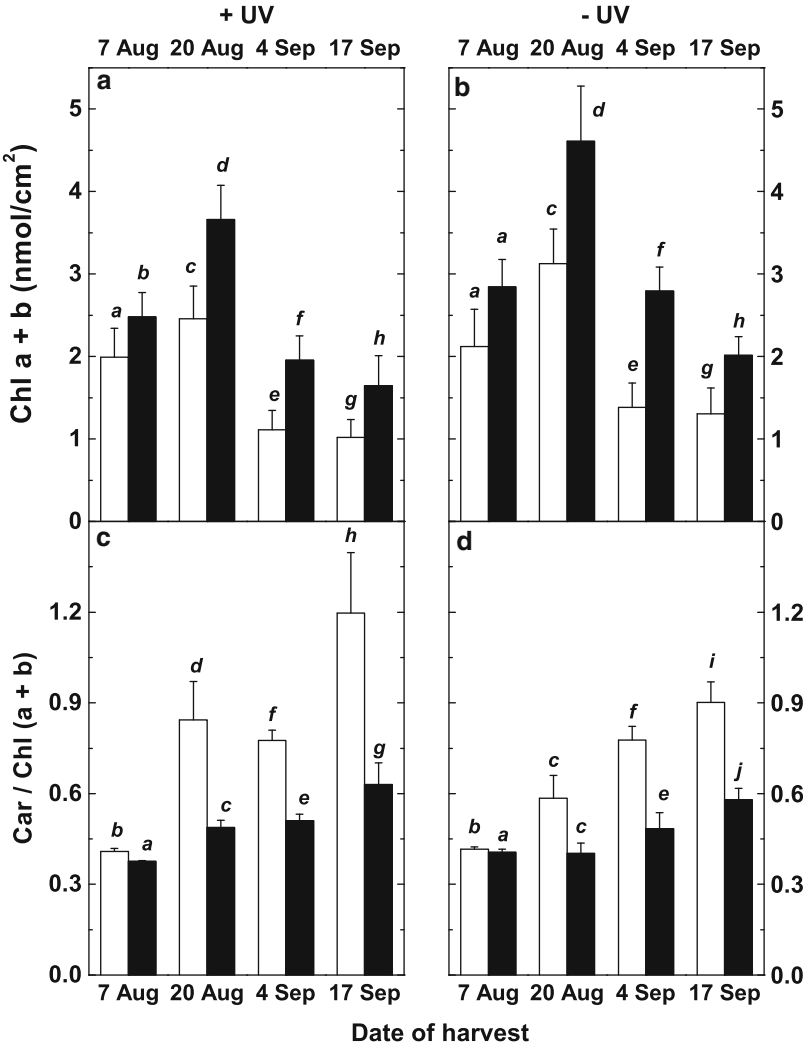

Fig. 3.3 Changes in chlorophyll content (**a**, **b**) and carotenoid-to-chlorophyll ratio (**c**, **d**) of sunlit (*open bars*) and shaded (*closed bars*) skin of ripening Braeburn fruits grown under unaltered sunlight (**a**, **c**) and with UV radiation filtered out (**b**, **d**). Significantly different values are labeled with *different letters*. (Reproduced from Solovchenko et al. (2005) with kind permission)

spectrum does not alter the trends of the changes in chlorophyll and carotenoid content induced by high PAR intensity (cf. open and closed bars in Fig. 3.3). However, this observation remains somewhat controversial. Thus, the exclusion of UV radiation from the incident solar radiation does not always lead to a significant increase in photosynthesis, growth, etc.

Differential induction in phenolic compounds represents a common response to elevated levels of solar radiation. Generally, vacuolar flavonols of epidermal cells

appear to be the most responsive to UV irradiation levels and spectral quality. In contrast, epidermal hydroxycinnamic acid esters and mesophyll-localized flavonoids are less responsive to irradiation; their content appears to be rather genetically programmed (Reuber et al. 1996). The role of phenolic acids such as hydroxycinnamic acid in protection against UV radiation and the relationships of their accumulation with irradiance in different UV ranges was the subject of recent debates (Burchard et al. 2000; Kolb et al. 2001, 2003, 2006; Kolb and Pfundel 2005). Depending on the species under consideration, hydroxycinnamic acid and its derivatives are either largely unaffected by the illumination conditions (Burchard et al. 2000) or their contents increase with irradiance (Kolb et al. 2001). In *Phillyrea latifolia* L., leaves fully exposed to sunlight accumulate flavonols in the vacuoles of epidermal cells, subepidermal layers, and trichomes, whereas less-exposed leaves accumulate hydroxycinnamates in these tissues (Agati et al. 2002). A light-induced decrease in the hydroxycinnamate-to-falvonol ratio was observed by others (Tattini et al. 2000). However, some plant species specifically accumulate hydroxycinnamic acid derivatives under strong sunlight, e.g., chlorogenic acid in *Mahonia repens* (Lindl.) Don (Grace et al. 1998) or echinacoside in *Ligustrum vulgare* L. (Agati et al. 2009).

Analysis of the absorption spectra of such extracts often provides a clue about the principal group of phenolics, the synthesis of which is induced by stress. For example, in leaves (*Vitis*×*vinifera*; Kolb et al. 2001; *Arabidopsis thaliana* Heyn, *Beta vulgaris* L., *Nicotiana tabacum* L., *Pisum sativum* L., *Phaseolus vulgaris* L., *Spinacia oleracea* L.; Cerovic et al. 2002) and fruit (*Malus*×*domestica* Borkh.; Solovchenko and Schmitz-Eiberger 2003) acclimated to strong sunlight, the content of flavonol (mainly quercetin and kaempferol) glycosides often increases several times in comparison with nonacclimated samples (for *M. domestica*, cf. spectra 1–3 in Fig. 3.4a). It should be noted that the content of screening phenolics, such as flavonol glycosides, could be 1–2 orders of magnitude higher than the content of photosynthetic pigments such as chlorophyll (Fig. 3.5).

It is important to note that when these compounds are accumulated in high amounts (up to 400 mmol L^{-1} in the case of flavonol glycosides in vacuoles of apple skin cells) (Lancaster et al. 1994), their tail absorption can contribute significantly to attenuation of light not only in the region(s) of their maxima (UV-A), but also in the short-wavelength range of the visible part of the spectrum. This contribution could be significant for photoprotection (Havaux and Kloppstech 2001), especially if flavonol tautomers are formed, resulting in a bathochromic shift of the absorption maximum (Smith and Markham 1998). Taking into account strong scattering of plant tissues, screening of short-wavelength visible radiation by phenolic compounds in planta could be even more efficient (for more details on phenolic spectroscopy in planta, see Chap. 5 and Markham 1989; Strack and Wray 1989). In vitro absorption by anthocyanins in the visible part of the spectrum could be several times higher than that by chlorophylls and carotenoids (but nevertheless lower than that of flavonol glycosides in the UV part; see Figs. 3.4b, 3.6, 7.3).

A buildup of UV-absorbing phenolics manifests itself, in particular, as a considerable increase in UV absorbance of extracts of the acclimated tissue in polar

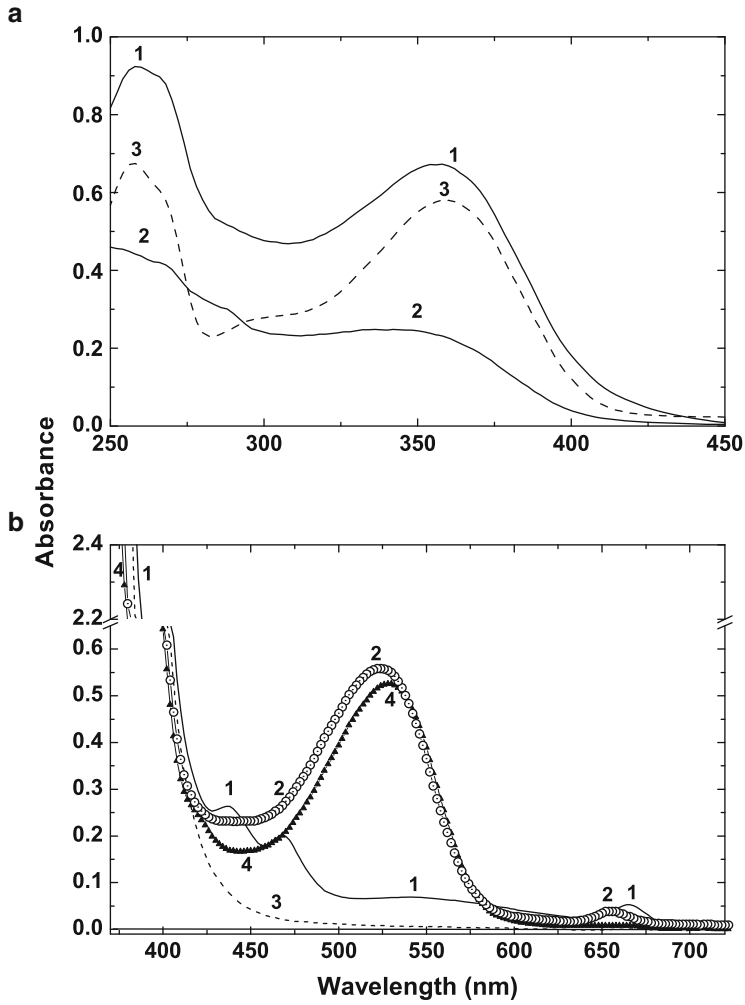

Fig. 3.4 Typical absorption spectra of the water–methanol fraction of Folch extracts from apple peel (diluted eightfold). **a** Spectra of peel extracts taken from (*1*) the sunlit and (*2*) shaded sides of an apple and the spectrum of the methanol solution of pure rutin (*3*). (**b**) Absorption spectra of the methanol extract (*1*, *2*) and the water–methanol fraction of a Folch extract (*3*, *4*). Spectra *2* and *4* were recorded after the addition of HCl. Spectra of undiluted extracts are shown. (Reproduced from Solovchenko et al. (2001) with kind permission from Springer Science+Business Media), Fig. 4

solvents (Fig. 3.4; see also Bidel et al. (2007), Bilger et al. (2007), Kolb et al. (2003), Liakoura et al. (2003)). Stress-induced accumulation of phenolic compounds absorbing in the visible part of the spectrum such as anthocyanins is immediately apparent as a change of plant coloration, from green usually to different shades

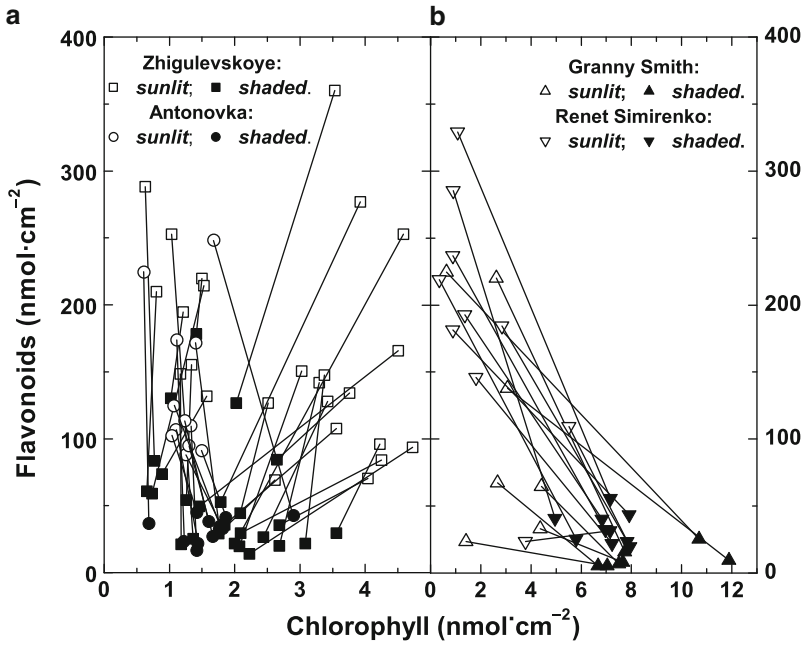

Fig. 3.5 Peel flavonoid glycosides versus chlorophyll content in shaded (*closed symbols*) and sunlit (*open symbols*) sides of apple fruits. The points obtained from the measurements of the same fruit are connected by *lines*. (Reprinted from Merzlyak et al. (2002) with permission from Elsevier)

of red and/or pink (Chalker-Scott 1999; Karageorgou and Manetas 2006; Merzlyak and Chivkunova 2000).

For the discussion of the photoprotective function of screening phenolics it is important to know what range of solar radiation efficiently induces their synthesis. In particular, anthocyanins are readily induced by UV radiation (Saure 1990) though they weakly absorb biologically important UV-B and UV-A radiation and provide a measurable screening in these ranges only when present in high amounts (Solovchenko and Schmitz-Eiberger 2003; Strack and Wray 1989). However, it is often overlooked that anthocyanins are often present in plant tissues together with flavonol glycosides in amounts which are 3–5 times higher than those of the former (cf. Figs. 3.5, 3.6). Therefore accumulation of anthocyanins in these cases could be a secondary consequence of the enhanced synthesis of the UV-absorbing flavonols and phenolic acids through the pathways simultaneously yielding anthocyanin precursors.

Screening phenolics are characterized by remarkable photostability. To the best of our knowledge, there have been no reports about photobleaching of phenolic compounds in vivo by physiologically relevant fluxes of PAR and/or UV radiation. In contrast, anthocyanins and flavonols withstand very high artificial (more than 2,500 μE m^{-2} s^{-1} PAR) irradiances (Merzlyak and Chivkunova 2000; Zeng et al.

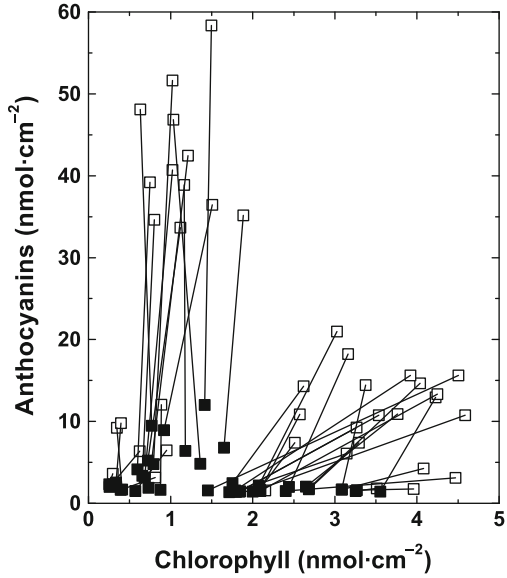

Fig. 3.6 Peel anthocyanin versus chlorophyll content in shaded (*closed symbols*) and sunlit (*open symbols*) sides of Zhigulevskoye apple. The points obtained from the measurements of the same fruit are connected by *lines*. (Reprinted from Merzlyak et al. (2002) with permission from Elsevier

2010). Notably, even in plant tissues suffering from severe photooxidative damage such as apple fruit affected by sunburn disorder, the amount of phenolics remained at the level of the intact fruit (Merzlyak et al. 2002).

One could note, in addition, that higher chlorophyll content is often recorded in the presence of anthocyanins (Fig. 3.6). Presumably, this could be explained by a lower risk of chlorophyll-mediated photooxidative damage when an anthocyanin sunscreen is in place. On the other hand, a higher amount of chlorophylls could be necessary to capture more light to maintain a sufficient level of photosynthesis since anthocyanins are able to intercept a considerable portion of PAR (Merzlyak et al. 2008a, b; see also Chap. 5).

3.2 Accumulation of Screening Pigments as a Result of Carotenogenesis

3.2.1 Carotenogenesis in Microalgae

In natural habitats, photosynthesizing microorganisms are subjected to harsh conditions: abrupt changes in temperature and irradiance as well as to deficiencies in mineral nutrition (Morgan-Kiss et al. 2006; Whitelam and Codd 1986). Coordinated synthesis of nonmembranal lipids such as triacylglycerols and carotenoids is an important mechanism for coping with the unfavorable conditions. This

mechanism was described in a considerable number of microalgal species, including chlorophytes *Dunaliella salina* (*Dunaliella bardawil*) (Ben-Amotz and Avron 1983; Borowitzka et al. 1990; Mendoza et al. 1999), *Haematococcus pluvialis* (Zhekisheva et al. 2002), *Parietochloris incisa* (Solovchenko et al. 2009), and *Scenedesmus komarekii* (Hanagata and Dubinsky 1999). The phenomenon of massive accumulation of carotenoids termed "carotenogenesis" is a well-known response of microalgae to stresses such as high levels of PAR and UV radiation (Mogedas et al. 2009), salinity, and extreme temperatures, especially of Chlorophyta (Borowitzka et al. 1990; Boussiba 2000; Wang et al. 2003). Carotenogenesis is thought to be an adaptive reaction allowing microalgae to cope with harsh environmental conditions. Indeed, one of the most illustrious examples of carotenogenic microalgae is given by so-called snow algae – species such as *Chlorella nivalis*, *H. pluvialis*, and *Chloromonas rubroleosa* that cause the spectacular phenomena known as "blood rain" and "blood snow." These microalgae are capable of growth on snowy mountain slopes under direct sunlight and at temperatures close to 0°C (Czygan 1970). Under unfavorable conditions, especially under high light, the microalgae change their coloration from green to red, brown, and orange owing to accumulation of high amounts of carotenoids. Common examples of carotenoids produced in abundance by carotenogenic algae include β-carotene in species of the genus *Dunaliella* (Ben-Amotz et al. 1982; Jahnke 1999; Pick 1998), astaxanthin in the genus *Haematococcus* (Wang et al. 2003), and certain other carotenoids (Hanagata and Dubinsky 1999).

Generally speaking, the accumulation of carotenoids in carotenogenic algae is induced under conditions when light is absorbed in excess i.e., when a considerable part of the chlorophyll excitation energy cannot be utilized in photochemical reactions (see Chap. 1). Such conditions are imposed by high fluxes of solar radiation and/or by other factors limiting the rate of CO_2 fixation (high salinity, mineral nutrition deficiencies, extremely high or low temperatures, etc.). These conditions often lead to an increase in ROS formation, suggesting their participation in triggering carotenogenesis (Asada 2006; Shaish et al. 1993). This suggestion is supported by the fact that a massive accumulation of carotenoids in certain microalgae can be induced by treatment with dyes – generators of singlet oxygen (methylene blue or Bengal rose) even under dim (approximately 100 $\mu E\ m^{-2}\ s^{-1}$ PAR) illumination. Interestingly, the addition of substances generating oxygen radicals did not induce carotenogenesis. In contrast, treatment with singlet oxygen quenchers such as histidine and eosin inhibited carotenogenesis in *H. pluvialis* even under strong illumination and mineral nutrition deficiency (Shaish et al. 1993).

Considering the above-mentioned circumstances, nonradical ROS, namely, singlet oxygen, seems to participate in the induction of carotenogenesis in microalgae under unfavorable conditions; the role of radical ROS is less evident. The ROS could supposedly play the role of secondary messengers (Bouvier et al. 1998; Shaish et al. 1993), whereas the exact mechanism of their participation in the transduction of the signal inducing carotenogenesis remains to be understood.

A hypothetical mechanism for the ROS-mediated induction of carotenogenesis in microalgae can be considered using *Dunaliella* as an example (Fig. 3.7; see also

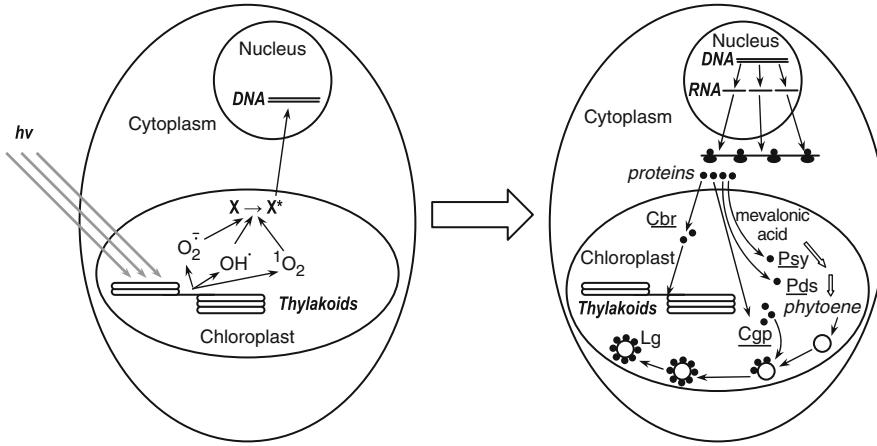

Fig. 3.7 Hypothetical mechanism of the induction of carotenogenesis in *Dunaliella* including two stages: activation (*left*) and massive accumulation of carotenoids (*right*). *X* hypothetical receptor, *Cbr* early light-induced protein homologue protein synthesized during carotenogenesis, *Psy* phytoene synthase, *Pds* phytoene desaturase, *Cgp* protein stabilizing lipid globules, *Lg* carotenoid-containing lipid globules stabilized by Cgp. (Adapted from Pick (1998) with kind permission)

Pick (1998); Shaish et al. (1993)). The massive accumulation of β-carotene in *Dunaliella* during acclimation to strong PAR irradiation involves the coordinated upregulation of, at least, three genes encoding the enzymes of the β-carotene biosynthesis pathway: phytoene synthase (*phy*), phytoene desaturase (*pds*), as well as protein-stabilizing lipid globules (*cgp*, carotene-globule-associated protein) and a gene encoding protein similar to those from early light-induced proteins (ELIP), the proteins synthesized at the early stages of high-light acclimation in higher plants and homologous to the chlorophyll *a* and chlorophyll *b* binding proteins of the photosynthetic apparatus (Lers et al. 1991) family (Levy et al. 1992, 1993) presumably forming complexes with molecules of β-carotene. Globules of similar composition but smaller size and in lower numbers were found in noncarotenogenic *Dunaliella* species. The above-mentioned genes were found in all representatives of the genus *Dunaliella* studied. The induction of β-carotene synthesis is accompanied by upregulation of *cbr*, another gene similar to the genes encoding proteins from the ELIP family. The product of this gene was fond among the major photosystem II light-harvesting complex (LHC) proteins and, according to Levy et al. (1992, 1993), is related to the induction of the violaxanthin cycle, playing an important role in high-light resistance of *Dunaliella*.

On the whole, microalgal mutants overproducing β-carotene display coordinated regulation of different high-light responses, including accumulation of extrathylakoid carotenoids, proteins stabilizing lipid globules which accommodate the bulk of extrathylakoid carotenoids synthesized under stress, violaxanthin deepoxidation and the expression of zeaxanthin-binding proteins of the LHC, a decline in the amount of chlorophyll, and adjustment of the proportion of the LHC and reaction centers in photosystem II (Levy et al. 1992, 1993; Steinbrenner and Linden 2003).

These process lead, in particular, to an increase in nonphotochemical quenching of excited states of chlorophyll, which provides additional photoprotection (Lohr and Wilhelm 1999).

Another possible mechanism of the induction of carotenogenesis is based on redox signaling and employs a hypothetical signal transduction cascade which detects changes in the redox state of the plastoquinone pool in the chloroplast and results in the transcriptional activation of the nuclear-localized carotenoid biosynthesis genes. Although the presence of such a signaling cascade has been generally accepted, the signaling components have not been identified. A similar mechanism is proposed for the induction of astaxanthin synthesis in stressed *H. pluvialis* (Steinbrenner and Linden 2003): upon the transfer of *H. pluvialis* cells from low-light to high-light conditions, the components of the photosynthetic electron transport chain, including the plastoquinone pool, are reduced. Specifically, the plastoquinone pool seems to function as the redox sensor; its reduction subsequently leads to the transcriptional activation of the genes involved in astaxanthin biosynthesis.

Collectively, the experimental evidence obtained to date confirms the existence of cross talk between different pathways of stress sensing and induction of various mechanisms of stress tolerance in microalgae. On the other hand, it strongly suggests the tight integration of radiation-screening-based mechanisms with other photoprotective mechanisms in algae. In addition, we note that the current literature contains scarce information about the participation of photoreceptors in the induction or control of carotenogenesis in microalgae.

Some lines of evidence suggest that accumulation of neutral lipids in cytoplasmic oil bodies facilitates adaptation to the unfavorable conditions by serving as the sink for the excessive photosynthates and as a source of energy (Thompson 1996) and polyunsaturated fatty acid moieties during growth restoration (Khozin-Goldberg et al. 2005). Apart from these functions, oil bodies also serve as the depot for the extraplastidic secondary carotenoids which are supposed to provide photoprotection via screening chloroplasts from the excessive light (Ben-Amotz et al. 1989; Hagen et al. 1994; Solovchenko et al. 2009).

The accumulation of secondary carotenoids manifests itself, in particular, as a conspicuous rise in the total carotenoid-to-chlorophyll ratio and characteristic changes in carotenoid composition. We shall consider these manifestations using *P. incisa* cultivated under high light as an example. The freshwater single-celled alga *P. incisa* comb. nov. (Trebouxiophyceae, Chlorophyta) features an exceptionally high content of valuable eicosatetraenoic (arachidonic) acid (Bigogno et al. 2002). A characteristic response of *P. incisa* to high PAR irradiation is induction of carotenoid synthesis dependent on the availability of nitrogen in the cultivation medium. Under nonstressful conditions [relatively low (35 μE m^{-2} s^{-1} PAR) light and ample nitrogen], cultures of *P. incisa* demonstrate high chlorophyll (up 80 nmol mL^{-1} suspension) content and proportionally high carotenoid content (Fig. 3.8a–c, closed symbols). Depriving the algae of nitrogen brought about cessation of chlorophyll accumulation and a limited increase in the amount of carotenoids, resulting in a small but distinct increase in the carotenoid-to-chlorophyll ratio (Fig. 3.8a–c, open symbols). Growing of the alga at higher irradiances

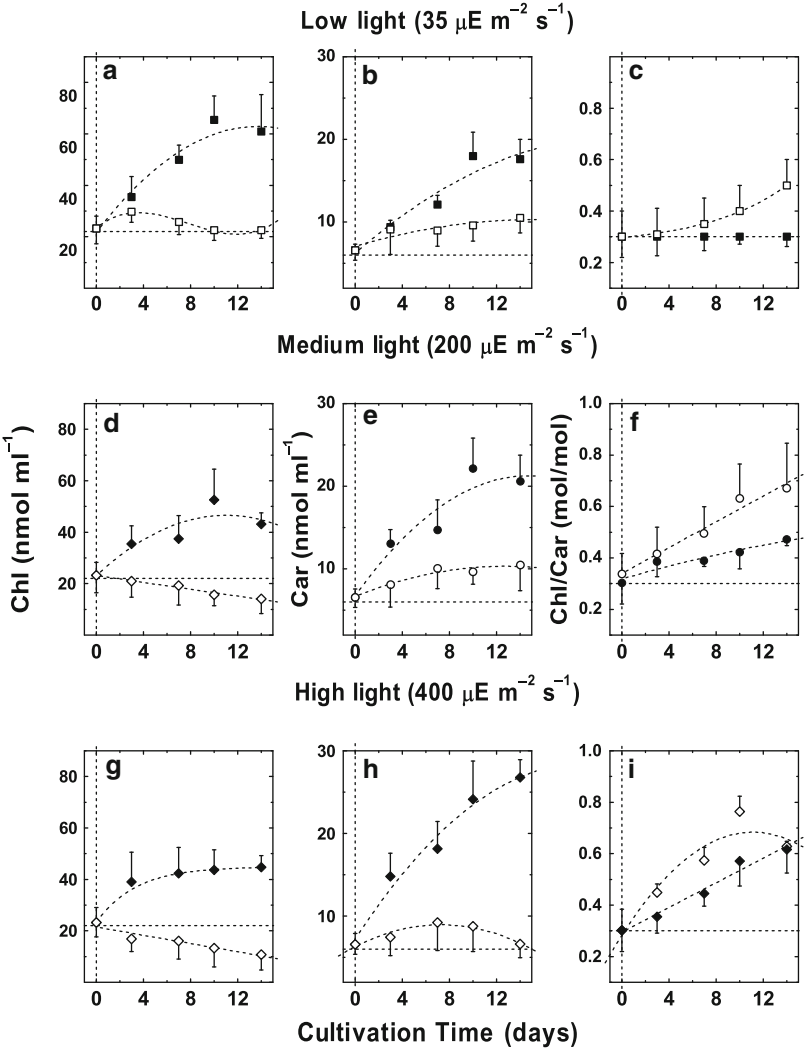

Fig. 3.8 Dynamics of chlorophyll (**a**, **d**, **g**), total carotenoid (**b**, **e**, **h**) contents, and their ratio (**c**, **f**, **i**) in *Parietochloris incisa* cells grown with (*closed symbols*) and without (*open symbols*) nitrogen in a medium under low (**a–c**), medium (**d–f**), and high (**g–i**) illumination intensity. (Reproduced from Solovchenko et al. (2008) with kind permission from Springer Science+Business Media), Fig. 1

(200–400 μE m^{-2} s^{-1} PAR) on complete medium caused a decline in the amount of chlorophyll on the background of an increase in the amount of carotenoids, with the net result of a progressive increase in the carotenoid-to-chlorophyll ratio (Fig. 3.2d–i, open symbols). Collectively, high-light stress induced notable irradiance-dependent accumulation of carotenoids in *P. incisa* grown on complete medium. Nitrogen deprivation at high light decreases the extent of carotenoid

accumulation in *P. incisa*, probably owing to overall limitation of biosynthetic processes in the cell and leads to a decline in the amount of chlorophyll. Regardless of the changes in individual pigment content, the net result under both types of stress was the increase of the carotenoid-to-chlorophyll ratio (Fig. 3.8i).

This type of stress response of the pigment apparatus appears to be common for photoautotrophic microalgae (see the references at the beginning of this section). The common interpretation of this response is that it aims to ameliorate a potentially high photodynamic effect of chlorophyll (which is a potent photosensitizer) by means of attaining a relatively high concentration of carotenoids – powerful antioxidants (Choudhury and Behera 2001). However, it is difficult to be certain about the mechanism of the photoprotective function of carotenoids accumulated under stress without knowing their composition.

According to the results of chromatographic analysis, the pattern of carotenoid changes recorded in *P. incisa* cultivated under stress (Fig. 3.9) could be ascribed to photoacclimation. The data on carotenoid content and composition in cultures grown on complete medium (Figs. 3.8, 3.9) suggest that the β-carotene and lutein accumulated during cultivation under high light are mostly synthesized de novo as suggested by an increase in absolute carotenoid content. This is unlikely in the case of nitrogen-starved cultures, since they display only a slight increase in the amount of carotenoids (Fig. 3.7). The cultures grown under high irradiances displayed a decline of 6–10% in the proportion of lutein, the xanthophyll localized predominantly in the LHC (Horton and Ruban 2005). The decrease in its content (along with the decline in the chlorophyll content) could be due to a decrease in the amount of LHC and absorption of light. However, no change in the ratio of chlorophyll *a* to chlorophyll *b*, which could be expected in this case, was recorded. A considerable increase in the proportion of β-carotene in total carotenoids is another characteristic response of *P. incisa* to the stresses. The incorporation of high amounts of β-carotene into the pigment–protein complexes of the photosynthetic apparatus is unlikely because these structures are highly conserved (Horton and Ruban 2005). Taking this into account, the β-carotene accumulated (relative to chlorophyll) under stress could hardly be localized within thylakoids and is deposited outside these structures. Similar reasoning applies to many cases of stress-induced carotenogenesis in microalgae.

A hint of possible role of extrathylakoid carotenoids in microalgae was given by an interesting observation of the tight interdependence between the syntheses of the pigments and the storage lipids (Fig. 3.10). The accumulation of the latter by algal cells is thought to be determined by a balance between carbon fixation and absorption of nitrogen from the medium (Mayzaud et al. 1989). During the stage of a rapid increase in biomass (typically, the first 3 days of cultivation) under low and moderate irradiances, the fatty acid content could even decrease. Under high light, the cellular carbon/nitrogen balance shifts toward lipid accumulation even when there is ample nitrogen in the medium, apparently because of the high rate of photosynthate formation, which is channeled to the pathways of lipid biosynthesis; under nitrogen starvation, the same events could take place under lower irradiances.

As can be seen from Fig. 3.10, the increase in the carotenoid and lipid contents relative to the chlorophyll content is highly correlated in *P. incisa*. Similar

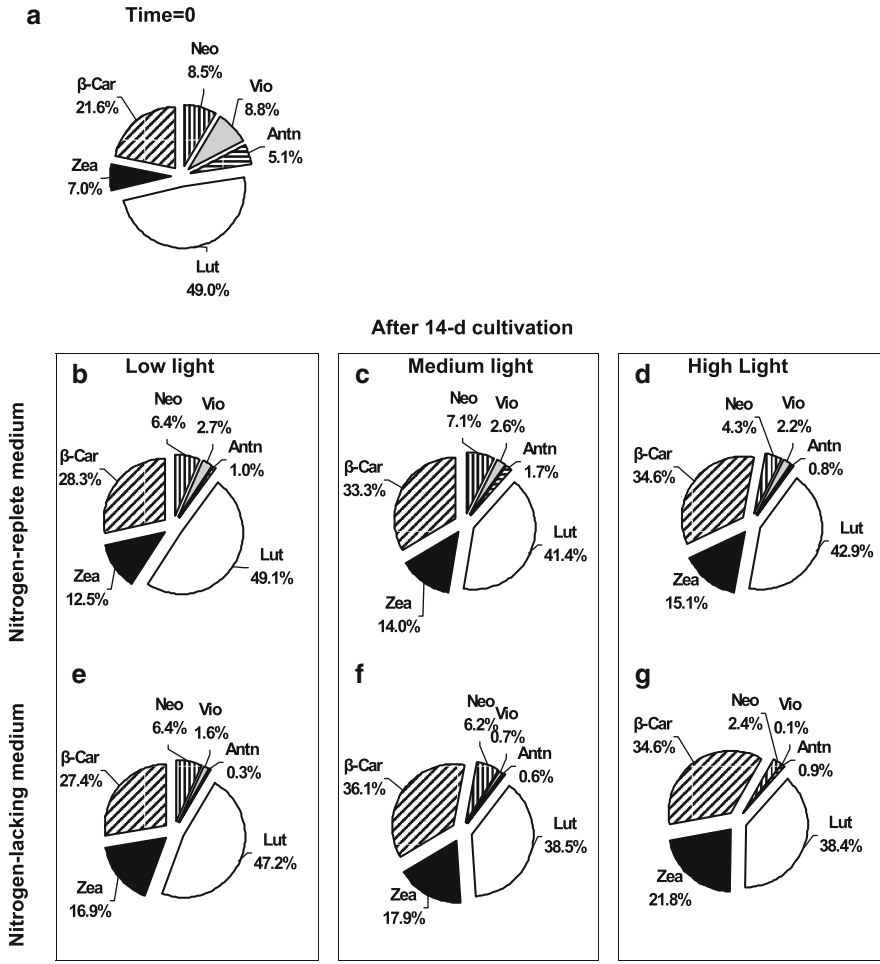

Fig. 3.9 The carotenoid composition of the initial culture of *P. incisa* and that after 14 days of cultivation with (**b**–**d**) and without (**e**–**g**) nitrogen under low (**b**, **e**), medium (**c**, **f**), and high (**d**, **g**) illumination intensity (see Fig. 3.1). *β-Car* β-carotene, *Neo* neoxanthin, *Vio* violaxanthin, *Antn* antheraxanthin, *Lut* lutein, *Zea* zeaxanthin. (Reproduced from Solovchenko et al. (2008) with kind permission from Springer Science+Business Media), Fig. 3

correlations were found in several carotenogenic algae, such as *H. pluvialis* (Zhekisheva et al. 2002), *Nannochloropsis oculata*, a *P. incisa* mutant, and *D. bardawil* (*D. salina*) (Mendoza et al. 1999; Rabbani et al. 1998). The lipids accumulated under stress are often deposited in the cytoplasm in the form of oil bodies, which appeared to be the depot for secondary carotenoids in many of the cases studied. Indeed, the analysis of isolated oil bodies showed that these cytoplasmic inclusions serve as a depot for the bulk of the extrathylakoid β-carotene (for more details on the localization of secondary carotenoids, see Chap. 4); similar

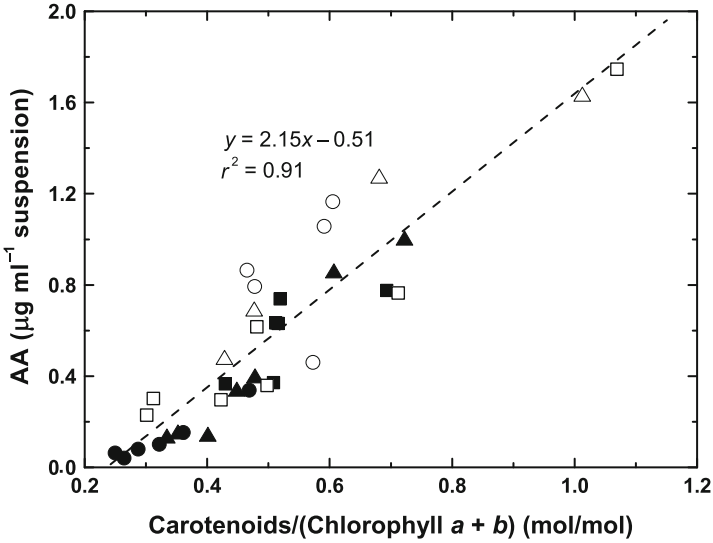

Fig. 3.10 Relationships between arachidonic acid volumetric content and carotenoid-to-chlorophyll ratio in *P. incisa* cells grown on complete (*closed symbols*) and nitrogen-free (*open symbols*) media under an irradiance of 35 μE m^{-2} s^{-1} (*filled squares*, *open square*), 200 μE m^{-2} s^{-1} (*open circles*, *filled circle*), or 400 μE m^{-2} s^{-1} (*filled triangles*, *open triangle*). (Reproduced from Solovchenko et al. (2009) with kind permission from Springer Science+Business Media), Fig. 2

data were obtained for the microalgae listed above. Moreover, in certain cases, a role of the oil body formation was established as a key driver of the synthesis and deposition of the extraplastidic carotenoids (Mendoza et al. 1999; Rabbani et al. 1998; Zhekisheva et al. 2005).

Collectively, the data on changes in pigment and lipid composition presented above and published in the literature strongly suggest that stress-induced carotenogenesis yields mainly extrathylakoid carotenoids, which most probably participate in screening the excessive PAR. At the same time, the upregulation of biosynthesis of storage lipids, apart from providing a sink for the excessive photosynthates, leads to the formation of cytoplasmic inclusions (oil bodies) – the structures which accommodate the bulk of the hydrophobic carotenoids within the hydrophilic environment of cytoplasm (see also Chap. 4). In addition, the carotenoids as powerful antioxidants can protect the polyunsaturated fatty acids within oil bodies against photooxidation. It appears that these processes facilitate the buildup of screening in microalgal cells in a cooperative manner.

3.2.2 Carotenogenesis in Higher Plants

Similarly to microalgae, higher plants often respond to high light and other kind of stress by profound changes in pigment composition, particularly by a decline in

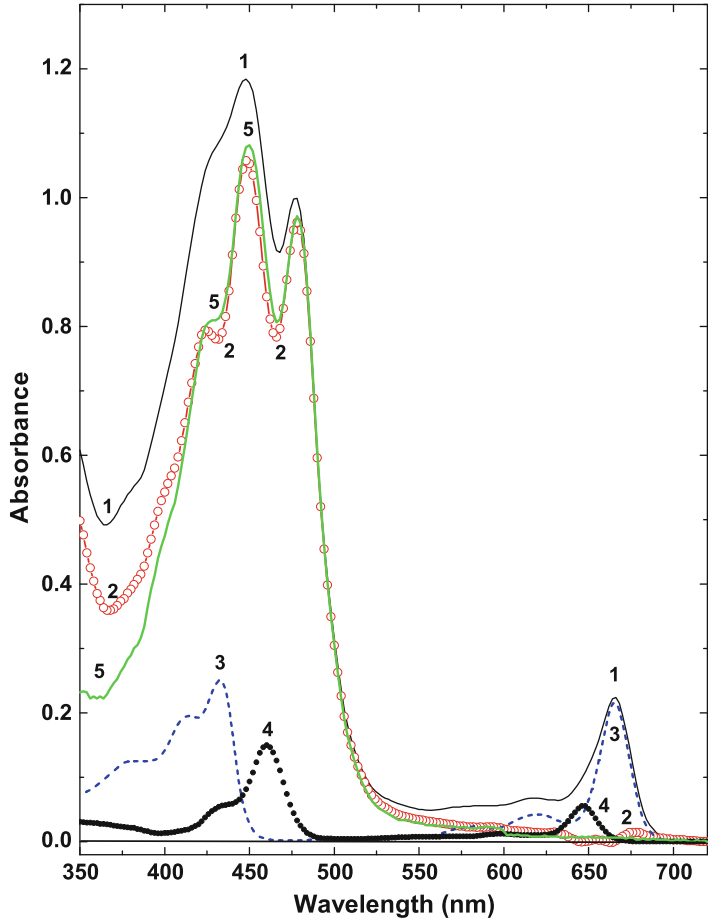

Fig. 3.11 Typical absorption spectrum of the chloroform fraction of a Folch extract from the apple tissue adapted to high fluxes of sunlight (*1*), its "residual" spectrum (*2*) obtained by subtracting the absorption spectrum of chlorophyll *a* (*3*) and that of chlorophyll *b* (*4*) from the original spectrum, and the absorption spectrum of the unsaponified fraction in chloroform (*5*). Note the high contribution of carotenoids (*curve 2* or *curve 5*). (Reproduced from Solovchenko et al. (2001) with kind permission from Springer Science+Business Media), Fig. 3

the chlorophyll content on the background of a retention or an increase in the amount of carotenoids (Merzlyak et al. 1999; Merzlyak and Solovchenko 2002; Fig. 3.11). The massive accumulation of carotenoids in plants both in response to stresses and as a genetically programmed event is suggested to be induced by ROS and/or redox signals originating from the photosynthetic electron transport chain (Bouvier et al. 1994, 1998; Vishnevetsky et al. 1999). Stress-induced imbalance between the amount of light energy absorbed and the plant's ability to utilize it for CO_2 assimilation (see Chap. 1; Asada 2006; Ort 2001) leads to the situation when certain components of the photosynthetic electron transport chain capable of

donating electrons to molecular oxygen are reduced most of the time. As a result, the steady-state concentration of ROS increases and shifts the equilibrium between reduced and oxidized forms of important metabolites in plant cells. This shift then could be sensed, e.g., via photosystem II or an intersystem electron carrier redox state (Ensminger et al. 2006; Huner et al. 1996, 1998), and transformed into a signal upregulating the carotenoid biosynthesis (Bouvier et al. 1998). For example, the redox state of the plastoquinone pool could serve as a trigger for synthesis of screening pigments such as extrathylakoid carotenoids (Bouvier et al. 1998).

Interestingly, the activation of the pathways of carotenoid biosynthesis, together with other processes accompanying photooxidative stress, results in changes in the pigment content and composition resembling the pigment pattern characteristic of accelerated senescence or (in the case of fruit) ripening (the pigment transformation in senescing and stressed assimilatory tissues is discussed in Sect. 3.3). This pattern includes, in particular, a pronounced increase of the carotenoid-to-chlorophyll ratio, mainly due to accumulation of extrathylakoid carotenoids (Breithaupt and Bamedi 2001; Gross 1987; Merzlyak and Solovchenko 2002; Solovchenko et al. 2006). In the leaves and fruit of many plant species, such as *M. domestica* (Merzlyak and Solovchenko 2002), *Capsicum annuum* (Hornero-Mendez and Minguez-Mosquera 2000), and *Aloe arborescens* (Diaz et al. 1990; Merzlyak et al. 2005a), the onset of these changes manifests itself in the chloroplast-to-chromoplast transition (for more details on plastid transformation and the role of this process in photoprotection via optical screening of radiation, see Chaps. 4, 5).

Chromoplasts possess the ability to synthesize a broad spectrum of different compounds, including carotenoids. Chromoplasts accumulating gross amounts of carotenoids are referred to as "carotenoidoplasts." The mechanisms of regulation of carotenoid synthesis in chromoplasts were shown to be different from those in chloroplasts. The key role in upregulation of carotenoid synthesis in chloroplasts is played by hormones, predominantly ethylene (Breithaupt and Bamedi 2001; Thelander et al. 1986). The differentiation of carotenoidoplasts is usually accompanied by an increase in the transcription of the genes encoding the key enzymes of carotenoid biosynthesis: phytoene synthase, phytoene desaturase, and lycopene β-cyclase; there have also been reports on posttranscriptional regulation of the induction of carotenogenesis (Cunningham and Gantt 1998; Hirschberg 2001).

In certain cases, a relatively stable total carotenoid content or one that increases as a result of carotenogenesis is accompanied by a profound alteration of the composition. Thus, new molecular species of carotenoids specific to stressed/senescing plant tissues are formed. Generally, the amount of "screening" carotenoids increases, often at the expense of "photosynthetic" carotenoids (the LHC-bound carotenoids that effectively transfer the absorbed light energy to chlorophyll). The latter are liberated from thylakoids and, in many cases, undergo chemical modification such as oxidation or esterification by fatty acids (which are also liberated from decomposing chloroplast membranes) (Gross 1987) (Fig. 3.12). For example, in assimilatory tissues of apple fruit adapted to strong sunlight, the proportion of violaxanthin can increase more than 50 times over, whereas the total carotenoid content remains relatively stable (Knee 1988; Solovchenko et al. 2006). Notably,

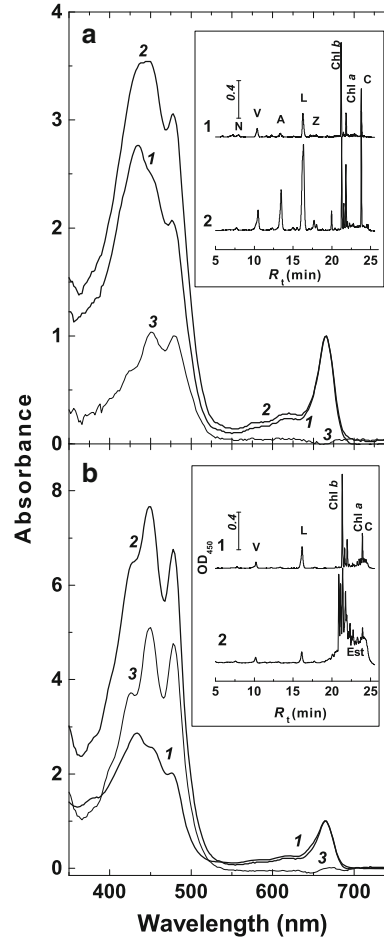

Fig. 3.12 Absorption spectra normalized to the red chlorophyll absorption maximum и and HPLC profiles (*insets*) of the extract from skin of shaded (*1*) and sunlit (*2*) apple fruit surfaces as well as their difference spectra (*3*) at the beginning of fruit development (**a**) and after 3 months of growth at the periphery of the canopy (**b**). Note the increase in xanthophyll ester content on the sunlit surface after the period of acclimation to strong sunlight. *N* neoxanthin, *V* violaxanthin, *A* antheraxanthin, *L* lutein, *Z* zeaxanthin, *C* β-carotene, *Est* fatty acid xanthophyll esters. (Solovchenko, unpublished)

the violaxanthin accumulated in this case does not get involved in the operation of the violaxanthin cycle but is deposited in the form of fatty acid esters, presumably within plastoglobuli (Merzlyak and Solovchenko 2002).

The gradual esterification of xanthophylls (Figs. 3.12, 3.13) accumulated as a result of stress and/or senescence-induced carotenogenesis is believed to have a distinct physiological significance: it facilitates the deposition of polar xanthophylls within the hydrophobic environment of plastoglobuli. Nonpolar carotenes could simply be dissolved in neutral lipids, which are the main constituents of plastoglobuli (Lichtenthaler 1969a, b; Steinmüller and Tevini 1985; Tevini and Steinmüller 1985).

