Fifty-Five Years of Research on Photosynthesis, Chloroplasts, and Stress Physiology of Plants: 1958–2013

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Contents

Abstract In the past 55 years, enormous scientific progress was made in many fields of plant physiology and plant biochemistry. Throughout these years, our knowledge on the photosynthetic light processes, the chemical composition and

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biosynthesis of the photosynthetic apparatus, the ultrastructure of chloroplasts, and their large adaptation capacity to high-light and low-light was extremely enhanced. The author of this article reviews the substantial scientific evolution in these and other fields in which he was actively involved together with his group. The topics that are reviewed also include forest decline research, the mode of action of herbicides in photosynthesis, and in blocking biosynthetic pathways of chloroplasts, such as de novo fatty acid and isoprenoid biosynthesis, as well as the application of chlorophyll fluorescence imaging in the fast noninvasive determination of photosynthetic activity and early detection of plant stress. Moreover, the detection, elucidation, and metabolic significance of the non-mevalonate chloroplast pathway for isopentenyl diphosphate and isoprenoid biosynthesis, the DOXP/MEP pathway, is reviewed. The author further documents that this extreme progress in plant science was largely due to the continuous development and application of new scientific methods and instruments.

1 Introduction

In the fall of 1958 when I started my Ph.D. thesis performing scientific research in the laboratory of the late Professor Dr. August Seybold, Botanical Institute of the University of Heidelberg, Germany, experimental research in plant physiology and photosynthesis in Europe and elsewhere was still in its infancy. Most of the modern scientific instruments and approaches applied as routine methods today did not yet exist. In fact, in 1958 our knowledge on photosynthetic light reactions, on the development and biosynthesis of functional chloroplasts, and on the genuine biosynthetic pathways of plastids and chloroplasts was very limited. By applying $^{14}CO_2$ Melvin Calvin (Nobel laureate 1961) and his group had elucidated already in 1956 the path of carbon in photosynthesis known today as Calvin–Benson cycle. It was yet unknown that there are two light reactions in photosynthesis with associated photosynthetic electron transport processes, that herbicides can specifically block photosynthesis, and that chloroplasts possess several other unique biosynthetic capacities, such as the non-mevalonate pathway of isoprenoid biosynthesis or the de novo fatty acid biosynthesis. Though the electron microscopy of leaves and other plant materials had started, the fine structure of chloroplasts, the biosynthesis and arrangements of their biomembranes and the processes during biosynthesis and degradation of the photosynthetic apparatus were not known.

Over the past 55 years, however, an enormous, steadily increasing progress has been made in science and particularly in plant science which dramatically increased our knowledge and understanding of the photosynthetic light reactions, of the pigment, prenylquinone, and lipid composition of the photosynthetic biomembrane, as well as of the mode of action of herbicides in blocking either photosynthetic electron transport or special metabolic activities of chloroplasts. Moreover, the

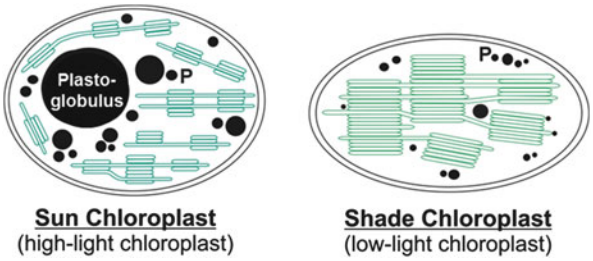


Fig. 1 Scheme showing the differences in the ultrastructure of sun and shade chloroplasts with regard to frequency, width, and stacking degree of thylakoids as well as size and frequency of osmiophilic plastoglobuli. Sun chloroplasts usually contain one or several large starch grains which are not shown here. P = osmiophilic plastoglobuli. The scheme is based on Lichtenthaler (1981), Lichtenthaler et al. (1981a, 1982a) and was presented in a similar form in the review Lichtenthaler (2007)

ultrastructure of functional chloroplasts and their light adaptation, i.e., the arrangement of thylakoids in either high and broad grana stacks (*shade chloroplasts*) or as low and narrow grana stacks (*sun chloroplasts*), has been evaluated (see Fig. 1). In addition, the osmiophilic plastoglobuli were recognized as regular chloroplast structures and as reservoirs for plastohydroquinone-9, α-tocopherol, and other excess plastid lipids. Furthermore, the special genuine, non-mevalonate chloroplast pathway for isopentenyl diphosphate (IPP), isoprenoid, and carotenoid biosynthesis, the DOXP/MEP pathway (see below paragraph 8), was detected between 1995 and 1998 by H. Lichtenthaler, Karlsruhe, in close cooperation with M. Rohmer, Strasbourg, as a special genuine metabolic activity of chloroplasts and all other plastid forms. Besides, the application of the red and far-red chlorophyll fluorescence and the fluorescence imaging technique to leaves and plants as a tool for the investigation of photosynthetic processes and for stress detection in plants were established. The enormous progress in all of these fields, to which my research activities (including those of my students and my cooperation partners from other laboratories) essentially contributed, is briefly summarized and reviewed in this report that also provides information on the general development of the entire field of plant science over the past 55 years.

2 Phylloquinone (Vitamin K_1), Its Localization and Function in Chloroplasts Including Results on Other Prenylquinones

Vitamin K_1, a 2-methyl-1,4-naphthoquinone with a phytyl side chain, had been detected in the late 1920s as a vitamin that is involved in the blood coagulation process and reduces the blood clotting time (see Dam 1942). Biological vitamin tests with animals (chickens) in those years had shown that K_1 is present

predominantly in green plant tissue such as leaves, yet even in the late 1950s a chemical analysis of vitamin K_1 in plant tissues had not yet been performed. This then became the topic of my Ph.D. thesis in the fall of 1958 when I started it with Professor August Seybold at the University of Heidelberg. Due to the fact