A remarkable and interesting peculiarity of certain plants (mainly evergreen winter-hardening conifers from the genera *Cryptomeria*, *Metasequoia*, *Taxodium*, *Chamaecyparis*, *Buxus*, and *Thuja*) and flowering plants (genus *Aloe*) which are unable to synthesize anthocyanins is the stress-induced transient reddening of

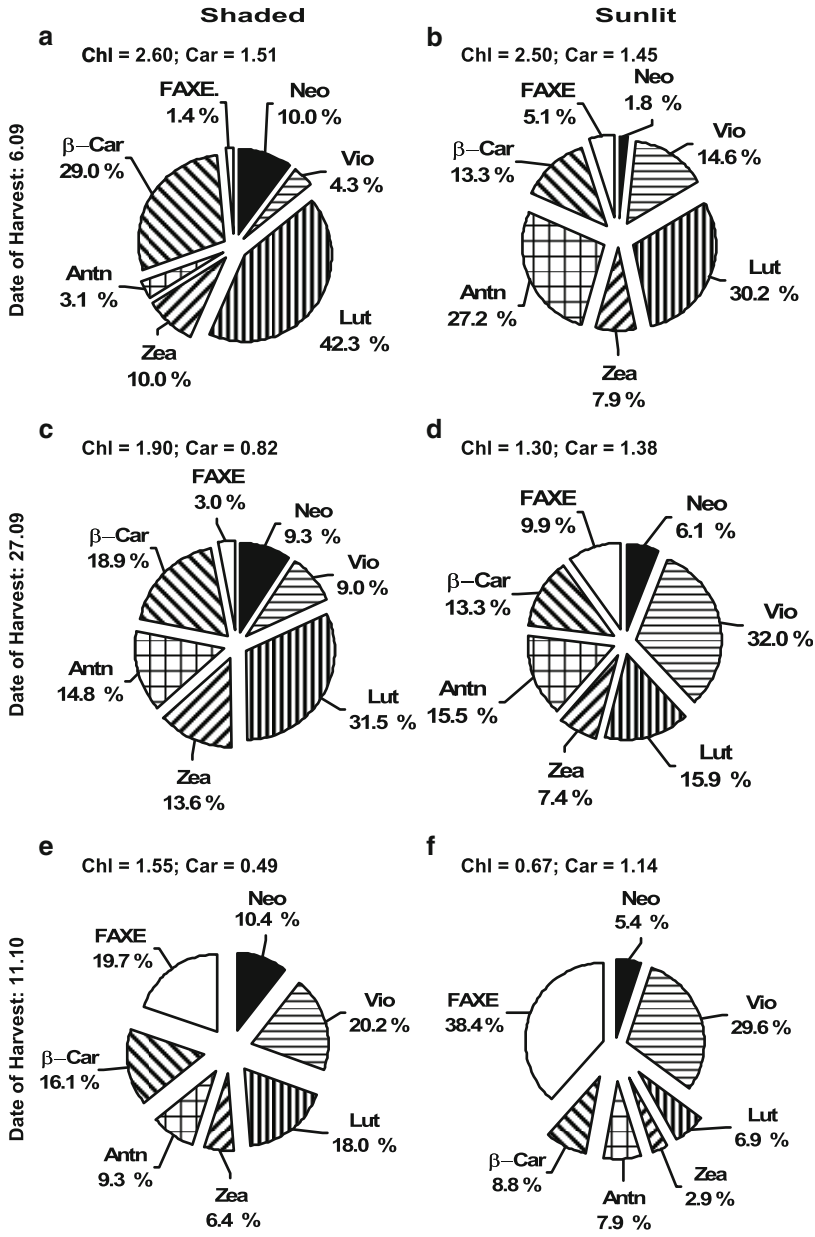

Fig. 3.13 Enhanced esterification of xanthophylls in sunlit (**a**, **c**, **e**) and shaded (**b**, **d**, **f**) skin of apple fruit. Chlorophyll and carotenoid contents (nmol cm^{-2}) and dates of harvest are shown. *β-Car* β-carotene, *FAXE* fatty acid esters of xanthophylls, *Neo* neoxanthin, *Vio* violaxanthin, *Lut* lutein, *Zea* zeaxanthin, *Antn* antheraxanthin. (Reprinted from Solovchenko et al. (2006) with permission from Elsevier)

leaves or needles owing to accumulation of high amounts of ketocarotenoids such as rhodoxanthin (Czeczuga 1987; Diaz et al. 1990; Han et al. 2003, 2004; Ida et al. 1991; Merzlyak et al. 2005a; Weger et al. 1993) and escholtzxanthin (Hormaetxe et al. 2005, 2007).

Aloe arborescens Mill. is a representative example of a species possessing a photoprotective mechanism based on radiation screening by rhodoxanthin. To the best of our knowledge, no evidence has been obtained on the involvement of rhodoxanthin in photoprotection within thylakoid membranes. It was reported that the light-harvesting chlorophyll–protein complex of *Cryptomeria japonica* does not retain rhodoxanthin (Han et al. 2003). Taking into account the changes of ultrastructure observed (Merzlyak et al. 2005a), it is likely that the main depot of rhodoxanthin in *Aloe* plastids is situated outside the thylakoid membranes in plastoglobuli as it occurs in the course of leaf senescence.

Under stressful conditions (strong sunlight and water shortage), *A. arborescens* plants change leaf color from green to reddish; upon restoration of normal conditions (after watering and/or covering the plants with a net) the leaf coloration is reversed to green. The chemical analysis of a red leaf extract did not reveal the presence of anthocyanin. In contrast, the presence of rhodoxanthin in amounts comparable to those of other carotenoids was revealed (Fig. 3.14).

As stated in Chap. 1, high photostability in planta is a prerequisite for a pigment to fulfill a radiation-screening function. However, carotenoids are highly susceptible to oxidation by molecular oxygen and its radicals and are readily destroyed by PAR irradiation in chlorophyll-containing aerated solutions. Irradiation of green fruit and plants with high PAR fluxes brought about a complete synchronous photobleaching of carotenoids and chlorophylls, obviously photosensitized by the latter (Merzlyak et al. 1998).

However, the extent of carotenoid photodegradation depends in plant samples on the initial chlorophyll content and carotenoid composition (Fig. 3.15). The results of experiments carried out in our laboratory (Merzlyak and Solovchenko 2002) suggest the existence of, at least, two pools of carotenoids which differ in their resistance to photodestruction. A plausible explanation is that the first pool, which disappears at a faster rate and completely after prolonged irradiation, represents the "photosynthetic" carotenoids bound to pigment–protein complexes within chloroplast thylakoids. The high efficiency of carotenoid degradation in this case can be explained by involvement of ROS generated in photosynthetic electron transport chains and/or by the photodynamic activity of chlorophyll (Asada 2006). The second carotenoid pool, which increases during acclimation to strong sunlight and exhibits higher photostability, is most likely localized in plastoglobuli of chloroplasts undergoing transformation to chromoplast/carotenoidoplasts (see also Chap. 4).

Although, to the best of our knowledge, no information is available on the chemical composition of apple fruit plastoglobuli, those from leaves contain only traces of chlorophyll and almost all leaf carotenoids together with other neutral lipids are their primary constituents (Steinmüller and Tevini 1985). The relative photostability of carotenoids in the absence of chlorophyll can be explained by

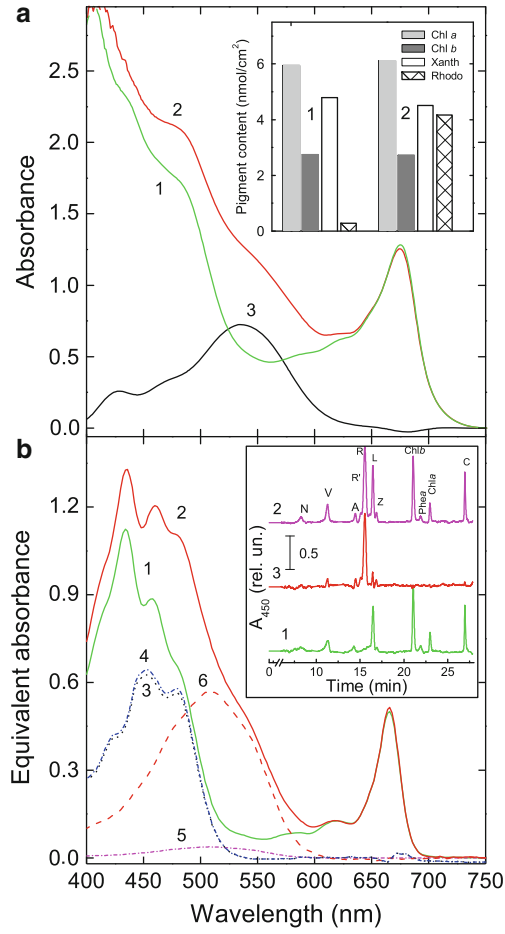

Fig. 3.14 Absorption spectra and pigment analysis of green and red *Aloe* adaxial mesophyll tissues (AMT). **a**. Absorption spectra of green (*1*) and red (*2*) tissues and their difference (*3*). *Inset*: Leaf chlorophyll, non-ketocarotenoid (NKC), and rodoxanthin content. **b**. Absorption spectra of pigments from green (*1*, *3*, *5*) and red (*2*, *4*, *6*) AMT (see **a**). Spectra *1* and *2* are the spectra of total chloroform extracts, whereas spectra *3–6* are results of spectral reconstruction analysis for NKC (*3*, *4*) and rhodoxanthin (*5*, *6*). *Inset*: High-performance liquid chromatography of *Aloe* pigments from green (*1*) and red (*2*) AMT. The chromatograms are normalized to the magnitude of chlorophyll *b* peak. *3* difference between chromatograms *2* and *1*. *N* neoxanthin, *V* violaxanthin, *A* antheraxanthin, *R′* rhodoxanthin derivative, *R* rhodoxanthin, *L* lutein, *Z* zeaxanthin, *Chla′* derivative of chlorophyll *a*, *C* β-carotene. (Reproduced from Merzlyak et al. (2005a) with permission from the Royal Society of Chemistry for the European Society for Photobiology, the European Photochemistry Association, and the Royal Society of Chemistry)

photophysical properties of their excited states: in higher-plant carotenoid molecules, the transition from ground to low-lying S_1 singlet states is forbidden and in the absence of a suitable energy donor, the probability of the formation of

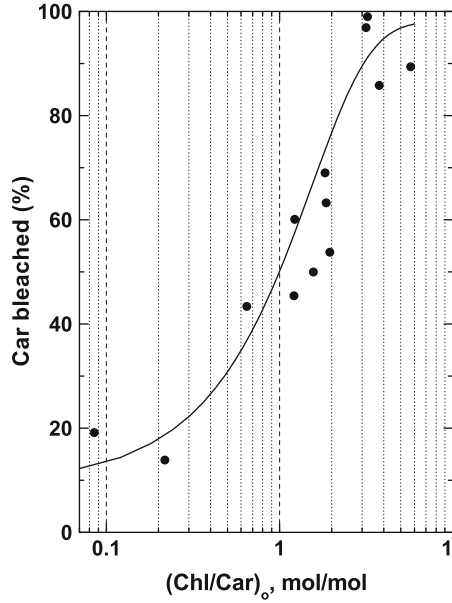

Fig. 3.15 The extent of carotenoid bleaching after prolonged irradiation versus chlorophyll-to-carotenoid molar ratios in intact apple fruits. Apples were irradiated for 100–170 min (Fig. 3.4b), which resulted in *green* and *greenish-yellow* fruit and a drop in peel chlorophyll content to about 0.1–0.2 nmol cm^{-2}. (Reprinted from Merzlyak and Solovchenko (2002) with permission from Elsevier)

carotenoid triplet states is very low (Mathis and Kleo 1973). In addition, the photostability of carotenoids in vivo could also be related to the presence in plastoglobuli of α-tocopherol, which possesses strong antiradical activity.

Accordingly, the physiological significance of carotenoid buildup in senescing leaves and ripening fruit occurring in the lipid environment of plastoglobuli could be ascribed both to the dominant contribution of carotenoids to light absorption (Bigogno et al. 2002; Bornman 1999; Breithaupt and Bamedi 2001; Cheynier 2006) and stability to photodestruction at the terminal stages of chlorophyll breakdown. Carotenoids together with α-tocopherol, which are present in plastoglobuli at very high local concentrations (Tevini and Steinmüller 1985) and possess antioxidant properties, could be involved in the protection of triglycerols, unsaturated lipids, and prenyl quinons (Lichtenthaler 1969a, b) stored in these structures from (photo)oxidation.

It should be noted that the processes described above often take place during senescence of plants when photosynthetic apparatus undergoing genetically controlled dismantling is especially vulnerable to photooxidative damage (Munné-Bosch and Alegre 2002; Munné-Bosch and Lalueza 2007). These circumstances allow one to speculate that the fluxes of solar radiation which are considered to be normal for a mature photosynthetic apparatus would probably be stressful for senescing leaves. This makes optical screening-based mechanisms and carotenogenesis in senescing plant tissues especially important for their protection against photooxidative damage. One could think that at lower fluxes of solar radiation the rate and pattern of senescence-induced pigment transformation in plants is controlled mainly by the balance of hormones promoting (ethylene) and retarding

(auxins) senescence. High fluxes of solar radiation trigger the events which locally accelerate the transformation of pigments in the affected tissues with likely involvement of ROS.

3.3 Concluding Remarks

The synthesis of screening pigments in plants and microalgae is induced by high fluxes of solar light and/or other environmental stresses with participation of a complex network of signal reception and transduction pathways. This network includes different photoreceptors and/or sensors of the redox state of the cell, which could be shifted owing to (1) overreduction of certain components of the photosynthetic electron transport chain and (2) increased ROS formation under stress. The massive accumulation of phenolic screening compounds appears to be a high-irradiance dose-dependent response which could be modulated by photoreceptors in an irradiance-independent manner.

The buildup of extrathylakoid sunscreen during acclimation to high fluxes of solar radiation often involves the accumulation of two groups of pigments: MAA (in microalgae) or phenolic compounds (in higher plants) and carotenoids (in all taxa). Its general, the pattern comprises a decrease of the proportion of thylakoid-bound "photosynthetic" carotenoids (the carotenoids which participate in light harvesting and transfer of excitation energy to chlorophyll) with a simultaneous increase in the amount of extrathylakoid (screening) carotenoids, often in the form of fatty acid esters.

It should be noted, in conclusion, that screening pigments, both of phenolic and of carotenoid nature, display remarkable photostability in planta under physiologically relevant fluxes of solar radiation, which is important for their function in plants.

References

Agati G, Galardi C, Gravano E, Romani A, Tattini M (2002) Flavonoid distribution in tissues of *Phillyrea latifolia* L. leaves as estimated by microspectrofluorometry and multispectral fluorescence microimaging. Photochem Photobiol 76:350–360

Agati G, Stefano G, Biricolti S, Tattini M (2009) Mesophyll distribution of 'antioxidant' flavonoid glycosides in *Ligustrum vulgare* leaves under contrasting sunlight irradiance. Ann Bot 104:853–861

Asada K (2006) Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiol 141:391–396

Beggs C, Wellmann E (1994) Photocontrol of flavonoid biosynthesis. In: Kendrick R, Kronenberg G (eds) Photomorphogenesis in plants, vol 2. Kluwer Academic Publishers, Dordrecht, pp 733–750

Beggs C, Schneider-Ziebert U, Wellmann E (1986) UV-B radiation and adaptive mechanisms in plants. In: Worrest R, Caldwell M (eds) Stratospheric ozone reduction. Solar ultraviolet radiation and plant life. Springer, Berlin, pp 235–250

Ben-Amotz A, Avron M (1983) On the factors which determine massive β-carotene accumulation in the halotolerant alga *Dunaliella bardawil*. Plant Physiol 72:593–597

Ben-Amotz A, Katz A, Avron M (1982) Accumulation of β-carotene in halotolerant alge: purification and characterization of β-carotene-rich globules from *Dunaliella bardawil* (Chlorophyceae). J Phycol 18:529–537

Ben-Amotz A, Shaish A, Avron M (1989) Mode of action of the massively accumulated β-carotene of *Dunaliella bardawil* in protecting the alga against damage by excess irradiation. Plant Physiol 86:1286–1291

Bidel L, Meyer S, Goulas Y, Cadot Y, Cerovic Z (2007) Responses of epidermal phenolic compounds to light acclimation: In vivo qualitative and quantitative assessment using chlorophyll fluorescence excitation spectra in leaves of three woody species. J Photochem Photobiol B Biol 88:163–179

Bigogno C, Khozin-Goldberg I, Boussiba S, Vonshak A, Cohen Z (2002) Lipid and fatty acid composition of the green oleaginous alga *Parietochloris incisa*, the richest plant source of arachidonic acid. Phytochemistry 60:497–503

Bilger W, Rolland M, Nybakken L (2007) UV screening in higher plants induced by low temperature in the absence of UV-B radiation. Photochem Photobiol Sci 6:190–195

Blanke M, Lenz F (1989) Fruit photosynthesis. Plant Cell Environ 12:31–46

Bornman J (1999) Localisation and functional significance of flavonoids and related compounds. In: Rozema J (ed) Stratospheric ozone depletion: the effects of enhanced UV-B radiation on terrestrial ecosystems. Backhuys, Leiden, pp 59–69

Bornman J, Reuber S, Cen Y, Weissenböck G (1997) Ultraviolet radiation as a stress factor and the role of protective pigments. In: Plants and UV-B: responses to environmental change. Cambridge University Press, Cambridge, pp 157–168

Borowitzka MA, Borowitzka LJ, Kessly D (1990) Effects of salinity increase on carotenoid accumulation in the green alga *Dunaliella salina*. J Appl Phycol 2:111–119

Boussiba S (2000) Carotenogenesis in the green alga *Haematococcus pluvialis*: cellular physiology and stress response. Physiol Planta 108:111–117

Bouvier F, Hugueney P, d'Harlingue A, Kuntz M, Camara B (1994) Xanthophyll biosynthesis in chromoplasts: isolation and molecular cloning of an enzyme catalyzing the conversion of 5, 6-epoxycarotenoid into ketocarotenoid. Plant J 6:45–54

Bouvier F, Backhaus R, Camara B (1998) Induction and control of chromoplast-specific carotenoid genes by oxidative stress. J Biol Chem 273:30651–30659

Breithaupt D, Bamedi A (2001) Carotenoid esters in vegetables and fruits: a screening with emphasis on β-cryptoxanthin esters. J Agric Food Chem 49:2064–2070

Burchard P, Bilger W, Weissenbock G (2000) Contribution of hydroxycinnamates and flavonoids to epidermal shielding of UV-A and UV-B radiation in developing rye primary leaves as assessed by ultraviolet-induced chlorophyll fluorescence measurements. Plant Cell Environ 23:1373–1380

Caldwell M, Bornman J, Ballare C, Flint S, Kulandaivelu G (2007) Terrestrial ecosystems, increased solar ultraviolet radiation, and interactions with other climate change factors. Photochem Photobiol Sci 6:252–266

Cecchi F, De Martino G, Bellincontro A, Botondi R, Mencarelli F (2005) Influence of sunlight exposure and postharvest ethylene control on carotenoids content of peach fruit. Acta Hortic 682:329–336

Cerovic Z et al (2002) The use of chlorophyll fluorescence excitation spectra for the non-destructive in situ assessment of UV-absorbing compounds in leaves. Plant Cell Environ 25:1663–1676

Chalker-Scott L (1999) Environmental significance of anthocyanins in plant stress responses. Photochem Photobiol 70:1–9

Cheynier V (2006) Flavonoids in wine. In: Andersen Ø, Markham K (eds) Flavonoids: chemistry, biochemistry, and applications. CRC Taylor & Francis, Boca Raton, pp 263–318

Choudhury N, Behera R (2001) Photoinhibition of photosynthesis: Role of carotenoids in photoprotection of chloroplast constituents. Photosynthetica 39:481–488
Close D, McArthur C (2002) Rethinking the role of many plant phenolics-protection from photodamage not herbivores? Oikos 99:166
Cockell C, Knowland J (1999) Ultraviolet radiation screening compounds. Biol Rev 74:311–345
Cunningham F Jr, Gantt E (1998) Genes and enzymes of carotenoid biosynthesis in plants. Annu Rev Plant Biol 49:557–583
Czeczuga B (1987) Different rhodoxanthin contents in the leaves of gymnosperms grown under various light intensities. Biochem Syst Ecol 15:531–533
Czygan F (1970) Blood-rain and blood-snow: nitrogen-deficient cells of *Haematococcus pluvialis* and *Chlamydomonas nivalis*. Arch Mikrobiol 74:69
DeLucia E, Day T, Vogelman T (1992) Ultraviolet-B and visible light penetration into needles of two species of subalpine conifers during foliar development. Plant Cell Environ 15:921–929
Diaz M, Ball E, Luttge U (1990) Stress-induced accumulation of the xanthophyll rhodoxanthin in leaves of *Aloe vera*. Plant Physiol Biochem 28:679–682
Dixon R, Paiva N (1995) Stress-induced phenylpropanoid metabolism. Plant Cell 7:1085–1097
Ensminger P, Schäfer E (1992) Blue and ultraviolet-B light photoreceptors in parsley cells. Photochem Photobiol 55:437–447
Ensminger I, Busch F, Huner N (2006) Photostasis and cold acclimation: sensing low temperature through photosynthesis. Physiol Planta 126:28–44
Foyer C, Noctor G (2005) Oxidant and antioxidant signalling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. Plant Cell Environ 28:1056–1071
Merzlyak M, Solovchenko A, Pogosyan S (2005) Optical properties of rhodoxanthin accumulated in Aloe arborescens Mill. leaves under high-light stress with special reference to its photoprotective function. Photochemical and Photobiological Sciences, 4, 333–40.
Grace S, Logan B, Adams W (1998) Seasonal differences in foliar content of chlorogenic acid, a phenylpropanoid antioxidant, in *Mahonia repens*. Plant Cell Environ 21:513–521
Gross J (1987) Carotenoids: pigments in fruits. Food science and technology. Series of monographs. Academic, London, pp 87–98
Hagen C, Braune W, Björn L (1994) Functional aspects of secondary carotenoids in *Haematococcus lacustris* (Volvocales) III. Action as a sunshade. J Phycol 30:241–248
Hahlbrock K, Scheel D (1989) Physiology and molecular biology of phenylpropanoid metabolism. Annu Rev Plant Biol 40:347–369
Han Q, Shinohara K, Kakubari Y, Mukai Y (2003) Photoprotective role of rhodoxanthin during cold acclimation in *Cryptomeria japonica*. Plant Cell Environ 26:715–723
Han Q, Katahata S, Kakubari Y, Mukai Y (2004) Seasonal changes in the xanthophyll cycle and antioxidants in sun-exposed and shaded parts of the crown of *Cryptomeria japonica* in relation to rhodoxanthin accumulation during cold acclimation. Tree Physiol 24:609
Hanagata N, Dubinsky Z (1999) Secondary carotenoid accumulation in *Scenedesmus komarekii* (Chlorophyceae, Chlorophyta). J Phycol 35:960–966
Harborne J (2001) Twenty-five years of chemical ecology. Nat Prod Rep 18:361–379
Havaux M, Kloppstech K (2001) The protective functions of carotenoid and flavonoid pigments against excess visible radiation at chilling temperature investigated in *Arabidopsis npq* and *tt* mutants. Planta 213:953–966
Hirschberg J (2001) Carotenoid biosynthesis in flowering plants. Curr Opin Plant Biol 4:210–218
Hoch W, Zeldin E, McCown B (2001a) Physiological significance of anthocyanins during autumnal leaf senescence. Tree Physiol 21:1
Hoch W, Zeldin E, McCown B (2001b) Physiological significance of anthocyanins during autumnal leaf senescence. Tree Physiol 21:1
Hoch W, Singsaas E, McCown B (2003) Resorption protection. Anthocyanins facilitate nutrient recovery in autumn by shielding leaves from potentially damaging light levels. Plant Physiol 133:1296–1305

Hormaetxe K, Becerril J, Fleck I, Pintó M, Garcia-Plazaola J (2005) Functional role of red (retro)-carotenoids as passive light filters in the leaves of *Buxus sempervirens* L.: increased protection of photosynthetic tissues? J Exp Bot 56:2629–2636

Hormaetxe K, Becerril J, Hernández A, Esteban R, Garcia-Plazaola J (2007) Plasticity of photoprotective mechanisms of *Buxus sempervirens* L. leaves in response to extreme temperatures. Plant Biol 9:59–68

Hornero-Mendez D, Minguez-Mosquera M (2000) Xanthophyll esterification accompanying carotenoid overaccumulation in chromoplast of *Capsicum annuum* ripening fruits is a constitutive process and useful for ripeness index. J Agric Food Chem 48:1617–1622

Horton P, Ruban A (2005) Molecular design of the photosystem II light-harvesting antenna: photosynthesis and photoprotection. J Exp Bot 56:365–373

Hughes N, Morley C, Smith W (2007) Coordination of anthocyanin decline and photosynthetic maturation in juvenile leaves of three deciduous tree species. New Phytol 175:675–685

Huner N et al (1996) Sensing environmental temperature change through imbalances between energy supply and energy consumption: redox state of photosystem II. Physiol Planta 98:358–364

Huner N, Öquist G, Sarhan F (1998) Energy balance and acclimation to light and cold. Trends Plant Sci 3:224–230

Ibdah M, Krins A, Seidlitz H, Heller W, Strack D, Vogt T (2002) Spectral dependence of flavonol and betacyanin accumulation in *Mesembryanthemum crystallinum* under enhanced ultraviolet radiation. Plant Cell Environ 25:1145–1154

Ida K, Saito F, Takeda S (1991) Isomers of rhodoxanthin in reddish brown leaves of gymnosperms and effect of daylight intensity on the contents of pigments during autumnal coloration. J Plant Res 104:157–169

Jahnke L (1999) Massive carotenoid accumulation in *Dunaliella bardawil* induced by ultraviolet-A radiation. J Photochem Photobiol B Biol 48:68–74

Jansen M, Gaba V, Greenberg B (1998) Higher plants and UV-B radiation: balancing damage, repair and acclimation. Trends Plant Sci 3:131–135

Jenkins GI (2009) Signal transduction in responses to UV-B radiation. Annu Rev Plant Biol 60:407–431

Karageorgou P, Manetas Y (2006) The importance of being red when young: anthocyanins and the protection of young leaves of *Quercus coccifera* from insect herbivory and excess light. Tree Physiol 26:613

Khozin-Goldberg I, Shrestha P, Cohen Z (2005) Mobilization of arachidonyl moieties from triacylglycerols into chloroplastic lipids following recovery from nitrogen starvation of the microalga *Parietochloris incisa*. Biochim Biophys Acta 1738:63–71

Klisch M, Häder D (2002) Wavelength dependence of mycosporine-like amino acid synthesis in *Gyrodinium dorsum*. J Photochem Photobiol B Biol 66:60–66

Knee M (1988) Carotenol esters in developing apple fruits. Phytochemistry 27:1005–1009

Kolb C, Pfundel E (2005) Origins of non-linear and dissimilar relationships between epidermal UV absorbance and UV absorbance of extracted phenolics in leaves of grapevine and barley. Plant Cell Environ 28:580–590

Kolb C, Kaser M, Kopecky J, Zotz G, Riederer M, Pfündel E (2001) Effects of natural intensities of visible and ultraviolet radiation on epidermal ultraviolet screening and photosynthesis in grape leaves. Plant Physiol 127:863–875

Kolb C, Kopecky J, Riederer M, Pfundel E (2003) UV screening by phenolics in berries of grapevine (*Vitis vinifera*). Funct Plant Biol 30:1177–1186

Kolb C, Wirth E, Kaiser W, Meister A, Riederer M, Pfundel E (2006) Noninvasive evaluation of the degree of ripeness in grape berries (*Vitis vinifera* L. cv. Bacchus and Silvaner) by chlorophyll fluorescence. J Agric Food Chem 54:299–305

Lancaster J, Grant J, Lister C, Taylor M (1994) Skin color in apples: influence of copigmentation and plastid pigments on shade and darkness of red color in five genotypes. J Am Soc Hortic Sci 119:63–69

Lancaster J, Reay P, Norris J, Butler R (2000) Induction of flavonoids and phenolic acids in apple by UV-B and temperature. J Hortic Sci Biotechnol 75:142–148

Lers A, Levy H, Zamir A (1991) Co-regulation of a gene homologous to early light-induced genes in higher plants and β-carotene biosynthesis in the alga *Dunaliella bardawil*. J Biol Chem 266:13698–13705

Levy H, Gokhman I, Zamir A (1992) Regulation and light-harvesting complex II association of a Dunaliella protein homologous to early light-induced proteins in higher plants. J Biol Chem 267:18831–18836

Levy H, Tal T, Shaish A, Zamir A (1993) Cbr, an algal homolog of plant early light-induced proteins, is a putative zeaxanthin binding protein. J Biol Chem 268:20892–20896

Liakoura V, Bornman J, Karabourniotis G (2003) The ability of abaxial and adaxial epidermis of sun and shade leaves to attenuate UV-A and UV-B radiation in relation to the UV absorbing capacity of the whole leaf methanolic extracts. Physiol Planta 117:33–43

Lichtenthaler H (1969a) Die plastoglobuli von spinat, ihre grösse, isolierung und lipochinonzusammensetzung. Protoplasma 68:65–77

Lichtenthaler H (1969b) Plastoglobuli und lipochinongehalt der chloroplasten von *Cereus peruvianus* (L.) Mill. Planta 87:304–310

Lingakumar K, Amudha P, Kulandaivelu G (1999) Exclusion of solar UV-B (280-315 nm) radiation on vegetative growth and photosynthetic activities in *Vigna unguiculata* L. Plant Sci 148:97–103

Logemann E, Tavernaro A, Schulz W, Somssich I, Hahlbrock K (2000) UV light selectively coinduces supply pathways from primary metabolism and flavonoid secondary product formation in parsley. Proc Natl Acad Sci USA 97:1903–1907

Lohr M, Wilhelm C (1999) Algae displaying the diadinoxanthin cycle also possess the violaxanthin cycle. Proc Natl Acad Sci USA 96:8784–8789

Mackerness S (2000) Plant responses to ultraviolet-B (UV-B: 280–320 nm) stress: what are the key regulators? Plant Growth Regul 32:27–39

Markham K (1989) Flavones, flavonols and their glycosides. In: Harborne J, Dey P (eds) Methods in plant biochemistry, vol 1. Academic, London, pp 197–235

Mathis P, Kleo J (1973) The triplet state of -carotene and of analog polyenes of different length. Photochem Photobiol 18:343–346

Mayzaud P, Chanut J, Ackman R (1989) Seasonal changes of the biochemical composition of marine particulate matter with special reference to fatty acids and sterols. Mar Ecol Prog Ser 56:189–204

Mendoza H, Martel A, Jimenez del Rio M, Garcia Reina G (1999) Oleic acid is the main fatty acid related with carotenogenesis in *Dunaliella salina*. J Appl Phycol 11:15–19

Merzlyak MN, Chivkunova OB (2000) Light-stress-induced pigment changes and evidence for anthocyanin photoprotection in apples. J Photochem Photobiol B Biol 55:155–163

Merzlyak M, Solovchenko A (2002) Photostability of pigments in ripening apple fruit: a possible photoprotective role of carotenoids during plant senescence. Plant Sci 163:881–888

Merzlyak M, Gitelson A, Pogosyan S, Lekhimena L, Chivkunova O (1998) Light-induced pigment degradation in leaves and ripening fruits studied in situ with reflectance spectroscopy. Physiol Planta 104:661–667

Merzlyak M, Gitelson A, Chivkunova O, Rakitin V (1999) Non-destructive optical detection of pigment changes during leaf senescence and fruit ripening. Physiol Planta 106:135–141

Merzlyak M, Solovchenko A, Chivkunova O (2002) Patterns of pigment changes in apple fruits during adaptation to high sunlight and sunscald development. Plant Physiol Biochem 40:679–684

Merzlyak M, Solovchenko A, Gitelson A (2003) Reflectance spectral features and non-destructive estimation of chlorophyll, carotenoid and anthocyanin content in apple fruit. Postharvest Biol Technol 27:197–212

Merzlyak M, Solovchenko A, Pogosyan S (2005a) Optical properties of rhodoxanthin accumulated in *Aloe arborescens* Mill. leaves under high-light stress with special reference to its photoprotective function. Photochem Photobiol Sci 4:333–340

Merzlyak MN, Solovchenko AE, Smagin AI, Gitelson AA (2005b) Apple flavonols during fruit adaptation to solar radiation: spectral features and technique for non-destructive assessment. J Plant Physiol 162:151–160

Merzlyak MN, Chivkunova OB, Solovchenko AE, Naqvi KR (2008a) Light absorption by anthocyanins in juvenile, stressed, and senescing leaves. J Exp Bot 59:3903–3911

Merzlyak MN, Melo TB, Naqvi KR (2008b) Effect of anthocyanins, carotenoids, and flavonols on chlorophyll fluorescence excitation spectra in apple fruit: signature analysis, assessment, modelling, and relevance to photoprotection. J Exp Bot 59:349–359

Mogedas B, Casal C, Forján E, Vílchez C (2009) β-Carotene production enhancement by UV-A radiation in *Dunaliella bardawil* cultivated in laboratory reactors. J Biosci Bioeng 108:47–51

Mol J, Jenkins G, Schäfer E, Weiss D, Walbot V (1996) Signal perception, transduction, and gene expression involved in anthocyanin biosynthesis. Crit Rev Plant Sci 15:525–557

Morgan-Kiss R, Priscu J, Pocock T, Gudynaite-Savitch L, Huner N (2006) Adaptation and acclimation of photosynthetic microorganisms to permanently cold environments. Microbiol Mol Biol Rev 70:222–252

Munné-Bosch S, Alegre L (2002) Plant aging increases oxidative stress in chloroplasts. Planta 214:608–615

Munné-Bosch S, Lalueza P (2007) Age-related changes in oxidative stress markers and abscisic acid levels in a drought-tolerant shrub, *Cistus clusii* grown under Mediterranean field conditions. Planta 225:1039–1049

Olsen K et al (2009) Temperature and nitrogen effects on regulators and products of the flavonoid pathway: experimental and kinetic model studies. Plant Cell Environ 32:286–299

Olsson L, Veit M, Weissenböck G, Bornman J (1998) Differential flavonoid response to enhanced UV-B radiation in *Brassica napus*. Phytochemistry 49:1021–1028

Ort D (2001) When there is too much light. Plant Physiol 125:29–32

Parisi A, Downs N (2004) Variation of the enhanced biologically damaging solar UV due to clouds. Photochem Photobiol Sci 3:643–647

Pick U (1998) *Dunaliella*: a model extremophilic alga. Isr J Plant Sci 46:131–139

Rabbani S, Beyer P, Lintig J, Hugueney P, Kleinig H (1998) Induced β-carotene synthesis driven by triacylglycerol deposition in the unicellular alga *Dunaliella bardawil*. Plant Physiol 116:1239–1248

Reuber S, Bornman J, Weissenböck G (1996) Phenylpropanoid compounds in primary leaf tissues of rye (*Secale cereale*). Light response of their metabolism and the possible role in UV-B protection. Physiol Planta 97:160–168

Rozema J, van de Staaij J, Bjrn L, Caldwell M (1997) UV-B as an environmental factor in plant life: stress and regulation. Trends Ecol Evol 12:22–28

Saure M (1990) External control of anthocyanin formation in apple: a review. Sci Hortic 42:181–218

Schnitzler J et al (1996) Tissue localization of UV-B-screening pigments and of chalcone synthase mRNA in needles of Scots pine seedlings. New Phytol 132:247–258

Shaish A, Avron M, Pick U, Ben-Amotz A (1993) Are active oxygen species involved in induction of β-carotene in *Dunaliella bardawil*? Planta 190:363–368

Shick J, Dunlap W (2002) Mycosporine-like amino acids and related gadusols: biosynthesis, accumulation, and UV-protective functions in aquatic organisms. Annu Rev Physiol 64:223–262

Sinha R, Klisch M, Gröniger A, Häder D (2001) Responses of aquatic algae and cyanobacteria to solar UV-B. Plant Ecol 154:219–236

Smith G, Markham K (1998) Tautomerism of flavonol glucosides: relevance to plant UV protection and flower colour. J Photochem Photobiol A Chem 118:99–105

Solovchenko A, Merzlyak M (2003) Optical properties and contribution of cuticle to UV protection in plants: experiments with apple fruit. Photochem Photobiol Sci 2:861–866
Solovchenko A, Schmitz-Eiberger M (2003) Significance of skin flavonoids for UV-B-protection in apple fruits. J Exp Bot 54:1977–1984
Solovchenko A et al (2001) Spectrophotometric analysis of pigments in apples. Russ J Plant Physiol 48:698
Solovchenko A, Matthes A, Schmitz-Eiberger M (2005) The role of solar UV in long-term adaptation of ripening apple fruits to high sunlight. J Appl Bot Food Qual 79:72–76
Solovchenko A, Avertcheva O, Merzlyak M (2006) Elevated sunlight promotes ripening-associated pigment changes in apple fruit. Postharvest Biol Technol 40:183–189
Solovchenko A et al (2008) Effects of light and nitrogen starvation on the content and composition of carotenoids of the green microalga *Parietochloris incisa*. Russ J Plant Physiol 55:457
Solovchenko A, Khozin-Goldberg I, Cohen Z, Merzlyak M (2009) Carotenoid-to-chlorophyll ratio as a proxy for assay of total fatty acids and arachidonic acid content in the green microalga *Parietochloris incisa*. J Appl Phycol 21:361–366
Steinbrenner J, Linden H (2003) Light induction of carotenoid biosynthesis genes in the green alga *Haematococcus pluvialis*: regulation by photosynthetic redox control. Plant Mol Biol 52:343–356
Steinmüller D, Tevini M (1985) Composition and function of plastoglobuli. Planta 163:201–207
Steyn W, Wand S, Holcroft D, Jacobs G (2002) Anthocyanins in vegetative tissues: a proposed unified function in photoprotection. New Phytol 155:349–361
Steyn WJ, Wand SJE, Jacobs G, Rosecrance RC, Roberts SC (2009) Evidence for a photoprotective function of low-temperature-induced anthocyanin accumulation in apple and pear peel. Physiol Planta 136:461–472
Strack D, Wray V (1989) Anthocyanins. In: Harborne J, Dey P (eds) Methods in plant biochemistry, vol 1. Academic, London, pp 325–356
Strack D, Vogt T, Schliemann W (2003) Recent advances in betalain research. Phytochemistry 62:247–269
Tanaka Y, Sasaki N, Ohmiya A (2008) Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids. Plant J 54:733
Tattini M, Gravano E, Pinelli P, Mulinacci N, Romani A (2000) Flavonoids accumulate in leaves and glandular trichomes of Phillyrea latifolia exposed to excess solar radiation. New Phytol 148:69–77
Tevini M, Steinmüller D (1985) Composition and function of plastoglobuli. Planta 163:91–96
Tevini M, Braun J, Fieser G (1991) The protective function of the epidermal layer of rye seedlings against ultraviolet-B radiation. Photochem Photobiol 53:329–333
Thelander M, Narita J, Gruissem W (1986) Plastid differentiation and pigment biosynthesis during tomato fruit ripening. Curr Top Plant Biochem Physiol 5:128–141
Thompson G (1996) Lipids and membrane function in green algae. Biochim Biophys Acta 1302:17–45
Turunen M, Heller W, Stich S, Sandermann H, Sutinen M, Norokorpi Y (1999) The effects of UV exclusion on the soluble phenolics of young Scots pine seedlings in the subarctic. Environ Pollut 106:219–228
Vishnevetsky M, Ovadis M, Zuker A, Vainstein A (1999) Molecular mechanisms underlying carotenogenesis in the chromoplast: multilevel regulation of carotenoid-associated genes. Plant J 20:423
Vogt T, Ibdah M, Schmidt J, Wray V, Nimtz M, Strack D (1999) Light-induced betacyanin and flavonol accumulation in bladder cells of *Mesembryanthemum crystallinum*. Phytochemistry 52:583–592
Wade H, Bibikova T, Valentine W, Jenkins G (2001) Interactions within a network of phytochrome, cryptochrome and UV-B phototransduction pathways regulate chalcone synthase gene expression in *Arabidopsis* leaf tissue. Plant J 25:675

Wang B, Zarka A, Trebst A, Boussiba S (2003) Astaxanthin accumulation in *Haematococcus pluvialis* (Chlorophyceae) as an active photoprotective process under high irradiance. J Phycol 39:1116–1124

Weger H, Silim S, Guy R (1993) Photosynthetic acclimation to low temperature by western red cedar seedlings. Plant Cell Environ 16:711–717

Weisshaar B, Jenkins G (1998) Phenylpropanoid biosynthesis and its regulation. Curr Opin Plant Biol 1:251–257

Whitelam G, Codd G (1986) Damaging effects of light on microorganisms. In: Herbert R, Codd G (eds) Microbes in extreme environments. Academic, London, pp 129–169

Zeliou K, Manetas Y, Petropoulou Y (2009) Transient winter leaf reddening in *Cistus creticus* characterizes weak (stress-sensitive) individuals, yet anthocyanins cannot alleviate the adverse effects on photosynthesis. J Exp Bot 60:3031–3042

Zeng X-Q, Chow WS, Su L-J, Peng X-X, Peng C-L (2010) Protective effect of supplemental anthocyanins on *Arabidopsis* leaves under high light. Physiol Planta 138:215–225

Zhekisheva M, Boussiba S, Khozin-Goldberg I, Zarka A, Cohen Z (2002) Accumulation of oleic acid in *Haematococcus pluvialis* (Chlorophyceae) under nitrogen starvation or high light is correlated with that of astaxanthin esters. J Phycol 38:325–331

Zhekisheva M, Zarka A, Khozin-Goldberg I, Cohen Z, Boussiba S (2005) Inhibition of astaxanthin synthesis under high irradiance does not abolish triacylglycerol accumulation in the green alga *Haematococcus pluvialis* (Chlorophyceae). J Phycol 41:819–826

Chapter 4
Localization of Screening Pigments Within Plant Cells and Tissues

Abstract Subcellular localization and distribution within tissues are crucial characteristics of plant screening pigments. General patterns of the localization and distribution of screening compounds in plant tissues are discussed in connection with their function, chemical properties, and biosynthetic origin. Special attention is paid to the vacuolar and cuticular phenolics and extrathylakoid carotenoids deposited in oil bodies of microalgae and plastoglobuli of higher plants.

The efficiency of photoprotection by screening pigments should strongly depend on their localization within plant tissue; it can be evaluated in terms of external filtering, when photoprotective pigments serve as a screen, or internal filtering, when such pigments compete with light absorption by chlorophyll within a leaf. A large body of evidence suggests that the bulk of high-light-stress-induced screening compounds are situated within the protective complex comprising the cuticle, the epidermis and its appendages, and derivative structures such as hairs and trichomes. The patterns of localization and distribution of different classes of screening pigments are discussed below.

4.1 Subcellular Localization of Screening Pigments in Plants: General Patterns

The screening pigments discovered in plants to date can be divided into several categories according to their subcellular localization (Fig. 4.1). The cellular compartment in which a pigment is or could be localized depends on the properties of its molecules (such as polarity), the site of its biosynthesis (e.g., chloroplasts or cytoplasm) and accumulation (e.g., endoplasmic reticulum or vacuole), as well as

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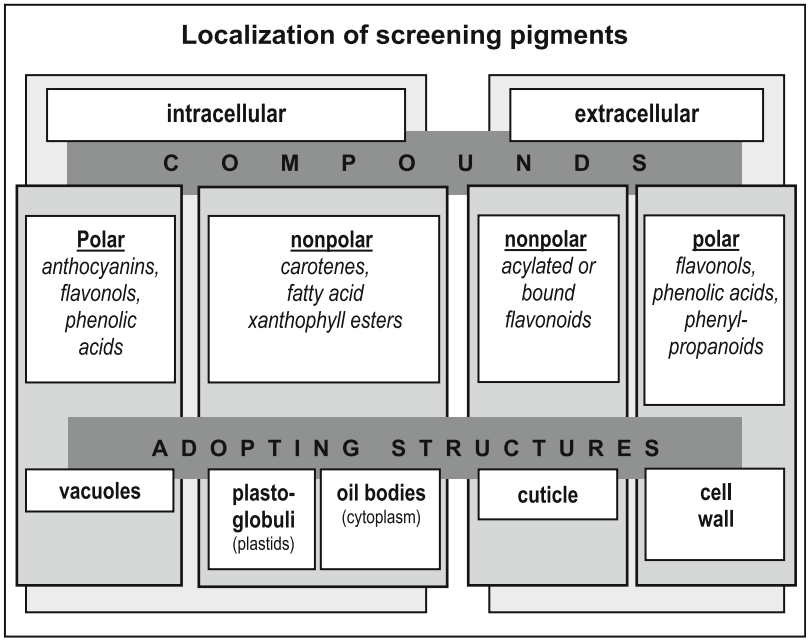

Fig. 4.1 Localization of screening pigments in higher plants. (Solovchenko, unpublished)

its effect on cellular metabolism. In particular, certain phenolics can be toxic to the cell when present in concentrations necessary for efficient screening of solar radiation. These phenolics are predominantly accumulated in the form of glycosides, which are less toxic, within the vacuole, where they could reach high local concentration without the risk of damage to other cell components; the predominant vacuolar localization of phenolics in the cell is also determined by their high polarity (Harborne 1980; Harborne and Williams 2000; Taiz 1992). There are notable exceptions, however. It was found recently that certain phenolic compounds can be accumulated in relatively high amounts in chloroplasts (Agati et al. 2007), e.g., in lumen (Georgieva et al. 2010). However, it is questionable whether these phenolics could contribute to radiation screening; it seems more likely for them to function as reactive oxygen species (ROS) scavengers (Takahama 1983). A considerable amount of phenolic compounds are also accumulated in endoplasmic reticulum (Markham 1989; Strack and Wray 1989) and/or are excreted to the apoplast, where it becomes involved in lignin biosynthesis or impregnates the cell wall matrix.

Highly hydrophobic extraplastidic carotenoids which cannot be accumulated in the vacuole are accumulated within lipid globules forming in cytoplasm (oil bodies, more details on oil bodies are given in Sect. 4.3) or the plastidic stroma (plastoglobuli). The formation of lipid globules comprising mostly neutral lipids appears to be among the key factors controlling accumulation of extrathylakoid carotenoids and their fatty

acid esters. Thus, the formation of cytoplasmic oil bodies and hence their capacity for carotenoid storage is an important factor regulating the synthesis of extrathylakoid β-carotene in *Dunaliella bardawil* (Mendoza et al. 1999).

4.2 Distribution of Phenolic Screening Compounds Within Plant Tissues

The bulk of phenolic compounds synthesized in plant cells are accumulated within the vacuoles of epidermal and/or mesophyll cells (Agati et al. 2009; Bidel et al. 2007; Markstädter et al. 2001) or, in the case of fruit, hypodermal cells (Awad et al. 2000; Solovchenko and Merzlyak 2003; Solovchenko and Schmitz-Eiberger 2003). At the same time, considerable amounts of phenolics are excreted from the cells and remain within or become covalently bound to the cell wall and/or cuticle (Baur et al. 1998; Krauss et al. 1997; Solovchenko and Merzlyak 2003). In certain "hairy" plant species featuring pubescence, trichomes, or similar structures, the cells which form these structures often contain high amounts of screening compounds, mostly of phenolic nature, within their vacuoles (Karabourniotis and Bornman 1999; Karabourniotis et al. 1992, 1998; Skaltsa et al. 1994). The functioning of the phenolic screening compounds localized in different structures and tissues of leaves and fruit is considered in this section.

4.2.1 Screening Phenolics in the Cuticle

Certain phenolic compounds synthesized by plants, such as flavonoids, undergo heavy methylation and/or acylation, attaining as a result a considerable hydrophobicity (Harborne 1980; Harborne and Williams 2000). These phenolics (represented mainly by flavonol and phenolic acid derivatives) are often incorporated in the cuticle in the form of very long chain fatty acid esters of cuticular waxes (Holloway et al. 1982; Kolattukudy 1970, 1980; Liakopoulos et al. 2001). The accumulation of phenolic compounds in plant leaf cuticle allows it to act similarly to a cutoff filter for UV, mainly UV-B, radiation (Krauss et al. 1997; Markstädter et al. 2001; Solovchenko and Merzlyak 2003). It is claimed that only a small part of incident solar UV radiation can penetrate the cuticle to reach the underlying epidermis and mesophyll tissue; additional details on the efficiency of screening pigments and the effect of their accumulation on the optical properties of the cuticle are given in Chap. 5.

It is important to note that the common feature of cuticular phenolics is very low, if any, metabolic turnover. This is especially true for the phenolics covalently bound to the cuticular constituents. Therefore, the UV-B shielding provided by cuticular

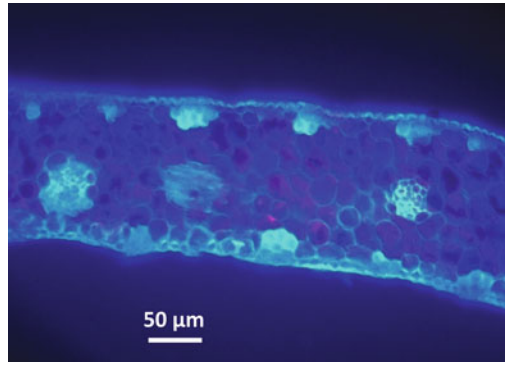

Fig. 4.2 Cross section of a palm (*Phoenix dactyliphera* L.) leaf under a fluorescence microscope. Note the *blue fluorescence* of phenolics bound to cell walls of epidermal cells and fiber vascular bundles. (Lobakova and Solovchenko, unpublished)

phenolics can be considered as a primary constitutive adaptation to solar short-wavelength radiation. The UV-screening compounds localized in the outermost structure of the epidermis protects not only assimilatory but all living tissues of a leaf (see Fig. 4.2), including epidermal cells, whose nuclei, membranes, and, in the case of guard cells, chloroplasts are vulnerable to UV damage (Krauss et al. 1997). However, an accumulation of hydroxycinnamic acids bound to the cuticle and cell walls hardly provides an additional UV screen at the leaf surface (Day et al. 1993).

4.2.2 Vacuolar Phenolics of Mesophyll and Epidermis

Anthocyanins, flavonol, and phenolic acid derivatives which are accumulated in vacuoles of epidermal and underlying mesophyll cells (Agati et al. 2002, 2009; Merzlyak et al. 2008; Steele et al. 2009) or hypodermal cells are distributed within leaf and fruit tissues according to diverse patterns (see Figs. 4.3–4.6).

In leaves acclimated to high sunlight, flavonoids were shown to occur mainly in the vacuoles of the adaxial (both epidermal and mesophyll) cells, whereas hydroxycinnamates, the efficient UV-B-screening compounds, are situated predominantly in vacuoles of mesophyll cells (Olsson et al. 1999; Tattini et al. 2004). Moreover, flavonols tend to localize almost exclusively in epidermal and palisade mesophyll cell vacuoles (Gould et al. 2000; Kolb et al. 2001; Tattini et al. 2004).

It should be noted that flavonols and other screening phenolics could be colocalized with anthocyanins. Thus, the epidermal and hypodermal cells of sunlit apple skin contain high amounts of both anthocyanins and flavonols in their vacuoles (Figs. 4.4, 4.5; see also Merzlyak et al. 2002; Solovchenko and Schmitz-Eiberger 2003). The vacuoles of parenchymal cells of apple pulp possess a low content of flavonoids in comparison with skin cells and accumulate mainly chlorogenic acid (Awad et al. 2000, 2001a, b; Escarpa and González 1998).

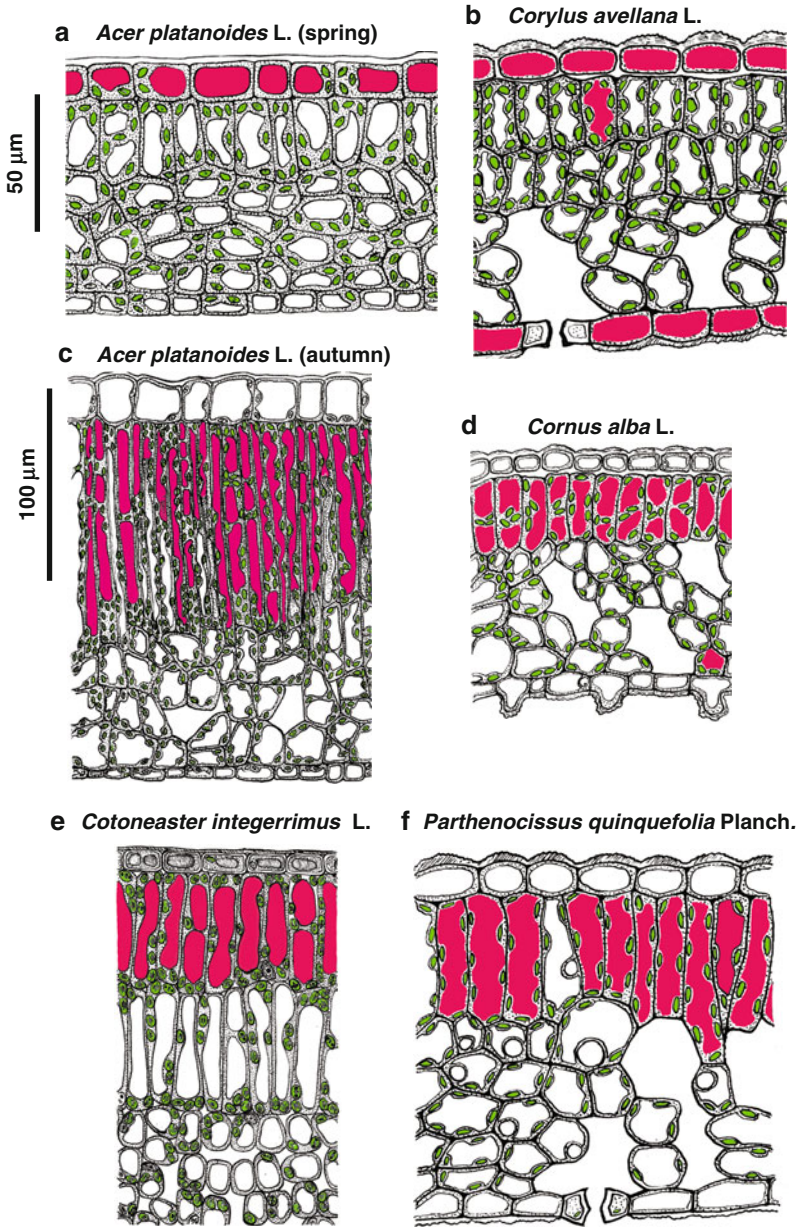

Fig. 4.3 The anatomy of spring (**a**, **b**) and autumn (**c**–**f**) leaves of the species studied. The chloroplasts and anthocyanin-containing vacuoles are shown in *green* and *magenta*, respectively. Note that in juvenile leaves anthocyanins are accumulated in epidermal cell vacuoles, whereas in autumn leaves these pigments are localized predominantly in mesophyll. (Reproduced from Merzlyak et al. (2008) with permission from Oxford University Press)

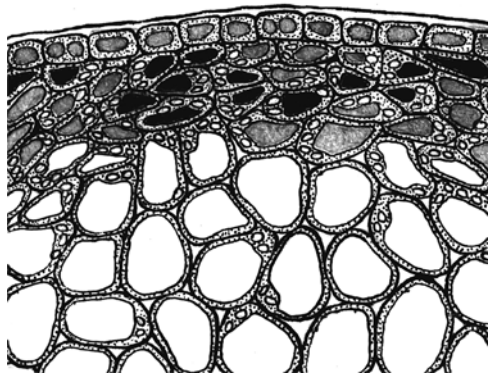

Fig. 4.4 The typical anatomy of apple (cultivar Zhiguliovskoe) fruit skin comprising (ordered from outside to inside) cuticle, epidermis, and hypoderm as well as parenchyma (×500). The vacuoles containing flavonols are *shaded with gray* and those containing both flavonols and anthocyanins are *shaded with black*. (Solovchenko and Buzulukova, unpublished)

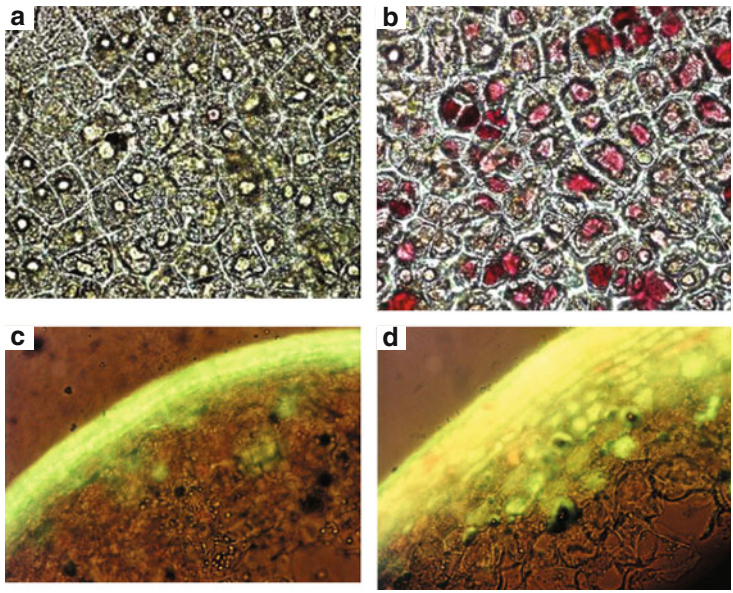

Fig. 4.5 Microphotographs of apple (*Malus* × *domestica* Borkh.) fruit tissues adapted to low (**a**, **c**) and high (**b**, **d**) light. Bright-field microscopy (×400) reveals *red anthocyanins* in the vacuoles of the cells of sunlit skin (**b**), but absent in *shaded tissue cells* (**a**). Fluorescence microscopy (**c**, **d**; ×200; λ_{ex} 365 nm, λ_{em} 450–600 nm) demonstrates an increase in the amount of flavonols (apparent as *bright-yellow fluorescence*) in the vacuoles of sunlit skin (**d**) over the amount in the shaded skin (**c**). Also note the presence of chlorogenic acid in the vacuoles of shaded skin cells (**c**) and the cuticle of both samples (**c**, **d**). (Solovchenko, unpublished)

As reviewed by Steyn et al. (2002), the distribution of anthocyanins within organs and tissues is genetically determined by tissue-specific expression of regulatory genes. These genes control expression of structural genes in response to environmental and developmental stimuli (Saure 1990; Winkel-Shirley 2001, 2002).

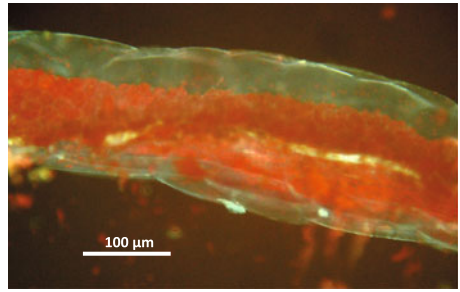

Fig. 4.6 Cross section of a sorrel (*Oxalis acetozella* L.) leaf under a fluorescence microscope. Chloroplasts localized in the mesophyll emit *red fluorescence*. Note the *blue-green fluorescence* of flavonols in vacuoles of epidermal cells and the *blue fluorescence* of cuticle-bound cinnamic acid derivatives. (Solovchenko, unpublished)

It is important to note in the context of the radiation screening function that anthocyanin synthesis is a cell-autonomous response, i.e., that color development is controlled at the level of the individual cell (Lancaster et al. 1994). This allows local accumulation of anthocyanin in the exposed tissues, resulting in a finely tailored light screen, in contrast to other leaf light-avoidance measures. Cells without anthocyanin are found dispersed throughout red-anthocyanin-rich apple peel (Lancaster et al. 1994; Fig. 4.5). There are species that accumulate anthocyanins only in adaxial (upper) epidermis such as in stressed juvenile leaves of Norway maple, *Acer platanoides* L. (Fig. 4.3a) or in both adaxaial and abaxial (lower) epidermis; juvenile walnut, *Corylus avellana* L., leaves could serve as an example (Fig. 4.3b). Other species accumulate anthocyanins in the vacuoles of predominantly palisade mesophyll cells (autumn leaves of *A. platanoides*, *Cornus alba* L., *Parthenocissus tricuspidata* Planch.; Figs. 4.3c–f) and occasionally in sponge mesophyll (*C. alba*, Fig. 4.3d).

It appears that accumulation of anthocyanins in epidermal vacuoles is characteristic of transient red pigmentation of juvenile leaves and stress-induced reddening of senescing autumn leaves (Merzlyak et al. 2008). By contrast, accumulation of these pigments in mesophyll cell vacuoles seems to occur more often in mature or senescing leaves. In certain cases, leaves of the same species can accumulate anthocyanins in different tissues at different stages of ontogenesis. Juvenile leaves of *A. platanoides* transiently accumulate anthocyanins in the vacuoles of epidermal cells in the spring (Fig. 4.3a); mature summer leaves of this species are usually green and essentially anthocyanin-free. In autumn, high solar light and low temperatures often trigger accumulation of anthocyanins, which occurs in the vacuoles of palisade mesophyll cells (Fig. 4.3c).

An interesting case is represented by many common understorey plants (Lee and Collins 2001) of the tropics which accumulate anthocyanins only in the vacuoles of abaxial epidermis or sponge mesophyll (Forsyth and Simmonds 1954; Hughes et al. 2008; Lee et al. 1979). The function of abaxially localized anthocyanins is a subject of debate. Some workers suggested that the pigments participate in photoprotection in plants where exposed abaxial leaf surfaces are vulnerable to high levels of incident light (Hughes and Smith 2007), whereas some argued against this point of view (Kyparissis et al. 2007). According to Hughes et al. (2008), the

photoprotective function of anthocyanins featuring abaxial localization such as in *Begonia heracleifolia* (Cham. & Schltdl.) may be significant in shade-adapted understorey plants, which photosynthetically saturate (and thus photoinhibit) at relatively low irradiances, and yet frequently encounter potentially damaging irradiance via high-intensity sunflecks and sunpatches.

It should be noted, in addition, that the effectiveness of epidermal UV screening depends not only on leaf anatomy and the content of UV-screening pigments, but also on their uniformity over the leaf area (Day et al. 1993; Meyer et al. 2009). Meyer et al. (2009) emphasized that adaxial and abaxial epidermises are not uniform UV screens for the developing mesophyll. The accumulation of flavonoids in the vacuole varies from cell to cell and is suggested to reflect the asynchronous development of epidermal cells (Avery 1933). Since immature leaves are sinks, importing sugar through phloem from tree storage to synthesize soluble phenolic compounds (Kleiner et al. 1999), the epidermal cells situated above veins accumulate the first flavonoids i.e., the epidermal cells close to the importing area might accumulate flavonoids in vacuoles earlier than distant epidermal cells. In mature leaves, epidermises on both surfaces of a leaf constitute a uniform UV screen for the mesophyll (Meyer et al. 2009).

4.2.3 Phenolics in Hairs and Trichomes

The adaptive significance of leaf hairs and trichomes was the subject of a considerable number of studies. It was suggested that these structures, besides other numerous functions, may participate in the screening of solar radiation (Karabourniotis and Bornman 1999; Karabourniotis et al. 1992; Morales et al. 2002; Tattini et al. 2000). Isolated trichomes strongly absorb UV-B radiation owing to the presence of polyphenols (mainly flavonoid aglycones such as kaempherol, luteolin, apigenin, and quercetin and their glycosides) in the trichomes (Karabourniotis et al. 1992, 1998; Skaltsa et al. 1994). The polyphenol compounds in the mature leaf hairs of olive (*Olea europaea* L.), as well as of oak (*Quercus ilex* L.), are diffusely located in the hair cell walls (Karabourniotis and Fasseas 1996). However, the cell wall is not the only site of screening pigment localization in trichomes and hairs. In certain plant species, phenolic screening compounds are accumulated in the vacuoles of hair cells; this is the case in *C. avellana* juvenile leaves (Fig. 4.7); see also (Tattini et al. 2000). Studies of the changes of subcellular localization of phenolics during development of *O. europaea* leaves performed with fluorescence microscopy (Karabourniotis et al. 1998) showed that despite the differences in morphology between *O. europaea* and *Q. ilex*, high concentrations of polyphenol compounds are initially located mainly in the cytoplasm of the developing nonglandular hairs, and their deposition on the cell walls takes place during the secondary cell wall thickening.

One should note, in addition, that the occurrence of a dense trichome layer, especially in young leaves, may provide protection against not only UV-B radiation, but also against high fluxes of visible radiation (Karabourniotis and Bornman

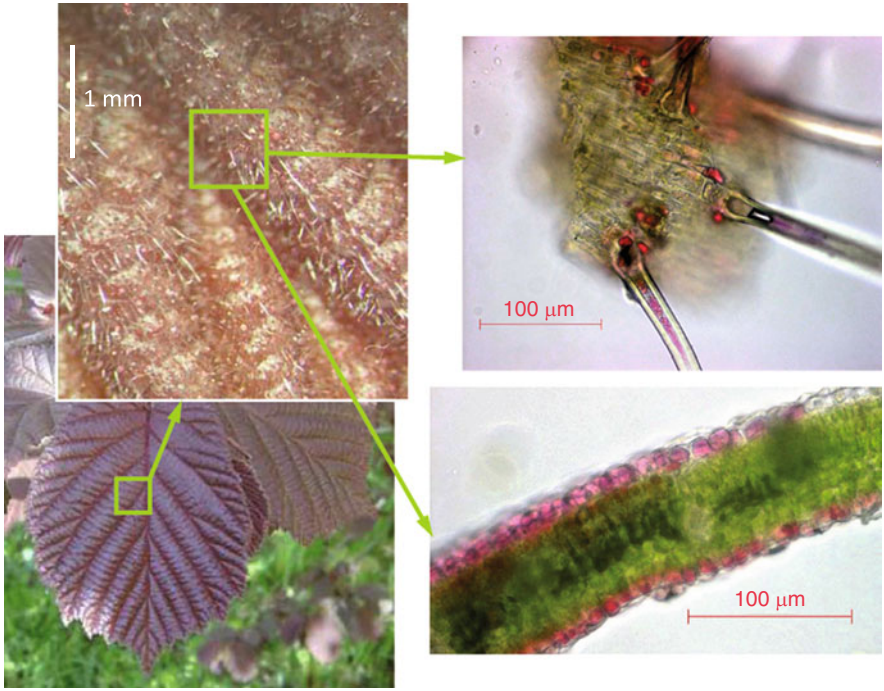

Fig. 4.7 Juvenile leaves of walnut (*Corylus avellana* L.) and localization of anthocyanins in their epidermal (*lower-right plate*) and hair (*upper-right plate*) cells. They appear *red* when observed in reflected light in spite of considerable chlorophyll content owing to the presence of anthocyanin-containing hairs which strongly scatter light on their surface. (Solovchenko, unpublished)

1999). This is especially evident in cases when hairs contain visible-absorbing anthocyanins in their vacuoles (Ntefidou and Manetas (1996; Fig. 4.7).

4.3 Depots of Secondary Carotenoids in Microalgae and Higher Plants

Acclimation to stressors, especially to high light levels, often brings about considerable changes in the ultrastructure of microalgal and higher-plant cells. In particular, conspicuous changes take place in chloroplasts. A decrease in the size of the plastids accompanied by degeneration of grana and lamellae, a reduction of stacking together, with an increase in the size and number of stromal and cytoplasmic inclusions, mainly of lipid nature, is often recorded in plant cells adapted to high fluxes of solar radiation (Figs. 4.6–4.9; Berner et al. 1989; Lichtenthaler 2007; Merzlyak et al. 2007; Merzlyak and Solovchenko 2002; Merzlyak et al. 2005). Still, the integrity of the remaining thylakoids is retained even at advanced stages of

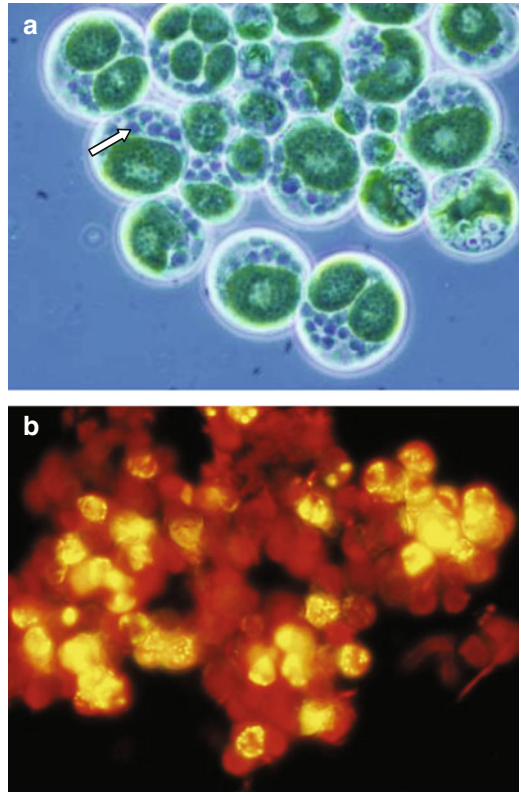

Fig. 4.8 *Parietochloris incisa* cells grown on nitrogen-deficient medium under light (**a**, ×400) and the same cells stained by Nile Red (**b**, ×200), the vital stain for neutral lipids, under a fluorescence microscope. Note oil bodies are apparent under phase contrast (**a**) as *bluish globules* and after staining (**b**) as the structures with *bright-yellow fluorescence*. (Khozin-Goldberg and Solovchenko, unpublished)

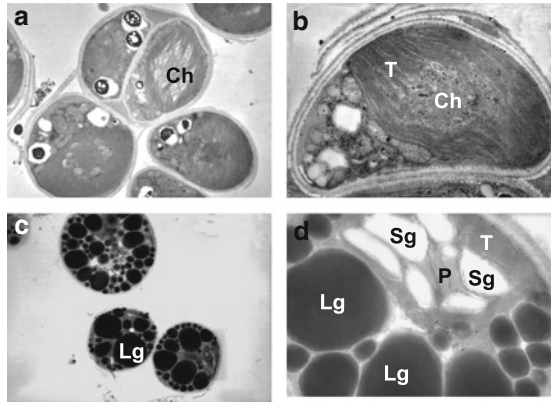

Fig. 4.9 Ultrastructure of *P. incisa* cells (**a**, **c**) and chloroplasts (**b**, **d**) after 5 weeks of growth on nitrogen-supplemented (**a**, **b**) and nitrogen-deficient (**c**, **d**) media. *Ch* chloroplast, *Lg* lipid globuli (oil bodies), *P* pyrenoid, *Sg* starch grains, *T* thylakoids. (Reproduced from Merzlyak et al. (2007) with kind permission from John Wiley and Sons)

acclimation, and chloroplasts do not show signs of a deep degradation, suggesting the reversibility of these changes. Indeed, *Parietochloris incisa* and other species, including those listed above, are able to recover their photosynthetic apparatus upon removal of the stress.

One of the most striking among common features of algal cells acclimating to high-light stress is a vast increase in the size and number of cytoplasmic lipid globuli (so-called oil bodies), which eventually could occupy most of the acclimated cell volume (Figs. 4.8, 4.9). At the same time, growing oil bodies often became more electron-dense owing to an increase in unsaturation of the lipids. The lipid inclusions, occurring mainly in cytoplasm, often become the depot for lipophilic screening pigments such as extrathylakoid carotenoids as is the case in a number of algal species such as *Haematococcus pluvialis* (Boussiba 2000), different species of the genus *Dunaliella* (Mendoza et al. 1999; Rabbani et al. 1998), *P. incisa* (Solovchenko et al. 2008), and a number of others. For example, *P. incisa* cells grown under nonstressful irradiances (about 35 μE m^{-2} s^{-1} photosynthetically active radiation) possess large chloroplasts with a well-formed thylakoid system and a moderate amount of starch grains (Figs. 4.8, 4.9), typical of chloroplasts with active photosynthetic function (Lichtenthaler 2007).

Deposition of screening carotenoids in microalgae could also take place within the chloroplast. This is the case in *Dunaliella salina* (*D. bardawil*), which accumulates high amounts of β-carotene within the stroma of chloroplasts in the form of lipid-containing granules stabilized by special proteins (Ben-Amotz and Avron 1983; Ben-Amotz et al. 1982, 1989; Katz et al. 1995).

Comparison of the high-light-stress-induced changes in the chloroplast ultrastructure of green algae and higher plants reveals their marked similarity (cf. Figs. 4.9, 4.10). As in microalgae, in higher-plant chloroplasts, a partial degeneration of chloroplast thylakoid membranes and the formation of lipid globules of large size and number is often observed under acclimation to high light (Lichtenthaler 2007; Merzlyak and Solovchenko 2002; Merzlyak et al. 2005); yet it should be emphasized that the lipid globules (oil bodies) in algal cells are characterized by extraplastidic localization (Fig. 4.9), whereas lipid globules in higher-plant cells (plastoglobuli) are localized within chloroplasts (Figs. 4.10, 4.11).

Evidence of the function of the lipid inclusions in the cytoplasm and stroma of the plastids as a depot for screening carotenoids was obtained in the course of isolation and pigment analysis of plastids and oil bodies from plant assimilatory tissues acclimated to high fluxes of solar radiation. In the case of *P. incisa,* the bulk (up to 66%) of β-carotene accumulated under stress induced by high light and nitrogen deficiency was localized in cytoplasmic oil bodies (Fig. 4.12). Extrathylakoid lipid inclusions discovered in *D. bardawil* were also shown to contain the bulk of β-carotene synthesized by this alga under stress (Rabbani et al. 1998). More polar xanthophylls, e.g., astaxanthin in *H. pluvialis*, are deposited in lipid globules in the form of fatty acid esters (Zhekisheva et al. 2002, 2005).

Electron microscopy showed that in the cells of sunlit skin of apple fruit, plastoglobuli occupied a volume 3 times larger than that in shaded skin (Merzlyak and Solovchenko 2002). Obviously, the changes in cell ultrastructure outlined above facilitate the accumulation of secondary carotenoids most probably providing photoprotection via screening of the excessive visible radiation. Another illustrious example is given by plastids from red leaves of *Aloe arborescence* (Figs. 4.13, 4.14). Ultrastructural observations showed that the adaptation of

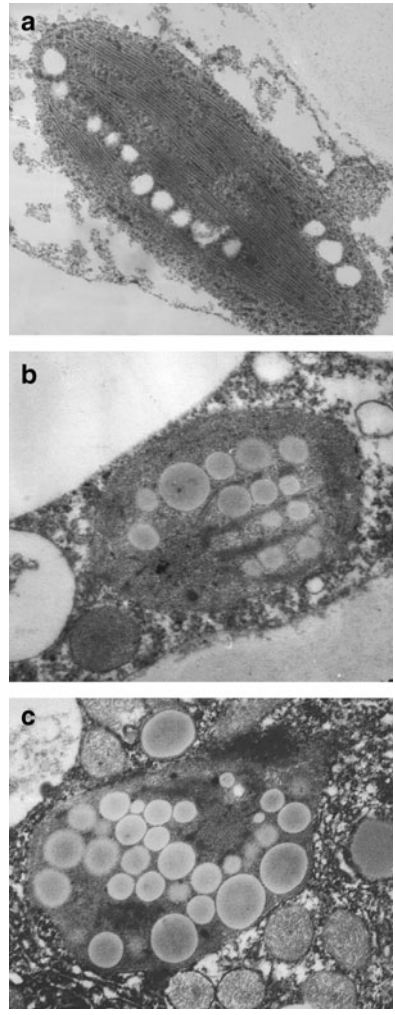

Fig. 4.10 Chloroplast–chromoplast (*from top to bottom*) transformation in the peel of Antonovka apples. *Top micrograph*: fixation in glutaraldehyde and $KMnO_4$, magnification: ×15,000. Other micrographs: fixation in *p*-formaldehyde, glutaraldehyde, and OsO_4, magnification: ×30,000. (Reprinted from Merzlyak and Solovchenko (2002) with permission from Elsevier)

A. arborescence to the stressful conditions is accompanied by a change of coloration (from green to red) and deep rearrangement of chloroplasts, including degradation of thylakoids and accumulation of globular electron-dense structures (probably of lipidic nature) resembling the osmiophilic globules encountered in plastids of senescing leaves (Fig. 4.11; Hudák 1981; Steinmüller and Tevini 1985; Tevini and Steinmüller 1985). Taking into account the ultrastructural details observed (Fig. 4.11), one can regard the plastids of the red aloe leaves as chromoplasts and, more specifically, carotenoidoplasts, accumulating rhodoxanthin, obviously providing photoprotection via screening of the excessive visible radiation (Diaz et al. 1990; Merzlyak et al. 2005). Similar changes in plastid ultrastructure were recorded in other

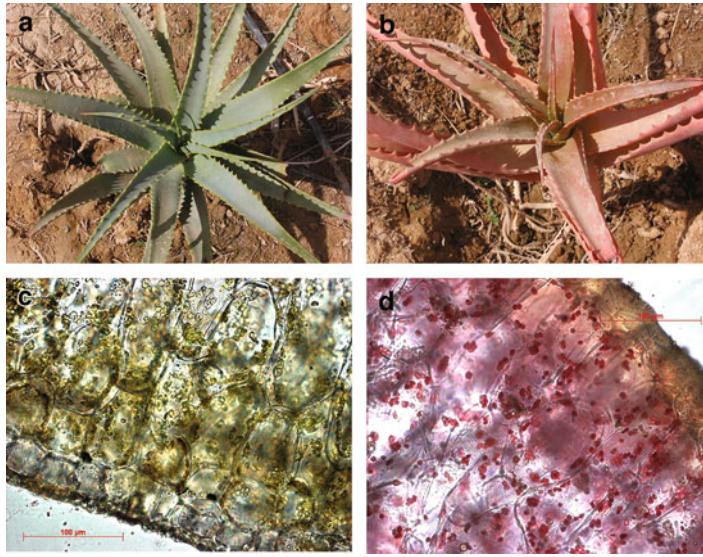

Fig. 4.11 The appearance of intact (**a**) and stressed (**b**) *A. arborescence* plants and their leaf cross sections (**c, d**). (Reproduced from Merzlyak et al. (2005) with permission from the Royal Society of Chemistry for the European Society for Photobiology, the European Photochemistry Association, and the Royal Society of Chemistry)

species accumulating ketocarotenoids such as rhodoxanthin and escholtzxanthin as screening pigments (Hormaetxe et al. 2005, 2007; Weger et al. 1993).

The analysis of the carotenoid composition of higher-plant tissues acclimated to high light revealed, in contrast to microalgae, a marked decrease in β-carotene content accompanied by a dramatic increase in the proportion of xanthophylls, which are considerably more polar than carotenes and cannot be directly incorporated into the highly hydrophobic interior of lipid globules (such as cytoplasmic oil bodies or plastoglobuli localizing in the stroma of plastids), with the interior comprising chiefly neutral lipids (Steinmüller and Tevini 1985; Tevini and Steinmüller 1985) surrounded by a thin layer of polar lipids and amphiphilic proteins (Austin et al. 2006; Bréhélin and Kessler 2008; Bréhélin et al. 2007; Kessler et al. 1999; Kessler and Vidi 2007). The lipid globules were initially suggested to be the site of storage of metabolic waste to be discarded with shedding of leaves (Biswal 1995). However, recent studies employing advanced methods of biochemistry and molecular biology showed that the function of lipid globules in plant cells is much more sophisticated. In particular, these structures could serve as a depot for the lipids (carotenoids, fatty acids, triacylglycerols, prenylquinones, etc.) liberated during decomposition of thylakoid membranes accompanying acclimation to strong sunlight. It important that these valuable molecules remain "at hand," i.e., can be quickly retrieved for rebuilding of thylakoids during reacclimation to low light or redifferentiation of chloroplasts in the course of greening

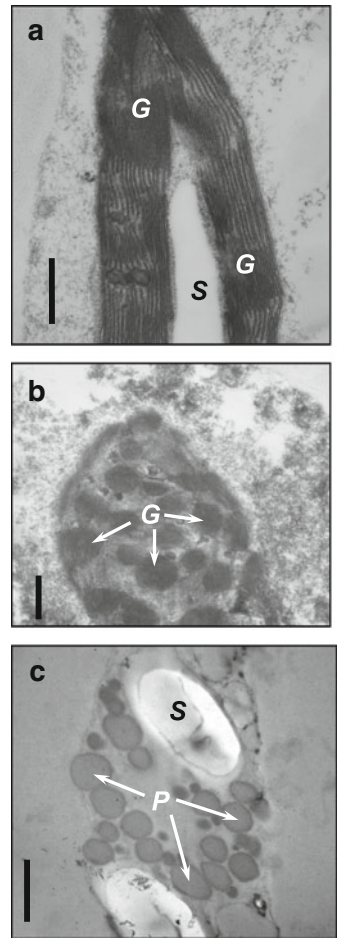

Fig. 4.12 Ultrastructure of plastids of *green* (**a**), *reddish-green* (**b**), and *red* (**c**) leaves of *Aloe arborescence*. **a**, **b** Fixation with glutaraldehyde and $KMnO_4$; **c** fixation with glutaraldehyde and OsO_4. *G* grana, *S* starch grain, *P* plastoglobuli. *Bar* 0.5 mm. (Reproduced from Merzlyak et al. (2005) with permission from the Royal Society of Chemistry for the European Society for Photobiology, the European Photochemistry Association, and the Royal Society of Chemistry)

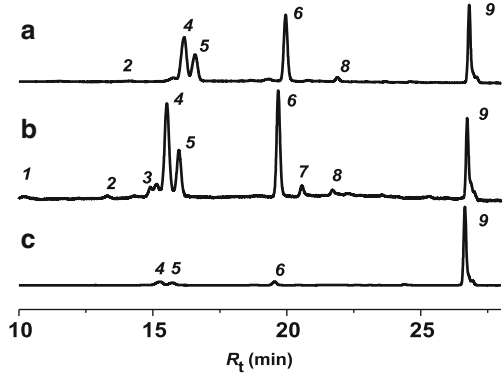

Fig. 4.13 High performance liquid chromatography chromatograms of pigments from *P. incisa* cells (**a**) grown under 400 mE m_2 s_1 photosynthetically active radiation and thylakoids (**b**) and oil bodies (**c**) isolated from the same cells. Detection at 455 nm. *1* neoxanthin, *2* violaxanthin, *3* lutein 5,6-epoxide, *4* lutein, *5* zeaxanthin, *6* chlorophyll *b*, *7* phaeophytin *a*, *8* chlorophyll *a*, *9* β-carotene. (Reproduced from Solovchenko et al. (2008) with kind permission from Springer Science+Business Media), Fig. 4

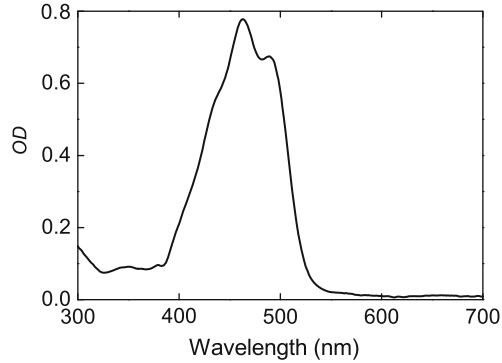

Fig. 4.14 Absorption spectrum of chloroform extract from oil bodies isolated from *P. incisa* cells grown under high (400 μE m^{-1} s^{-1} photosynthetically active radiation) light. Note the characteristic maximum position and shape of the spectrum similar to that of β-carotene (Britton 1995b). (Solovchenko and Khozin-Goldberg, unpublished)

(Kessler and Vidi 2007). As stated above, the lipids synthesized de novo could also be deposited in and become the main constituents of the lipid globules as often occurs in microalgae (Bigogno et al. 2002a; Mendoza et al. 1999; Zhekisheva et al. 2005).

Carotenol (xanthophyll) accumulation within lipid globules containing less polar triacylglycerols is limited by the relatively high polarity of these pigments (Bréhélin and Kessler 2008; Bréhélin et al. 2007; Britton 1995a). In plants accumulating xanthophylls as screening pigments, this obstacle is usually overcome by esterification of the carotenoids by fatty acids, e.g., in the microalga *H. pluvialis* (Zhekisheva et al. 2002), ripening apple fruit (Knee 1988), or senescing leaves of deciduous woody plants (Lichtenthaler 1969a, b; Lichtenthaler and Weinert 1970; Steinmüller and Tevini 1985; Tevini and Steinmüller 1985). The free fatty acids necessary for the synthesis of carotenol esters could be formed de novo or liberated upon the ordered decomposition of photosynthetic membranes during photoacclimation or in the course of dismantling of the photosynthetic apparatus during senescence (Gross 1987; Hornero-Mendez and Minguez-Mosquera 2000) of assimilatory tissues (Bigogno et al. 2002b; Knee 1988). There are also reports on the acceleration of synthesis of specific xanthophylls and their fatty acid esters in plants by acclimation to strong sunlight with simultaneous increase in lipid globule size and number (Merzlyak and Solovchenko 2002; Solovchenko et al. 2006). There could be other mechanisms of carotenoid buildup in plants acclimating to strong solar light. For example, fatty acid xanthophyll esters were not detected in red *A. arborescence* leaves accumulating high amounts of rhodoxanthin, though numerous large lipid globules were revealed (Fig. 4.12; Merzlyak et al. 2005).

4.4 Concluding Remarks

Collectively, the findings presented in this chapter suggest that different groups of screening pigments are characterized by certain patterns of subcellular localization (Fig. 4.1) and, in the case of multicellular plants, distribution within tissues (see

Sects. 4.2, 4.3). Specific sites of screening pigment localization are determined by their biosynthetic origin, the polarity of the molecules, the effect(s) on cellular metabolism, etc. Several groups of screening pigments can be present in plant cells simultaneously, occupying different cell compartments.

Unicellular algae accumulate UV-protective compounds represented mainly by mycosporine-like amino acids in the cytoplasm (Moisan and Mitchell 2001; Shick and Dunlap 2002) and in the cell wall (Shick and Dunlap 2002). In terrestrial plants, the bulk of UV-protective compounds which comprise phenolic compounds (for more details on the diversity of screening phenolics, see Chap. 1) are situated in surface-protective structures, including the cuticle, epidermis, hairs, and trichomes, the first-line defense against adverse environmental factors including solar UV radiation, as well as in underlying mesophyll cells (Agati et al. 2002; Awad et al. 2000; Baur et al. 1998; Bornman 1999; Caldwell et al. 1983; Chalker-Scott 1999; Karabourniotis et al. 1992, 1998, 2001; Karageorgou and Manetas 2006; Kolb and Pfundel 2005; Manetas 2003; Mazza et al. 2000; Ntefidou and Manetas 1996; Skaltsa et al. 1994; Solovchenko and Schmitz-Eiberger 2003; Steyn et al. 2002). Epidermal cells excrete the phenolics synthesized by them into the apoplast, where they could be incorporated into the cell wall and/or cuticle (Holloway et al. 1982; Kolb et al. 2001; Kolb and Pfundel 2005; Krauss et al. 1997). Betalains, which are similar to anthocyanins in function and spectral properties, also resemble them in subcellular localization and tissue distribution. They usually occur in the vacuoles of epidermal or special repository cells (Ibdah et al. 2002; Vogt et al. 1999), such as in stressed *Mesembryanthemum crystallinum* plants.

Elevated solar irradiation often induces, apart from an increase in the amount of phenolic screening compounds, the buildup of extrathylakoid (secondary) carotenoids, which do not transfer the energy of absorbed light quanta to chlorophyll in microalgae (Boussiba 2000; Mogedas et al. 2009; Rabbani et al. 1998; Solovchenko et al. 2009) and higher plants (Hagen et al. 1993, 1994; Hormaetxe et al. 2005, 2007; Merzlyak and Solovchenko 2002; Pick 1998; Rabbani et al. 1998). These carotenoids are represented by carotenes (such as β-carotene) and xanthophylls (carotenols) esterified by fatty acids (see Chap. 1). These pigments are localized in lipid globules, which could be situated in the cytoplasm (oil bodies of microalgae; Figs. 4.8, 4.9) or within the stroma of plastids (plastoglobuli of higher plants; Figs. 4.10, 4.11). The amount of extrathylakoid carotenoids could be comparable with or superior to the amount of photosynthetic (involved in light harvesting) carotenoids.

Acclimation to high fluxes of solar radiation is often accompanied by ultrastructural changes of plant cells expanding their capacity for accumulation of screening pigments by means of an increase in the size and number of the structures accumulating these compounds. Thus, elevated UV levels often cause thickening of the epidermis and cuticle (Beggs et al. 1986; Caldwell et al. 1983; Jansen et al. 1998) and the formation of more dense hair layers (Karabourniotis et al. 1992, 1998). It also important to note that in microalgal and higher-plant cells, high light induces the formation of numerous large lipid globules – the versatile structures playing a multifaceted role, including storage of screening carotenoids, antioxidants, and

lipids, the building blocks for membranes facilitating rapid recovery of the photosynthetic apparatus and its protection against photooxidative damage (Austin et al. 2006; Bréhélin and Kessler 2008; Bréhélin et al. 2007; Kessler and Vidi 2007; Khozin-Goldberg et al. 2005; Pick 1998). The massive synthesis of lipids in the course of the formation of lipid globules provides a sink for excessive photosynthates, which is also important for prevention of overreduction of the chloroplast electron transport chain and ROS formation under excessive light (Asada 2006).

References

Agati G, Galardi C, Gravano E, Romani A, Tattini M (2002) Flavonoid distribution in tissues of *Phillyrea latifolia* L. leaves as estimated by microspectrofluorometry and multispectral fluorescence microimaging. Photochem Photobiol 76:350–360

Agati G, Matteini P, Goti A, Tattini M (2007) Chloroplast-located flavonoids can scavenge singlet oxygen. New Phytol 174:77–89

Agati G, Stefano G, Biricolti S, Tattini M (2009) Mesophyll distribution of 'antioxidant' flavonoid glycosides in *Ligustrum vulgare* leaves under contrasting sunlight irradiance. Ann Bot 104:853–861

Asada K (2006) Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiol 141:391–396

Austin J, Frost E, Vidi P, Kessler F, Staehelin L (2006) Plastoglobules are lipoprotein subcompartments of the chloroplast that are permanently coupled to thylakoid membranes and contain biosynthetic enzymes. Plant Cell 18:1693–1703

Avery G Jr (1933) Structure and development of the tobacco leaf. Am J Bot 20:565–592

Awad M, de Jager A, van Westing L (2000) Flavonoid and chlorogenic acid levels in apple fruit: characterisation of variation. Sci Hortic 83:249–263

Awad M, de Jager A, van der Plas L, van der Krol A (2001a) Flavonoid and chlorogenic acid changes in skin of 'Elstar'and 'Jonagold' apples during development and ripening. Sci Hortic 90:69–83

Awad M, Wagenmakers P, de Jager A (2001b) Effects of light on flavonoid and chlorogenic acid levels in the skin of 'Jonagold' apples. Sci Hortic 88:289–298

Baur P, Stulle K, Uhlig B, Schönherr J (1998) Absorption von Strahlung im UV-B und Blaulichtbereich von Blattkutikeln ausgewählter Nutzpflanzen. Gartenbauwissenschaft 63:145–152

Beggs C, Schneider-Ziebert U, Wellmann E (1986) UV-B radiation and adaptive mechanisms in plants. In: Worrest R, Caldwell M (eds) Stratospheric ozone reduction. Solar ultraviolet radiation and plant life. Springer, Berlin, pp 235–250

Ben-Amotz A, Avron M (1983) On the factors which determine massive β-carotene accumulation in the halotolerant alga *Dunaliella bardawil*. Plant Physiol 72:593–597

Ben-Amotz A, Katz A, Avron M (1982) Accumulation of β-carotene in halotolerant alge: purification and characterization of β-carotene-rich globules from *Dunaliella bardawil* (Chlorophyceae). J Phycol 18:529–537

Ben-Amotz A, Shaish A, Avron M (1989) Mode of action of the massively accumulated β-carotene of *Dunaliella bardawil* in protecting the alga against damage by excess irradiation. Plant Physiol 86:1286–1291

Berner T, Dubinsky Z, Wyman K, Falkowski P (1989) Photoadaptation and the "package" effect in *Dunaliella tertiolecta* (Chlorophyceae). J Phycol 25:70–78

Bidel L, Meyer S, Goulas Y, Cadot Y, Cerovic Z (2007) Responses of epidermal phenolic compounds to light acclimation: in vivo qualitative and quantitative assessment using

chlorophyll fluorescence excitation spectra in leaves of three woody species. J Photochem Photobiol B Biol 88:163–179
Bigogno C, Khozin-Goldberg I, Boussiba S, Vonshak A, Cohen Z (2002a) Lipid and fatty acid composition of the green oleaginous alga *Parietochloris incisa*, the richest plant source of arachidonic acid. Phytochemistry 60:497–503
Bigogno C, Khozin-Goldberg I, Cohen Z (2002b) Accumulation of arachidonic acid-rich triacylglycerols in the microalga *Parietochloris incisa* (Trebuxiophyceae, Chlorophyta). Phytochemistry 60:135–144
Biswal B (1995) Carotenoid catabolism during leaf senescence and its control by light. J Photochem Photobiol B Biol 30:3–13
Bornman J (1999) Localisation and functional significance of flavonoids and related compounds. In: Rozema J (ed) Stratospheric ozone depletion: the effects of enhanced UV-B radiation on terrestrial ecosystems. Backhuys, Leiden, pp 59–69
Boussiba S (2000) Carotenogenesis in the green alga *Haematococcus pluvialis*: cellular physiology and stress response. Physiol Planta 108:111–117
Bréhélin C, Kessler F (2008) The plastoglobule: a bag full of lipid biochemistry tricks. Photochem Photobiol 84:1388–1394
Bréhélin C, Kessler F, van Wijk K (2007) Plastoglobules: versatile lipoprotein particles in plastids. Trends Plant Sci 12:260–266
Britton G (1995a) Structure and properties of carotenoids in relation to function. FASEB J 9:1551–1558
Britton G (1995b) UV/visible spectroscopy. In: Britton G, Liaaen-Jensen S, Pfander H (eds) Carotenoids, vol 1B. Birkhauser, Basel, pp 13–62
Caldwell M, Robberecht R, Flint S (1983) Internal filters: prospects for UV-acclimation in higher plants. Physiol Planta 58:445–450
Chalker-Scott L (1999) Environmental significance of anthocyanins in plant stress responses. Photochem Photobiol 70:1–9
Day T, Martin G, Vogelmann T (1993) Penetration of UV-B radiation in foliage: evidence that the epidermis behaves as a non-uniform filter. Plant Cell Environ 16:735–741
Diaz M, Ball E, Luttge U (1990) Stress-induced accumulation of the xanthophyll rhodoxanthin in leaves of *Aloe vera*. Plant Physiol Biochem 28:679–682
Escarpa A, González M (1998) High-performance liquid chromatography with diode-array detection for the determination of phenolic compounds in peel and pulp from different apple varieties. J Chromatogr A 823:331–337
Forsyth W, Simmonds N (1954) A survey of the anthocyanins of some tropical plants. Proc R Soc Lond B Biol Sci 142:549–564
Georgieva K, Sarvari E, Keresztes A (2010) Protection of thylakoids against combined light and drought by a lumenal substance in the resurrection plant *Haberlea rhodopensis*. Ann Bot 105:117–126
Gould K, Markham K, Smith R, Goris J (2000) Functional role of anthocyanins in the leaves of *Quintinia serrata* A. Cunn. J Exp Bot 51:1107–1115
Gross J (1987) Carotenoids: pigments in fruits. Food science and technology. Series of monographs. Academic, London, pp 87–98
Hagen C, Braune W, Greulich F (1993) Functional aspects of secondary carotenoids in *Haematococcus lacustris* [Girod] Rostafinski (Volvocales). IV: protection from photodynamic damage. J Photochem Photobiol B Biol 20:153–160
Hagen C, Braune W, Björn L (1994) Functional aspects of secondary carotenoids in *Haematococcus lacustris* (Volvocales) III. Action as a sunshade. J Phycol 30:241–248
Harborne J (1980) Plant phenolics. In: Bell E, Charlwood B (eds) Secondary plant products. Springer, Berlin, pp 329–402
Harborne J, Williams C (2000) Advances in flavonoid research since 1992. Phytochemistry 55:481–504

Holloway P, Cutler D, Alvin K, Price C (1982) Structure and histochemistry of plant cuticular membranes: an overview. In: Cutler D, Alvin K, Price C (eds) The plant cuticle. Academic, London, pp 1–32

Hormaetxe K, Becerril J, Fleck I, Pintó M, Garcia-Plazaola J (2005) Functional role of red (retro)-carotenoids as passive light filters in the leaves of *Buxus sempervirens* L.: increased protection of photosynthetic tissues? J Exp Bot 56:2629–2636

Hormaetxe K, Becerril J, Hernández A, Esteban R, Garcia-Plazaola J (2007) Plasticity of photoprotective mechanisms of *Buxus sempervirens* L. leaves in response to extreme temperatures. Plant Biol 9:59–68

Hornero-Mendez D, Minguez-Mosquera M (2000) Xanthophyll esterification accompanying carotenoid overaccumulation in chromoplast of *Capsicum annuum* ripening fruits is a constitutive process and useful for ripeness index. J Agric Food Chem 48:1617–1622

Hudák J (1981) Plastid senescence I: changes of chloroplast structure during natural senescence in cotyledons of *Sinapis alba* L. Photosynthetica 15:174–178

Hughes N, Smith W (2007) Attenuation of incident light in *Galax urceolata* (Diapensiaceae): concerted influence of adaxial and abaxial anthocyanic layers on photoprotection. Am J Bot 94:784

Hughes N, Vogelmann T, Smith W (2008) Optical effects of abaxial anthocyanin on absorption of red wavelengths by understorey species: revisiting the back-scatter hypothesis. J Exp Bot 59:3435–3442

Ibdah M, Krins A, Seidlitz H, Heller W, Strack D, Vogt T (2002) Spectral dependence of flavonol and betacyanin accumulation in *Mesembryanthemum crystallinum* under enhanced ultraviolet radiation. Plant Cell Environ 25:1145–1154

Jansen M, Gaba V, Greenberg B (1998) Higher plants and UV-B radiation: balancing damage, repair and acclimation. Trends Plant Sci 3:131–135

Karabourniotis G, Bornman J (1999) Penetration of UV-A, UV-B and blue light through the leaf trichome layers of two xeromorphic plants, olive and oak, measured by optical fibre microprobes. Plant Physiol 105:655–661

Karabourniotis G, Fasseas C (1996) The dense indumentum with its polyphenol content may replace the protective role of the epidermis in some young xeromorphic leaves. Can J Bot 74:347–351

Karabourniotis G, Papadopoulos K, Papamarkou M, Manetas Y (1992) Ultraviolet-B radiation absorbing capacity of leaf hairs. Physiol Planta 86:414–418

Karabourniotis G, Kofidis G, Fasseas C, Liakoura V, Drossopoulos I (1998) Polyphenol deposition in leaf hairs of *Olea europaea* (Oleaceae) and *Quercus ilex* (Fagaceae). Am J Bot 85:1007–1012

Karabourniotis G, Tzobanoglou D, Nikolopoulos D, Liakopoulos G (2001) Epicuticular phenolics over guard cells: exploitation for in situ stomatal counting by fluorescence microscopy and combined image analysis. Ann Bot 87:631

Karageorgou P, Manetas Y (2006) The importance of being red when young: anthocyanins and the protection of young leaves of *Quercus coccifera* from insect herbivory and excess light. Tree Physiol 26:613

Katz A, Jimenez C, Pick U (1995) Isolation and characterization of a protein associated with carotene globules in the alga *Dunaliella bardawil*. Plant Physiol 108:1657–1664

Kessler F, Vidi P (2007) Plastoglobule lipid bodies: their functions in chloroplasts and their potential for applications. Adv Biochem Eng/Biotechnol 107:153

Kessler F, Schnell D, Blobel G (1999) Identification of proteins associated with plastoglobules isolated from pea (*Pisum sativum* L.) chloroplasts. Planta 208:107–113

Khozin-Goldberg I, Shrestha P, Cohen Z (2005) Mobilization of arachidonyl moieties from triacylglycerols into chloroplastic lipids following recovery from nitrogen starvation of the microalga *Parietochloris incisa*. Biochim Biophys Acta 1738:63–71

Kleiner K, Raffa K, Dickson R (1999) Partitioning of 14 C-labeled photosynthate to allelochemicals and primary metabolites in source and sink leaves of aspen: evidence for secondary metabolite turnover. Oecologia 119:408–418

Knee M (1988) Carotenol esters in developing apple fruits. Phytochemistry 27:1005–1009

Kolattukudy PE (1970) Biosynthesis of cuticular lipids. Annu Rev Plant Physiol 21:163–192
Kolattukudy P (1980) Cutin, suberin, and waxes. In: Stumpf P, Conn E (eds) The biochemistry of plants, vol 4. Academic, New York, pp 571–645
Kolb C, Pfundel E (2005) Origins of non-linear and dissimilar relationships between epidermal UV absorbance and UV absorbance of extracted phenolics in leaves of grapevine and barley. Plant Cell Environ 28:580–590
Kolb C, Kaser M, Kopecky J, Zotz G, Riederer M, Pfündel E (2001) Effects of natural intensities of visible and ultraviolet radiation on epidermal ultraviolet screening and photosynthesis in grape leaves. Plant Physiol 127:863–875
Krauss P, Markstadter C, Riederer M (1997) Attenuation of UV radiation by plant cuticles from woody species. Plant Cell Environ 20:1079–1085
Kyparissis A, Grammatikopoulos G, Manetas Y (2007) Leaf morphological and physiological adjustments to the spectrally selective shade imposed by anthocyanins in *Prunus cerasifera*. Tree Physiol 27:849
Lancaster J, Grant J, Lister C, Taylor M (1994) Skin color in apples: influence of copigmentation and plastid pigments on shade and darkness of red color in five genotypes. J Am Soc Hortic Sci 119:63–69
Lee D, Collins T (2001) Phylogenetic and ontogenetic influences on the distribution of anthocyanins and betacyanins in leaves of tropical plants. Int J Plant Sci 162:1141–1153
Lee D, Lowry J, Stone B (1979) Abaxial anthocyanin layer in leaves of tropical rain forest plants: enhancer of light capture in deep shade. Biotropica 11:70–77
Liakopoulos G, Stavrianakou S, Karabourniotis G (2001) Analysis of epicuticular phenolics of *Prunus persica* and *Olea europaea* leaves: evidence for the chemical origin of the UV-induced blue fluorescence of stomata. Ann Bot 87:641
Lichtenthaler H (1969a) Die Plastoglobuli von Spinat, ihre Grösse, Isolierung und Lipochinonzusammensetzung. Protoplasma 68:65–77
Lichtenthaler H (1969b) Plastoglobuli und Lipochinongehalt der Chloroplasten von *Cereus peruvianus* (L.) Mill. Planta 87:304–310
Lichtenthaler HK (2007) Biosynthesis, accumulation and emission of carotenoids, alpha-tocopherol, plastoquinone, and isoprene in leaves under high photosynthetic irradiance. Photosynth Res 92:163–179
Lichtenthaler H, Weinert H (1970) Die Beziehungen zwischen Lipochinonsynthese und Plastoglobulibildung in den Chloroplasten von *Ficus elastica*. Z Naturforsch 25:619–623
Manetas Y (2003) The importance of being hairy: the adverse effects of hair removal on stem photosynthesis of *Verbascum speciosum* are due to solar UV-B radiation. New Phytol 158:503–508
Markham K (1989) Flavones, flavonols and their glycosides. In: Harborne J, Dey P (eds) Methods in plant biochemistry, vol 1. Academic, London, pp 197–235
Markstädter C, Queck I, Baumeister J, Riederer M, Schreiber U, Bilger W (2001) Epidermal transmittance of leaves of *Vicia faba* for UV radiation as determined by two different methods. Photosynth Res 67:17–25
Mazza C, Boccalandro H, Giordano C, Battista D, Scopel A, Ballare C (2000) Functional significance and induction by solar radiation of ultraviolet-absorbing sunscreens in field-grown soybean crops. Plant Physiol 122:117–126
Mendoza H, Martel A, Jimenez del Rio M, Garcia Reina G (1999) Oleic acid is the main fatty acid related with carotenogenesis in *Dunaliella salina*. J Appl Phycol 11:15–19
Merzlyak M, Solovchenko A (2002) Photostability of pigments in ripening apple fruit: a possible photoprotective role of carotenoids during plant senescence. Plant Sci 163:881–888
Merzlyak M, Solovchenko A, Chivkunova O (2002) Patterns of pigment changes in apple fruits during adaptation to high sunlight and sunscald development. Plant Physiol Biochem 40:679–684
Merzlyak M, Solovchenko A, Pogosyan S (2005) Optical properties of rhodoxanthin accumulated in *Aloe arborescens* Mill. leaves under high-light stress with special reference to its photoprotective function. Photochem Photobiol Sci 4:333–340

Merzlyak M et al (2007) Effect of nitrogen starvation on optical properties, pigments, and arachidonic acid content of the unicellular green alga *Parietochloris incisa* (Trebouxiophyceae, Chlorophyta). J Phycol 43:833–843

Merzlyak MN, Chivkunova OB, Solovchenko AE, Naqvi KR (2008) Light absorption by anthocyanins in juvenile, stressed, and senescing leaves. J Exp Bot 59:3903–3911

Meyer S, Louis J, Moise N, Piolot T, Baudin X, Cerovic ZG (2009) Developmental changes in spatial distribution of in vivo fluorescence and epidermal UV absorbance over *Quercus petraea* leaves. Ann Bot 104:621–633

Mogedas B, Casal C, Forján E, Vílchez C (2009) β-Carotene production enhancement by UV-A radiation in *Dunaliella bardawil* cultivated in laboratory reactors. J Biosci Bioeng 108:47–51

Moisan T, Mitchell B (2001) UV absorption by mycosporine-like amino acids in *Phaeocystis antarctica* Karsten induced by photosynthetically available radiation. Mar Biol 138:217–227

Morales F, Abadía A, Abadía J, Montserrat G, Gil-Pelegrín E (2002) Trichomes and photosynthetic pigment composition changes: responses of *Quercus ilex* subsp. ballota (Desf.) Samp. and *Quercus coccifera* L. to Mediterranean stress conditions. Trees Struct Funct 16:504–510

Ntefidou M, Manetas Y (1996) Optical properties of hairs during the early stages of leaf development in *Platanus orientalis*. Aust J Plant Physiol 23:535–538

Olsson L, Veit M, Bornman J (1999) Epidermal transmittance and phenolic composition in leaves of atrazine-tolerant and atrazine-sensitive cultivars of *Brassica napus* grown under enhanced UV-B radiation. Plant Physiol 107:259–266

Pick U (1998) *Dunaliella*: a model extremophilic alga. Isr J Plant Sci 46:131–139

Rabbani S, Beyer P, Lintig J, Hugueney P, Kleinig H (1998) Induced β-carotene synthesis driven by triacylglycerol deposition in the unicellular alga *Dunaliella bardawil*. Plant Physiol 116:1239–1248

Saure M (1990) External control of anthocyanin formation in apple: a review. Scientia Horticulturae 42:181–218

Shick J, Dunlap W (2002) Mycosporine-like amino acids and related gadusols: biosynthesis, accumulation, and UV-protective functions in aquatic organisms. Annu Rev Physiol 64:223–262

Skaltsa H, Verykokidou E, Harvala C, Karabourniotis G, Manetas Y (1994) UV-B protective potential and flavonoid content of leaf hairs of *Quercus ilex*. Phytochemistry 37:987–990

Solovchenko A, Merzlyak M (2003) Optical properties and contribution of cuticle to UV protection in plants: experiments with apple fruit. Photochem Photobiol Sci 2:861–866

Solovchenko A, Schmitz-Eiberger M (2003) Significance of skin flavonoids for UV-B-protection in apple fruits. J Exp Bot 54:1977–1984

Solovchenko A, Avertcheva O, Merzlyak M (2006) Elevated sunlight promotes ripening-associated pigment changes in apple fruit. Postharvest Biol Technol 40:183–189

Solovchenko A, Khozin-Goldberg I, Didi-Cohen S, Cohen Z, Merzlyak M (2008) Effects of light and nitrogen starvation on the content and composition of carotenoids of the green microalga *Parietochloris incisa*. Russ J Plant Physiol 55:455–462

Solovchenko A, Khozin-Goldberg I, Cohen Z, Merzlyak M (2009) Carotenoid-to-chlorophyll ratio as a proxy for assay of total fatty acids and arachidonic acid content in the green microalga *Parietochloris incisa*. J Appl Phycol 21:361–366

Steele M, Gitelson A, Rundquist D, Merzlyak M (2009) Nondestructive estimation of anthocyanin content in grapevine leaves. Am J Enol Vitic 60:87

Steinmüller D, Tevini M (1985) Composition and function of plastoglobuli. Planta 163:201–207

Steyn W, Wand S, Holcroft D, Jacobs G (2002) Anthocyanins in vegetative tissues: a proposed unified function in photoprotection. New Phytol 155:349–361

Strack D, Wray V (1989) Anthocyanins. In: Harborne J, Dey P (eds) Methods in plant biochemistry, vol 1. Academic, London, pp 325–356

Taiz L (1992) The plant vacuole. J Exp Bot 172:113–122

Takahama U (1983) Redox reactions between kaempferol and illuminated chloroplasts. Plant Physiol 71:598–601

Tattini M, Gravano E, Pinelli P, Mulinacci N, Romani A (2000) Flavonoids accumulate in leaves and glandular trichomes of Phillyrea latifolia exposed to excess solar radiation. New Phytol 148:69–77

Tattini M, Galardi C, Pinelli P, Massai R, Remorini D, Agati G (2004) Differential accumulation of flavonoids and hydroxycinnamates in leaves of Ligustrum vulgare under excess light and drought stress. New Phytol 163:547–561

Tevini M, Steinmüller D (1985) Composition and function of plastoglobuli. Planta 163:91–96

Vogt T, Ibdah M, Schmidt J, Wray V, Nimtz M, Strack D (1999) Light-induced betacyanin and flavonol accumulation in bladder cells of *Mesembryanthemum crystallinum*. Phytochemistry 52:583–592

Weger H, Silim S, Guy R (1993) Photosynthetic acclimation to low temperature by western red cedar seedlings. Plant Cell Environ 16:711–717

Winkel-Shirley B (2001) Flavonoid biosynthesis. A colorful model for genetics, biochemistry, cell biology, and biotechnology. Am Soc Plant Biol.

Winkel-Shirley B (2002) Biosynthesis of flavonoids and effects of stress. Current Opinion in Plant Biology 5:218–223

Zhekisheva M, Boussiba S, Khozin-Goldberg I, Zarka A, Cohen Z (2002) Accumulation of oleic acid in *Haematococcus pluvialis* (Chlorophyceae) under nitrogen starvation or high light is correlated with that of astaxanthin esters. J Phycol 38:325–331

Zhekisheva M, Zarka A, Khozin-Goldberg I, Cohen Z, Boussiba S (2005) Inhibition of astaxanthin synthesis under high irradiance does not abolish triacylglycerol accumulation in the green alga *Haematococcus pluvialis* (Chlorophyceae). J Phycol 41:819–826

Chapter 5
Manifestations of the Buildup of Screening Pigments in the Optical Properties of Plants

Abstract Accumulation of screening pigments manifests itself as directional changes in plant optical properties. Understanding the relationships between the magnitude and spectral quality of these changes and the extent of the underlying buildup of screening pigments could provide valuable insights into the status of screening-related photoprotection in plants. This chapter focuses on manifestations of the induction of screening pigments in reflectance and absorption spectra of microalgae and plants and lays a foundation for nondestructive quantification of screening compounds and their efficiency in plants.

Plant cells and tissues comprising many components and structures of different morphology, chemical composition, and physical properties (such as refraction index) and containing high amounts of pigments are intricate, inhomogeneous optical systems (Butler and Norris 1960; Fukshansky 1981; Oborne and Raven 1986; Ustin et al. 2001; Vogelmann 1993). The efficiency of light absorption by both photoprotective pigments depends to a considerable extent, apart from their content, on a number of other factors exerting profound effects on the pigment spectra in planta (Butler and Norris 1960; Gonnet 1999, 2003; Smith and Markham 1998). For example, more than a half of the variation of light absorption by chlorophyll *a* in the cells of the green microalga *Dunaliella tertiolecta* in the course of its acclimation to irradiation intensity is explained by changes in the degree of thylakoid membrane stacking and less than a half is explained by changes in chlorophyll content per se (Berner et al. 1989).

Numerous studies (see, e.g., Merzlyak et al. 2005a, b, 2008a; Solovchenko and Merzlyak 2003, 2008) showed that the rearrangements of the plant pigment apparatus, including buildup of screening pigments during adaptation to illumination conditions, inevitably manifests itself in plant optical properties. Accordingly, the analysis of reflectance and absorption spectra of algae and plants provides valuable

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insights into the status of the photoprotective mechanisms of these organisms. Characteristic changes in absorption and reflection of light induced by accumulation of different screening pigments are considered in the following sections.

5.1 The Factors Affecting In Planta Spectra of Screening Pigments and Radiation Screening Efficiency

To understand the functioning of optical screening of solar radiation in living plants, it is important to realize that the in vivo absorption properties of pigments differ considerably from those of isolated pigments in vitro (Naqvi et al. 1997). Flattening of absorption bands and bathochromic shifts of the maxima are evident in vivo in comparison with absorption spectra of pigments recorded in organic solutions (Fukshansky 1981; Fukshansky et al. 1993; Gitelson et al. 2003b, 2006).

The spectrum shape and the positions of the maxima in pigment spectra are also influenced by the environment (polarity and chemical composition) of the pigment molecules, the organization of the structures containing the pigments, and other effects, such as packaging (Berner et al. 1989; Britton 1995; Gonnet 1999; Merzlyak et al. 2009; Naqvi et al. 1997, 2004; Smith and Markham 1998). Spectral properties of photosynthetic pigments (chlorophylls and carotenoids) are also affected in vivo by proteins which bind them to form pigment–protein complexes of the photosynthetic apparatus (Britton 1995; Green and Durnford 1996).

The spectra of vacuole-contained pigments can be changed significantly as a result of intra- and intermolecular copigmentation, the formation of various complexes, and the effects of pH, metal ion chelation, tautomerism, etc. (Asen et al. 1972; Lancaster et al. 1994; Smith and Markham 1998). These effects lead to a significant hyperchromic effect and profound bathochromic shifts in the absorption spectra of vacuolar contents.

The in planta spectra of screening pigments are also greatly influenced by scattering (multiple internal reflection/refraction) which arises from the complex morphology of plant cell tissues and causes a considerable increase in the effective path length of solar radiation absorption within plant tissues (Butler and Norris 1960; Fukshansky 1981; Vogelmann 1993). As a result, the same amount of a pigment in planta absorbs several times more strongly than in an organic extract (Butler and Norris 1960).

The above-mentioned mechanisms and processes can significantly influence the efficiency of radiation screening in certain wavebands. For example, the long-wavelength absorption maximum of flavonols in vitro is situated in the UV-A region (Markham 1989), but in planta the absorption band is flatter than in solution and its maximum can be shifted 20–30 nm toward longer wavelengths (Smith and Markham 1998). As a result, flavonols obviously absorbing in the UV range (the maximum is at 350–360 nm) begin to exert detectable screening in the broad band from the violet to the blue-green region of the spectrum (Havaux and Kloppstech 2001; Merzlyak et al. 2005b). This is especially significant for protection against

UV-A radiation, which is not so harmful as UV-B radiation but reaches Earth's surface in much higher fluxes in comparison with UV-B radiation (Bjorn and Murphy 1985). Mechanisms similar to those outlined above also influence the in vivo spectra of anthocyanins. As a result, these pigments can gain the ability to intercept visible radiation from the blue-green to the orange part of the spectrum (Merzlyak et al. 2008a, b). This could be of special importance for the protection of senescing leaves and ripening fruit.

The concentration-dependent broadening of screening pigment absorption bands often causes even more profound bathochromic shifts of the long-wavelength absorption slope of phenolic and carotenoid in vivo absorption, enhancing the ability of phenolics and carotenoids to intercept radiation at longer wavelengths, where they possess low absorption coefficients (Markham 1989; Strack and Wray 1989). This effect could be additionally enhanced owing to the lengthening of the absorption optical path due to light scattering (see above).

Collectively, the considerations presented above suggest that the effects influencing the pigment spectra in planta should be taken into account for the correct estimation of real photoprotective capacity of the screening pigments.

5.2 Contribution of Secondary Carotenoids to Absorption of Light by Microalgae

Many microalgae respond to stresses, especially to high light, by changes of the pigment composition, mainly by an increase in the amount of secondary carotenoids on the background of a decline in the amount of chlorophylls (for more details, see Chap. 3) apparent as a change in the suspension coloration toward more yellowish hues (see Fig. 5.1). Even more profound changes in cell suspension color from green to brownish and eventually to reddish could be found in microalgae accumulating red secondary carotenoids (Boussiba 2000; Czygan 1970).

The analysis of the stress-induced changes in scattering-corrected (Merzlyak and Naqvi 2000) suspension absorption spectra normalized to the red chlorophyll maximum (see Figs. 5.2 and 5.3) suggests that these manifestation are accompanied by a considerable increase in the relative contribution of carotenoids to light absorption by the cells of microalgae (Merzlyak et al. 2007; Solovchenko et al. 2009).

Similar effects on light absorption by microalgal cells can be exerted by different trends of changes in pigment composition. An increase in the content of carotenoids on the background of a constant content of chlorophylls such as occurs in *Parietochloris incisa* grown on complete medium under high light (Figs. 3.8 and 5.2a, c) and a decrease in the content of chlorophylls on the background of constant carotenoid content (Figs. 3.8 and 5.2b, d) are apparent as a rise of the carotenoid contribution to the absorption (Fig. 5.2c, d). The spectral contribution of secondary xanthophylls and carotenes has a characteristic three-headed shape (Fig. 5.2c, d; Britton 1995). The contribution of reddish ketocarotenoids exhibits a shoulder or a single maximum in the green region of the spectrum (such as recorded in *Haematococcus pluvialis*;

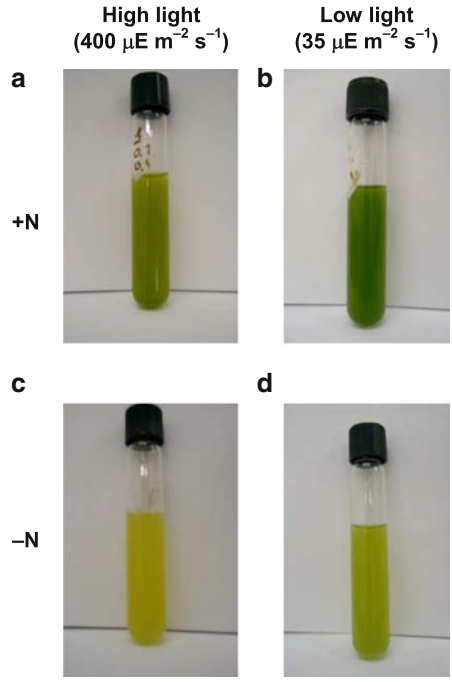

Fig. 5.1 *Parietochloris incisa* cell suspensions grown under different conditions. Note more *yellowish coloration* of the suspension grown under high light (cf. **a** and **b**, **c** and **d**) which is exacerbated by additional stress imposed by nitrogen deficiency (cf. **a** and **d**). (Solovchenko, unpublished)

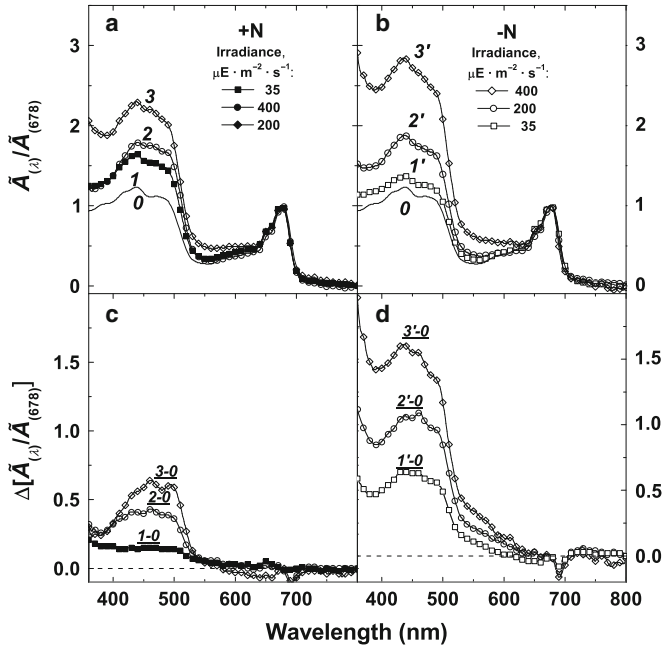

Fig. 5.2 Average scattering-corrected absorption spectra (*1–3*) of *P. incisa* cell suspensions differing in carotenoid-to-chlorophyll ratio grown on complete (**a**) and nitrogen-free (**b**) media and their difference spectra (*2–1*, *3–1*). (Solovchenko, unpublished)

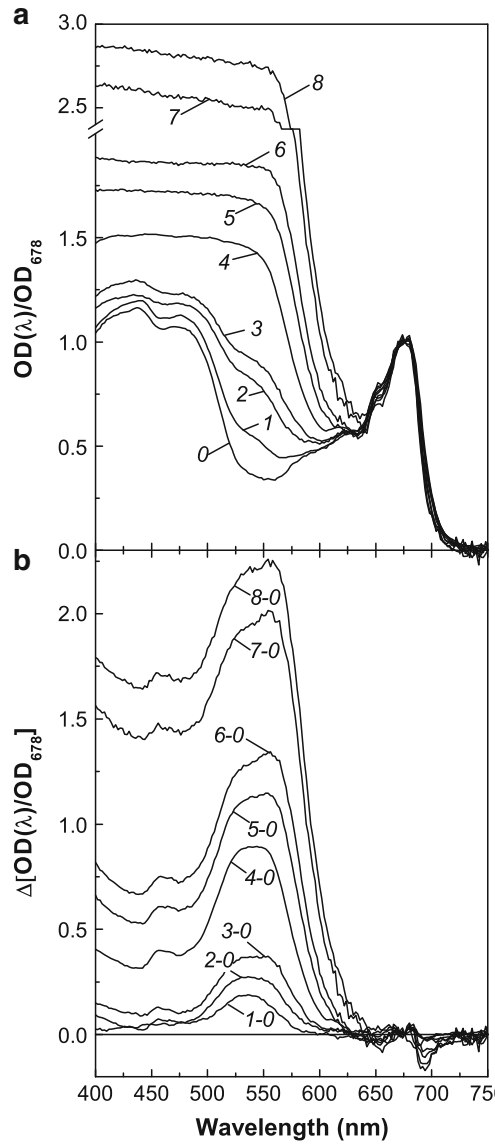

Fig. 5.3 (**a**) Typical changes in absorption spectra of *green vegetative cells* of *Haematococcus pluvialis* (**a**, *curve 0*) during 8 days of cultivation under high (350 μE m^{-2} s^{-1} photosynthetically active radiation) light and (**b**) corresponding difference spectra. The culture age (days) is indicated near the corresponding curves. Note the considerable increase of the absorption in the *green part* of the spectrum (the maximum near 550 nm in the difference spectra). (Solovchenko, unpublished)

Fig. 5.3). Notably, accumulation of high amounts of these pigments brings about the disappearance of the fine structure and a considerable flattening of the whole-cell suspension spectrum (Fig. 5.3, curves 4–8). Comparison of whole-cell, cell homogenate (Fig. 5.4), and pigment extract absorption spectra suggests that these changes are due to strong effects of packaging, stemming presumably from high

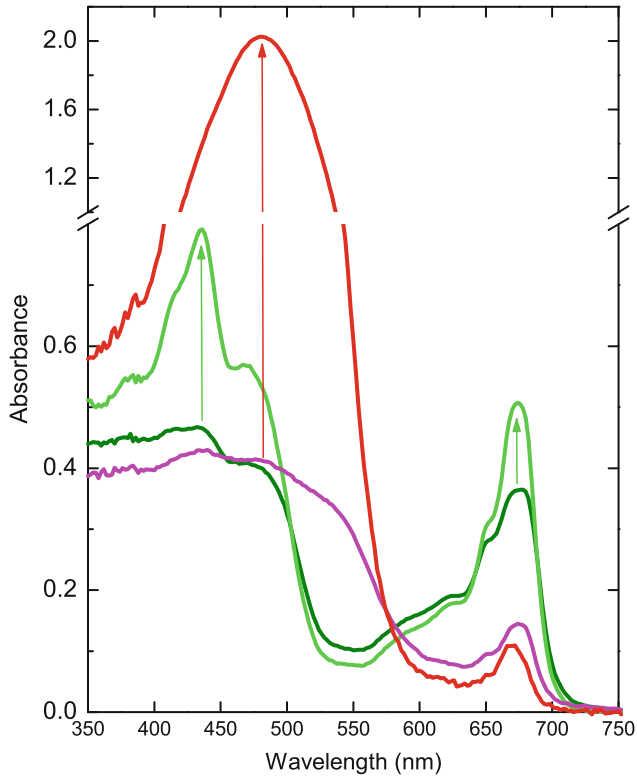

Fig. 5.4 The effect of homogenization on absorbance of *H. pluvialis* cells. The spectra of vegetative cells (*dark green*) and astaxanthin-accumulating cysts (*magenta*) before and after homogenization (*green* and *red*, respectively) are shown. Note the increase in the absorbance of the *red cell sample* in the *green region* of spectrum after homogenization. (Merzlyak and Solovchenko, unpublished)

local concentration of astaxanthin in the algal cell. On the other hand, these measurements clearly demonstrate the difficulties associated with the assessment of the in vivo screening efficiency from spectral measurements carried out on whole-cell suspensions. Probably, fluorescence-excitation-based techniques (see Chap. 6) will yield more realistic estimations in this case.

The analysis of individual carotenoid composition (Fig. 3.9) and localization (Figs. 4.9, 4.13) suggests the observed changes in whole-cell absorbance could be ascribed to an increase in absorption of light by secondary (extrathylakoid) carotenoids, namely, to the β-carotene localized within cytoplasmic oil bodies. A similar reasoning applies to the interpretation of stress-induced changes in light absorption observed in a number of other algal species accumulating red ketocarotenoids outside thylakoids such as *H. pluvialis* (Fig. 5.3; see also (Boussiba 2000; Wang et al. 2003; Chap. 2).

It is important to note that the combined stress such as that imposed by cultivation under high light with simultaneous deprivation of algae of nitrogen often causes more profound stress in comparison with single stress but qualitatively the same changes in the spectral absorption of secondary carotenoids (Fig. 5.2). One could think that combined stress impairs the ability of algal cells to utilize photosynthates more significantly than a single stress causing higher steady-state reactive oxygen species levels which eventually lead to the enhanced accumulation of screening carotenoids (Fabregas et al. 2003; Steinbrenner and Linden 2003; Vidhyavathi et al. 2008); see also Chap. 3.

It is important to note that, under stressful conditions, especially under high light, the increase in the contribution of carotenoids is often accompanied by an increase in absorption in the 400–410-nm band (Fig. 5.2d). Similar absorption changes were recorded in autotrophic microorganisms accumulating high amounts of mycosporine-like amino acids (MAA) (Singh et al. 2008; Sinha and Häder 2007).

5.3 Stress-Induced Changes in Optical Properties of Cell Structures Containing Screening Pigments

Knowledge of the optical properties of subcellular structures where the screening pigments are deposited (Chap. 4) facilitates the interpretation of the effects of screening pigment buildup observed on light absorption by plant assimilatory organs or algal cell suspensions. These measurements provide more direct information on the spectral properties of screening pigments in situ (phenolics dissolved in vacuolar sap or carotenoids distributed in the environment of lipid globules). On the other hand, reservations should be made about the assessment of screening efficiency solely from the in vivo spectra of plastids and/or vacuoles since it do not necessarily reflect the influence of scattering by complex structures of plant tissue (see Sect. 5.1).

5.3.1 Anthocyanin-Containing Vacuoles

Microspectrophotometric measurements of vacuolar anthocyanin absorption (Merzlyak et al. 2008a) revealed its close similarity in shape and maxima positions to the contribution of anthocyanins to light absorption by whole leaves obtained by comparing selected anthocyanic and acyanic leaves with similar chlorophyll absorption in the red region (see Fig. 5.5; for a discussion of anthocyanin effects on plant reflectance, see Sect. 5.5.2). The increased absorption at wavelengths shorter than 440 nm frequently recorded in vacuoles of anthocyanic leaves is consistent with the presence of UV-absorbing flavonols and other phenolics (Cerovic et al. 2002). The analysis of chlorophyll and anthocyanin absorption shown a sharp distinction in the absorption by chloroplast chlorophylls and

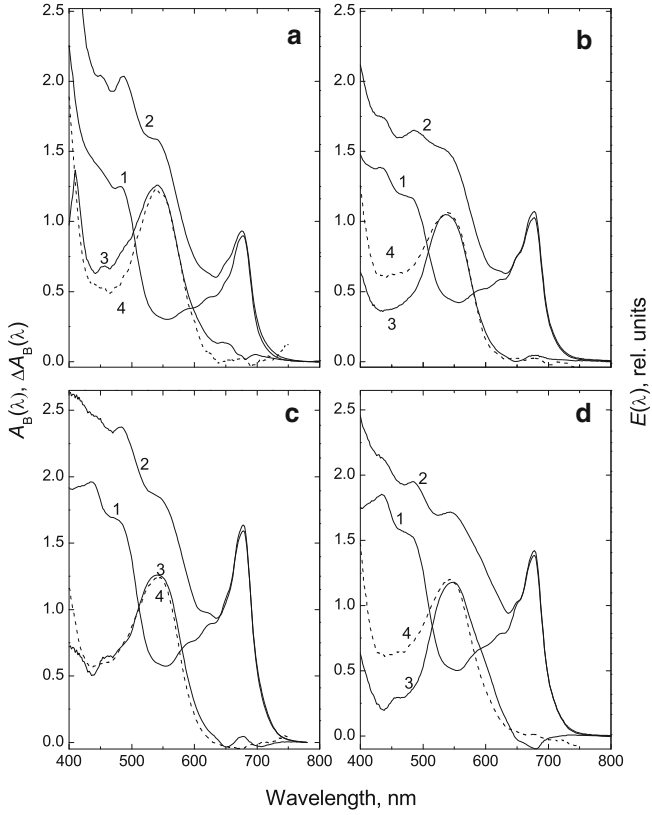

Fig. 5.5 Attenuation plots of *green* (*1*) and *red* (*2*) autumn leaves and their difference (anthocyanic minus acyanic) spectra (*3*; *left scale*). Scaled attenuation plots of vacuoles measured in cross sections of anthocyanic leaves are plotted on the *right scale* (*broken lines*). Note the similarity of spectra *3* and *4*. (**a**) *Acer platanoides*, (**b**) *Parthenocissus quinquefolia*, (**c**) *Cotoneaster alaunica*, and (**d**) *Cornus alba*. (Reproduced from Merzlyak et al. (2008a, b) with permission from Oxford University Press)

vacuolar anthocyanins and strongly suggests independence of their contributions in light absorption by the leaves. Interestingly, the quantitative data on anthocyanin absorption suggest that (in contrast to chlorophyll) light absorption by vacuolar anthocyanins in leaves behaves similarly to that in concentrated pigment solution as affected by pigment aggregation and copigmentation and, to a large extent, follows a form of the Lambert–Beer law (Merzlyak et al. 2008a).

5.3.2 Carotenoid-Accumulating Plastids

Comparison of the single chloroplast absorption spectra of *P. incisa* grown under low light on complete medium and under high light under nitrogen deprivation stress (Fig. 5.6) displayed changes similar to those recorded in whole-cell suspension.

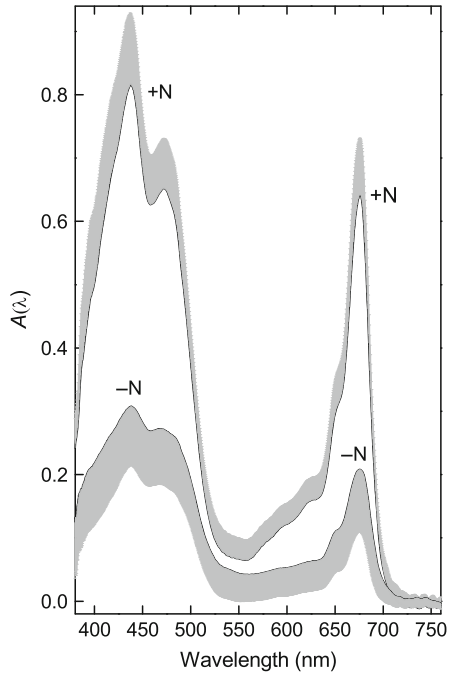

Fig. 5.6 Microspectrophotometry of *P. incisa* cells with nitrogen and deprived of nitrogen. Averaged ($n = 17$) absorption spectra ± standard deviation are given for cells with nitrogen and deprived of nitrogen, respectively. (Reproduced from Merzlyak et al. (2007) with kind permission from John Wiley and Sons)

In the red-orange range, the spectra contained details characteristic of chlorophyll *a* and *b* absorption; in the blue region, the spectra contained distinct maxima near 437 and 467–471 nm. The difference spectra obtained by subtraction of the normalized spectra shown in Fig. 5.6 revealed maxima near 461 and 496 nm (not shown), which suggest an increase in the contribution of carotenoids to the absorption of photosynthetically active radiation (PAR) by the chloroplasts relative to that of chlorophylls. Notably, the chloroplasts of stressed algal cells (Fig. 5.6) possess, unlike whole-cell suspensions (Fig. 5.2), low absorption in the blue-violet range of the spectrum, suggesting the absence of UV-absorbing compounds, presumably MAA, in chloroplasts. This is consistent with current knowledge on MAA localization in algal cells (see Chap. 4; Shick and Dunlap 2002).

Generally, higher-plant plastids undergoing chloroplast-to-chromoplast transformation higher gradually lose chlorophyll, turn yellowish or reddish, and spectral features of carotenoid absorption appear in their spectra (see Fig. 5.7), strongly suggesting retention of carotenoids over chlorophylls. An interesting case from the standpoint of in situ spectroscopy of single plastids is that of *Aloe arborescence* accumulating rhodoxanthin under stressful conditions (see Chaps. 2–4). At advanced stages of chlorophyll degradation and leaf reddening, two types of carotenoid absorption become apparent in the spectra of *A. arborescence* plastids. Comparison of plastid spectra with reconstructed absorption spectra of carotenoids in leaf and fruit extracts (see Fig. 3.11) makes it possible to distinguish two main types of carotenoid absorption in plastids: attributable to non-ketocarotenoids

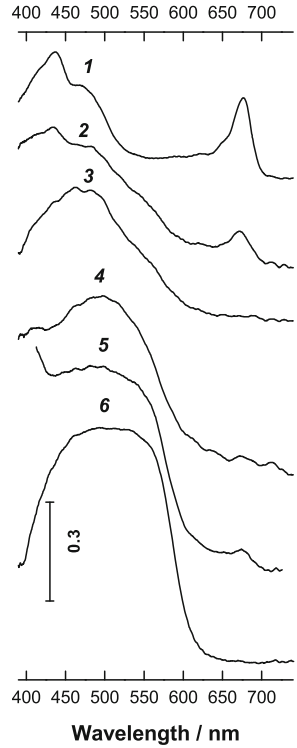

Fig. 5.7 Typical absorption spectra of aloe plastids of green-to-red leaves. (Reproduced from Merzlyak et al. (2005a, b) with permission from the Royal Society of Chemistry for the European Society for Photobiology, the European Photochemistry Association, and the Royal Society of Chemistry)

(photosynthetic carotenoids, in the range 460–500 nm) and to rhodoxanthin at longer wavelengths, appearing first as shoulders in the 520–550-nm region and than as a broad band centered at 500 nm (Fig. 5.7, curve 6). Such a large shift of the absorption maximum in vivo compared with solutions (see Fig. 3.14 ; Britton 1995; Diaz et al. 1990) may involve aggregation of the pigment owing to its high local concentration presumably in lipid globules. The contributions of non-ketocarotenoids and rhodoxanthin to the absorption of individual plastids are different even in uniformly colored leaf samples and frequently the spectral features of rhodoxanthin appear on the background of non-ketocarotenoid absorption (Fig. 5.7, curves 3–6).

5.4 Selective Screening of PAR and UV Radiation by Cuticle and Epidermis

The buildup of phenolic compound is a basic response of higher plants to elevated levels of solar radiation (Barnes et al. 2000; Caldwell et al. 2007). The bulk of the phenolics accumulated under high-sunlight stress are situated within a superficial protective complex comprising cuticle, epidermis, and several underlying cell layers (see Chap. 2; Bornman 1999; Kolb and Pfundel 2005; Solovchenko and Merzlyak 2003; Solovchenko and Schmitz-Eiberger 2003).

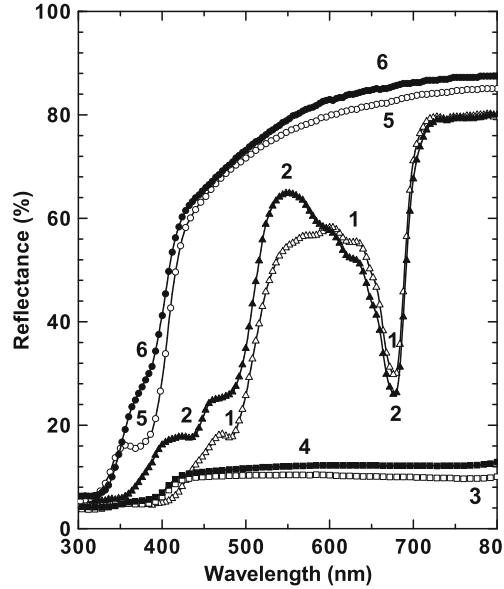

Fig. 5.8 Representative reflectance spectra of a whole apple fruit (*1* and *2*) and its cuticles (*3–6*). Sunlit (*1*, *3*, and *5*) and shaded (*2*, *4*, *6*) surfaces of a fruit and its cuticles are shown as *open symbols* and *closed symbols*, respectively. Spectra *3* and *4* and *5* and *6* were recorded on *black* and *white* backgrounds, respectively. (Reproduced from Solovchenko and Merzlyak (2003) with permission from the Royal Society of Chemistry for the European Society for Photobiology, the European Photochemistry Association, and the Royal Society of Chemistry)

According to measurements carried out on preparations (Baur et al. 1998; Krauss et al. 1997; Solovchenko and Merzlyak 2003), plant cuticles do not exhibit measurable absorption in the near-IR and visible parts of the spectrum and transmit nonreflected PAR nearly completely (80–98% of incident radiation), reflecting, on average, 15–17% (Fig. 5.8), which is consistent with high demands for light in photosynthesis.

Compared with PAR, cuticular reflectance and transmittance of UV radiation were much lower owing to the presence of UV-absorbing constituents. In particular, the phenolics accumulating in the cuticle modify its optical properties, making it a nonuniform filter selectively absorbing radiation in the UV region (Fig. 5.8). This effect is especially apparent in the reflectance spectra of cuticle preparations recorded on the highly reflective background (Fig. 5.8. curves 5, 6) as well as in transmittance spectra (Fig. 5.9).

Transmittance spectra of cuticles usually contain no bands attributable to chromophore absorption in the visible range displaying a monotonous decrease with wavelength, suggesting a strong influence of scattering in this range. In the UV-A range, cuticular transmittance spectra possess spectral features near 350–370 nm (shoulders or pronounced maxima in the case of samples adapted to low of high fluxes of solar radiation, respectively). Regardless of the illumination conditions, the cuticle possesses low transmittance in the UV-B range. Transmission of cuticles in the UV-A range is comparable to (in apple) or considerably higher than (in leaves) that in the UV-B range. Taking in account light losses due to scattering, apple cuticles transmit approximately 55% (shaded) and 40% (sunlit) of nonreflected radiation in the UV-A range (near 360 nm) and less than 20% in the UV-B range.

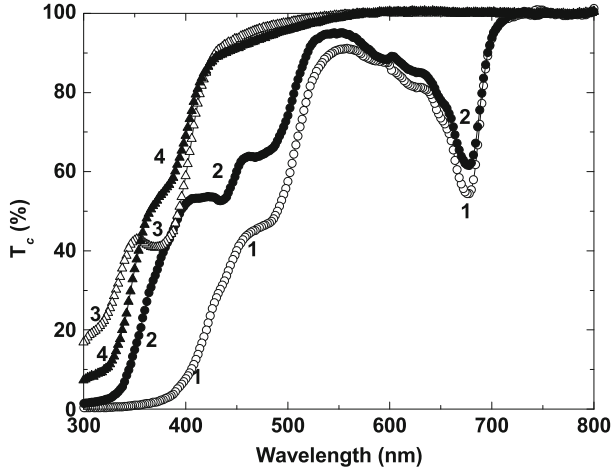

Fig. 5.9 Representative scattering-corrected transmittance spectra of skin (including cuticle, epidermis and three to five hypodermal cell layers, see Fig. 4.4) samples (*1*, *2*) and cuticles from sunlit (*1*, *3*) and shaded (*2*, *4*) fruit surfaces. Whole-fruit reflectance spectra are shown in Fig. 5.8. (Reproduced from Solovchenko and Merzlyak (2003) with permission from the Royal Society of Chemistry for the European Society for Photobiology, the European Photochemistry Association, and the Royal Society of Chemistry)

It should be noted that transmission of UV radiation, especially UV-A radiation, by preparations of apple skins which include cuticle, epidermal,, and several hypodermal cell layers is considerably lower in comparison with that of cuticles obtained from the same skin preparations (cf. 1–4 in Fig. 5.9). For example, transmittance of the skin isolate from a sunlit apple surface was less than 2.5% at wavelengths shorted than 360 nm, whereas the skin from shaded apple surface transmits, on average, 20% of UV-A radiation. The skin preparations possessed very low transmittance (less than 2%) in the UV-B region regardless of the illumination conditions.

In the case of apple fruit cuticle, methanol-extractable flavonoids are responsible for attenuation of nonreflected UV-A radiation by 60–95% (Fig. 5.10). In contrast, compounds responsible for 80–90% of absorption of UV-B radiation (the band centered near 300 nm; Fig. 5.9) are not readily extractable by methanol (spectra 2 and 4 in Fig. 5.10) and their spectral features were retained in the spectra of the cuticle even after repeated extraction. According to data in the literature, cuticular UV-B-absorbing compounds are covalently bound phenolics. In leaf cuticles hydroxycinnamic acid derivatives are esterified by fatty acids and covalently bound to cutin (Baur et al. 1998). Higher UV-B absorption by phenolics in shaded versus sunlit cuticles does not seem to be controversial, since the synthesis of hydroxycinnamic acid is considered to be largely unaffected by ambient radiation conditions (Burchard et al. 2000).

Quantitative analysis did not show a gross difference in flavonoid content between shaded and sunlit cuticles (Fig. 5.11) as was found in the skin (Merzlyak et al. 2002; Solovchenko and Schmitz-Eiberger 2003). The analysis of methanolic

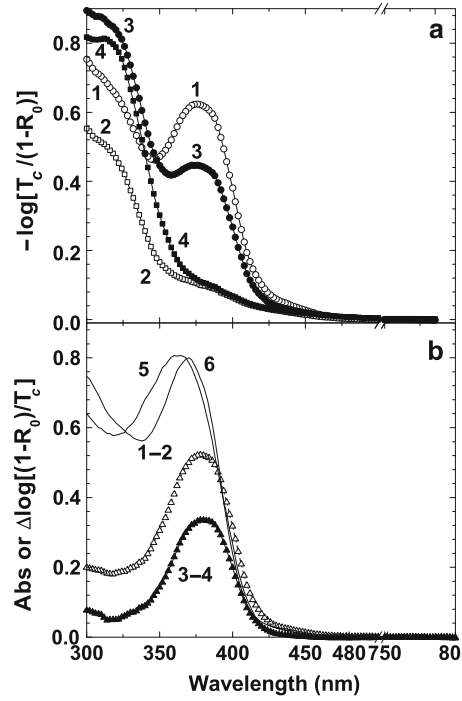

Fig. 5.10 Absorption spectra of apple fruit cuticles and of cuticular methanolic extracts. **a** Average corrected spectra of *sunlit* (*1*, *2*) and *shaded* (*3*, *4*) cuticles before (*1*, *3*) and after (*2*, *4*) extraction with methanol. **b** Difference "nonextracted minus extracted" absorption spectra of sunlit (*1–2*) and shaded (*3–4*) cuticles (see **a**). Representative spectra of methanolic extract of cuticles before (*5*) and after (*6*) acid hydrolysis. *Abs* absorbance of extract. (Reproduced from Solovchenko and Merzlyak (2003) with permission from the Royal Society of Chemistry for the European Society for Photobiology, the European Photochemistry Association, and the Royal Society of Chemistry)

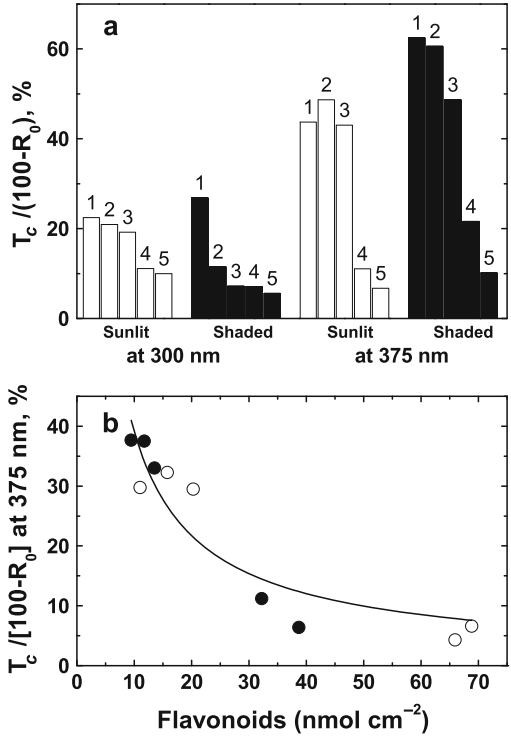

Fig. 5.11 Relationship between nonreflected light transmittance by cuticle and cuticular flavonoid content. **a** Transmittance at 300 and 375 nm of *sunlit* and *shaded* cuticles isolated from five apple samples ordered according to the increase in cuticular flavonoid content. **b** Relationship between transmittance at 375 nm and flavonoid content for cuticles isolated from sunlit (*open symbols*) and shaded (*closed symbols*) fruit surfaces The *line* is the best-fit function. (Reproduced from Solovchenko and Merzlyak (2003) with permission from the Royal Society of Chemistry for the European Society for Photobiology, the European Photochemistry Association, and the Royal Society of Chemistry)

extracts revealed that the principal UV-A-absorbing compound was a quercetin glycoside, highly abundant in apple fruit skin (Awad et al. 2000). Cuticular flavonoids were characterized by long-wavelength absorption maximum located at 375 nm in situ (Fig. 5.10a) and at 368 nm in methanol (Fig. 5.10b), whereas the broad maximum of skin flavonoids in the same solvent is situated near 360 nm (Solovchenko et al. 2001).

Spectrophotometry, acid hydrolysis (Fig. 5.10), and thin-layer chromatography of the cuticular and skin extracts showed that methanol-extractable cuticular and skin flavonoids have the same aglycone, quercetin. Transmittance of a cuticle at 375 nm correlated exponentially with flavonoids in the whole range of its content. However, an increase in flavonoid content over 40 nmol cm^{-2} did not affect significantly cuticular transmittance, comprising 5–7% (Fig. 5.11). It is noteworthy that the UV-screening efficacy of apple fruit cuticle is quantitatively comparable with that of detached leaf epidermis: cf. Fig. 5.9 and Fig. 1 in Markstädter et al. (2001).

Collectively, the data presented and referred to above suggest that phenolics accumulating in plant cuticles are able to attenuate a significant part of UV-A radiation and an even more significant part of UV-B radiation before they reach sensitive structures and components of epidermal and hypodermal cells. The screening ability of the cuticle depends on the UV range. In the UV-B region, it serves as a quite efficient external cutoff filter; in the UV-A region, its shielding is somewhat less efficient. One may speculate that this is the consequence of the limited capacity of hydrophobic cuticular membranes to accommodate relatively polar molecules of flavonols. These compounds simply could not accumulate there in the amounts necessary for efficient UV-A screening (the cuticular flavonol content recorded in our experiments did not exceed 70 nmol cm^{-2} or 20% of the total flavonol content in the fruit skin).

Comparison of cuticles isolated from shaded and sunlit fruit surfaces (adapted to low and high fluxes of solar radiation, respectively) did not reveal a distinct effect that could be attributed to an effective adaptation to solar UV radiation, although the flavonoid content tended to increase in fruits exposed to strong sunlight (Fig. 5.11). Therefore, it is possible to suggest a limited potential of cuticular phenolics for UV adaptation (see above). It appears that the UV-absorbing components of the plant cuticle, including phenolics covalently bound to it, provide reliable constitutive protection against UV-B radiation, whereas the content of vacuolar flavonols of epidermal and hypodermal cells is much more responsible for protection against UV-A radiation and evidently plays a key role in acclimation to it.

5.5 The Influence of Screening Pigment Accumulation on Whole-Plant Optical Spectra

The acclimation of plants to high fluxes of solar radiation causes significant rearrangements of the pigment apparatus (including both photosynthetic chlorophylls and carotenoids and screening pigments), inevitably apparent as changes in

their reflectance spectra. Understanding the relationships between stress-induced changes in plant reflectance and the underlying changes in pigment composition is a prerequisite for the development of techniques for nondestructive quantification and monitoring of screening pigments in situ (for more information on optical reflectance-based estimation of screening pigment contents, see Chap. 6). Knowledge of these relationships also makes it possible to estimate the state of acclimation of plants to strong sunlight and other stresses inducing the buildup of screening pigments (Merzlyak et al. 2002, 2005a, b, 2008a; Solovchenko and Merzlyak 2003, 2008).

Specifically, the observed changes of the reflectance spectra depend on the ability of plants to synthesize different groups of screening pigments and the patterns of long-term adaptation of photosynthetic pigments to high (sun)light. Briefly, a characteristic and ubiquitous feature of acclimation to strong sunlight is a decrease of the reflectance coefficients in the UV region and the blue-violet part of the visible spectrum (Fig. 5.12) owing to accumulation of high amounts of phenolic compounds, mainly flavonols or phenylpropanoids (see Chap. 3; Sect. 5.5.1). In species accumulating anthocyanins or other screening pigments with similar spectral properties (such as betalains and red ketocarotenoids) a decrease of reflectance in the green region of the spectrum is observed (as in the apple cultivars developing red blush on a sunlit surface; Fig. 5.12b, curve 2). In species deprived of the ability to synthesize anthocyanins or similar pigments, an increase in extrathylakoid carotenoids is often observed, causing a decrease of reflectance and the appearance of characteristic spectral features of carotenoids in the blue-green region of the spectrum (as in apple cultivars with green-yellow fruit; Fig. 5.12a). These basic responses are often superimposed (Fig. 5.12b, curve 3), making the overall picture of stress-induced reflectance changes quite complicated.

It should be emphasized that both photoacclimation and photodamage can bring about a similar decline in chlorophyll content and a corresponding increase in reflectance (Fig. 5.12a, c). However, photodamage induces a simultaneous reflectance increase in the blue region owing to synchronous bleaching of chlorophylls and carotenoids. In contrast, the reflectance in the blue-green range displays a decrease during photoacclimation owing to accumulation of extrathylakoid carotenoids (cf. curves 3 in Fig. 5.12a, c; see also Merzlyak and Solovchenko 2002; Merzlyak et al. 2002).

The next sections are devoted to more detailed analysis of the effect of accumulation of key groups of screening pigments and laying a foundation for the development of methods for their assessment in situ described in detail in Chap. 6.

5.5.1 Manifestations of the Buildup of Flavonols in Reflectance Spectra

The analysis of the effects of solar-radiation-induced flavonol buildup in plant tissues, which exhibit low reflectance in the UV region, particularly in the UV-A

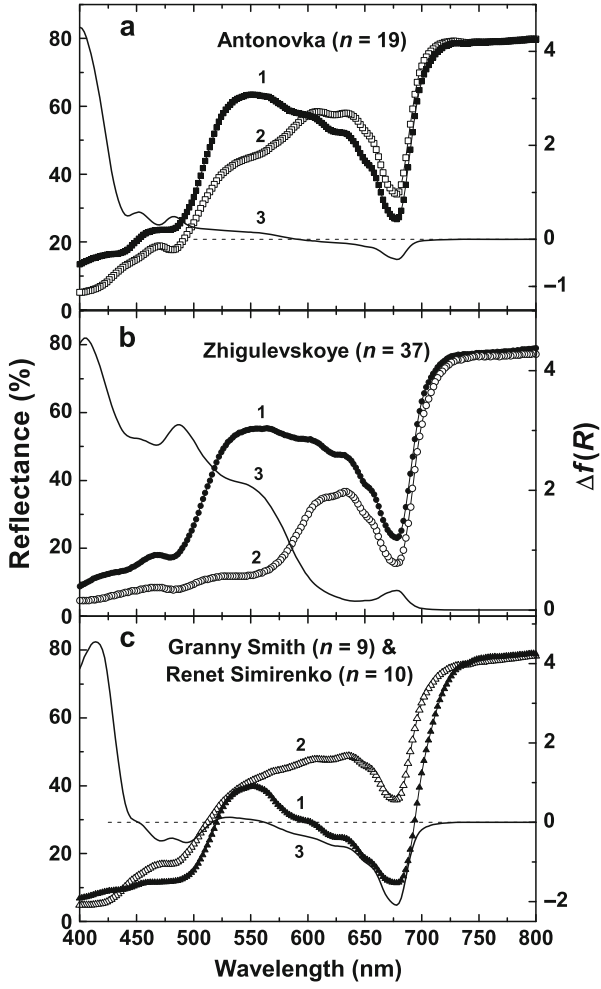

Fig. 5.12 Average reflectance spectra recorded from sunlit and shaded surfaces in (**a**) and (**b**) and from undamaged and sunburn-affected zones in (**c**). *1 shaded* (or undamaged) and *2 sunlit* (or sunburn-affected) sides of apple fruit (*left scale*) and *3* the difference analogous to the absorption remission function, *f*(*R*), calculated for the spectra *1* and *2* (*right scale*). Note a decrease of reflectance in the *blue region* [apparent as a positive peak in the difference *f*(*R*) spectra] in (**a**–**c**) and a decrease of reflectance in the *broad blue-green-to-orange part* in anthocyanin-containing fruit (**b**). (Reprinted from Merzlyak et al. (2002) with permission from Elsevier)

region, is greatly complicated by overlapping of their absorption with the absorption of several pigments: chlorophylls, carotenoids, and UV-B-absorbing phenolics (such as catechins and phenolic acids), which are less inducible by solar UV radiation (Awad et al. 2001; Burchard et al. 2000). In addition, scattering exerts a strong influence on UV reflectance: it was reported that the scattering coefficients of whole fruit (Taroni et al. 2003) and the skin and isolated cuticles of apples

(Merzlyak et al. 2005b) are wavelength-dependent and undergo an increase with wavelength decrease. Apple fruit possessing well-resolved reflectance spectra and displaying a pronounced buildup of flavonols in response to strong sunlight appears to be a convenient model to study the reflectance changes in the course of flavonol accumulation (Merzlyak et al. 2005b).

In fruits with low flavonol content, the reflectance between 440 nm (in vivo maximum of chlorophyll *a* absorption) and 400 nm was flat and then showed a monotonous decline reaching its minimum near 360 nm (Fig. 5.13a), close to the rutin absorption maximum in solution (see Fig. 3.4). These spectral features in the UV region could, to a certain degree, be related to the optical properties of cuticles: those isolated from shaded surfaces of Antonovka apples contained a considerable amount of skin flavonols as well as other phenolics and contributed appreciably to whole-fruit reflectance in this spectral range (see Sect. 5.4).

The accumulation of flavonols occurring mainly in the vacuoles of subcuticular (epidermal and hypodermal) cell layers of the skin (see Chap. 4) is accompanied by a sharp decrease of fruit reflectance and flattening of the spectrum in the broad band between 360 and 420 nm (Fig. 5.13a). Interestingly, whole-fruit reflectance in this spectral range did not drop below 4–5% even for high flavonol content. This effect is attributable to reflectance from surface fruit structures, in particular, to the cuticle, which contains a relatively low proportion (about 20%) of skin flavonols and exhibits considerable scattering (Merzlyak et al. 2005b).

In apple fruit, reflectance near 360 nm is quickly saturated, reaching its minimal values at relatively low skin flavonol content. The buildup of flavonol brought about a broadening of the absorption band and manifested itself as the extension of the region with very low reflectance from the near-UV region to the violet part of the visible spectrum (Fig. 5.13a). An increase in skin flavonol content up to about 140 nmol cm^{-2} was accompanied by movement of the edge of the fruit reflectance spectrum toward longer wavelengths (Fig. 5.13b), with the shift in the position of the inflection point from about 364 to 425 nm (Fig. 5.13c). Qualitatively similar changes in reflectance have been observed in leaves and fruits in the presence of high amounts of chlorophyll (Gitelson et al. 2003a, b; Merzlyak et al. 2003) and anthocyanins (Gitelson et al. 2009; Steele et al. 2009) in the red and green regions of the spectrum, respectively.

The flavonol-dependent shift of the edge of the reflectance spectrum in apple fruit was as high as 60 nm (Fig. 5.13). In addition to the concentration-dependent effect of flavonols on spectral reflectance, the changes observed could be related to their intermolecular interactions, such as copigmentation and aggregation, resulting in a considerable bathochromic shift of their absorption band as suggested as an explanation for the yellow coloration exhibited by flower petals of certain plant species (Smith and Markham 1998). This mechanism is quite possible since the local concentration of flavonols in vacuoles of apple skin cells is extremely high, reaching 1.7×10^{-2} mol L^{-1} (Lancaster et al. 1994). As a result of accumulation of high amounts of flavonols, the spectral features of chlorophyll *a* in the Soret band were masked by flavonol absorption both in whole apple fruit reflectance (Fig. 5.13a, curves 4 and 5) and in absorption spectra of skin extracts.

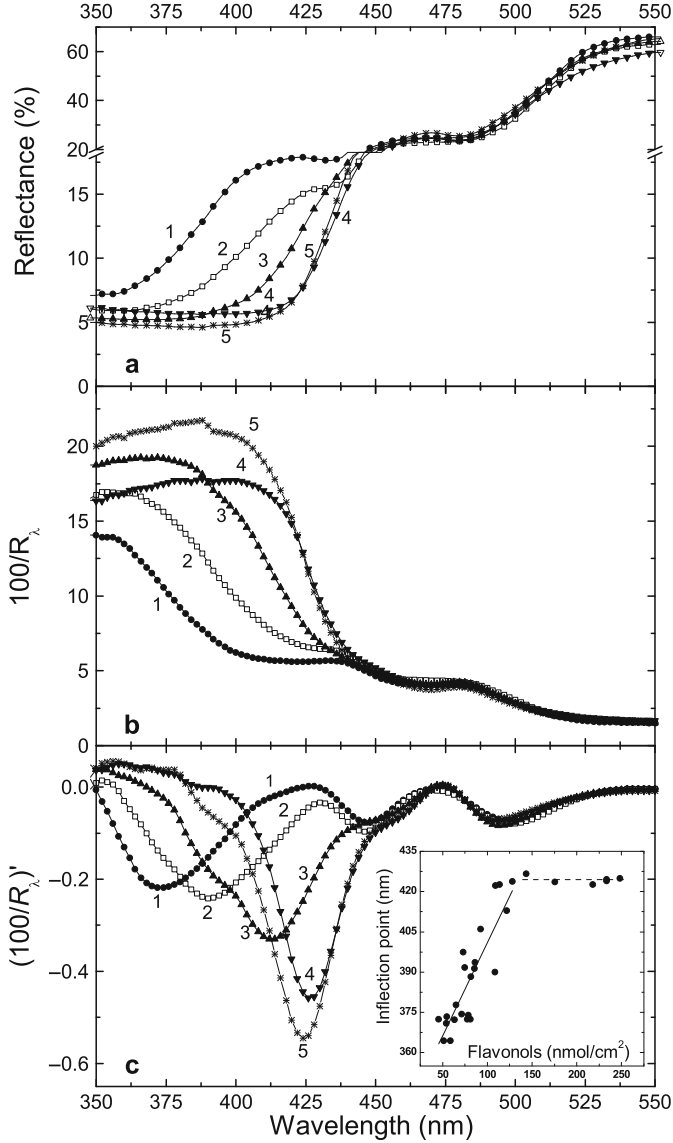

Fig. 5.13 (**a**) Reflectance spectra of Antonovka apple fruits with different skin flavonol content (*1* 45.7, *2* 108.5, *3* 121.8, *4* 143.5, and *5* 233.8 nmol cm^{-2}). (**b**, **c**) Corresponding reciprocal reflectance spectra and their first derivatives, respectively. (Reprinted from Merzlyak et al. (2005a, b) with permission from Elsevier)

The influence of high flavonol content on fruit reflectance in the 350–430-nm band is also evident in the spectra as *r*, the correlation coefficient calculated for the relationship "reflectance versus pigment content." An increase of *r* for flavonol

coincided with a sharp decrease of correlation both for chlorophylls and carotenoids (Fig. 5.14). Distinct positive peaks of correlation between reflectance and flavonol content (Fig. 5.14a) were observed even in the region (green) of high reflectance (maxima near 530 nm) as well as corresponding negative peaks in the case of chlorophyll (Fig. 5.14b) and carotenoids (Fig. 5.14c). The presence of these

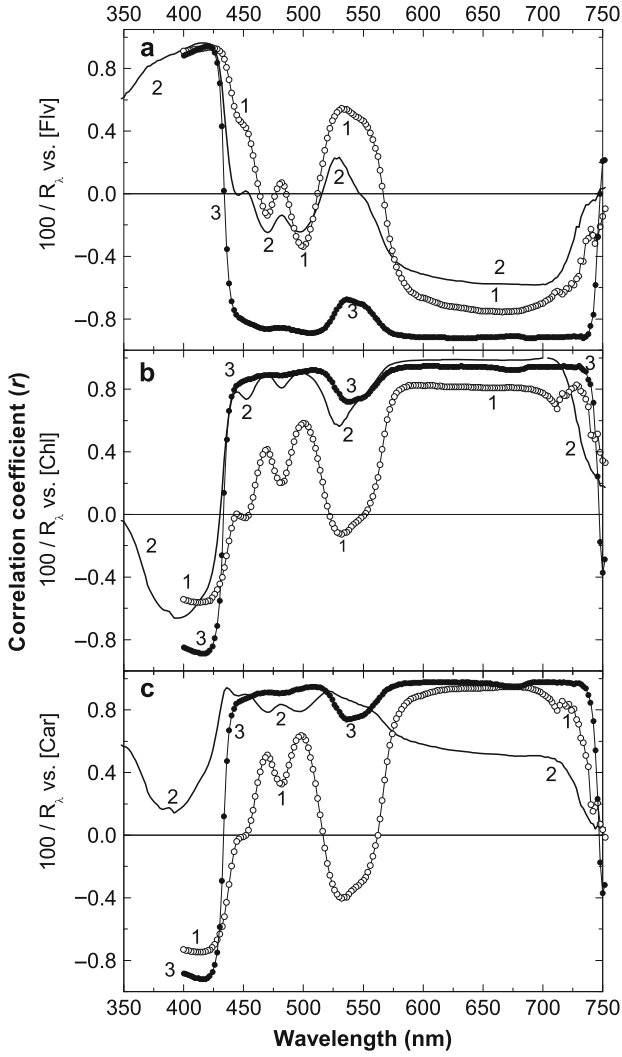

Fig. 5.14 Spectral dependencies of the correlation coefficient, *r*, between the reciprocal reflectance, $100/R_\lambda$, and flavonol (**a**), chlorophyll (**b**), and carotenoid (**c**) content for Golden Delicious (*1*), Antonovka (*2*), and Renet Simirenko (*3*) apple fruits. (Reprinted from Merzlyak et al. (2005a, b) with permission from Elsevier)

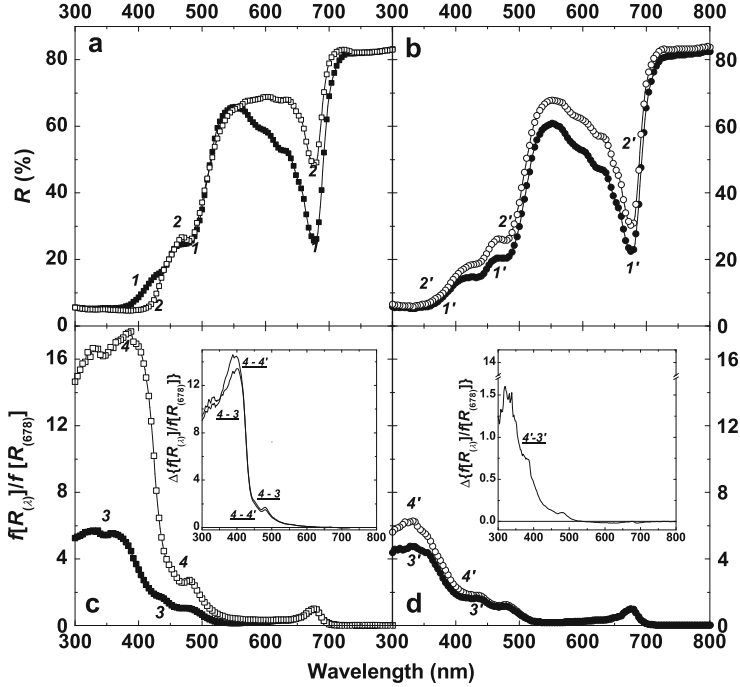

Fig. 5.15 Effect of solar UV radiation and its exclusion on the reflectance spectra of apple fruit (**a**, **b**) and the reflectance spectra of apple fruit calculated from the remission function, $f(R)$, (**c**, **d**). The spectra for shaded (*1*, *1′*, *3*, *3′*) and sunlit (*2*, *2′*, *4*, *4′*) surfaces of the fruit (cultivar Antonovka) grown under a full solar spectrum (*1–4*) and with UV radiation filtered out (*1′–4′*). *Insets*: Difference $f(R_\lambda)/f(R_{678})$ spectra. Note the absence of a high contribution of sunlight-induced flavonols (*inset* **c**, spectrum *4–3*) in apples grown without UV irradiation (*inset in* **d**, spectrum *4′–3′*). (Solovchenko, unpublished)

features suggests that flavonols in anthocyanin-free fruits exert a contribution to light absorption and hence to the screening of solar radiation not only in the UV-A region but also in the visible part of the spectrum.

Additional evidence for the crucial role of accumulation of screening pigments induced by solar UV radiation in the development of characteristic changes in reflectance spectra was obtained in experiments with exclusion of UV radiation from the solar radiation spectrum (Fig. 5.15; see also Chap. 3). The reflectance spectra taken from the sunlit surface of fruit grown under a full solar spectrum and possessing a high flavonol content (see Fig. 3.5) displayed features attributable to flavonol buildup (Fig. 5.15a, curve 2, c, curve 4). By contrast, the spectra of the sunlit surface of fruit grown in the absence of UV radiation and possessing a low flavonol content (see Fig. 3.5) did not exhibit these features (Fig. 5.16b, curve 2′; b, curve 2′) and were similar to the spectra of shaded surfaces of fruit grown in either illumination condition (cf. spectra 1, 1′, 3, 3′, and 2′, 4′in Fig. 5.15; see also the insets in Fig. 5.15c, d).

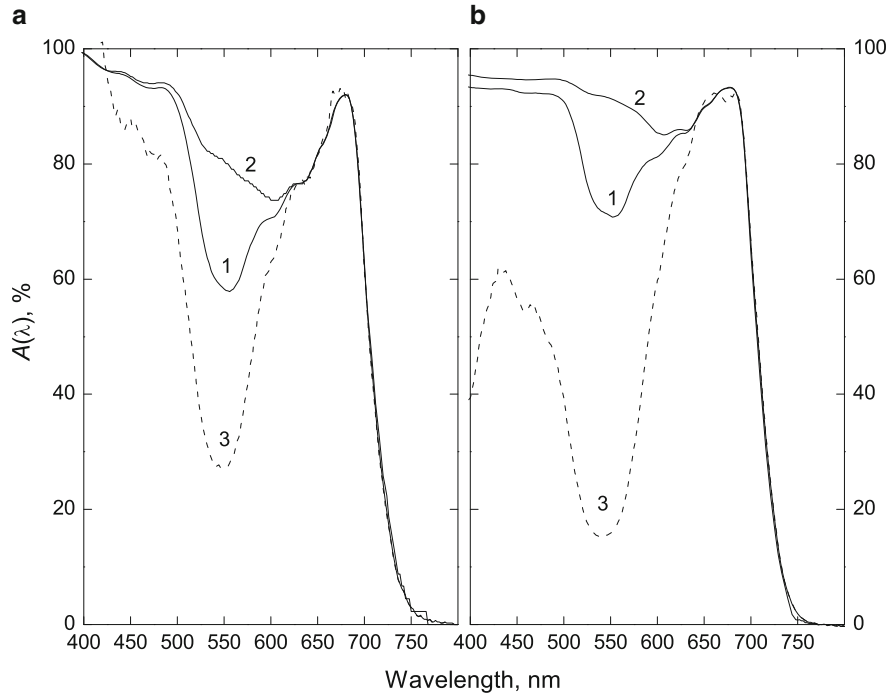

Fig. 5.16 Absorptance plots of spring green (1) and red (2) *A. platanoides* (**a**) and *Corylus avellana* (**b**) leaves. Spectrum *3* is the model of chloroplast thylakoid pigment absorption in anthocyanic leaves as screened by anthocyanins. (Reproduced from Merzlyak et al. (2008a, b) with permission from Oxford University Press)

5.5.2 *Effect of Anthocyanins on Leaf and Fruit Spectra*

The effects of anthocyanins on leaf and fruit optical properties such as reflection and absorption of light for samples with different contents of the pigments have been documented in the literature for various plant species (Feild et al. 2001; Gitelson et al. 2001; Hughes et al. 2005; Hughes and Smith 2007; Pietrini et al. 2002; Pietrini and Massacci 1998). A remarkable feature of higher-plant leaves is that only chlorophyll pigments contribute to light absorption in the red region of the visible spectrum; absorption at shorter wavelengths, particularly in the orange to blue region, can be attributed to other principal leaf pigments: carotenoids and flavonoids, including anthocyanins (Cerovic et al. 2002; Gitelson et al. 2001; Merzlyak et al. 2005b, 2008a). A comparison of the absorptance[1] plots of anthocyanin-free and anthocyanin-containing leaf specimens with similar chlorophyll contents showed that anthocyanin peaks are located around 550 nm in *Acer platanoides*,

[1]Defined as $A(\lambda) = 1 - T(\lambda) - R(\lambda)$, where $R(\lambda)$ and $T(\lambda)$ are the reflectance and transmittance at wavelength λ.

Cotoneaster alaunica, *Cornus alba,* and *Pelargonium zonale* (Gitelson et al. 2001). Furthermore, it was found that leaf absorptance near 550 nm (Gitelson et al. 2001) and that in the 400–600-nm band (Pietrini and Massacci 1998) is linearly related to anthocyanin content.

A convenient and efficient (though oversimplified in that it presumes that the entire leaf surface is covered by epidermal anthocyanins) approach for the analysis of leaf optical properties is based on paired comparison of anthocyanin-containing and anthocyanin-free specimens with similar chlorophyll absorption in the red region of the spectrum (rather than similar chlorophyll content), taking into account some uncertainties in routine leaf spectral measurements (Merzlyak et al. 2004, 2008a). This approach allowed Merzlyak et al. (2008a) to quantify the screening effect of anthocyanins on light absorption by the chloroplast pigments (Figs. 5.16 and 5.17).

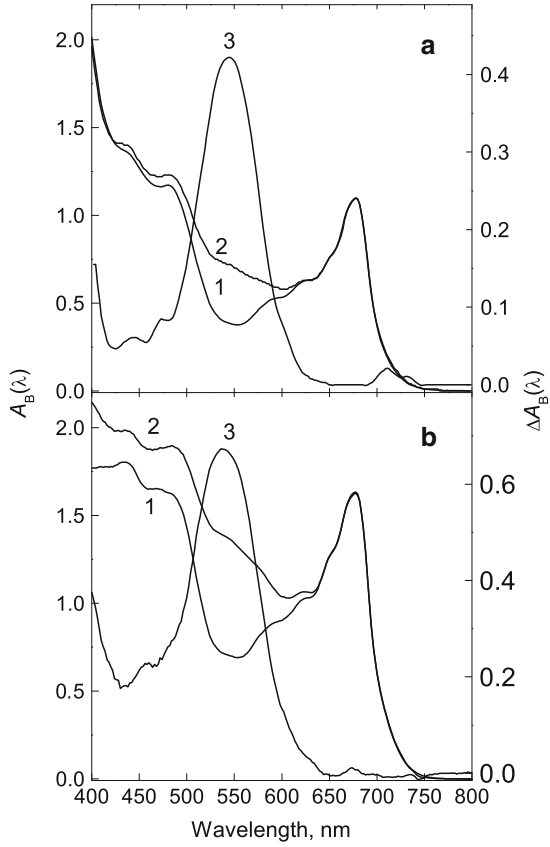

Fig. 5.17 Attenuation [defined as $A_B(\lambda) = -\log(T(\lambda)/[1-R(\lambda)])$, where $R(\lambda)$ and $T(\lambda)$ are the reflectance and transmittance; Merzlyak et al. 2008a] plots of *green* (1) and *red* (2) spring *A. platanoides* (**a**) and *C. avellana* (**b**) leaves (*left scales*) and their difference (anthocyanic minus acyanic) spectra (*curves 3*, *right scale*). (Reproduced from Merzlyak et al. (2008a, b) with permission from Oxford University Press)

According to the difference spectroscopy data, anthocyanin absorption maxima in leaves with appreciable chlorophyll content were located between 537 and 544 nm. In senescing chlorophyll-free *Parthenocissus quinquefolia* leaves, anthocyanin maxima were found between 530 and 542 nm, suggesting the accumulation of several spectral forms of anthocyanins. The bathochromic shift of the anthocyanin maxima in vivo by 5–20 nm and broadening of the absorption band as compared with solution spectra may involve effects of self-association, copigmentation by flavonols and protoanthocyanidins, metal chelation, etc. (see Sect. 5.1).

The analysis of leaf optical properties (Figs. 5.16 and 5.17) shows that anthocyanin pigments compete strongly with chlorophyll for light absorption in the green range and with chlorophyll *b* and carotenoids for absorption at shorter wavelengths. In chlorophyll-free leaves, when the anthocyanin content is as high as 40–50 nmol cm^{-2}, the absorptance at 550 nm and in the 500–600-nm band reaches 95%. Furthermore, for high anthocyanin content, their contribution to light absorption could be profound, even in the 600–650-nm band (Merzlyak et al. 2008a).

The independence of anthocyanin and chlorophyll absorption as well as the spectral features of anthocyanins in leaves (see above) provide a means for quantifying the difference between the incident flux and the reduced flux that reaches the thylakoids. Figure 5.16 (curves 3) demonstrates the estimated chloroplast thylakoid pigment absorption plots of the anthocyanin-containing leaves when illuminated from the adaxial surface only (*A. platanoides*) and from both adaxial and abaxial surfaces (*Corylus avellana*) (see Fig. 4.3). According to estimates by Merzlyak et al. (2008a), anthocyanins reduce considerably leaf light absorption between 400 and 600 nm and around 550 nm, more than 2 and 4 times in *A. platanoides* and *C. avellana*, respectively.

5.5.3 *Effect of Red Carotenoids on Leaf Reflectance*

Similarly to the buildup of anthocyanins, the accumulation of red ketocarotenoids such as rhodoxanthin in *A. arborescence* under strong light stress induces remarkable changes in spectral light reflection and absorption by leaves (Fig. 5.18). Interestingly, in the case of *A. arborescence*, all specimens investigated possessed characteristic chlorophyll spectral features in the red region, though microspectrophotometry revealed chlorophyll-free plastids in stressed leaves (Fig. 5.7, curves 4–6). This indicates that aloe leaf optical properties are determined by the proportion of plastids with different types of absorption and their distribution within tissue.

The accumulation of rhodoxanthin under stress conditions brought about a decrease of leaf reflectance along with an increase of absorption in the green region of the visible spectrum. The spectra of green-to-red leaves showed a significant variation in the orange-red region (with a minimum near 678 nm due to saturation of absorption for high content of the pigment; Gitelson et al. 2003b), indicating considerable changes in leaf chlorophyll content. At the same time, low and almost invariable absorption was recorded below 500 nm in the spectral region governed

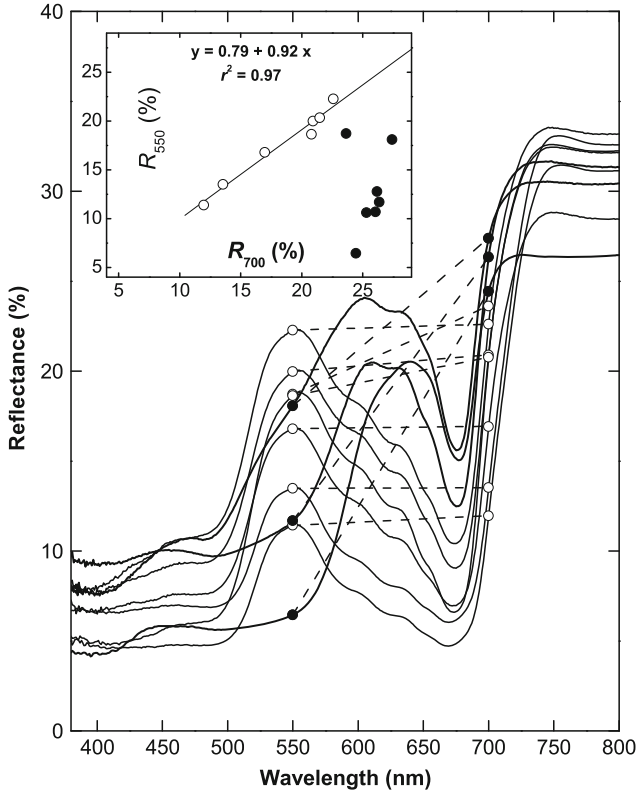

Fig. 5.18 Representative reflection spectra of whole aloe leaves. Reflectances at 500 and 678 nm are shown as *symbols* and connected by *dashed lines* to show the difference between them. *Inset*: Relationship between reflectances at 500 and 678 nm for visually *green* (*open symbols*) and *reddish to red* (*closed symbols*) leaves. (Reproduced from Merzlyak et al. (2005a, b) with permission from the Royal Society of Chemistry for the European Society for Photobiology, the European Photochemistry Association, and the Royal Society of Chemistry)

by combined absorption of chlorophylls and non-ketocarotenoids. Comparison of absorption spectra of selected red and green aloe leaves exhibiting similar optical properties in the red region as well as chlorophyll and non-ketocarotenoid content also strongly suggests that rhodoxanthin absorption in vivo occurs as a band in the blue-green range; accordingly, the maximum of rhodoxanthin absorption in aloe leaves is located near 540 nm (Merzlyak et al. 2005a).

The spectral properties of rhodoxanthin in aloe leaves closely resemble those of anthocyanins in plant species accumulating these pigments (Gitelson et al. 2001; Merzlyak et al. 2003, 2008a, b). Similarly to the leaves of anthocyanin-free species, green aloe leaves possessed similar R_{550} and R_{700} in a wide range of their changes. The reddening of the leaves manifests itself as lowering of R_{550} in comparison with R_{700} (Fig. 5.18, inset). In winter aloe plants suffering from combined stress induced by light and drought, the decrease of R_{550} occurred at higher R_{700} values,suggesting

that under these conditions the adaptation of aloe involved a considerable decrease in chlorophyll content probably to reduce the amount of light absorbed by the photosynthetic apparatus.

Another response has been observed in mature plants with established root system. The relationship "R_{550} versus R_{700}" in Fig. 5.18, leaf absorption spectra, and the pigment chemical analysis data in Fig. 3.14 indicate that the accumulation of rhodoxanthin frequently took place in leaves with relatively high chlorophyll content and even green leaves contained noticeable amounts of rhodoxanthin. It is tempting to speculate that in this case the level of protection provided by the buildup of rhodoxanthin and, probably, by other mechanisms was sufficient to prevent a dramatic decrease in chlorophyll content under high-light stress.

Collectively, the analysis of aloe leaf reflectance indicates that accumulation of rhodoxanthin in chromoplasts of light-stressed aloe leaves is able to provide a considerable attenuation of light absorbed by plant tissue in the green range of the visible spectrum. These findings are consistent with a proposed photoprotective function of rhodoxanthin (Han et al. 2003, 2004; Weger et al. 1993) which is accomplished via efficient internal light trapping aimed at diminishing the amount of radiation absorbed by chlorophyll in the photosynthetic apparatus under stressful conditions. In addition, it is possible to suggest that in plastids devoid of chlorophyll, rhodoxanthin and other carotenoids are able to protect lipids contained in lipid globules from deleterious effects of irradiation in a broad spectral band.

5.6 Concluding Remarks

Taken together, the data presented in this chapter clearly demonstrate that the optical properties of screening pigments in planta differ considerably from those of isolated pigments owing to a number of factors (see Sect. 5.1). As a result, the effective screening ability of the pigments within plant cells and tissues could be considerably higher than one would expect from studying their spectra in solutions. In particular, broadening of absorption bands and bathochromic shifts considerably expand the spectral region where screening pigments could potentially provide photoprotection in vivo. These circumstances emphasize the importance of investigations on the in planta spectra of screening pigments to gain an insight into their real photoprotective efficiency.

Generally, natural sunscreens and internal light traps comprising structures with high screening pigment content (such as cuticle and epidermis or cytoplasmic lipid globules) appear to attenuate or even block the harmful UV radiation and excessive visible radiation quite effectively. According to measurements of the internal light gradient within the leaf blade made with an optical microfiber (Day et al. 1992, 1993, 1994), in conifer plants adapted to high fluxes of solar radiation the depth of UV radiation penetration into leaves does not exceed 2 μm and UV-B radiation is blocked almost completely by the epidermis (DeLucia et al. 1992).

Pigments absorbing in the visible part of the spectrum, such as anthocyanins and red ketocarotenoids, are able to effectively shield the photosynthetic apparatus, intercepting up to 60% of the PAR otherwise absorbed by chlorophyll *b* or "photosynthetic" carotenoids. It is also remarkable that anthocyanin pigments absorb strongly in the 500–600-nm region, close to the solar energy maximum in the gap between the region of strong absorption by chlorophyll and carotenoids at one end of the visible spectrum (400–500 nm) and the other, red end, where chlorophyll captures the light that penetrates deep into plant tissue and efficiently drives CO_2 fixation (Nishio 2000; Sun and Nishio 2001; Sun et al. 1998).

Finally, as already mentioned, the effect of plastidic rhodoxanthin on light absorption by leaves closely resembles that of vacuolar anthocyanins. It appears that both pigments are able to serve as effective broad-band internal traps for radiation in the green range, in which light penetrates deeply into leaf tissues. Therefore, it is remarkable that anthocyanins and rhodoxanthin, pigments disparate in terms of their biosynthesis, photochemistry, and subcellular localization, but with similar in vivo optical properties, are relied upon by different plant species for the purposes of long-term adaptation to and protection against strong solar irradiation in the visible range.

References

Asen S, Stewart R, Norris K (1972) Co-pigmentation of anthocyanins in plant tissues and its effect on color. Phytochemistry 11:1139–1144

Awad M, de Jager A, van Westing L (2000) Flavonoid and chlorogenic acid levels in apple fruit: characterisation of variation. Sci Hortic 83:249–263

Awad M, de Jager A, van der Plas L, van der Krol A (2001) Flavonoid and chlorogenic acid changes in skin of 'Elstar' and 'Jonagold' apples during development and ripening. Sci Hortic 90:69–83

Barnes P, Searles P, Ballare C, Ryel R, Caldwell M (2000) Non-invasive measurements of leaf epidermal transmittance of UV radiation using chlorophyll fluorescence: field and laboratory studies. Physiol Planta 109:274–283

Baur P, Stulle K, Uhlig B, Schönherr J (1998) Absorption von Strahlung im UV-B und Blaulichtbereich von Blattkutikeln ausgewählter Nutzpflanzen. Gartenbauwissenschaft 63: 145–152

Berner T, Dubinsky Z, Wyman K, Falkowski P (1989) Photoadaptation and the "packace" effect in *Dunaliella tertiolecta* (Chlorophyceae). J Phycol 25:70–78

Bjorn L, Murphy T (1985) Computer calculation of solar ultraviolet radiation at ground level. Physiol Veg 23:555–561

Bornman J (1999) Localisation and functional significance of flavonoids and related compounds. In: Rozema J (ed) Stratospheric ozone depletion: the effects of enhanced UV-B radiation on terrestrial ecosystems. Backhuys, Leiden, pp 59–69

Boussiba S (2000) Carotenogenesis in the green alga *Haematococcus pluvialis*: cellular physiology and stress response. Physiol Planta 108:111–117

Britton G (1995) UV/visible spectroscopy. In: Britton G, Liaaen-Jensen S, Pfander H (eds) Carotenoids, vol 1B. Birkhauser, Basel, pp 13–62

Burchard P, Bilger W, Weissenbock G (2000) Contribution of hydroxycinnamates and flavonoids to epidermal shielding of UV-A and UV-B radiation in developing rye primary leaves as

assessed by ultraviolet-induced chlorophyll fluorescence measurements. Plant Cell Environ 23:1373–1380
Butler W, Norris K (1960) The spectrophotometry of dense light-scattering material. Arch Biochem Biophys 87:31
Caldwell M, Bornman J, Ballare C, Flint S, Kulandaivelu G (2007) Terrestrial ecosystems, increased solar ultraviolet radiation, and interactions with other climate change factors. Photochem Photobiol Sci 6:252–266
Cerovic Z et al (2002) The use of chlorophyll fluorescence excitation spectra for the non-destructive in situ assessment of UV-absorbing compounds in leaves. Plant Cell Environ 25:1663–1676
Czygan F (1970) Blood-rain and blood-snow: nitrogen-deficient cells of *Haematococcus pluvialis* and *Chlamydomonas nivalis*. Arch Mikrobiol 74:69
Day T, Vogelmann T, DeLucia E (1992) Are some plant life forms more effective than others in screening out ultraviolet-B radiation? Oecologia 92:513–519
Day T, Martin G, Vogelmann T (1993) Penetration of UV-B radiation in foliage: evidence that the epidermis behaves as a non-uniform filter. Plant Cell Environ 16:735–741
Day T, Howells B, Rice W (1994) Ultraviolet absorption and epidermal-transmittance spectra in foliage. Physiol Planta 92:207–218
DeLucia E, Day T, Vogelman T (1992) Ultraviolet-B and visible light penetration into needles of two species of subalpine conifers during foliar development. Plant Cell Environ 15: 921–929
Diaz M, Ball E, Luttge U (1990) Stress-induced accumulation of the xanthophyll rhodoxanthin in leaves of *Aloe vera*. Plant Physiol Biochem 28:679–682
Fabregas J, Dominguez A, Maseda A, Otero A (2003) Interactions between irradiance and nutrient availability during astaxanthin accumulation and degradation in *Haematococcus pluvialis*. Appl Microbiol Biotechnol 61:545–551
Feild T, Lee D, Holbrook N (2001) Why leaves turn red in autumn. The role of anthocyanins in senescing leaves of red-osier dogwood. Plant Physiol 127:566–574
Fukshansky L (1981) Optical properties of plant tissue. In: Smith H (ed) Plants and the daylight spectrum. Springer, Berlin, pp 37–303
Fukshansky L, Remisowsky A, McClendon J, Ritterbusch A, Richter T, Mohr H (1993) Absorption spectra of leaves corrected for scattering and distributional error: a radiative transfer and absorption statistics treatment. Photochem Photobiol 57:538–555
Gitelson AA, Merzlyak MN, Chivkunova OB (2001) Optical properties and nondestructive estimation of anthocyanin content in plant leaves. Photochem Photobiol 74:38–45
Gitelson A, Gritz Y, Merzlyak M (2003a) Non destructive chlorophyll assessment in higher plant leaves: algorithms and accuracy. J Plant Physiol 160:271–282
Gitelson AA, Gritz Y, Merzlyak MN (2003b) Relationships between leaf chlorophyll content and spectral reflectance and algorithms for non-destructive chlorophyll assessment in higher plant leaves. J Plant Physiol 160:271–282
Gitelson A, Keydan G, Merzlyak M (2006) Three-band model for noninvasive estimation of chlorophyll, carotenoids, and anthocyanin contents in higher plant leaves. Geophys Res Lett 33:L11402
Gitelson A, Chivkunova O, Merzlyak M (2009) Nondestructive estimation of anthocyanins and chlorophylls in anthocyanic leaves. Am J Bot 96:1861
Gonnet J (1999) Colour effects of co-pigmentation of anthocyanins revisited-2. A colorimetric look at the solutions of cyanin co-pigmented by rutin using the CIELAB scale. Food Chem 66:387–394
Gonnet J (2003) Origin of the color of cv. Rhapsody in blue rose and some other so-called “blue” roses. J Agric Food Chem 51:4990–4994
Green B, Durnford D (1996) The chlorophyll-carotenoid proteins of oxygenic photosynthesis. Annu Rev Plant Biol 47:685–714
Han Q, Shinohara K, Kakubari Y, Mukai Y (2003) Photoprotective role of rhodoxanthin during cold acclimation in *Cryptomeria japonica*. Plant Cell Environ 26:715–723

Han Q, Katahata S, Kakubari Y, Mukai Y (2004) Seasonal changes in the xanthophyll cycle and antioxidants in sun-exposed and shaded parts of the crown of *Cryptomeria japonica* in relation to rhodoxanthin accumulation during cold acclimation. Tree Physiol 24:609

Havaux M, Kloppstech K (2001) The protective functions of carotenoid and flavonoid pigments against excess visible radiation at chilling temperature investigated in *Arabidopsis npq* and *tt* mutants. Planta 213:953–966

Hughes N, Smith W (2007) Attenuation of incident light in *Galax urceolata* (Diapensiaceae): concerted influence of adaxial and abaxial anthocyanic layers on photoprotection. Am J Bot 94:784

Hughes N, Neufeld H, Burkey K (2005) Functional role of anthocyanins in high-light winter leaves of the evergreen herb *Galax urceolata*. New Phytol 168:575–587

Kolb C, Pfundel E (2005) Origins of non-linear and dissimilar relationships between epidermal UV absorbance and UV absorbance of extracted phenolics in leaves of grapevine and barley. Plant Cell Environ 28:580–590

Krauss P, Markstadter C, Riederer M (1997) Attenuation of UV radiation by plant cuticles from woody species. Plant Cell Environ 20:1079–1085

Lancaster J, Grant J, Lister C, Taylor M (1994) Skin color in apples: influence of copigmentation and plastid pigments on shade and darkness of red color in five genotypes. J Am Soc Hortic Sci 119:63–69

Markham K (1989) Flavones, flavonols and their glycosides. In: Harborne J, Dey P (eds) Methods in plant biochemistry, vol 1. Academic, London, pp 197–235

Markstädter C, Queck I, Baumeister J, Riederer M, Schreiber U, Bilger W (2001) Epidermal transmittance of leaves of *Vicia faba* for UV radiation as determined by two different methods. Photosynth Res 67:17–25

Merzlyak MN, Naqvi KR (2000) On recording the true absorption spectrum and the scattering spectrum of a turbid sample: application to cell suspensions of the cyanobacterium *Anabaena variabilis*. J Photochem Photobiol B 58:123–129

Merzlyak M, Solovchenko A (2002) Photostability of pigments in ripening apple fruit: a possible photoprotective role of carotenoids during plant senescence. Plant Sci 163:881–888

Merzlyak M, Solovchenko A, Chivkunova O (2002) Patterns of pigment changes in apple fruits during adaptation to high sunlight and sunscald development. Plant Physiol Biochem 40:679–684

Merzlyak M, Solovchenko A, Gitelson A (2003) Reflectance spectral features and non-destructive estimation of chlorophyll, carotenoid and anthocyanin content in apple fruit. Postharvest Biol Technol 27:197–212

Merzlyak MN, Melo TB, Razi Naqvi K (2004) Estimation of leaf transmittance in the near infrared region through reflectance measurements. J Photochem Photobiol B Biol 74: 145–150

Merzlyak M, Solovchenko A, Pogosyan S (2005a) Optical properties of rhodoxanthin accumulated in *Aloe arborescens* Mill. leaves under high-light stress with special reference to its photoprotective function. Photochem Photobiol Sci 4:333–340

Merzlyak MN, Solovchenko AE, Smagin AI, Gitelson AA (2005b) Apple flavonols during fruit adaptation to solar radiation: spectral features and technique for non-destructive assessment. J Plant Physiol 162:151–160

Merzlyak M et al (2007) Effect of nitrogen starvation on optical properties, pigments, and arachidonic acid content of the unicellular green alga *Parietochloris incisa* (Trebouxiophyceae, Chlorophyta). J Phycol 43:833–843

Merzlyak MN, Chivkunova OB, Solovchenko AE, Naqvi KR (2008a) Light absorption by anthocyanins in juvenile, stressed, and senescing leaves. J Exp Bot 59:3903–3911

Merzlyak MN, Melo TB, Naqvi KR (2008b) Effect of anthocyanins, carotenoids, and flavonols on chlorophyll fluorescence excitation spectra in apple fruit: signature analysis, assessment, modelling, and relevance to photoprotection. J Exp Bot 59:349–359

Merzlyak M, Chivkunova O, Zhigalova T, Naqvi K (2009) Light absorption by isolated chloroplasts and leaves: effects of scattering and 'packing'. Photosynth Res 102:31–41
Naqvi K, Mely T, Raji B (1997) Assaying of chromophore composition of photosynthetic systems by spectral resolution: application to the light-harvesting complex (LHC II) and total pigment content of higher plants. Spectrochim Acta 53:2229–2234
Naqvi KR, Merzlyak MN, Melo TB (2004) Absorption and scattering of light by suspensions of cells and subcellular particles: an analysis in terms of Kramers–Kronig relations. Photochem Photobiol Sci 3:132–137
Nishio J (2000) Why are higher plants green? Evolution of the higher plant photosynthetic pigment complement. Plant Cell Environ 23:539–548
Oborne B, Raven J (1986) Light absorption by plants and its implications for photosynthesis. Biol Rev 61:1–60
Pietrini F, Massacci A (1998) Leaf anthocyanin content changes in *Zea mays* L. grown at low temperature: Significance for the relationship between the quantum yield of PS II and the apparent quantum yield of CO_2 assimilation. Photosynth Res 58:213–219
Pietrini F, Iannelli M, Massacci A (2002) Anthocyanin accumulation in the illuminated surface of maize leaves enhances protection from photo-inhibitory risks at low temperature, without further limitation to photosynthesis. Plant Cell Environ 25:1251–1259
Shick J, Dunlap W (2002) Mycosporine-like amino acids and related gadusols: biosynthesis, accumulation, and UV-protective functions in aquatic organisms. Annu Rev Physiol 64:223–262
Singh S, Klisch M, Sinha R, Hader D (2008) Effects of abiotic stressors on synthesis of the mycosporine-like amino acid shinorine in the cyanobacterium *Anabaena variabilis* PCC 7937. Photochem Photobiol 84:1500–1505
Sinha R, Häder D (2007) UV-protectants in cyanobacteria. Plant Sci 174:278–289
Smith G, Markham K (1998) Tautomerism of flavonol glucosides: relevance to plant UV protection and flower colour. J Photochem Photobiol A Chem 118:99–105
Solovchenko A, Merzlyak M (2003) Optical properties and contribution of cuticle to UV protection in plants: experiments with apple fruit. Photochem Photobiol Sci 2:861–866
Solovchenko A, Merzlyak M (2008) Screening of visible and UV radiation as a photoprotective mechanism in plants. Russ J Plant Physiol 55:719–737
Solovchenko A, Schmitz-Eiberger M (2003) Significance of skin flavonoids for UV-B-protection in apple fruits. J Exp Bot 54:1977–1984
Solovchenko A, Chivkunova O, Merzlyak M, Reshetnikova I (2001) A spectrophotometric analysis of pigments in apples. Russ J Plant Physiol 48:693–700
Solovchenko A, Khozin-Goldberg I, Cohen Z, Merzlyak M (2009) Carotenoid-to-chlorophyll ratio as a proxy for assay of total fatty acids and arachidonic acid content in the green microalga *Parietochloris incisa*. J Appl Phycol 21:361–366
Steele M, Gitelson A, Rundquist D, Merzlyak M (2009) Nondestructive estimation of anthocyanin content in grapevine leaves. Am J Enol Vitic 60:87
Steinbrenner J, Linden H (2003) Light induction of carotenoid biosynthesis genes in the green alga *Haematococcus pluvialis*: regulation by photosynthetic redox control. Plant Mol Biol 52: 343–356
Strack D, Wray V (1989) Anthocyanins. In: Harborne J, Dey P (eds) Methods in plant biochemistry, vol 1. Academic, London, pp 325–356
Sun J, Nishio J (2001) Why abaxial illumination limits photosynthetic carbon fixation in spinach leaves. Plant Cell Physiol 42:1–8
Sun J, Nishio J, Vogelmann T (1998) Green light drives CO_2 fixation deep within leaves. Plant Cell Physiol 39:1020–1026
Taroni P, Pifferi A, Torricelli A, Comelli D, Cubeddu R (2003) In vivo absorption and scattering spectroscopy of biological tissues. Photochem Photobiol Sci 2:124–129
Ustin S, Jacquemoud S, Govaerts Y (2001) Simulation of photon transport in a three-dimensional leaf: implications for photosynthesis. Plant Cell Environ 24:1095–1103

Vidhyavathi R, Venkatachalam L, Sarada R, Ravishankar G (2008) Regulation of carotenoid biosynthetic genes expression and carotenoid accumulation in the green alga *Haematococcus pluvialis* under nutrient stress conditions. J Exp Bot 59:1409–1418

Vogelmann T (1993) Plant tissue optics. Annu Rev Plant Biol 44:231–251

Wang B, Zarka A, Trebst A, Boussiba S (2003) Astaxanthin accumulation in *Haematococcus pluvialis* (Chlorophyceae) as an active photoprotective process under high irradiance. J Phycol 39:1116–1124

Weger H, Silim S, Guy R (1993) Photosynthetic acclimation to low temperature by western red cedar seedlings. Plant Cell Environ 16:711–717

Chapter 6
Quantification of Screening Pigments and Their Efficiency In Situ

Abstract This chapter deals with nondestructive quantification of screening pigment content and estimation of the efficiency of screening pigments. The first part of the chapter describes the approaches for the employment of the relationships between changes in screening pigment content and composition and the effects screening pigments exert on reflection of light by plants (considered in detail in the previous chapter) for quantification of screening pigments in situ. The second part considers the current approaches for estimating the efficiency of screening by different pigments in planta.

The investigation of the physiological significance of screening pigments in plants requires information about pigment content and the photoprotective capability of the pigments. The traditional biochemical procedures are often not suitable for the solution of this problem since they do not provide information on the in vivo screening efficiency and do not take into account the issues related to in planta spectroscopy of pigments (see Sect. 5.1). During recent decades, a number of alternative approaches for nondestructive quantification of screening pigments and estimation of their efficiency in situ have evolved. Roughly, they can be divided into two major groups (1) reflectance-based, i.e., employing optical reflectance spectroscopy, and (2) fluorescence-based, i.e., using chlorophyll as an internal fluorescent probe. Both techniques are briefly considered below with an emphasis on the advances made in the laboratories of the author and his colleagues.

6.1 Optical Reflectance-Based Techniques for Nondestructive Screening Pigment Assessment

Spectrophotometry of tissue extracts is a common method for the analysis of plant pigments in physiological and biochemical studies. The application of this method inevitably involves destruction of the sample; it is time-consuming and is prone to

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artifacts due to pigment instability, incomplete extraction, the presence of light-absorbing impurities, etc. (Lichtenthaler 1987; Merzlyak et al. 1996; Solovchenko et al. 2001). These circumstances make the nondestructive estimation of pigment content with reflectance spectroscopy of intact tissues an attractive alternative to "wet" chemical methods. Indeed, both qualitative and quantitative changes in pigment content of plant tissues are inevitably apparent in tissue optical properties, as shown in Chap. 5. The application of nondestructive optical methods for pigment quantification is advantageous since they possess a high throughput, i.e., allow rapid measurements of a large number of samples, which thereafter remain intact and can be used for further analysis. This is especially important for experiments involving serial measurement of the same object, for example, for monitoring stress-induced screening pigment buildup. The development of nondestructive optical reflectance-based techniques for pigment quantification was greatly facilitated by the advent of fiber-optics reflectometers suitable for field measurements providing reliable spectral data from very small samples or whole plants (Gamon and Surfus 1999; Penuelas and Filella 1998; Richardson et al. 2002). Furthermore, reflectance spectroscopy is widely used in remote sensing for global monitoring of agro- and phytocenoses; in recent years, these approaches have also been implemented in "precision agriculture" technologies (Gitelson et al. 2003a, b, 2006; Gitelson and Merzlyak 1998; Merzlyak et al. 2003a; Penuelas and Filella 1998). A significant amount of research was dedicated to the development of techniques for nondestructive analysis of screening pigments in plants over the last 20 years (Filella and Penuelas 1999; Gitelson et al. 2009; Merzlyak et al. 2003b, 2005a, b, 2008a; Penuelas and Filella 1998; Solovchenko and Merzlyak 2003; Solovchenko et al. 2010b). It should be noted that successful application of reflectance spectroscopy for nondestructive analysis of plant pigments requires a thorough understanding of the patterns of their changes during physiological processes in plants (Chap. 3), their localization within tissues (Chap. 4), as well as in vivo spectroscopy (Chap. 5). A brief review of techniques for quantitative estimation of carotenoids, anthocyanins, and flavonols in leaves and fruits with reflectance spectroscopy is presented in this section.

6.1.1 The General Approach

The foundation of the optical reflectance-based approach successfully applied for in situ quantification of both photosynthetic and screening pigments was laid in the works of Gitelson et al. (2002, 2003b, 2006, 2009). It was found that the reciprocal reflectance of leaves (Gitelson et al. 2003b, 2006, 2009) and apple fruit (Merzlyak et al. 2003b) at a certain wavelength depends on pigment contents. This feature was used in the development of models which relate reflectance and pigment content. On the basis of these models, algorithms for estimation of chlorophyll and other pigments in leaves and fruits were developed (for a review, see Merzlyak et al. (2003a)).

Briefly, a conceptual semianalytical three-band model (Gitelson et al. 2003a, 2006) relating reflectance and the content of pigment of interest [P] was suggested in the form:

$$[\mathrm{P}] \propto (R_{\lambda_1}^{-1} - R_{\lambda_2}^{-1}) \times R_{\lambda_3} \tag{6.1}$$

The model contains reflectances in three spectral bands (λ_1, λ_2, λ_3). The reflectance in spectral band λ_1 is maximally sensitive to the pigment of interest; however, it is also affected by absorption by other pigments contained in plant tissue and scattering by plant tissue. To eliminate the effect of absorption by other pigments on reflectance R_{λ_1}, the reflectance in spectral band λ_2 (R_{λ_2}) has been used. R_{λ_2} is affected by absorption of other pigments and is minimally affected by absorption of the pigment of interest. Thus, the difference $(R_{\lambda_1}^{-1} - R_{\lambda_2}^{-1})$ in (6.1) is related to the pigment of interest; however, is still affected by the scattering. To minimize this effect, the reflectance in spectral band λ_3 should be governed mainly by scattering of the sample studied.

The following strategy allows one to overcome some of the complications inherent in nondestructive analysis of plant pigments using reflectance spectra and to employ the model in (6.1):

1. The detection of reflectance spectral bands governed predominantly by absorption of an individual pigment and sensitive to the content of this pigment
2. The development of algorithms relating reflectance at certain wavelengths to pigment content in the entire range of its variation
3. Finding a way to eliminate the contribution of chlorophyll to the reflectance, which is necessary for analyses of other pigments

As a result of signature analysis of reflectance spectra, the bands of in situ absorption of leaves of different species and apple skin pigments were established (Gitelson and Merzlyak 1996; Gitelson et al. 2001, 2002, 2006; Merzlyak et al. 2003b). The results obtained provided evidence that the conceptual model is applicable for an accurate nondestructive estimation of the contents of certain screening pigments in fruits and leaves. The algorithms developed turned to be (1) sensitive mainly to the pigment of interest and minimally sensitive to the contents of other pigments or morphological–anatomical features of plant samples, and (2) applicable to independently obtained data sets (Gitelson et al. 2002, 2003a, 2009; Solovchenko et al. 2010a; Steele et al. 2009).

Apple fruit possessing resolved reflectance spectra and pronounced responses in terms of accumulation of screening pigments such as flavonols, anthocyanins, and extrathylakoid carotenoids are a suitable model for demonstration of the development of reflectance-based methods for quantification of screening pigments (for a detailed description of corresponding techniques for leaves, see Gitelson et al. (2001, 2002, 2003b, 2006, 2009), Merzlyak et al. (2003a)). Similarly to leaves, fruit optics is determined by the overall content of pigments, their local

concentration, interactions and distribution within cell structures, as well as the role played by scattering in determining internal optical properties. Apple fruit contain plastidic chlorophyll and carotenoids as well as vacuolar flavonoids (including anthocyanins) as principal pigments absorbing in the visible range. In addition, cuticle-bound phenolic acids and vacuolar flavonoids contribute to light absorption at shorter wavelengths (see Chaps. 3–5).

Fruits with low chlorophyll and anthocyanin contents exhibit high reflectance (about 65–80%) at wavelengths beyond 600 nm. The presence of the pigments in low amounts, hardly assessable analytically, manifests itself as distinct troughs in reflectance spectra in the bands of chlorophyll and carotenoid and anthocyanin absorption. With an increase in pigment content, the spectra become less resolved and flatter (Fig. 6.1). Notably, fruit reflectance in the main bands of chlorophyll *a* absorption (near 440–450 and 670–680 nm) is low and is not sensitive to chlorophyll content exceeding 5–6 nmol cm^{-2} (Fig. 6.2a; Knee 1980; Merzlyak et al. 2003b). The reflectance at the edges of the red chlorophyll absorption band (located 20–30 nm from the absorption maximum) displayed a considerable variation as the chlorophyll content varied (Fig. 6.1; see also Merzlyak et al. (2003b)).

Distinct bands attributable to carotenoid absorption could be distinguished only in reflectance spectra of ripe anthocyanin-free fruit (see the uppermost curve in Fig. 6.1a). Anthocyanin absorption manifests itself as a shoulder or a trough near 540–550 nm, usually superimposed on a considerable chlorophyll and carotenoid background. In the presence of a moderate chlorophyll content (5–8 nmol cm^{-2}), anthocyanins, when accumulated in high amounts (over 30 nmol cm^{-2}), govern the reflectance of apple fruit, resulting in very low reflectance (below 5%) in the green part of the spectrum (Fig. 6.1b).

A high correlation between reflectances at 550 and 700 nm, representing the fundamental feature of the reflectance spectra of anthocyanin-free leaves of diverse plants (Gitelson et al. 2001, 2002, 2006), was also found in anthocyanin-free fruit, where R_{550} and R_{700} correlated very closely ($r^2 = 0.95$, Fig. 6.2b) regardless of chlorophyll content and maturity stage (Merzlyak et al. 2003b). In contrast, in anthocyanin-containing apple fruit, the strong correlation between R_{550} and R_{700} in red fruit was affected as a result of anthocyanin absorption in the green range (Fig. 6.2b). Thus, in the green range of the spectrum, both pigments, anthocyanins and chlorophylls, absorb, whereas chlorophylls *a* and *b* are the only absorbers in the red-edge region – this is the case for both leaf and fruit (Gitelson et al. 2001, 2002; Merzlyak et al. 2003b). This fundamental feature of the reflectance of plant assimilatory tissues is exploited in the development of reflectance-based methods for screening pigment quantification (see below).

The analysis of the relationship "$R(\lambda)$ versus pigment content" showed that spectral regions where the reflectance coefficients are sensitive to wide-range variations of chlorophyll content (from 0 to 50–60 nmol cm^{-2}) are situated aside from the red maximum of chlorophyll absorption: in the green (broad band near 550–600 nm) and red (narrow band at 700–705 nm) parts of the spectrum (see Fig. 6.3a). It was found that the reflectances in these bands were hyperbolically related to chlorophyll content (Gitelson and Merzlyak 1996, 1998; Lichtenthaler

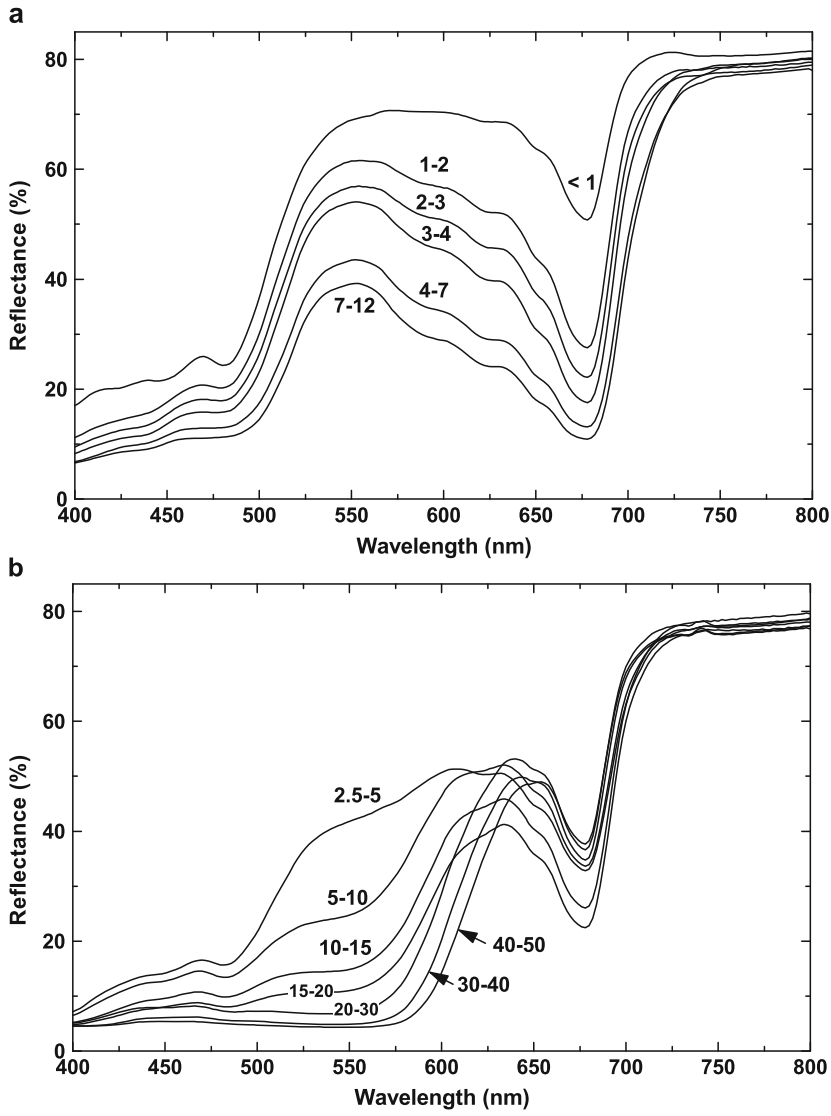

Fig. 6.1 Average reflectance spectra of anthocyanin-free green to yellow-green apple fruit (**a**) and Zhigulevskoe apples accumulating anthocyanins on sun-exposed sides of a fruit (**b**). *Numbers* indicate the ranges of chlorophyll (**a**) and anthocyanin (**b**) content (nmol cm^{-2}) in the peel of fruits. (Reprinted from Merzlyak et al. (2003a, b) with permission from Elsevier)

et al. 1996). It should be noted that the chlorophyll extinction coefficients are very low in these bands. This apparently universal feature of plant reflectance spectra (the linear dependence of the inverse reflectance coefficient in certain spectral regions versus pigment content) was used as a basis for devising the algorithms for chlorophyll and other pigment assays.

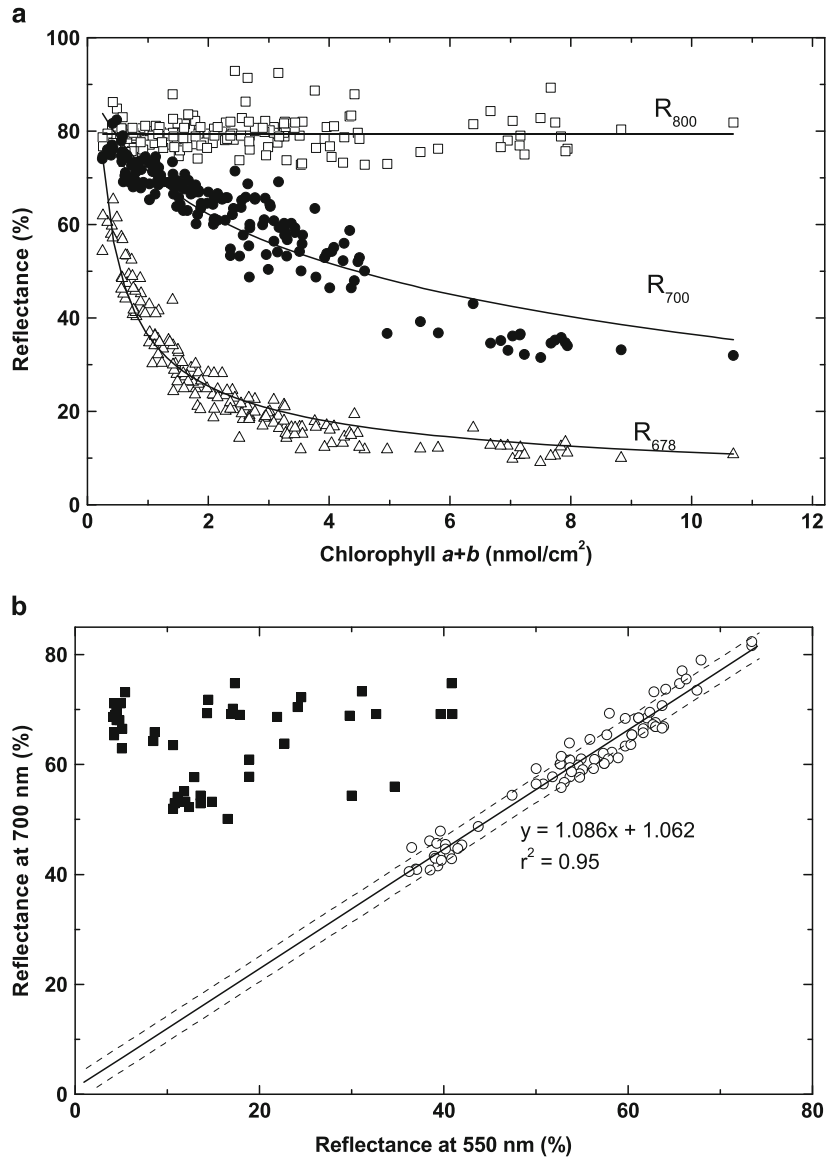

Fig. 6.2 **(a)** Reflectances at 678, 700, and 800 nm versus chlorophyll content and **(b)** reflectance at 700 nm versus reflectance at 550 nm in apple fruits. For green to green-yellow fruits, R_{550} versus R_{700} is linear with a determination coefficient higher than 0.95, whereas for anthocyanin-containing fruits, $R_{550} < R_{700}$ and the fair relationship between them was disturbed. *Solid lines* represent the best-fit functions; *dashed lines* represent standard deviation in **(b)**. (Reprinted from Merzlyak et al. (2003a, b) with permission from Elsevier)

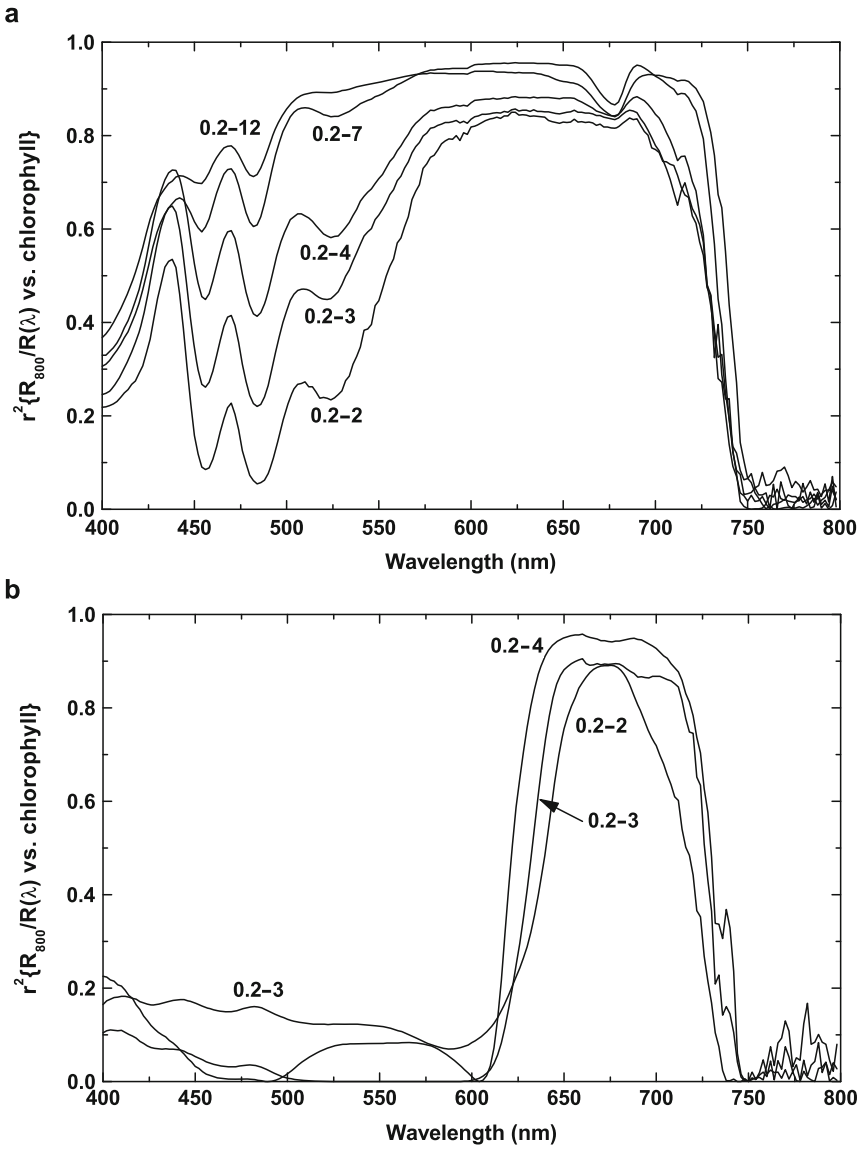

Fig. 6.3 The spectra of the determination coefficient of the relationship between $R_{800}/R(\lambda)$ and chlorophyll content for green to yellow (**a**) and red (**b**) apple fruits. Chlorophyll content ranges (nmol cm^{-2}) are indicated on each curve. (Reprinted from Merzlyak et al. (2003a, b) with permission from Elsevier)

One of the requirements for reliable algorithms for pigment analysis is their low sensitivity to morphological and anatomical traits of plant tissues (Merzlyak et al. 2003a). For leaves and fruits differing in pigment content, the lowest variation of reflectance at wavelengths longer than 500 nm was found in the near-IR (NIR)

region (Gitelson and Merzlyak 1994; Lichtenthaler et al. 1996). Since leaf pigments possess no measurable absorption in the NIR region, the tissue reflectance in this region is apparently determined by "internal" optical properties related to leaf thickness, water content, and light scattering. The scattering within plant tissues arises at interfacial boundaries separating phases with different refractive indices (Buschmann and Nagel 1993; Fukshansky 1981; Merzlyak et al. 2002b).

The algorithms for estimation of chlorophyll content were suggested in the form of simple ratios of reflectance coefficients at certain wavelengths taking into account the above-mentioned circumstances: $R_{NIR} \times R_{550}^{-1}$ and $R_{NIR} \times R_{700}^{-1}$, where R_{NIR} is insensitive and R_{550}^{-1} and R_{700}^{-1} are highly sensitive to chlorophyll content. Both ratios were highly sensitive to chlorophyll content in a wide range of its changes in leaves and fruits of diverse plant species and depended linearly on the pigment content (Gitelson and Merzlyak 1993, 1994, 1998; Lichtenthaler et al. 1996; Merzlyak et al. 2003a).

6.1.2 Anthocyanins

As stated in the previous section, the $R_{NIR} \times R_{550}^{-1}$ and $R_{NIR} \times R_{700}^{-1}$ ratios possess similar sensitivity to chlorophyll content, which is due to high correlation between reflectance coefficients at 550 and 700 nm characteristic of healthy anthocyanin-free leaves (Gitelson et al. 2001) and fruits (Fig. 6.2b). Accumulation of anthocyanins leads to a significant decrease in R_{550} relative to R_{700} (cf. closed and open symbols in Fig. 6.2b). This greatly complicates the application of the $R_{NIR} \times R_{550}^{-1}$ index for chlorophyll determination in red leaves. Further studies (Gitelson et al. 2001; Merzlyak et al. 2003b) showed that the $R_{NIR} \times R_{700}^{-1}$ index could be used for chlorophyll assessment even with high anthocyanin content. Thus, the lack of close correlation between the reflectance coefficients at 550 and 700 nm in red leaves became the basis of an effective approach to nondestructive determination of anthocyanins. It should be stressed that the decrease in correlation between R_{550} and R_{700} takes place even for very low anthocyanin content (about 1–2 nmol cm^{-2}), so the sensitive assay of these screening pigments in situ is feasible with this technique.

The main challenge of nondestructive anthocyanin determination is that in the green range of the spectrum, where anthocyanins absorb in vivo (Fig. 6.4a), the reflectance is also affected by absorption of chlorophylls (Figs. 6.1b, 6.3b). So, the goal of tuning the conceptual three-band model (6.1) for anthocyanin determination was to find spectral band λ_2 where reflectance is governed only by chlorophyll absorption and is not affected by anthocyanin content. Such a band was found using the minimal error of anthocyanin estimation for λ_2 in the red-edge range around 700 nm (Gitelson et al. 2001; Merzlyak et al. 2003a). As a result, the anthocyanin reflectance index (ARI) index for anthocyanin assessment was defined as

$$\mathrm{ARI} = (R_{550}^{-1} - R_{700}^{-1}) \times R_{800}, \tag{6.2}$$

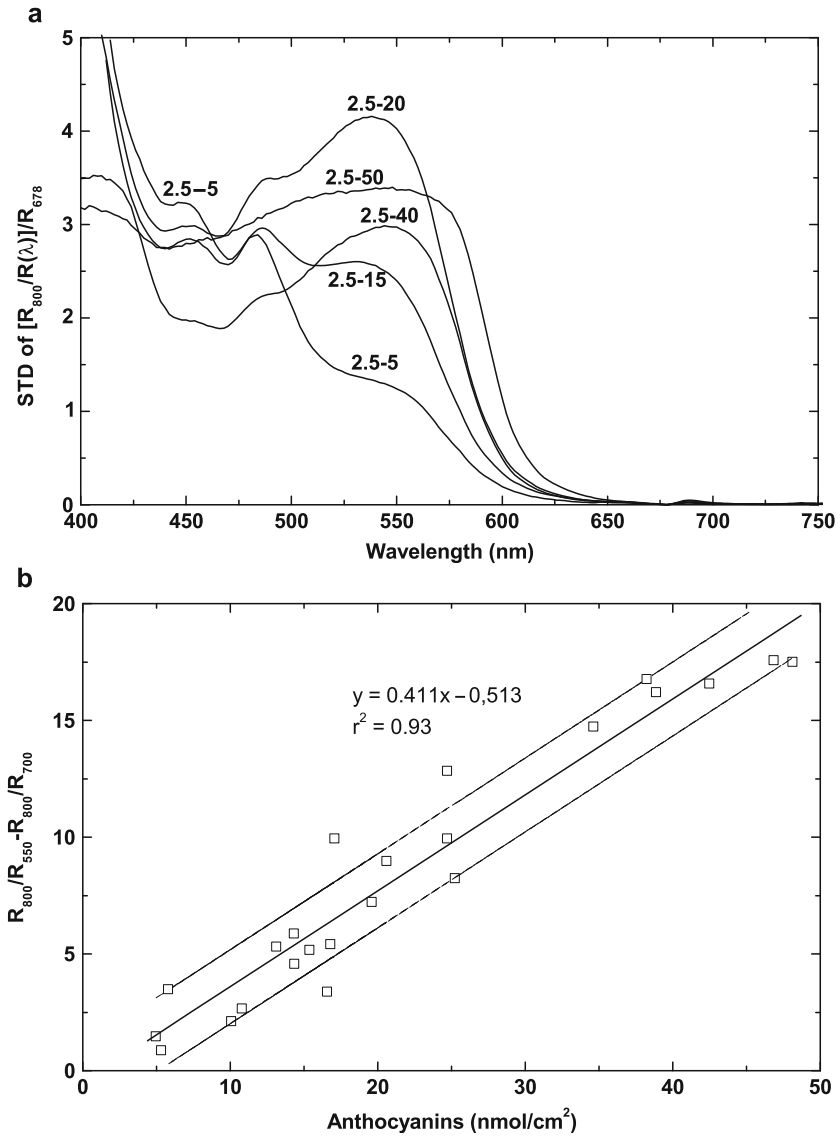

Fig. 6.4 **(a)** The STD spectra of the function $[R_{800} \times R(\lambda)^{-1}] \times R_{678}^{-1}$ for fruits with various anthocyanin content indicated in nanomoles per square centimeter near the curves. **(b)** The relationship of the reflectance ratio $(R_{550}^{-1} - R_{700}^{-1}) \times R_{800}$ and anthocyanin content. The *solid line* represents the best-fit function; *dashed lines* represent STD. (Reprinted from Merzlyak et al. (2003a, b) with permission from Elsevier)

where the first term is associated with combined absorption by anthocyanin and chlorophyll, the second one is related to chlorophyll absorption only, and the third one is not affected by pigment absorption and depends solely upon scattering. In

leaves of many plant species and apple fruit (Fig. 6.4b), the ARI and derived indices proved to be a highly sensitive linear indicators of anthocyanin content (Gitelson et al. 2001, 2003a, 2009; Steele et al. 2009). It should be underlined that the algorithms developed are able to provide accurate pigment estimation owing to precise subtraction of the chlorophyll contribution from the reflectance in the green range of reciprocal reflectance in the red-edge range.

6.1.3 Flavonols

The accumulation of flavonols occurring mainly in the vacuoles of subcuticular cell layers of the peel (see Chap. 4) is accompanied by a sharp decrease of fruit reflectance and flattening of the spectrum in the broad band between 350 and 420 nm (Fig. 5.13). The nondestructive assessment of flavonols in plant tissues, which exhibit low reflectance in the UV-A region, is complicated by overlapping of their absorption with the absorption of several pigments: chlorophyll and carotenoids, as well as other phenolics (catechins and phenolic acids) possessing main absorption bands in the UV-B region (Burchard et al. 2000; Krauss et al. 1997). In addition, scattering exerts a strong influence on UV reflectance: it was reported that the scattering coefficients of whole fruit (Cubeddu et al. 2001) and the skin and isolated cuticles of apples (Solovchenko and Merzlyak 2003) are wavelength-dependent and undergo an increase with wavelength decrease.

The application of the conceptual model (6.1) for quantitative estimation of flavonol content required finding optimal λ_1, λ_2, and λ_3 (see Sect. 6.1.1). Remarkably, at wavelengths shorter than 380 nm the reciprocal reflectance of apple fruits showed a weak correlation with flavonol content (Fig. 5.14). This could result from the saturation of the relationship "$100R(\lambda)^{-1}$ versus flavonol content" as well as interference by different phenolic substances (Krauss et al. 1997). The reflectance band of the highest sensitivity to flavonols in the whole range of its changes was found between 380 and 420 nm, peaking near 410 nm (band λ_1; Fig. 5.14). However, in this spectral band chlorophylls and carotenoids also strongly absorb. Therefore, it was not surprising that the linear relationship between $R_{800} \times R_{410}^{-1}$ and flavonol content was not significant (Fig. 6.5a), owing to the interference by chlorophylls and carotenoids. To remove the contribution of these pigments, one needs to find band λ_2 in (6.1), where the reflectance is closely related to the chlorophyll and carotenoid contents and is minimally affected by flavonol absorption. This band was selected using the criterion of minimal error of flavonol estimation around 460 nm. As a result, the flavonol reflectance index (FRI) was suggested in the form (Merzlyak et al. 2005b)

$$\mathrm{FRI} = (R_{410}^{-1} - R_{460}^{-1}) \times R_{800}. \tag{6.3}$$

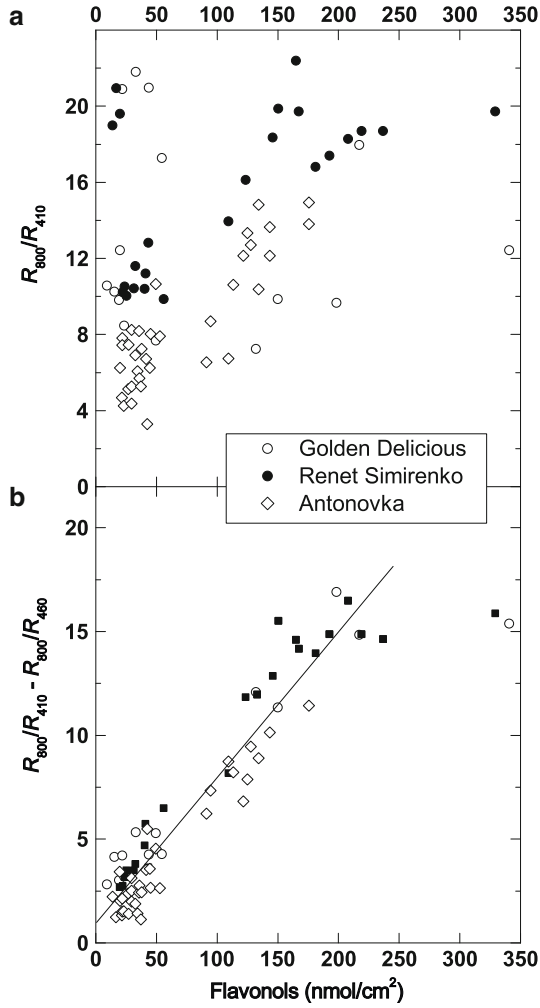

Fig. 6.5 Relationships between reciprocal reflectance at 410 nm (**a**), flavonol reflectance index, $(R_{410}^{-1} - R_{460}^{-1}) \times R_{800}$, (**b**), and apple skin flavonol content. In **b** the *solid line* represents the linear fit for flavonol content in the range 8–220 nmol cm^{-2}. (Reprinted from Merzlyak et al. (2005a, b) with permission from Elsevier)

The FRI allowed accurate assessment ($r^2 = 0.92$, error 0.05 nmol cm^{-2}) of skin flavonol content ranging from 0.08 to 2.20 nmol cm^{-2} for all apple fruit varieties studied.

The development of the FRI for nondestructive quantification of flavonols in apple fruit represents an example of spectral tuning a conceptual model developed for terrestrial plant leaves. It provides evidence that fine-tuning of the conceptual model can be carried out knowing the spectral characteristics of the specific medium of interest.

Another important conclusion is that the influence of flavonols on optical spectra of apple fruits and higher-plant leaves might extend quite far into the visible spectrum. Therefore, when using reflectances for nondestructive determination of

higher-plant pigments absorbing in the visible range, one should be aware of obstacles which could be caused by flavonols when they are present in high amounts.

6.1.4 Carotenoids

The analysis of carotenoids absorbing in the blue region of the spectrum is greatly complicated by overlapping absorption of chlorophyll present in high amounts in plant tissues (Gitelson et al. 2002; Merzlyak et al. 1999, 2002b). Additional obstacles to the analysis of carotenoids in plants are due to the presence of several photosynthetic (thylakoid-bound) and photoprotective (extrathylakoid) xanthophyll species whose pools undergo disproportional changes during leaf ontogeny and upon adaptation of leaves to variable light conditions (Gross 1987; Knee 1988; Merzlyak and Solovchenko 2002). To estimate the effect of carotenoids on reflectance spectra, one needs to remove the significant effect of chlorophyll absorption. Normalization of the reciprocal reflectance to reflectance at 678 nm (red chlorophyll absorption band) removes to a certain degree the chlorophyll effect, since the amplitudes of the normalized spectra depend on factors other than chlorophyll (Fig. 6.6; Gitelson et al. 2002).

The quantitative estimation of carotenoids in apple turned out to be feasible using the same three-band model (6.1) with λ_1 in the range 510–520 nm (Figs. 6.6,

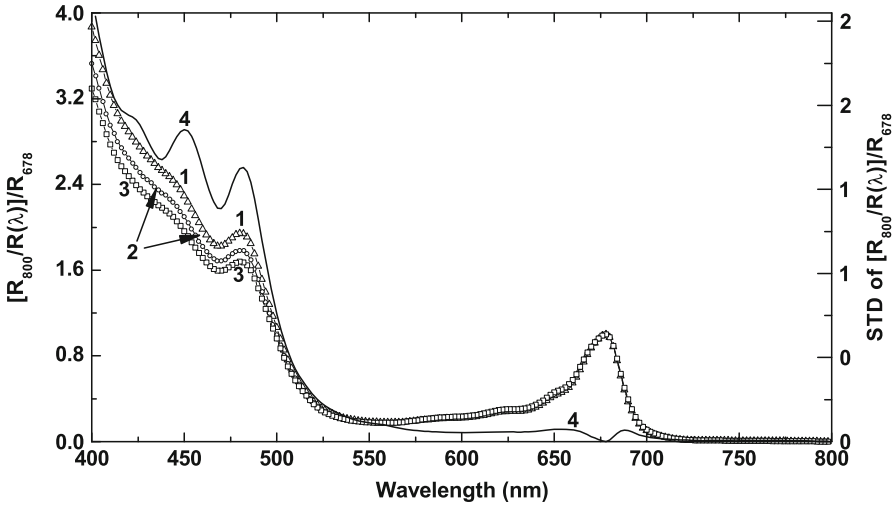

Fig. 6.6 The in vivo maxima of carotenoid absorption in anthocyanin-free apple fruit. The average spectra of the function $(R_{800} \times R_{(\lambda)}^{-1}) \times R_{678}^{-1}$ (*left scale*) for anthocyanin-free apple fruits with different ranges of carotenoids (*1* 0–1 nmol cm^{-2}, *2* 0–3 nmol cm^{-2}, and *3* 0–4 nmol cm^{-2}) and their STD (*right scale*) spectrum. (Reprinted from Merzlyak et al. (2005a, b) with permission from Elsevier)

6.7; Gitelson et al. 2002; Merzlyak et al. 2003b). To subtract the contribution of chlorophyll absorption to reflectance in spectral band λ_1, λ_2 was found to be optimal in either the green range (around 550 nm) or the red-edge range (700 nm). As for chlorophyll and anthocyanin determination, the optimal λ_3 was in the NIR range beyond 750 nm. Two carotenoid reflectance indexes (CRI) developed earlier for leaves (Gitelson et al. 2002) were suggested as

$$\mathrm{CRI}_1 = (R_{520}^{-1} - R_{700}^{-1}) \times R_{800}, \tag{6.4}$$

or

$$\mathrm{CRI}_2 = (R_{520}^{-1} - R_{550}^{-1}) \times R_{800}, \tag{6.5}$$

where the first term in the parentheses is associated with combined absorption by carotenoids and chlorophylls, and the second one relates to chlorophyll absorption only. The applications of a tuned version of these algorithms (Fig. 6.7b) to several apple cultivars have confirmed its efficiency for estimation of carotenoids in a wide range of their changes (Merzlyak et al. 2003b). It should be mentioned, however, that the CRI is not applicable to anthocyanin-pigmented samples.

6.2 Approaches to Estimation of the Photoprotective Pigment Efficiency In Planta

The efficiency of photoprotection provided by screening pigments in planta is determined by the ratio of the amount of radiation intercepted by screening pigments and the amount of radiation absorbed by photosynthetic pigments and other photosensitizers present in the plant cell (see Chap. 1). Numerous approaches characterized by distinct advantages and drawbacks are currently employed for quantification of screening pigments and estimation of their efficiency. A common approach involves the spectrophotometric analysis and comparison of the absorption spectra of extracts of algal cells or higher-plant tissues grown under contrasting conditions (i.e., normal and stressful conditions promoting the accumulation of screening pigments). For instance, the absorption of methanolic extracts from UV-irradiated cyanobacterial and microalgal cells in the band near 300 nm increases considerably owing to accumulation of mycosporine-like amino acids (Cockell and Knowland 1999; Karsten et al. 2005). Irradiation of leaves and fruit peel by elevated fluxes of UV and visible radiation caused an increase of the absorption of their extract owing to accumulation of phenolic screening compounds (flavonols and/or anthocyanins) and, often, carotenoids (Bidel et al. 2007; Merzlyak et al. 2002a; Solovchenko and Schmitz-Eiberger 2003). The more advanced variations of this method presume the elimination of the contribution of chlorophyll to the absorption of the extract, which could be achieved by alteration of the extraction procedure (Solovchenko et al. 2001), subtraction of the spectral contribution of

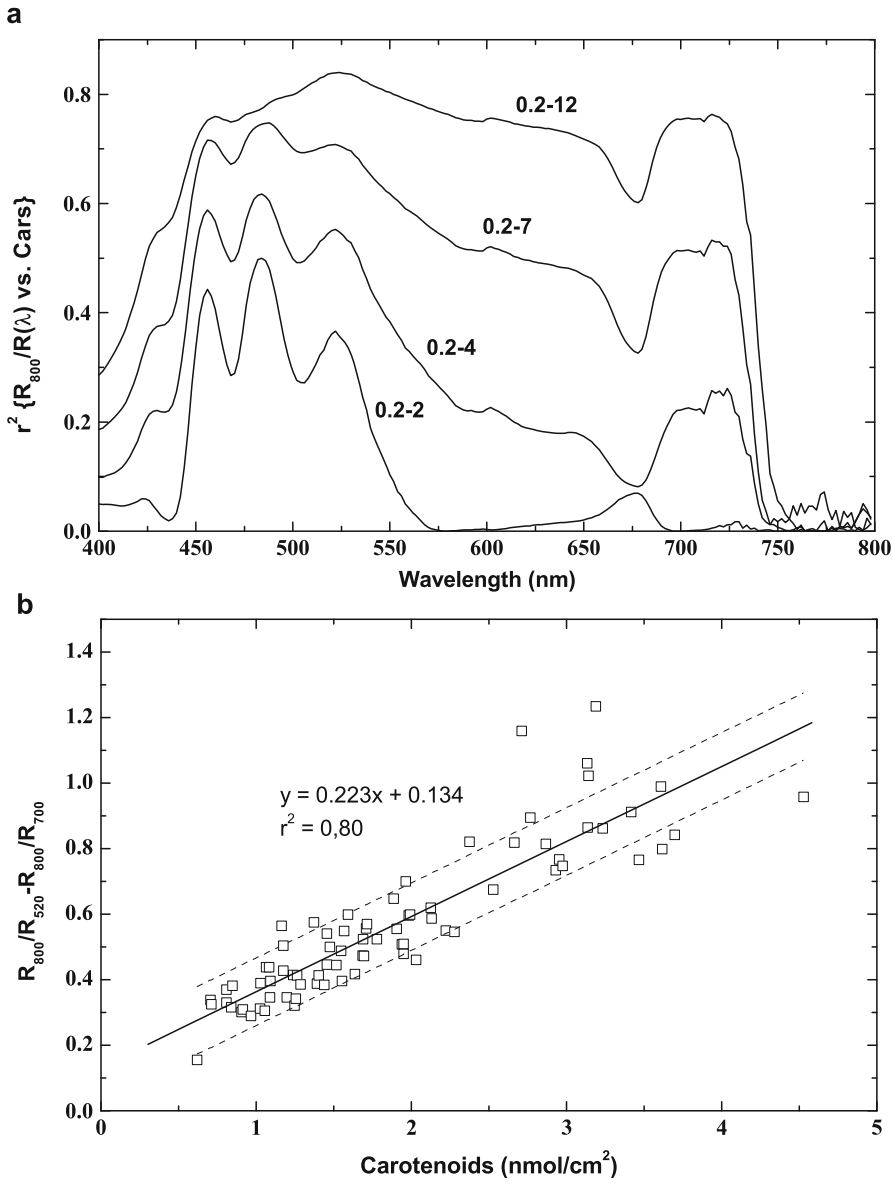

Fig. 6.7 **(a)** The determination coefficient spectra of the relationship "reflectance ratio $R_{800}R(\lambda)^{-1}$ versus carotenoid content" for fruits with different chlorophyll content. *Numbers* indicate the chlorophyll content range (nmol cm^{-2}). The *solid line* represents the best-fit function; *the dashed lines* represent STD. **(b)** The carotenoid reflectance index versus peel carotenoid content for anthocyanin-free fruits. *Solid lines* represent the best-fit function; *dashed lines* represent STD

chlorophylls from the spectra of total extracts (Cerovic et al. 2002; Solovchenko et al. 2001), or using chromatographic techniques. These approaches are relatively simple to implement but they are able to provide only limited information on the function of photoprotective pigments since their absorption in solution differs considerably from that in planta owing to a number of factors (see Chap. 5).

A number of works dedicated to investigation of the spectral absorption of light by superficial structures and epidermal tissues of leaves and fruits were carried out on preparations of isolated cuticle and epidermis (Baur et al. 1998; Krauss et al. 1997; Markstädter et al. 2001; Solovchenko and Merzlyak 2003). This method provides information on the attenuation of light by plant superficial structures but suffers from uncertainties related to the isolation and nativity of the preparation. Therefore, experiments with optical microfibers introduced to the mesophyll of otherwise intact leaf are of a considerable interest; the findings obtained using this technique allowed the light gradients within the leaf blade to be described for different plant species (Day et al. 1993; Vogelmann and Han 2000).

Recently, nondestructive techniques were developed based on the analysis of optical reflectance (Gitelson et al. 2009; Merzlyak 2006; Merzlyak et al. 2008a, b; Solovchenko et al. 2010b) or fluorescence excitation spectra of chlorophyll, employing the latter as the endogenous fluorescent probe (Bengtsson et al. 2006; Bilger et al. 1997, 2001, 2007; Burchard et al. 2000; Hagen et al. 2006, 2007; Markstädter et al. 2001). These techniques possess a number of important advantages over the methods described above and will be considered in detail in the next paragraphs.

The ratio of the intensities of chlorophyll fluorescence excited in the UV-B region to that in the blue-green regions of the spectrum was found to be proportional to the UV-B transmittance of the epidermis samples and their phenolic content (Barnes et al. 2000; Bidel et al. 2007; Bilger et al. 1997; Burchard et al. 2000). In leaves the ratio of chlorophyll fluorescence excited by UV-B radiation to that excited by blue-green light showed a negative correlation with the concentration of whole-leaf UV-B-absorbing pigments, and a positive correlation with the transmittance of isolated epidermal tissue, where flavonoids accumulate (Barnes et al. 2000). In these studies, screening by flavonoids was quantified, on the leaf/fruit level, by using a chlorophyll fluorescence excitation (CFE) ratio, e.g., the ratio of the chlorophyll fluorescence yields for different excitation wavelengths. Further progress can be made only by relating a CFE spectrum to specific spectral features of chlorophyll and individual light-screening and/or internally trapping pigments in the specimen under examination.

The ratio analysis of the CFE spectra provides the in situ information on the chromophore(s) absorbing, together with chlorophylls and carotenoids, radiation in the UV region and/or visible region (Cerovic et al. 2002; Hagen et al. 2006; Kolb et al. 2001; Merzlyak et al. 2008b). This approach was successfully used for estimation of flavonol and anthocyanin contents in fruits of apple (Hagen et al. 2007), grape (Agati et al. 2008), and olive (Agati et al. 2005) fruit as well as of broccoli leaves (Bengtsson et al. 2006). The analysis of the CFE spectra recorded from the adaxial and abaxial leaf surfaces revealed the epidermal UV-protective

phenolics (Cerovic et al. 2002). It was found that, owing to the strong absorption by chlorophyll at wavelengths shorter than 450 nm, differences in leaf optical properties exert only a minor influence on the shape of UV-excitation spectra, and chlorophyll behaves as a photon counter. These methods could also be implemented with the use of commercially available pulse-amplitude modulated fluorometers (such as PAM 2000) routinely employed for measurement of variable chlorophyll fluorescence (Hagen et al. 2006).

Another effective technique for in planta quantification of screening efficiency recently developed by Merzlyak et al. (2008a, 2008b) employs a simple approach similar to that used for reconstruction of pigment extract spectra (see, e.g., Naqvi et al. 2004). Briefly, the measured reflectance spectra in the form of the reciprocal reflectance $R(\lambda)^{-1}$ are represented as a linear combination of the contributions, $F(\lambda)$, of individual apple pigment pools and scattering according to the model

$$M_{(\lambda)} = \alpha_0 + \alpha_1 F_{\mathrm{T}}(\lambda) + \alpha_2 F_{\mathrm{X}}(\lambda) + \alpha_3 F_{\mathrm{P}}(\lambda) + \alpha_4 s(\lambda), \tag{6.6}$$

where $M_{(\lambda)}$ is the modeled reciprocal reflectance spectrum, $F_{\mathrm{T}}(\lambda)$ is the contribution of photosynthetic pigments (chlorophylls and carotenoids) tightly associated with thylakoid membranes (obtained in photobleaching experiments; see Merzlyak and Solovchenko 2002; Merzlyak 2006), $F_{\mathrm{X}}(\lambda)$ is the contribution of extrathylakoid carotenoids (mainly xanthophylls and fatty acid xanthophyll esters; see Chap. 4), $F_{\mathrm{P}}(\lambda)$ is the "tail" absorption by cuticular and vacuolar phenolics, $s(\lambda)$ is the contribution of light losses due to scattering, and $\alpha_1 - \alpha_4$ are constants.

Accordingly, the efficiency of interception of photosynthetically active radiation (PAR) by a pool of screening pigments, e.g., extrathylakoid carotenoids, at a given wavelength was estimated as the ratio of the amounts of light intercepted by these pigments and photosynthetic carotenoids and chlorophylls,

$$S_{(\lambda)} = F_{\mathrm{X}}(\lambda)/F_{\mathrm{T}}(\lambda) - 1, \tag{6.7}$$

or, in the whole PAR range, as

$$S^{\mathrm{PAR}} = \int_{\lambda=400}^{\lambda=750} \left(\frac{F_{\mathrm{X}}(\lambda)}{F_{\mathrm{T}}(\lambda)} - 1\right) \mathrm{d}\lambda. \tag{6.8}$$

The quantification of screening or internal trapping of radiation by flavonols, carotenoids, and anthocyanins using reconstruction of reflectance and CFE spectra according to Merzlyak et al. (2008b) could be demonstrated for the example of apple fruit acclimated to strong sunlight (Figs. 6.8, 6.9).

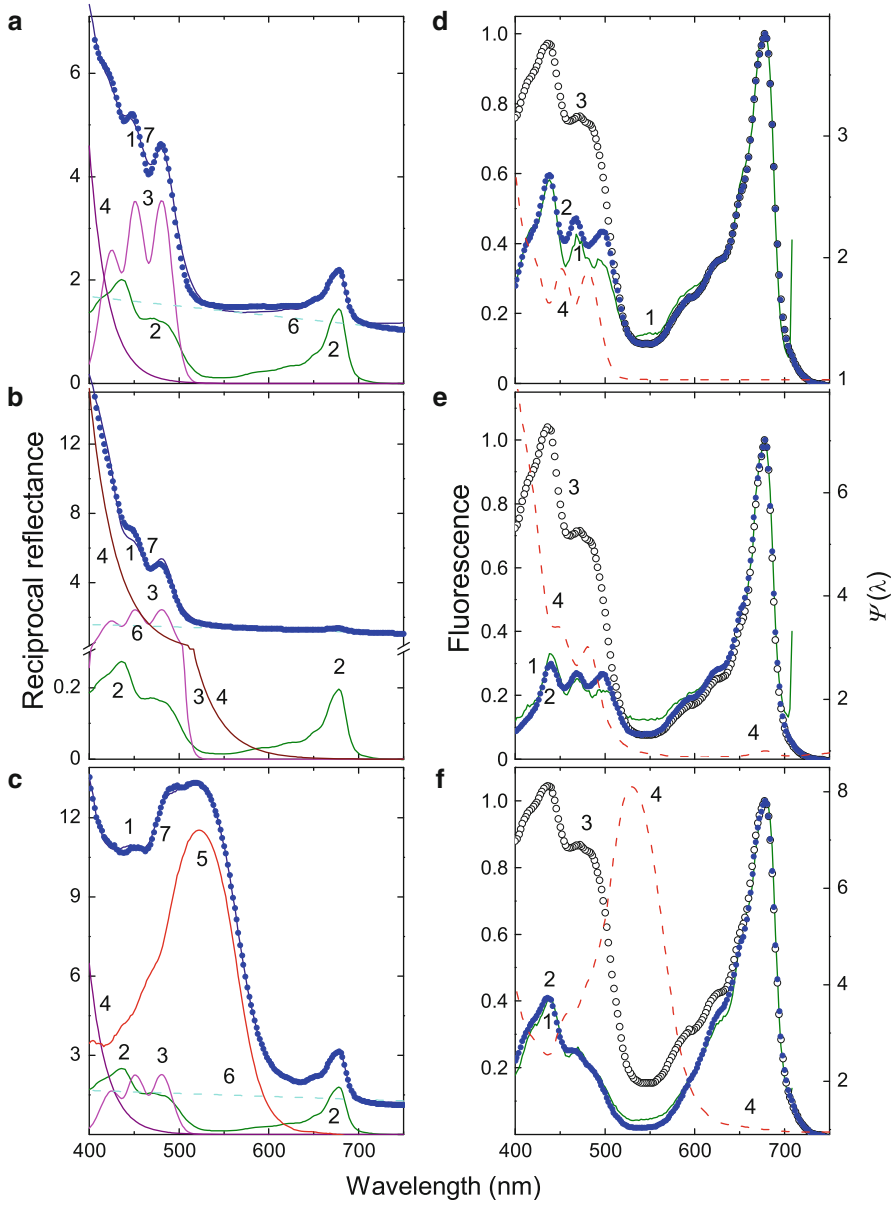

Fig. 6.8 Spectral reconstruction of reciprocal reflectance spectra (**a**–**c**) and chlorophyll fluorescence excitation (CFE) spectra (**d**–**f**) of apple fruit. In (**a**–**c**) (*right scale*), *curve 1* (*solid line*) is the reciprocal reflectance and *curve 7* (*symbols*) is the corresponding model (for details, see Merzlyak et al. (2008b), *curve 2* is the contribution from photosynthetic (thylakoid-bound) pigments, *curve 3* is the contribution from extrathylakoid carotenoids, *curve 4* is the tail absorption by phenolic compounds, *curve 5* is the contribution from anthocyanins, and *curve 6* is scattering. In (**d**–**f**) (*left scale*), *curve 1* (*solid line*) is the measured CFE spectrum (in the presence of screening pigments)

Comparison of CFE spectra of sunlit and shaded fruit surfaces of Golden Delicious apples differing in flavonol content displayed a remarkable difference below 500 nm (cf. Fig. 6.8d, e). Notably, in these apples the influence of flavonols was pronounced even in the blue range of the spectrum. In addition, the "shaded-to-sunlit" ratio spectrum revealed two bands near 455 and 485 nm attributable to carotenoids (Merzlyak and Solovchenko 2002), whose content was reported to increase in sun-exposed apple fruit (Ma and Cheng 2004; Solovchenko et al. 2006). The modeling of the CFE spectrum of a Golden Delicious fruit with increased flavonol content indicated that near 400 nm internal trapping by these pigments (and, to some extent, by carotenoids) causes an almost eightfold decrease of chlorophyll fluorescence (Fig. 6.8b). The increase in anthocyanins in Summer Red fruit was accompanied by a content-dependent decrease of chlorophyll fluorescence in a broad band up to 650 nm, and at high anthocyanin content only a weak chlorophyll *a* peak at 440 nm was detected (Fig. 6.8c, f).

Extrathylakoid carotenoids in fruit acclimated to strong sunlight contribute considerably to interception of PAR (Figs. 6.8a–c, 6.9) and exert a significant effect on CFE (Fig. 6.8d–f). According to estimates by Merzlyak et al. (2008b) and Solovchenko et al. (2010b), light trapping attributable to the extrathylakoid carotenoids in the 440–490-nm band in unripe apples with high chlorophyll content was low, but increased significantly at advanced stages of ripening and/or acclimation to strong sunlight (Fig. 6.9). Taking into account downregulation of "active" photoprotective mechanisms such as quenching of chlorophyll fluorescence (Solovchenko et al. 2010b), the buildup of "passive" optical screening (Fig. 6.9) demonstrates the "switching" from energy-dependent mechanisms to photoprotection via optical screening of the excessive PAR, which could be of considerable importance at the advanced stages of ripening, when "active" photoprotection mechanisms such as the violaxanthin cycle operate with low efficiency.

As shown in Agati et al. (2005), Barthod et al. (2007), Bengtsson et al. (2006), Cerovic et al. (2002), Hagen et al. (2006), and Merzlyak et al. (2008b), the application of chlorophyll fluorescence for nondestructive analysis of plant constituents able to compete with chlorophyll in light absorption is a promising tool. In anthocyanin-free fruit, the assessment of flavonols turned out to be feasible using the CFE ratio at 440 nm. For Summer Red apples, the CFE ratio at 700 and 580 nm provided efficient assessment of anthocyanins even in fruit with a high content of

Fig. 6.8 (continued) and *curve 2* (*symbols*) is the corresponding model, *curve 3* is the reconstructed CFE spectrum as it would be in the absence of screening pigments, and *curve 4* (*right scale*) is the ratio of *curve 3* and *curve 2* representing screening efficiency. Pigment content (nmol cm^{-2}) in (**a**) and (**d**) (Granny Smith) 1.59 (chlorophylls), 1.64 (carotenoids), and 11.5 (flavonols), in (**b**) and (**e**) (Golden Delicious) 0.59, (chlorophylls), 1.45 (carotenoids), and 102.9 (flavonols), and in (**c**) and (**f**) (Summer Red) 1.12 (chlorophylls) and 19.2 (anthocyanins). (Reproduced from Merzlyak et al. (2008b) with permission from Oxford University Press)

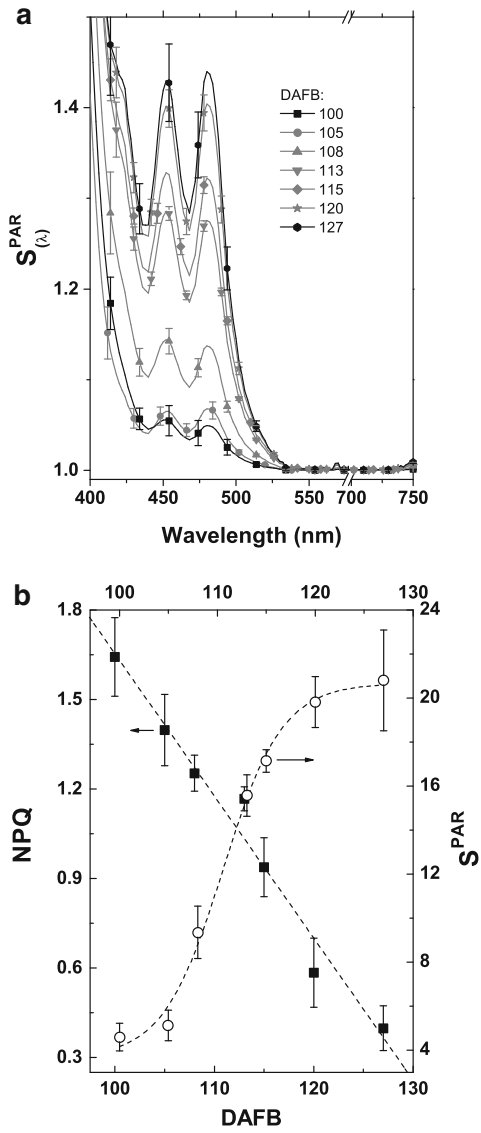

Fig. 6.9 The changes in **(a)** spectral efficiency of screening of photosynthetically active radiation, $S_{(\lambda)}$, by extrathylakoid carotenoids in the course of Antonovka apple ripening (indicated as days passed after full bloom occurred on May 25) and **(b)** time course of integral screening (for details, see Solovchenko et al. (2010b)) and nonphotochemical quenching changes in the same fruit. (Reprinted from Solovchenko et al. (2010a, b) with permission from Elsevier)

the pigments. Similar precision for anthocyanin and total flavonol analysis was reported in recent measurements with a PAM chlorophyll fluorometer in Aroma apples (Hagen et al. 2006). Although quantitative analysis of total carotenoids in anthocyanin-free apple fruit with reflectance spectroscopy was developed (see Sect. 6.1.4), attempts to use the fluorescence technique for this purpose were

unsuccessful, maybe due to the involvement of different carotenoid pools in light harvesting and in screening (Merzlyak and Solovchenko 2002; Merzlyak et al. 2008b; Solovchenko et al. 2006). It appears that at present spectral reflectance with simpler quantitative measurements of (re)emitted light and simple relations to the content of a pigment of interest represents a more reliable and flexible technique for nondestructive pigment assessments, although the specific advantages of the fluorescence analysis pointed out in the literature (Agati et al. 2005; Barthod et al. 2007; Bengtsson et al. 2006; Cerovic et al. 2002; Hagen et al. 2006; Merzlyak et al. 2008b) are of considerable importance.

6.3 Concluding Remarks

The results obtained during the last two decades considerably extended the possible applications of reflectance spectroscopy for estimation of screening pigment content and for assessment of the physiological state of plants. These achievements are really impressive, because some time ago reflectance spectroscopy was considered inadequate to provide useful information about plant organisms owing to their low reflectance and poorly resolved spectra that seemed similar in different species (Gamon and Surfus 1999).

The data presented in this chapter show that reflectance spectroscopy could be a useful and efficient tool for quantification of screening pigments in plants. Remarkably, the approaches for nondestructive assessment of carotenoids, anthocyanins, and flavonols discussed here require knowledge of reflectance only at a few certain wavelengths. However, the possibilities of application of this technique to leaves and other organs of other plant species need further verification. Still, the progress achieved so far facilitates the extensive application of reflectance spectroscopy for solving various issues of screening pigment physiology on the level of individual leaves and fruits as well as on the whole-plant scale.

The more advanced reflectance- and fluorescence-based techniques turned out to be efficient tools for the investigation of the screening-based photoprotective mechanisms in planta. The tuning of the basic model (6.1) allowed its application to leaves and fruits with a wide variation in pigment content and composition. Since the approach decreases the uncertainties related to the contributions of individual pigments to reflectance and estimating the effect of internal fruit properties on reflectance, it is able to improve both the precision and selectivity of nondestructive pigment determination. Finally, modeling of CFE spectra has confirmed that flavonols, carotenoids, and anthocyanins are able to exert strong photoprotective effects in specific spectral regions in planta. It is remarkable that the acclimation of plants to high-light stress involves the accumulation of pigments with strongly overlapping absorption that provide screening and internal light trapping of solar radiation in broad spectral ranges extending from the UV region to the green and, in anthocyanin-accumulating species, to the red regions of the visible spectrum.

References

Agati G, Pinelli P, Ebner S, Romani A, Cartelat A, Cerovic Z (2005) Nondestructive evaluation of anthocyanins in olive (*Olea europaea*) fruits by in situ chlorophyll fluorescence spectroscopy. J Agric Food Chem 53:1354–1363

Agati G, Traversi M, Cerovic Z (2008) Chlorophyll fluorescence imaging for the noninvasive assessment of anthocyanins in whole grape (*Vitis vinifera* L.) bunches. Photochem Photobiol 84:1431–1434

Barnes P, Searles P, Ballare C, Ryel R, Caldwell M (2000) Non-invasive measurements of leaf epidermal transmittance of UV radiation using chlorophyll fluorescence: field and laboratory studies. Physiol Planta 109:274–283

Barthod S, Cerovic Z, Epron D (2007) Can dual chlorophyll fluorescence excitation be used to assess the variation in the content of UV-absorbing phenolic compounds in leaves of temperate tree species along a light gradient? J Exp Bot 58:1753–1760

Baur P, Stulle K, Uhlig B, Schönherr J (1998) Absorption von Strahlung im UV-B und Blaulichtbereich von Blattkutikeln ausgewählter Nutzpflanzen. Gartenbauwissenschaft 63:145–152

Bengtsson G, Schöner R, Lombardo E, Schöner J, Borge G, Bilger W (2006) Chlorophyll fluorescence for non-destructive measurement of flavonoids in broccoli. Postharvest Biol Technol 39:291–298

Bidel L, Meyer S, Goulas Y, Cadot Y, Cerovic Z (2007) Responses of epidermal phenolic compounds to light acclimation: in vivo qualitative and quantitative assessment using chlorophyll fluorescence excitation spectra in leaves of three woody species. J Photochem Photobiol B Biol 88:163–179

Bilger W, Veit M, Schreiber L, Schreiber U (1997) Measurement of leaf epidermal transmittance of UV radiation by chlorophyll fluorescence. Physiol Planta 101:754–763

Bilger W, Johnsen T, Schreiber U (2001) UV-excited chlorophyll fluorescence as a tool for the assessment of UV-protection by the epidermis of plants. J Exp Bot 52:2007–2014

Bilger W, Rolland M, Nybakken L (2007) UV screening in higher plants induced by low temperature in the absence of UV-B radiation. Photochem Photobiol Sci 6:190–195

Burchard P, Bilger W, Weissenbock G (2000) Contribution of hydroxycinnamates and flavonoids to epidermal shielding of UV-A and UV-B radiation in developing rye primary leaves as assessed by ultraviolet-induced chlorophyll fluorescence measurements. Plant Cell Environ 23:1373–1380

Buschmann C, Nagel E (1993) In vivo spectroscopy and internal optics of leaves as basis for remote sensing of vegetation. Int J Remote Sens 14:711–722

Cerovic Z et al (2002) The use of chlorophyll fluorescence excitation spectra for the non-destructive in situ assessment of UV-absorbing compounds in leaves. Plant Cell Environ 25:1663–1676

Cockell C, Knowland J (1999) Ultraviolet radiation screening compounds. Biol Rev 74:311–345

Cubeddu R et al (2001) Time-resolved reflectance spectroscopy applied to the non-destructive monitoring of the internal optical properties in apples. Appl Spectrosc 55:1368–1374

Day T, Martin G, Vogelmann T (1993) Penetration of UV-B radiation in foliage: evidence that the epidermis behaves as a non-uniform filter. Plant Cell Environ 16:735–741

Filella I, Penuelas J (1999) Altitudinal differences in UV absorbance, UV reflectance and related morphological traits of *Quercus ilex* and *Rhododendron ferrugineum* in the Mediterranean region. Plant Ecol 145:157–165

Fukshansky L (1981) Optical properties of plant tissue. In: Smith H (ed) Plants and the daylight spectrum. Springer, Berlin, pp 37–303

Gamon J, Surfus J (1999) Assessing leaf pigment content and activity with a reflectometer. New Phytol 143:105–117

Gitelson A, Merzlyak M (1993) Spectral reflectance changes associated with autumn senescence of *Aesculus hippocastanum* L. and *Acer platanoides* L. leaves. Spectral features and relation to chlorophyll estimation. J Plant Physiol 143:286–292

Gitelson A, Merzlyak M (1994) Quantitative estimation of chlorophyll-a using reflectance spectra: experiments with autumn chestnut and maple leaves. J Photochem Photobiol B Biol 22:247–252

Gitelson A, Merzlyak M (1996) Signature analysis of leaf reflectance spectra: algorithm development for remote sensing of chlorophyll. J Plant Physiol 148:494–500

Gitelson A, Merzlyak M (1998) Remote sensing of chlorophyll concentration in higher plant leaves. Adv Space Res 22:689–692

Gitelson AA, Merzlyak MN, Chivkunova OB (2001) Optical properties and nondestructive estimation of anthocyanin content in plant leaves. Photochem Photobiol 74:38–45

Gitelson AA, Zur Y, Chivkunova OB, Merzlyak MN (2002) Assessing carotenoid content in plant leaves with reflectance spectroscopy. Photochem Photobiol 75:272–281

Gitelson A, Gritz Y, Merzlyak M (2003a) Non destructive chlorophyll assessment in higher plant leaves: algorithms and accuracy. J Plant Physiol 160:271–282

Gitelson AA, Gritz Y, Merzlyak MN (2003b) Relationships between leaf chlorophyll content and spectral reflectance and algorithms for non-destructive chlorophyll assessment in higher plant leaves. J Plant Physiol 160:271–282

Gitelson A, Keydan G, Merzlyak M (2006) Three-band model for noninvasive estimation of chlorophyll, carotenoids, and anthocyanin contents in higher plant leaves. Geophys Res Lett 33:L11402

Gitelson A, Chivkunova O, Merzlyak M (2009) Nondestructive estimation of anthocyanins and chlorophylls in anthocyanic leaves. Am J Bot 96:1861

Gross J (1987) Carotenoids: pigments in fruits. Food science and technology. Series of monographs. Academic, London, pp 87–98

Hagen S, Solhaug K, Bengtsson G, Borge G, Bilger W (2006) Chlorophyll fluorescence as a tool for non-destructive estimation of anthocyanins and total flavonoids in apples. Postharvest Biol Technol 41:156–163

Hagen S et al (2007) Phenolic contents and other health and sensory related properties of apple fruit (*Malus domestica* Borkh., cv. Aroma): effect of postharvest UV-B irradiation. Postharvest Biol Technol 45:1–10

Karsten U, Friedl T, Schumann R, Hoyer K, Lembcke S (2005) Mycosporine-like amino acids and phylogenies in green algae: *Prasiola* and its relatives from the Trebouxiophyceae (Chlorophyta). J Phycol 41:557–566

Knee M (1980) Methods of measuring green colour and chlorophyll content of apple fruit. Int J Food Sci Technol 15:493–500

Knee M (1988) Carotenol esters in developing apple fruits. Phytochemistry 27:1005–1009

Kolb C, Kaser M, Kopecky J, Zotz G, Riederer M, Pfündel E (2001) Effects of natural intensities of visible and ultraviolet radiation on epidermal ultraviolet screening and photosynthesis in grape leaves. Plant Physiol 127:863–875

Krauss P, Markstadter C, Riederer M (1997) Attenuation of UV radiation by plant cuticles from woody species. Plant Cell Environ 20:1079–1085

Lichtenthaler H (1987) Chlorophyll and carotenoids: pigments of photosynthetic biomembranes. Methods Enzymol 148:331–382

Lichtenthaler H, Gitelson A, Lang M (1996) Non-destructive determination of chlorophyll content of leaves of a green and an aurea mutant of tobacco by reflectance measurements. J Plant Physiol 148:483–493

Ma FW, Cheng LL (2004) Exposure of the shaded side of apple fruit to full sun leads to up-regulation of both the xanthophyll cycle and the ascorbate-glutathione cycle. Plant Sci 166:1479–1486

Markstädter C, Queck I, Baumeister J, Riederer M, Schreiber U, Bilger W (2001) Epidermal transmittance of leaves of *Vicia faba* for UV radiation as determined by two different methods. Photosynth Res 67:17–25

Merzlyak MN (2006) Modeling pigment contributions to spectral reflection of apple fruit. Photochem Photobiol Sci 5:748–754

Merzlyak M, Solovchenko A (2002) Photostability of pigments in ripening apple fruit: a possible photoprotective role of carotenoids during plant senescence. Plant Sci 163:881–888
Merzlyak M, Khozin I, Cohen Z (1996) Spectrophotometric analysis of carotenoids in plant extracts based on elimination of chlorophyll absorption. Phytochem Anal 7:294–299
Merzlyak M, Gitelson A, Chivkunova O, Rakitin V (1999) Non-destructive optical detection of pigment changes during leaf senescence and fruit ripening. Physiol Planta 106:135–141
Merzlyak M, Solovchenko A, Chivkunova O (2002a) Patterns of pigment changes in apple fruits during adaptation to high sunlight and sunscald development. Plant Physiol Biochem 40:679–684
Merzlyak MN, Chivkunova OB, Melo TB, Naqvi KR (2002b) Does a leaf absorb radiation in the near infrared (780–900 nm) region? A new approach to quantifying optical reflection, absorption and transmission of leaves. Photosynth Res 72:263–270
Merzlyak M, Gitelson A, Chivkunova O, Solovchenko A, Pogosyan S (2003a) Application of reflectance spectroscopy for analysis of higher plant pigments. Russ J Plant Physiol 50:704–710
Merzlyak M, Solovchenko A, Gitelson A (2003b) Reflectance spectral features and non-destructive estimation of chlorophyll, carotenoid and anthocyanin content in apple fruit. Postharvest Biol Technol 27:197–212
Merzlyak M, Solovchenko A, Pogosyan S (2005a) Optical properties of rhodoxanthin accumulated in *Aloe arborescens* Mill. leaves under high-light stress with special reference to its photoprotective function. Photochem Photobiol Sci 4:333–340
Merzlyak MN, Solovchenko AE, Smagin AI, Gitelson AA (2005b) Apple flavonols during fruit adaptation to solar radiation: spectral features and technique for non-destructive assessment. J Plant Physiol 162:151–160
Merzlyak MN, Chivkunova OB, Solovchenko AE, Naqvi KR (2008a) Light absorption by anthocyanins in juvenile, stressed, and senescing leaves. J Exp Bot 59:3903–3911
Merzlyak MN, Melo TB, Naqvi KR (2008b) Effect of anthocyanins, carotenoids, and flavonols on chlorophyll fluorescence excitation spectra in apple fruit: signature analysis, assessment, modelling, and relevance to photoprotection. J Exp Bot 59:349–359
Naqvi K, Hassan T, Naqvi Y (2004) Expeditious implementation of two new methods for analysing the pigment composition of photosynthetic specimens. Spectrochim Acta A Mol Biomol Spectrosc 60:2783–2791
Penuelas J, Filella I (1998) Visible and near-infrared reflectance techniques for diagnosing plant physiological status. Trends Plant Sci 3:151–156
Richardson A, Duigan S, Berlyn G (2002) An evaluation of noninvasive methods to estimate foliar chlorophyll content. New Phytol 153:185–194
Solovchenko A, Merzlyak M (2003) Optical properties and contribution of cuticle to UV protection in plants: experiments with apple fruit. Photochem Photobiol Sci 2:861–866
Solovchenko A, Schmitz-Eiberger M (2003) Significance of skin flavonoids for UV-B-protection in apple fruits. J Exp Bot 54:1977–1984
Solovchenko A, Chivkunova O, Merzlyak M, Reshetnikova I (2001) A spectrophotometric analysis of pigments in apples. Russ J Plant Physiol 48:693–700
Solovchenko A, Avertcheva O, Merzlyak M (2006) Elevated sunlight promotes ripening-associated pigment changes in apple fruit. Postharvest Biol Technol 40:183–189
Solovchenko A, Chivkunova O, Gitelson A, Merzlyak M (2010a) Non-destructive estimation pigment content, ripening, quality and damage in apple fruit with spectral reflection in the visible range. Fresh Prod 4:91–102
Solovchenko AE, Merzlyak MN, Pogosyan SI (2010b) Light-induced decrease of reflectance provides an insight in the photoprotective mechanisms of ripening apple fruit. Plant Sci 178:281–288
Steele M, Gitelson A, Rundquist D, Merzlyak M (2009) Nondestructive estimation of anthocyanin content in grapevine leaves. Am J Enol Vitic 60:87
Vogelmann T, Han T (2000) Measurement of gradients of absorbed light in spinach leaves from chlorophyll fluorescence profiles. Plant Cell Environ 23:1303–1311

Chapter 7
Buildup of Screening Pigments and Resistance of Plants to Photodamage

Abstract In this concluding chapter, the relationships between accumulation of screening pigments in microalgae and plants and the corresponding increase in their resistance to photodamage by radiation in different ranges of the spectrum are considered. According to the evidence presented in this chapter, screening pigments can efficiently protect the photosynthetic apparatus from bleaching under harsh environmental conditions, including irradiation by strong photosynthetically active radiation, and alleviate or prevent almost completely the photoinhibition that develops in plants under stresses of various nature. It is noted, in conclusion, that accumulation of screening pigments represents in many cases an important factor of plant stress tolerance.

As emphasized already (see Chap. 1), necessary evidence confirming the photoprotective effect of a screening compound is constituted by an increase in the resistance to photodamage of the organism accumulating this pigment in response to high-light stress. Currently, a large body of experimental data is available on the participation of screening pigments in protection of photoautotrophs against damage by high fluxes of solar radiation (Close and McArthur 2002; Cockell and Knowland 1999; Gould et al. 2000; Solovchenko and Merzlyak 2008). At the same time, direct evidence of and quantitative information about the relationships between the amount of screening pigments and the extent of plant resistance to photodamage is relatively scarce. In the brief summary of works on the physiological significance of screening pigments presented below, emphasis is placed on the quantitative evidence of photoprotective effects of screening pigments.

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7.1 Accumulation of Mycosporine-Like Amino Acids and Scytonemin Increases UV Resistance of Photoautotrophs

The UV-protective role of mycosporin-like amino acids (MAA) and scytonemin in prokaryotic photoautotrophs and eukaryotic microalgae is relatively well established (for reviews, see Cockell and Knowland (1999), Shick and Dunlap (2002), Sinha et al. (2001, 2002)). These studies have documented MAA-concentration-dependent protection of growth and photosynthesis in algae. In particular, MAA alleviated irradiation-induced chlorophyll photobleaching and photosynthesis inhibition in desiccated cyanobacteria. Also, higher concentration of MAA (and other UV-absorbing materials) in the hosts' cells protect the photosynthesis of symbiotic microalgae in the host, whereas UV irradiation inhibits photosynthesis in the freshly isolated endosymbionts (Shick and Dunlap (2002)).

One of the most harmful effects of UV irradiation is the damage to DNA (formation of pyrimidine dimers) resulting in abnormal gene expression or mutations through incorrect DNA replication. There is an inverse correlation between UV screening pigment content and UV-B-induced DNA damage in several species of red marine algae (Misonou et al. 2003; van de Poll et al. 2001). The importance of MAA for the protection of nucleic acids was confirmed by measurements of the fluorescence of 4′,6-diamidino-2-phenylindole -labeled cells (Garcia-Pichel and Castenholz 1993).

As evidenced by Garcia-Pichel et al. (1992), in terrestrial cyanobacteria, MAA and scytonemin provide combined protection which is often more efficient in comparison with that offered only by MAA. Scytonemin per se, when present in high amounts, efficiently reduces photosynthesis inhibition by UV-A radiation (measured by oxygen evolution) and photobleaching of chlorophyll *a*. Generally, the scytonemin-containing cells feature higher growth rates under elevated UV fluxes. In particular, UV-A irradiation retards the growth of the terrestrial cyanobacterium *Chlorogloeopsis* sp. The growth of the culture under elevated UV-A irradiation was resumed only after accumulation of scytonemin in the extracellular envelopes.

Scytonemin-synthesizing cultures were more resistant to photoinhibition of photosynthesis by UV-A irradiation than cultures lacking scytonemin. In the presence of this screening compound, this was correlated with the inability of UV-A radiation to induce strong photosynthetic pigment fluorescence (685-nm emission), regardless of the specific content of photosynthetic pigments (Garcia-Pichel et al. 1992).

Importantly, the protective function of this compound is more evident under conditions imposing physiological inactivity such as desiccation, when "active" photoprotective mechanisms are less efficient. The physical removal of the scytonemin-containing extracellular envelopes brought about the loss of UV-A resistance (Garcia-Pichel et al. 1992).

It should be noted, however, that the studies referred to above revealed that the protection by MAA is often incomplete (according to Garcia-Pichel et al. (1993),

the measured sunscreen factor of MAA for single cyanobacterial cells was 0.3, i.e., the MAA prevented three out of ten photons from hitting potential cytoplasmic targets). Therefore, MAA should be appropriately considered as a component of the UV-protective system of an organism. Furthermore, is it not clear that all of the protection attributed to MAA is indeed derived from them, because in some of the test organisms MAA were induced by UV preirradiation, which might have enhanced other protective mechanisms such as antioxidant systems. Still there are a number of unambiguous reports on protection against acute deleterious effects of UV radiation, e.g., in a dinoflagellate (Neale et al. 1998).

7.2 Buildup of UV-Absorbing Phenolics and UV Resistance of Plants

There is a large body of evidence about UV-protective effects of different phenolic compounds in photoautotrophic organisms (Bornman et al. 1997; Caldwell et al. 2007; Close and McArthur 2002; Rozema et al. 2002; Sinha et al. 2001, 2002; Solovchenko and Merzlyak 2008). Numerous studies (including the analysis of photobleaching kinetics, photosystem II inactivation, and DNA damage) indicated that the resistance (or susceptibility) to UV-induced damage is to a considerable extent correlated with the content of phenolic screening compounds.

An important role in UV protection of plants is played by UV-absorbing phenolics (see Chap. 2) localized in superficial structures of leaves (Krauss et al. 1997), fruit (Baur et al. 1998; Solovchenko and Merzlyak 2003), and other plant organs (see Chap. 4). Plants grown in glasshouses blocking most of the solar UV radiation contain lower amounts of flavonols and phenolic acids in comparison with plants of the same species grown outdoors. Accordingly, the former plants displayed an increased UV susceptibility (Caldwell 1981; Caldwell et al. 2007; Jansen et al. 1998; Reuber et al. 1996b; Tevini et al. 1991).

Investigations of a mutant deficient in the synthesis of flavonoids with different spectral characteristics and localizations (Havaux and Kloppstech 2001; Reuber et al. 1996a) as well as transgenic plants (Ryan et al. 2002) also confirmed the importance of the phenolics in UV defense. For example, *Arabidopsis thaliana* mutants lacking the key phenolic group involved in UV screening appeared to be more sensitive to UV radiation in comparison with the wild type (Li et al. 1993). Specifically, transparent testa 5 mutants (*tt5*), lacking flavonols, and ferulic acid hydroxylase deficient mutants (*fah1*), lacking ferulic acid esters, exhibited more UV-B-induced physiological injury (growth inhibition and foliar lesions) in comparison with wild-type plants (Landry et al. 1995; Li et al. 1993). As revealed by Landry et al. (1995), despite its ability to accumulate UV-absorptive flavonoid compounds, the ferulic acid hydroxylase mutant *fah1* was more susceptible to UV-B damage than either the wild type or *tt5*. The extreme UV-B sensitivity of *fahl* suggests the importance of hydroxycinnamate esters as UV-B protectants

which protect *A. thaliana* against UV-B radiation more efficiently than flavonoids. Barley (*Hordeum vulgare* L.) mutants deficient in glycosylated flavonols such as apigenin and luteolin were readily damaged by UV radiation. By contrast, wild-type plants were resistant under the same conditions, displaying at the same time a fivefold increase in the content of these flavonoids (Reuber et al. 1996a).

Havaux and Kloppstech (2001) observed that light absorption by mesophyll cells in chilled *Arabidopsis* leaves was noticeably reduced in the blue-green spectral region compared with the red region owing to the presence of anthocyanin and also to blue-light-absorbing compounds. The increase in leaf epidermis absorptance in the blue spectral region was detected in all genotypes except *tt5*, suggesting that flavonols and/or dihydroflavonols were responsible for this phenomenon. Although flavonols and dihydroflavonols are known mainly as UV absorbers, quercetin and kaempferol, which are both absent in *tt5*, have a strong tail absorption in the blue spectral region (Harborne 1976; Li et al. 1993; Markham 1989) extending *in planta* till the blue-green region of the spectrum (Merzlyak et al. 2005b). Those UV/blue-light absorbers seem to be more important for photoprotection than anthocyanins (see Sect. 7.3.2), at least under our light-stress conditions. Indeed, the absence of those compounds in the *tt5* mutant was associated with an increased sensitivity to lipid peroxidation and photodestruction, whereas the loss of anthocyanins in *tt3* did not result in significant lipid photooxidation and photoinhibition compared with the wild type. The flavonoid mutant *tt5* appeared to be much more photosensitive than the xanthophyll-cycle mutant *npql*: whereas *Arabidopsis* exposed to chilling stress in high light was able to compensate for the defect in *npql*, the absence of flavonoids in the *tt5* mutant could not be fully overcome by compensatory changes and resulted in increased photooxidation of the leaves (Havaux and Kloppstech 2001).

Further evidence of tight relationships between UV resistance and the ability to synthesize certain screening phenolics was provided by inhibitory analysis with the phenylalanine ammonia lyase (PAL) inhibitor 2-aminoindan-2-phosphonic acid (AIP) and red cabbage (*Brassica oleracea* L.) plants (Gitz et al. 1998). Application of AIP in concentrations ranging from 0.5 to 50 μM efficiently inhibited PAL did not affect plant weight, total chlorophylls, and plant architecture, suggesting there was no toxic effect of AIP in red cabbage seedlings at levels highly effective at inhibiting PAL. According to Gitz et al. (1998), plants grown with 50 μM AIP were about twice as sensitive as control plant to UV-B damage of photosystem II, suggesting that phenylpropanoids carried over from the seed, as well as flavonoids, serve as UV screens in young red cabbage seedlings.

The photosynthetic apparatus of higher plants is particularly sensitive to damage by UV-B radiation (Kulandaivelu and Noorudeen 1983). The primary targets of UV-B radiation in photosystem II are the 32-kDa D1 protein of the reaction center and the water-oxidizing system (Jansen et al. 1998). Damage to those components results in a decrease in the variable fluorescence level (Skórska 2000), making the measurement of chlorophyll fluorescence parameters such as Fo, Fm, and Fv/Fm convenient for estimation of UV-B-induced damage to plants (Schmitz-Eiberger and Noga 2001; Solovchenko and Schmitz-Eiberger 2003). Still, it was found that natural UV irradiance rarely causes damage to plants but instead triggers

genetically programmed defense mechanisms, including biosynthesis of screening compounds (Brosche and Strid 2003; Ryan et al. 2002). Consequently, plants are usually able to sustain a sufficient photosynthesis level under natural fluxes of solar radiation (Caldwell et al. 2007; Jansen et al. 1998). Indeed, assimilatory tissues containing high amounts of screening compounds display remarkable resistance to photodamage.

The above-mentioned circumstances often require application of elevated fluxes of artificial UV radiation to assess UV-protective capacity in real time. Thus, in experiments with apple fruit designed to estimate the significance of different groups of screening phenolics for the resistance of the photosynthetic apparatus to elevated UV-B levels, fruits acclimated to different fluxes of solar radiation and vastly differing in skin flavonoid contents (see Chap. 3) were subjected to a high flux of UV-B radiation and UV-B-induced damage to photosystem II was monitored via chlorophyll fluorescence measurements. The analysis of UV-B-induced Fo, Fm, and Fv/Fm changes revealed that the resistance of the photosynthetic apparatus of apple fruit to UV-B radiation correlated with skin phenolic content (Figs. 7.1 and 7.2). In the case of moderate flavonol content, UV-B irradiation induced severe damage to the photosynthetic machinery of apple fruit, which was apparent as a decline in Fm and Fv/Fm values (see Fig. 7.1a, c, e and closed symbols in b, d, f). An increase in the Fo level on the shaded (adapted to low fluxes of solar radiation, hence possessing low flavonol content) surfaces indicates that, in apple, UV-B radiation damages the reaction centers of photosystem II.

Remarkably, the extent of the UV-B-induced decrease in the Fm and Fv/Fm parameters exhibited a high correlation with apple skin flavonol content (Solovchenko and Schmitz-Eiberger 2003). Photosystem II in sunlit surface tissue of an apple cultivar with a somewhat limited potential for flavonol accumulation (such as Granny Smith) featured similar susceptibility to UV damage as in shaded surface tissues (cf. open and closed symbols in Fig. 7.1a, c, e). Sun-exposed skin of Braeburn apples possessing a high flavonol content (see Fig. 3.2) demonstrated remarkably high UV-B resistance of photosystem II, which did not show signs of damage at doses up to 97 kJ m^{-2}, which are significantly higher than natural doses (see the open symbols in Fig. 7.1b, d, f).

The correlation between Fv/Fm and skin flavonol (quercetin glycoside) content, negligible in intact fruits, significantly increases with an increase in UV dose (Fig. 7.2, curve 1) – i.e., in the situation when the integrity of photosystem II becomes dependent on the screening exerted by quercetin glycosides contained in the apple skin. At the same time, only a weak correlation was found between apple skin anthocyanin content and Fv/Fm during UV-B irradiation (Fig. 7.2, curve 2). This is in agreement with the data on the spectral properties of anthocyanins, which are characterized by low extinction coefficients in the UV-B region (Strack and Wray 1989); the contribution of quercetin glycosides to the UV absorbance of apple skin extracts must be much higher than that of anthocyanins (Fig. 7.3; for a detailed discussion of the physiological significance of screening provided by anthocyanins, see the next section).

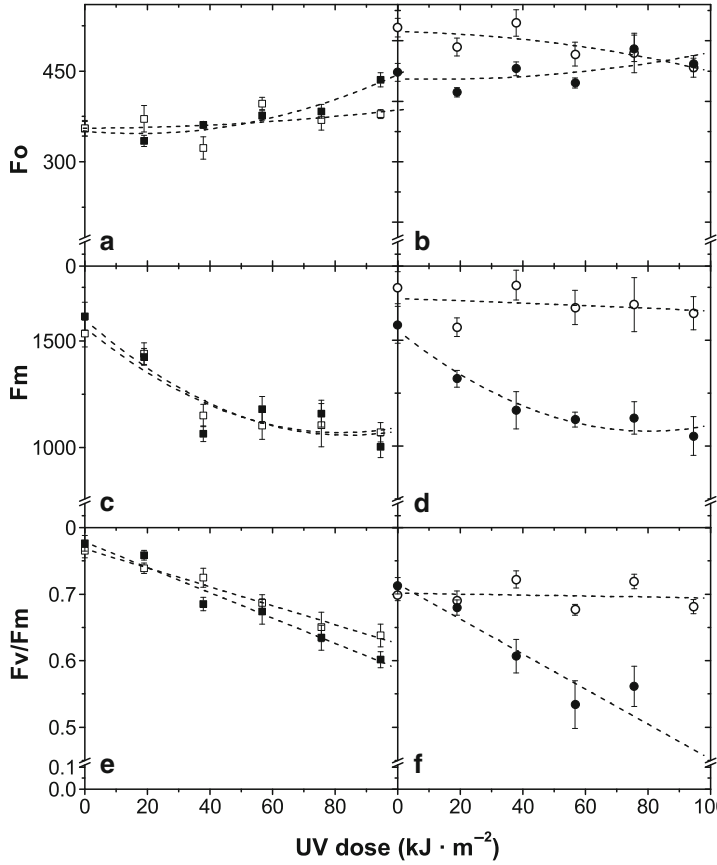

Fig. 7.1 Changes in chlorophyll fluorescence in shaded (*closed symbols*) and sun-exposed (*open symbols*) skin in the course of UV-B irradiation of Granny Smith (**a**, **c**, **e**) and Braeburn (**b**, **d**, **f**) apples; mean ± standard error, $n = 10$. In Granny Smith fruit susceptible to UV-B damage, a strong solar-light-induced increase in flavonol content is much less pronounced in comparison with Braeburn fruit highly resistant to UV-B damage (see also Fig. 3.2). (Reprinted from Solovchenko and Schmitz-Eiberger (2003) with permission from Oxford University Press)

The phenomenon of UV-B-dependent inhibition of the maximum photochemical yield of photosystem II inversely correlated with the buildup of epidermal screening for UV-B radiation was also recorded in the leaves of a number of plant species (Kolb et al. 2001). A marked reduction in the efficiency of photosystem II was recorded after exposing artificially dehaired leaves (in which screening phenolics are localized predominantly in the hairs) to UV-B radiation (Karabourniotis et al. 1993).

Apart from the photosynthetic apparatus, nucleic acids such as DNA represent an important target of UV-B-induced damage. UV-B radiation passing the epidermis will be able to induce DNA damage, which can be repaired by DNA photolyase and nucleotide excision repair (Sinha and Häder 2002). One may expect that the

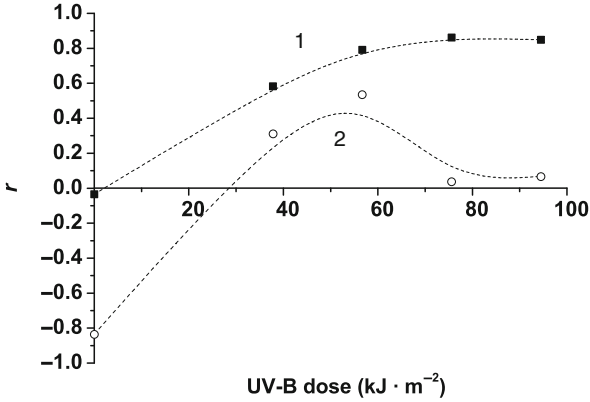

Fig. 7.2 Changes in correlation between Fv/Fm and quercetin glycoside (*1*) or anthocyanin (*2*) content in the course of UV-B irradiation of Braeburn apples ($n = 10$). Note that the correlation of UV-B-induced damage to photosystem II and flavonol content increases along with an increase in UV dose (*1*), but this is not the case for anthocyanins (*2*). (Reprinted from Solovchenko and Schmitz-Eiberger (2003)1981 with permission from Oxford University Press)

enzymatic repair processes are more inhibited under stressful conditions (e.g., at low temperature) than the photochemical damage processes (see Chap. 1). This situation could be alleviated by increased accumulation of screening pigments (Bilger et al. 2007; Cockell and Knowland 1999; Stapleton and Walbot 1994).

The widespread inducibility of the synthesis of UV-screening compounds by environmental stress points to an important function of these compounds under these conditions (Bilger et al. 2007). In the vast majority of cases, UV-B screening is believed to be determined by the illumination environment of a plant. Bilger et al. (2007) showed that temperature is another important factor modulating UV-B screening and possibly also UV-B resistance. Generally, lower temperatures decreased epidermal UV transmittance of greenhouse-grown *Vicia faba* L. plants and seven other crop plant species and *A. thaliana*, obviously owing to accumulation of flavonoids. Interestingly, the epidermal transmittance responded to temperature changes in developing but not in mature leaves of *V. faba*. Bolink et al. (2001) demonstrated that pretreatment with UV-B radiation can harden the plants against photoinhibition by high light, in particular, owing to accumulation of radiation-screening compounds: the decline of Fv/Fm by high-light stress was significantly slower in leaf discs of UV-B-treated plants than in those of control plants.

7.3 Anthocyanins and Other Phenolics as a Shield Against Excessive PAR

Anthocyanins appear to be one of the most investigated groups of "stress pigments" (Chalker-Scott 1999). The induction of their synthesis represents a well-known, obvious and common high-light-induced response (see Chap. 3). The physiological

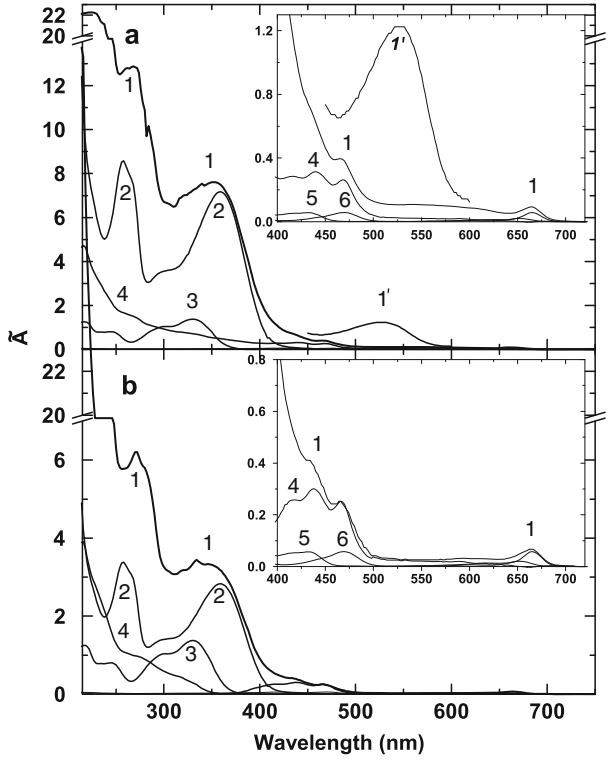

Fig. 7.3 The disparate contributions by flavonols and anthocyanins to specific absorbance by screening pigments ([Ã] in optical density units per square centimeter of the fruit surface) in the UV region. Characteristic absorption spectra of methanolic extracts (*1*) of sunlit (**a**) and shaded (**b**) tissues of apple fruit with pronounced buildup of flavonols and anthocyanins in response to strong sunlight irradiation. Spectral contributions from rutin (*2*), chlorogenic acid (*3*), carotenoids (*4*), chlorophyll *a* (*5*), and chlorophyll *b* (6) are presented for spectra *1*. Spectrum *1′* was obtained after acidification of the extract (*1*) with 0.1% HCl. (Solovchenko, unpublished)

significance of anthocyanins remains a subject of vigorous debate. There are numerous experimentally confirmed examples of an increase in the resistance of plant photoassimilatory tissues to photodamage as a result of anthocyanin accumulation. Anthocyanin-containing dogwood (*Cornus sericea* L.) leaves were less susceptible to photoinhibition and displayed higher efficiency of photosystem II in comparison with acyanic leaves of the same species (Feild et al. 2001; Krause et al. 1995). A considerable problem in studies of the physiological significance of anthocyanin accumulation under stress is due to the difficulty in finding samples differing in anthocyanin content. This obstacle could be circumvented by selecting samples with similar absorption in the red region (rather than similar chlorophyll content) and different absorption in the green region governed by anthocyanins (Merzlyak et al. 2008a, b). For example, Smillie and Hetherington (1999) used white, red, or blue–green light to subject pods of red (anthocyanin-containing) and

green (anthocyanin-free) *Bauhinia variegata* L. phenotypes to photoinhibitory conditions. Red light, which is not absorbed by anthocyanins, induced a similar degree of photoinhibition in pods of both colors. The increased tolerance of red pods for blue-green and white light irradiation compared with green pods was attributed to the presence of anthocyanins. This was, according to Steyn et al. (2002), the first conclusive evidence supporting a photoprotective function for anthocyanins that was not obviously confounded by other photoprotective measures. A similar approach was used by Feild et al. (2001) to demonstrate that anthocyanins reduced photodamage in red leaves compared with yellow senescing leaves of red-osier dogwood and by Merzlyak et al. (2008a, b) to estimate the efficiency of radiation screening by anthocyanins.

It should be noted though that photoprotection by anthocyanins could come at a cost: the absorption of light by anthocyanins in the visible region of the spectrum causes some reduction in the net carbon gain under limiting light, whereas under saturating light this effect could turn out to be negligible (Burger and Edwards 1996). Mesophyll cells located below a light-filter comprising anthocyanin-containing epidermal cells assumed the characteristic photosynthetic features of shade-type cells. As a result, red leaves showed a 23% reduction in CO_2 assimilation under light-saturating conditions, and a lower threshold irradiance for light saturation, relative to those of green leaves (Gould et al. 2002). Limitation of light penetration by anthocyanins could, in certain cases, limit the efficiency of photorepair cyclobutane pyrimidine dimers formed as a result of UV-B irradiation (Hada et al. 2003).

7.3.1 Are Anthocyanins Involved in UV Protection?

Anthocyanins are often considered as protective agents against harmful effects of UV radiation. Indeed, green-leaved plants were in some cases more susceptible to damage than red-leaved ones under exposure to UV-B and UV-C radiation (Burger and Edwards 1996). However, experiments with red and green leaves (Woodall and Stewart 1998) and apple fruit (Solovchenko and Schmitz-Eiberger 2003; see also Figs. 7.2, 7.3) did not confirm the participation of anthocyanins in protection against radiation in this range of the spectrum. In many works aimed at assessing the significance of anthocyanins for UV protection, a considerable buildup of UV-absorbing flavonoids, which could accumulate simultaneously with anthocyanins (Solovchenko and Schmitz-Eiberger 2003)) and actually provide the UV protection, is often overlooked. These considerations point to a rather limited significance of anthocyanins in the UV protection of plants, at least when they occur in low or moderate amounts (Hada et al. 2001, 2003; Smillie and Hetherington 1999). Little attention was paid to their involvement in the defense against damage caused by visible radiation until recently. Burger and Edwards (1996) found no difference in photoinhibition between leaves of red and green coleus (*Coleus blumei* Benth.) varieties exposed to severe photoinhibitory treatment (2 h at 1,800 μmol m^{-2} s^{-1} PAR).

Many researchers have noted the presence of anthocyanins in the upper epidermal layer of leaves belonging to a number of different plant species and, speculatively enough, associated them with UV-B protection (Burger and Edwards 1996; Lee and Lowry 1980; Tuohy and Choinski 1990; Woodall et al. 1998; Woodall and Stewart 1998). Still, as Beggs and Wellmann (1985) observed, anthocyanins possess no strong absorption in the UV-B range. This assessment was confirmed in a number of other species (Brandt et al. 1995; Solovchenko and Schmitz-Eiberger 2003; Teramura 1983), adding that anthocyanins often occur in very low concentrations compared with other UV-B-absorbing compounds and require a long exposure to UV-B radiation to be synthesized (Brandt et al. 1995). Nonetheless, there is evidence that anthocyanins do prevent UV-B damage in some instances (for additional information, see the review by Chalker-Scott (1999)). Cell cultures of cornflower (*Centaurea cyanus* L.) were apparently protected from UVB-induced DNA damage by anthocyanins (Takahashi et al. 1991). Hada et al. (1996) associated decreased levels of anthocyanins with increased DNA damage to sorghum (*Sorghum bicolor* L.) seedlings irradiated with UV-B radiation. It is important to know in this connection that anthocyanins esterified with cinnamic acids do absorb UV-B radiation (Tevini et al. 1991).

7.3.2 Anthocyanin and Cross-Resistance to Stress

Many researchers noted the similarity among the physiological and morphological responses to various abiotic stresses, including high visible light, elevated levels of UV-B radiation, cold, and drought. Generally, an increase in the production of lignin, tannins, suberin, anthocyanins, and other secondary compounds, including those involved in screening of radiation, occurs simultaneously with exposure to environmental stress. In many cases induced cross-resistance may be due to cell wall modifications and upregulation of other protective mechanisms; it is more likely that developing leaves (which necessarily lack these modifications) rely on vacuolar screening compounds in attenuating radiation and modifying water relations (Chalker-Scott 1999).

A comprehensive review of cross-resistance to abiotic stresses stemming from anthocyanin accumulation was compiled by Chalker-Scott (1999), who concluded that anthocyanins are "good general protectors" for a number of reasons. Firstly, anthocyanins are extremely soluble in water as they occur almost exclusively as glycosides (Strack and Wray 1989) and will therefore readily accumulate in vacuoles. Secondly, anthocyanins are glycosylated and therefore can bind and transport reactive monosaccharides during developmentally or environmentally critical stages. Thirdly, anthocyanins have the ability to attenuate UV-B radiation if they are acylated with hydroxycinnamic acids. It appears that even without acylation anthocyanins when present in high amounts can significantly attenuate UV and visible radiation, which might be of adaptive significance for juvenile leaf

tissues that lack adequate structural protection to avoid photooxidation from high levels of blue light.

According to Chalker-Scott (1999), anthocyanins in leaf tissues have a dual function as absorbers of harmful levels and/or wavelengths of radiation and as osmotic adjusters. The second function has at least two environmentally important consequences – when the water potential of the epidermis is lowered, two environmental stresses can be avoided: ice nucleation via freezing events on the leaf surface and drought. As speculated by Krol et al. (2002), the phenomenon of anthocyanin buildup in young *Pinus* seedlings "may somehow help them establish under a suite of suboptimal environmental conditions including photooxidation, low temperature, water and nutrient stress." Steyn et al. (2009) argued that anthocyanins afford photoprotection to peel during low-temperature-induced light stress in apple (*Malus*×*domestica* L.) and pear (*Pyrus communis* L.) and that the protection is not a fortuitous side effect of light absorption by anthocyanin. Apple and pear peel show considerable short-term fluctuation in redness in response to temperature, with the red color increasing rapidly in response to low temperature and just as quickly fading in response to high temperature. Shading pears and apples during cold conditions for 2 days reduced the accumulation of anthocyanin and increased the photosensitivity of peel. Subsequent shading during warm conditions did not affect the accumulation of anthocyanin or the photosensitivity of peel, indicating that the response at low temperature was not due to shade adaptation. Thus, anthocyanins may facilitate protection against damage caused, directly or indirectly, by cold temperatures, drought, and excessive visible and UV radiation (Chalker-Scott 1999).

7.3.3 Anthocyanins Prevent Photoinhibition and Photobleaching

The capability of anthocyanins to protect photosynthetic pigments (chlorophylls and carotenoids) against photobleaching was also confirmed in "acute" experiments involving irradiation of plant samples with very high fluxes of PAR. In apple fruit featuring a high (more than 50 nmol cm^{-2}) anthocyanin content, chlorophylls showed little, if any, photodestruction after several hours of irradiation with 2,500 W m^{-2} PAR, whereas in anthocyanin-lacking fruit, chlorophylls were bleached within about 100 min (Fig. 7.4); the rate of chlorophyll photobleaching was inversely related to the anthocyanin content (Merzlyak and Chivkunova 2000).

As in the case of UV-absorbing screening compounds (see the previous section), stress-induced accumulation of anthocyanins leads to formation of cross-resistance to high-light-induced photoinhibition and photobleaching. In particular, anthocyanins provided efficient protection for maize (*Zea mays* L.) leaves and chloroplasts under low-temperature conditions; the correlation between anthocyanin content and winter hardiness is also documented in many species (Chalker-Scott 1999; Hughes et al. 2005; Steponkus and Lanphear 1969; Steyn et al. 2002). Additional evidence for the participation of anthocyanins in the development of cross-resistance to high

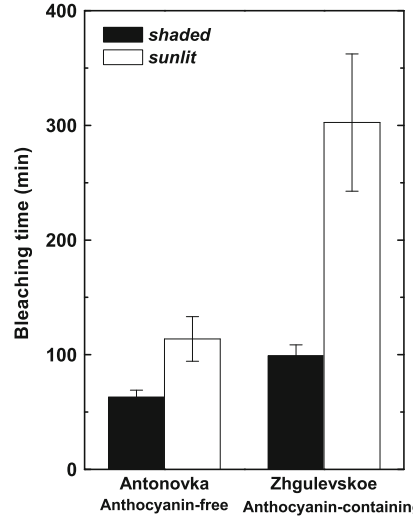

Fig. 7.4 Characteristic times of photobleaching of 50% chlorophylls in shaded (*closed bars*) and sunlit (*open bars*) apple fruit lacking anthocyanins (cultivar Antonovka) and accumulating these pigments in response to strong sunlight (cultivar Zhigulevskoye). (Solovchenko, unpublished)

light under chilling stress was obtained from studies on jack pine (*Pinus banksiana* Lamb.) seedlings subjected to variable excitation pressures (Krol et al. 2002). Seedlings acclimated at 5°C accumulated anthocyanins in needles exposed to 250 μmol m^{-2} s^{-1} PAR and UV radiation. Needles from the same seedlings shaded from direct light did not accumulate anthocyanin and were more susceptible to photoinhibition at moderate (600 μmol m^{-2} s^{-1}) irradiance in the same range. Seedlings kept at 20°C did not accumulate anthocyanin as well and, upon exposure to high irradiance (1,200 μmol m^{-2} s^{-1}), were twice as susceptible to photoinhibition as seedlings acclimated at 5°C.

The presence of anthocyanins relieves the strain on the violaxanthin cycle, dissipating the energy of solar radiation when it is absorbed in excess (see Chap. 1). An inverse correlation between anthocyanin and violaxanthin cycle contents was recorded in dog rose (*Rosa canina* L.), castor oil plant (*Ricinus communis* L.), and a number of other species (Manetas et al. 2002). Furthermore, at equal actinic PAR irradiances, the extent of violaxanthin deepoxidation was lower in anthocyanin-containing tissues in comparison with anthocyanin-lacking samples (Pietrini et al. 2002; Pietrini and Massacci 1998).

As summarized by Steyn et al. (2002), the failure to observe differences in photoinhibition at high irradiance leads us to believe that photoinhibition reaches a maximum at subsaturating irradiance and is not a good indicator of additional photooxidative stress at supersaturating irradiance. In estimating the extent to which anthocyanins reduce light capture by chlorophyll, one should take into account the spectral distribution of the radiation and localization of the pigment in tissues, that is, whether it is located in single or multiple layers in the epidermis, mesophyll, or both (see Chap. 4; Merzlyak et al. 2008a, b).

The photoprotective function served by anthocyanins appeared to be especially important during leaf senescence for the protection of foliar nutrient resorption via shielding photosynthetic tissues from excess light (Hoch et al. 2003). In wild-type plants and anthocyanin-deficient mutants of three deciduous woody species, *C. sericea*, *Vaccinium elliottii* Chapmn, and *Viburnum sargentii* Koehne, outdoors the appearance of anthocyanins in senescing leaves of wild-type plants coincided with the development of photoinhibition in mutant plants of all three anthocyanin-producing species. Under stress conditions, wild-type plants sustained higher photochemical efficiency in comparison with the mutants and were able to recover upon transfer to a low-stress environment. By contrast, the leaves of mutant plants were shed while still green and with signs of irreversible photooxidative damage. The nitrogen resorption efficiencies of all mutants were significantly lower than those of the wild-type counterparts.

The involvement of anthocyanins accumulating in the cells of abaxial epidermis and lower (sponge) mesophyll seems to be especially controversial, although this localization pattern has been documented for numerous species (see, e.g., Lee and Lowry 1980; Lee et al. 1979). Hughes and Smith (2007) demonstrated that abaxial anthocyanin could function as a screening pigment preventing photoinhibition in high-light environments or during light-sensitive developmental stages where leaf orientation and/or substrate albedo are variable.

7.4 Carotenoid Screening Pigments Protect Against Photodamage

Unlike that of anthocyanins and UV-absorbing phenolics, the physiological significance of carotenoids as screening pigments has been much less studied. Nevertheless, there are reports on the participation of extrathylakoid carotenoids in screening of excessive radiation in the blue-green part of the visible spectrum (Boussiba 2000; Han et al. 2003, 2004; Hormaetxe et al. 2005, 2007; Ida 1981; Ida et al. 1991, 1995; Wang et al. 2003; Weger et al. 1993).

The screening by secondary carotenoids was initially documented under stressful conditions in carotenogenic microalgae (Czygan 1970; Hanagata and Dubinsky 1999; Pick 1998). The secondary carotenoids rendered the algal cells less susceptible to photodamage by elevated PAR and UV radiation fluxes as well as to exogenous photosensitizers (Fan et al. 1994, 1998). C ells of the chlorophyte *Haematococcus pluvialis* featuring high contents of astaxanthin esters retained high efficiency of photosystem II even under high PAR irradiance, causing photoinhibition in green astaxanthin-free cells (Boussiba 2000; Wang et al. 2003). The formation and deposition of astaxanthin seems to prevent a profound reduction in the D1 protein level, enabling the cell to maintain photosystem II function and structural integrity (Wang et al. 2003). Interestingly, in the course of recovery of the cells from the high-light stress, the astaxanthin globules concentrated around the nucleus, indicating that the

pigment also serves as a physicochemical barrier, protecting the replicating DNA from oxidative damage during cell division (see also Boussiba (2000)).

Astaxanthin accumulating in *H. pluvialis* seems to protect the alga not only against photoinhibition but also against photodestruction of chlorophylls and photosynthetic carotenoids. Thus, the carotenoids and chlorophylls in green cells virtually devoid of astaxanthin undergo rapid, complete, and synchronous bleaching upon irradiation by high fluxes of PAR, whereas in brown and reddish astaxanthin-containing cells the extent of photobleaching is considerably lower (Fig. 7.5). High and inverse correlation was found between the carotenoid-to-chlorophyll ratio, which increases predominantly owing to accumulation of astaxanthin on the background of a decline in chlorophyll (and hence with $OD_{480} \times OD_{678}^{-1}$) and the rate of pigment photobleaching in *H. pluvialis* (Fig. 7.6).

In the green microalga *Parietochloris incisa* grown on a nitrogen-replete medium and accumulating high amounts of secondary β-carotene, the violaxanthin cycle was less engaged under high-light stress, whereas in nitrogen-starved cells the strain on the violaxanthin cycle was considerably higher (Solovchenko et al. 2008). As consequence, the efficiency of photochemical utilization of the absorbed light energy (as inferred from Fv/Fm measurements) in the *P. incisa* cells rich in secondary β-carotene intercepting a considerable amount of PAR (Solovchenko et al. 2009) was higher than in the nitrogen-starved cells with a low absolute content of the pigment (Fig. 7.7).

Ben-Amotz et al. (1989) demonstrated that the massively accumulated, e.g., in *Dunaliella bardawil*, β-carotene protects against photoinhibition by visible radiation in the bands strongly absorbed by β-carotene (i.e., in the blue region). No photoprotection is observed during irradiation with red light, which is not absorbed

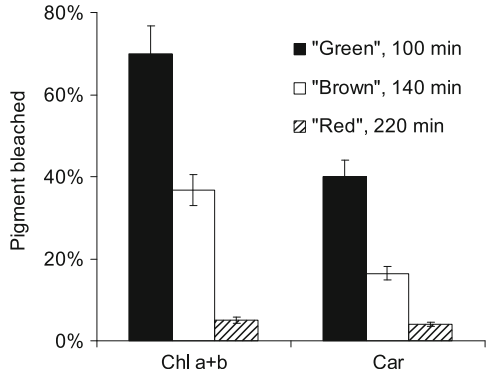

Fig. 7.5 The extent of pigment bleaching in different types of *Haematococcus pluvialis* cells irradiated by 2,500 W m^{-2} photosynthetically active radiation (PAR) during the time indicated. Note the profound bleaching of chlorophylls and carotenoids in the *green* (astaxanthin-lacking) *cells*, whereas in the cells containing the ketocarotenoid, photosynthetic pigments were more resistant to photodestruction. (Reprinted from Solovchenko et al. (2008) with kind permission from Springer Science+Business Media), Fig. 3

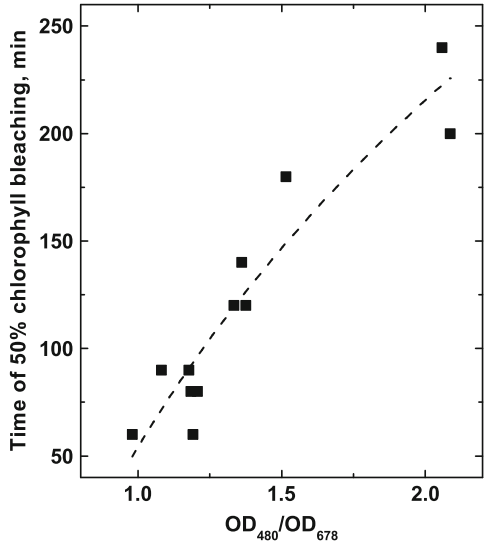

Fig. 7.6 The relationship between the rate of pigment photobleaching and the extent of interception of PAR by astaxanthin (estimated as the optical density at 480 nm, normalized to the red chlorophyll absorption maximum) in cells of *H. pluvialis* irradiated by 2,500 W m^{-2} PAR. (Reprinted from Solovchenko et al. (2008) with kind permission from Springer Science+Business Media), Fig. 4

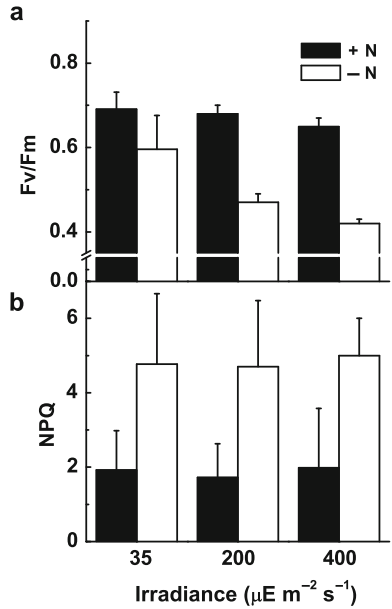

Fig. 7.7 The parameters of chlorophyll fluorescence in cells of *Parietochloris incisa* grown for 14 days on complete medium (**a**) and nitrogen-free medium (**b**) at three irradiances. (Reprinted from Solovchenko et al. (2008) with kind permission from Springer Science+Business Media), Fig. 5

by β-carotene. This is in agreement with the observation on the location of the β-carotene globules, distant from the thylakoid-bound chlorophyll, and with the mechanism of the photoprotection by massively accumulated carotene.

In higher plants, the radiation-screening function of secondary carotenoids appears to be even less studied than in microalgae. Evidence for the existence of

carotenoid-based screening in higher plants appeared only recently (Hormaetxe et al. 2005, 2007; Ida et al. 1995; Merzlyak and Solovchenko 2002). The potential participation of red *retro*-carotenoids in photoprotection via screening was tested in *Buxus sempervirens*, *Aloe arborescence* (Merzlyak et al. 2005a) leaves, and in the needles of some gymnosperms (Han et al. 2003; Weger et al. 1993).

The mechanisms of the photoprotective function of astaxanthin and other secondary carotenoids have been debated until now (Wang et al. 2003). Some lines of evidence suggest that, in microalgae, the carotenoids provide photoprotection via screening under physiologically relevant conditions (Czygan 1970; Hagen et al. 1994). Secondary carotenoids, including astaxanthin, are efficient antioxidants (Kobayashi and Sakamoto 1999; Krinsky 1979; Palozza and Krinsky 1992), and could be important for photoprotection (Boussiba 2000; Hu et al. 2008) and detoxication of reactive oxygen species (Kobayashi 2000). On the other hand, the reactive oxygen species photogenerated in the cell would attack, e.g., polyunsaturated lipids of chloroplast membranes before they could be detoxified by extrathylakoid carotenoids (Asada 2006). These circumstances make it difficult to explain the photoprotective effect of secondary carotenoids in vivo exclusively in terms of oxygen radical scavenging and/or singlet oxygen quenching. The results presented above strongly suggest that screening is the important mechanism involved in the photoprotective effects of secondary carotenoids. The radiation intercepted by secondary carotenoids is harmlessly dissipated as heat instead of being transferred to chlorophylls and eventually to reaction centers as evidenced by a decrease in chlorophyll fluorescence excitation by radiation in the bands attenuated by screening carotenoids during the buildup of the latter (Bidigare et al. 1993).

7.5 Concluding Remarks

As shown in this chapter, numerous lines of evidence point to the great physiological significance of screening pigments in microalgae and higher plants. There were earlier indications of the possible involvement of numerous compounds in radiation screening, but solid evidence began to stream only during last 15 years. More important, in many cases quantitative relationships between the amount and/or spectral properties of accumulated screening pigments and an increase in the resistance to photodamage have been established.

Taking into account prominent achievements in the research on UV-screening compounds, the existence and operation of the screening-based mechanisms in all major taxa of photoautotrophs, including cyanobacteria and plants, now seems to be established. The screening in the visible part of the spectrum, especially in the case of secondary carotenoids, is much less certain and is a more controversial issue. Nevertheless, there has been considerable progress in unraveling the role of visible-radiation-screening compounds in protecting plants against photodamage; this is

especially true for anthocyanin pigments, which are drawing increasing attention from researchers.

Summarizing the current results of the physiological role of screening pigments, one could think that the net effect of their presence, especially under long-term sink-limitation conditions, is the reestablishment of a balance between light capture, CO_2 assimilation, and photosynthate utilization while mitigating the risk of photo-oxidative damage in cells experiencing high excitation pressure (Steyn et al. 2002).

References

Asada K (2006) Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiol 141:391–396

Baur P, Stulle K, Uhlig B, Schönherr J (1998) Absorption von Strahlung im UV-B und Blaulichtbereich von Blattkutikeln ausgewählter Nutzpflanzen. Gartenbauwissenschaft 63:145–152

Beggs CJ, Wellmann E (1985) Analysis of light-controlled anthocyanin formation in coleoptiles of *Zea mays* L.: the role of UV-B, blue, red and far-red light. Photochem Photobiol 41:481–486

Ben-Amotz A, Shaish A, Avron M (1989) Mode of action of the massively accumulated β-carotene of *Dunaliella bardawil* in protecting the alga against damage by excess irradiation. Plant Physiol 86:1286–1291

Bidigare R et al (1993) Evidence a photoprotective for secondary carotenoids of snow algae. J Phycol 29:427–434

Bilger W, Rolland M, Nybakken L (2007) UV screening in higher plants induced by low temperature in the absence of UV-B radiation. Photochem Photobiol Sci 6:190–195

Bolink E, van Schalkwijk I, Posthumus F, van Hasselt P (2001) Growth under UV-B radiation increases tolerance to high-light stress in pea and bean plants. Plant Ecol 154:147–156

Bornman J, Reuber S, Cen Y, Weissenböck G (1997) Ultraviolet radiation as a stress factor and the role of protective pigments. In: Plants and UV-B: responses to environmental change. Cambridge University Press, Cambridge, pp 157–168

Boussiba S (2000) Carotenogenesis in the green alga *Haematococcus pluvialis*: cellular physiology and stress response. Physiol Planta 108:111–117

Brandt K, Giannini A, Lercari B (1995) Photomorphogenic responses to UV radiation III: a comparative study of UVB effects on anthocyanin and flavonoid accumulation in wild-type and aurea mutant of tomato (*Lycopersicon esculentum* Mill.). Photochem Photobiol 62:1081–1087

Brosche M, Strid A (2003) Molecular events following perception of ultraviolet-B radiation by plants. Physiol Planta 117:1–10

Burger J, Edwards G (1996) Photosynthetic efficiency, and photodamage by UV and visible radiation, in red versus green leaf coleus varieties. Plant Cell Physiol 37:395–399

Caldwell M (ed) (1981) Plant response to solar ultraviolet radiation. Springer, Berlin

Caldwell M, Bornman J, Ballare C, Flint S, Kulandaivelu G (2007) Terrestrial ecosystems, increased solar ultraviolet radiation, and interactions with other climate change factors. Photochem Photobiol Sci 6:252–266

Chalker-Scott L (1999) Environmental significance of anthocyanins in plant stress responses. Photochem Photobiol 70:1–9

Close D, McArthur C (2002) Rethinking the role of many plant phenolics-protection from photodamage not herbivores? Oikos 99:166

Cockell C, Knowland J (1999) Ultraviolet radiation screening compounds. Biol Rev 74:311–345

Czygan F (1970) Blood-rain and blood-snow: nitrogen-deficient cells of *Haematococcus pluvialis* and *Chlamydomonas nivalis*. Arch Mikrobiol 74:69

Fan L, Vonshak A, Boussiba S (1994) Effect of temperature and irradiance on growth of *Haematococcus pluvialis* (Chlorophyceae). J Phycol 30:829–833

Fan L, Vonshak A, Zarka A, Boussiba S (1998) Does astaxanthin protect *Haematococcus* against light damage? Z Naturforsch C 53:93

Feild T, Lee D, Holbrook N (2001) Why leaves turn red in autumn? The role of anthocyanins in senescing leaves of red-osier dogwood. Plant Physiol 127:566–574

Garcia-Pichel F, Castenholz R (1993) Occurrence of UV-absorbing, mycosporine-like compounds among cyanobacterial isolates and an estimate of their screening capacity. Appl Environ Microbiol 59:163–169

Garcia-Pichel F, Sherry ND, Castenholz RW (1992) Evidence for an ultraviolet sunscreen role of the extracellular pigment scytonemin in the terrestrial cyanobacterium *Chlorogloeopsis* sp. Photochem Photobiol 56:17–23

Garcia-Pichel F, Wingard C, Castenholz R (1993) Evidence regarding the UV sunscreen role of a mycosporine-like compound in the cyanobacterium *Gloeocapsa* sp. Appl Environ Microbiol 59:170–176

Gitz D, Liu L, McClure J (1998) Phenolic metabolism, growth, and UV-B tolerance in phenylalanine ammonia-lyase-inhibited red cabbage seedlings. Phytochemistry 49:377–386

Gould K, Markham K, Smith R, Goris J (2000) Functional role of anthocyanins in the leaves of *Quintinia serrata* A Cunn. J Exp Bot 51:1107–1115

Gould K, Vogelmann T, Han T, Clearwater M (2002) Profiles of photosynthesis within red and green leaves of *Quintinia serrata*. Physiol Planta 116:127–133

Hada M, Tsurumi S, Suzuki M, Wellmann E, Hashimoto T (1996) Involvement and non-involvement of pyrimidine dimer formation in UV-B effects on *Sorghum bicolor* Moench seedlings. J Plant Physiol 148:92–99

Hada M, Hino K, Takeuchi Y (2001) Development of UV defense mechanisms during growth of spinach seedlings. Plant Cell Physiol 42:784–787

Hada H, Hidema J, Maekawa M, Kumagai T (2003) Higher amounts of anthocyanins and UV-absorbing compounds effectively lowered CPD photorepair in purple rice (*Oryza sativa* L.). Plant Cell Environ 26:1691–1701

Hagen C, Braune W, Björn L (1994) Functional aspects of secondary carotenoids in *Haematococcus lacustris* (Volvocales) III. Action as a sunshade. J Phycol 30:241–248

Han Q, Shinohara K, Kakubari Y, Mukai Y (2003) Photoprotective role of rhodoxanthin during cold acclimation in *Cryptomeria japonica*. Plant Cell Environ 26:715–723

Han Q, Katahata S, Kakubari Y, Mukai Y (2004) Seasonal changes in the xanthophyll cycle and antioxidants in sun-exposed and shaded parts of the crown of *Cryptomeria japonica* in relation to rhodoxanthin accumulation during cold acclimation. Tree Physiol 24:609

Hanagata N, Dubinsky Z (1999) Secondary carotenoid accumulation in *Scenedesmus komarekii* (Chlorophyceae, Chlorophyta). J Phycol 35:960–966

Harborne J (1976) Functions of flavonoids in plants. In: Goodwin T (ed) Chemistry and biochemistry of plant pigments. Academic, New York, pp 736–778

Havaux M, Kloppstech K (2001) The protective functions of carotenoid and flavonoid pigments against excess visible radiation at chilling temperature investigated in *Arabidopsis npq* and *tt* mutants. Planta 213:953–966

Hoch W, Singsaas E, McCown B (2003) Resorption protection. Anthocyanins facilitate nutrient recovery in autumn by shielding leaves from potentially damaging light levels. Plant Physiol 133:1296–1305

Hormaetxe K, Becerril J, Fleck I, Pintó M, Garcia-Plazaola J (2005) Functional role of red (retro)-carotenoids as passive light filters in the leaves of *Buxus sempervirens* L.: increased protection of photosynthetic tissues? J Exp Bot 56:2629–2636

Hormaetxe K, Becerril J, Hernández A, Esteban R, Garcia-Plazaola J (2007) Plasticity of photoprotective mechanisms of *Buxus sempervirens* L. leaves in response to extreme temperatures. Plant Biol 9:59–68

Hu Z, Li Y, Sommerfeld M, Chen F, Hu Q (2008) Enhanced protection against oxidative stress in an astaxanthin-overproduction *Haematococcus* mutant (Chlorophyceae). Eur J Phycol 43:365–376

Hughes N, Smith W (2007) Attenuation of incident light in *Galax urceolata* (Diapensiaceae): concerted influence of adaxial and abaxial anthocyanic layers on photoprotection. Am J Bot 94:784

Hughes N, Neufeld H, Burkey K (2005) Functional role of anthocyanins in high-light winter leaves of the evergreen herb *Galax urceolata*. New Phytol 168:575–587

Ida K (1981) Eco-physiological studies on the response of taxodiaceous conifers to shading with special reference to the behavior of leaf pigments. J Plant Res 94:181–196

Ida K, Saito F, Takeda S (1991) Isomers of rhodoxanthin in reddish brown leaves of gymnosperms and effect of daylight intensity on the contents of pigments during autumnal coloration. J Plant Res 104:157–169

Ida K, Masamoto K, Maoka T, Fujiwara Y, Takeda S, Hasegawa E (1995) The leaves of the common box, *Buxus sempervirens* (Buxaceae), become red as the level of a red carotenoid, anhydroeschscholtzxanthin, increases. J Plant Res 108:369–376

Jansen M, Gaba V, Greenberg B (1998) Higher plants and UV-B radiation: balancing damage, repair and acclimation. Trends Plant Sci 3:131–135

Karabourniotis G, Kyparissis A, Manetas Y (1993) Leaf hairs of *Olea europeae* protect underlying tissues against ultraviolet-B radiation damage. Environ Exp Bot 33:341–345

Kobayashi M (2000) In vivo antioxidant role of astaxanthin under oxidative stress in the green alga *Haematococcus pluvialis*. Appl Microbiol Biotechnol 54:550–555

Kobayashi M, Sakamoto Y (1999) Singlet oxygen quenching ability of astaxanthin esters from the green alga *Haematococcus pluvialis*. Biotechnol Lett 21:265–269

Kolb C, Kaser M, Kopecky J, Zotz G, Riederer M, Pfündel E (2001) Effects of natural intensities of visible and ultraviolet radiation on epidermal ultraviolet screening and photosynthesis in grape leaves. Plant Physiol 127:863–875

Krause G, Virgo A, Winter K (1995) High susceptibility to photoinhibition of young leaves of tropical forest trees. Planta 197:583–591

Krauss P, Markstadter C, Riederer M (1997) Attenuation of UV radiation by plant cuticles from woody species. Plant Cell Environ 20:1079–1085

Krinsky N (1979) Carotenoid protection against oxidation. Pure Appl Chem 51:649–660

Krol M, Hurry V, Maxwell D, Malek L, Ivanov A, Huner N (2002) Low growth temperature inhibition of photosynthesis in cotyledons of jack pine seedlings (*Pinus banksiana*) is due to impaired chloroplast development. Botany 80:1042–1051

Kulandaivelu G, Noorudeen A (1983) Comparative study of the action of ultraviolet-C and ultraviolet-B radiation on photosynthetic electron transport. Physiol Planta 58:389–394

Landry L, Chapple C, Last R (1995) Arabidopsis mutants lacking phenolic sunscreens exhibit enhanced ultraviolet-B injury and oxidative damage. Plant Physiol 109:1159–1166

Lee D, Lowry J (1980) Young-leaf anthocyanin and solar ultraviolet. Biotropica 12:75–76

Lee D, Lowry J, Stone B (1979) Abaxial anthocyanin layer in leaves of tropical rain forest plants: enhancer of light capture in deep shade. Biotropica 11:70–77

Li J, Ou-Lee T, Raba R, Amundson R, Last R (1993) Arabidopsis flavonoid mutants are hypersensitive to UV-B irradiation. Plant Cell 5:171–179

Manetas Y, Drinia A, Petropoulou Y (2002) High contents of anthocyanins in young leaves are correlated with low pools of xanthophyll cycle components and low risk of photoinhibition. Photosynthetica 40:349–354

Markham K (1989) Flavones, flavonols and their glycosides. In: Harborne J, Dey P (eds) Methods in plant biochemistry, vol 1. Academic, London, pp 197–235

Merzlyak MN, Chivkunova OB (2000) Light-stress-induced pigment changes and evidence for anthocyanin photoprotection in apples. J Photochem Photobiol B Biol 55:155–163

Merzlyak M, Solovchenko A (2002) Photostability of pigments in ripening apple fruit: a possible photoprotective role of carotenoids during plant senescence. Plant Sci 163:881–888

Merzlyak M, Solovchenko A, Pogosyan S (2005a) Optical properties of rhodoxanthin accumulated in *Aloe arborescens* Mill. leaves under high-light stress with special reference to its photoprotective function. Photochem Photobiol Sci 4:333–340

Merzlyak MN, Solovchenko AE, Smagin AI, Gitelson AA (2005b) Apple flavonols during fruit adaptation to solar radiation: spectral features and technique for non-destructive assessment. J Plant Physiol 162:151–160

Merzlyak MN, Chivkunova OB, Solovchenko AE, Naqvi KR (2008a) Light absorption by anthocyanins in juvenile, stressed, and senescing leaves. J Exp Bot 59:3903–3911

Merzlyak MN, Melo TB, Naqvi KR (2008b) Effect of anthocyanins, carotenoids, and flavonols on chlorophyll fluorescence excitation spectra in apple fruit: signature analysis, assessment, modelling, and relevance to photoprotection. J Exp Bot 59:349–359

Misonou T et al (2003) UV-absorbing substance in the red alga Porphyra yezoensis (Bangiales, Rhodophyta) block thymine photodimer production. Mar Biotechnol 5:194–200

Neale P, Banaszak A, Jarriel C (1998) Ultraviolet sunscreens in *Gymnodinium sanguineum* (Dinophyceae): mycosporine-like amino acids protect against inhibition of photosynthesis. J Phycol 34:928–938

Palozza P, Krinsky N (1992) Antioxidant effects of carotenoids in vivo and in vitro: an overview. In: Packer L (ed) Methods in enzymology. Carotenoids. Part A. Chemistry, quantitation, and antioxidation, vol 213. Academic, San Diego, pp 403–420

Pick U (1998) *Dunaliella*: a model extremophilic alga. Isr J Plant Sci 46:131–139

Pietrini F, Massacci A (1998) Leaf anthocyanin content changes in *Zea mays* L. grown at low temperature: significance for the relationship between the quantum yield of PS II and the apparent quantum yield of CO_2 assimilation. Photosynth Res 58:213–219

Pietrini F, Iannelli M, Massacci A (2002) Anthocyanin accumulation in the illuminated surface of maize leaves enhances protection from photo-inhibitory risks at low temperature, without further limitation to photosynthesis. Plant Cell Environ 25:1251–1259

Reuber S, Bornman J, Weissenbock G (1996a) A flavonoid mutant of barley (*Hordeum vulgare* L.) exhibits increased sensitivity to UV-B radiation in the primary leaf. Plant Cell Environ 19:593–601

Reuber S, Bornman J, Weissenböck G (1996b) Phenylpropanoid compounds in primary leaf tissues of rye (*Secale cereale*). Light response of their metabolism and the possible role in UV-B protection. Physiol Planta 97:160–168

Rozema J et al (2002) The role of UV-B radiation in aquatic and terrestrial ecosystems – an experimental and functional analysis of the evolution of UV-absorbing compounds. J Photochem Photobiol B Biol 66:2–12

Ryan K, Swinny E, Markham K, Winefield C (2002) Flavonoid gene expression and UV photoprotection in transgenic and mutant *Petunia* leaves. Phytochemistry 59:23–32

Schmitz-Eiberger M, Noga G (2001) Reduction of paraquat-induced oxidative stress in *Phaseolus vulgaris* and *Malus domestica* leaves by α-tocopherol. Sci Hortic 91:153–167

Shick J, Dunlap W (2002) Mycosporine-like amino acids and related gadusols: biosynthesis, accumulation, and UV-protective functions in aquatic organisms. Annu Rev Physiol 64:223–262

Sinha R, Häder D (2002) UV-induced DNA damage and repair: a review. Photochem Photobiol Sci 1:225–236

Sinha R, Klisch M, Gröniger A, Häder D (2001) Responses of aquatic algae and cyanobacteria to solar UV-B. Plant Ecol 154:219–236

Sinha R, Richter P, Faddoul J, Braun M, Häder D (2002) Effects of UV and visible light on cyanobacteria at the cellular level. Photochem Photobiol Sci 1:553–559

Skórska E (2000) Responses of pea and triticale photosynthesis and growth to long-wave UV-B radiation. Biol Planta 43:129–131
Smillie R, Hetherington S (1999) Photoabatement by anthocyanin shields photosynthetic systems from light stress. Photosynthetica 36:451–463
Solovchenko A, Merzlyak M (2003) Optical properties and contribution of cuticle to UV protection in plants: experiments with apple fruit. Photochem Photobiol Sci 2:861–866
Solovchenko A, Merzlyak M (2008) Screening of visible and UV radiation as a photoprotective mechanism in plants. Russ J Plant Physiol 55:719–737
Solovchenko A, Schmitz-Eiberger M (2003) Significance of skin flavonoids for UV-B-protection in apple fruits. J Exp Bot 54:1977–1984
Solovchenko A, Khozin-Goldberg I, Didi-Cohen S, Cohen Z, Merzlyak M (2008) Effects of light and nitrogen starvation on the content and composition of carotenoids of the green microalga *Parietochloris incisa*. Russ J Plant Physiol 55:455–462
Solovchenko A, Khozin-Goldberg I, Cohen Z, Merzlyak M (2009) Carotenoid-to-chlorophyll ratio as a proxy for assay of total fatty acids and arachidonic acid content in the green microalga *Parietochloris incisa*. J Appl Phycol 21:361–366
Stapleton A, Walbot V (1994) Flavonoids can protect maize DNA from the induction of ultraviolet radiation damage. Plant Physiol 105:881–889
Steponkus P, Lanphear F (1969) The relationship of anthocyanin content to cold hardiness of Hedera helix. Hortscience 4:55–56
Steyn W, Wand S, Holcroft D, Jacobs G (2002) Anthocyanins in vegetative tissues: a proposed unified function in photoprotection. New Phytol 155:349–361
Steyn WJ, Wand SJE, Jacobs G, Rosecrance RC, Roberts SC (2009) Evidence for a photoprotective function of low-temperature-induced anthocyanin accumulation in apple and pear peel. Physiol Planta 136:461–472
Strack D, Wray V (1989) Anthocyanins. In: Harborne J, Dey P (eds) Methods in plant biochemistry, vol 1. Academic, London, pp 325–356
Takahashi A, Takeda K, Ohnishi T (1991) Light-induced anthocyanin reduces the extent of damage to DNA in UV-irradiated *Centaurea cyanus* cells in culture. Plant Cell Physiol 32:541
Teramura A (1983) Effects of ultraviolet-B radiation on the growth and yield of crop plants. Physiol Planta 58:415–427
Tevini M, Braun J, Fieser G (1991) The protective function of the epidermal layer of rye seedlings against ultraviolet-B radiation. Photochem Photobiol 53:329–333
Tuohy JM, Choinski JS (1990) Comparative photosynthesis in developing leaves of *Brachystegia spiciformis* Benth. J Exp Bot 41:919–923
van de Poll W, Eggert A, Buma A, Breeman A (2001) Effects of UV-B-induced DNA damage and photoinhibition on growth of temperate marine red macrophytes: habitat-related differences in UV-B tolerance. J Phycol 37:30–37
Wang B, Zarka A, Trebst A, Boussiba S (2003) Astaxanthin accumulation in *Haematococcus pluvialis* (Chlorophyceae) as an active photoprotective process under high irradiance. J Phycol 39:1116–1124
Weger H, Silim S, Guy R (1993) Photosynthetic acclimation to low temperature by western red cedar seedlings. Plant Cell Environ 16:711–717
Woodall G, Stewart G (1998) Do anthocyanins play a role in UV protection of the red juvenile leaves of *Syzygium*? J Exp Bot 49:1447–1450
Woodall G, Dodd I, Stewart G (1998) Contrasting leaf development within the genus *Syzgium*. J Exp Bot 49:79–87

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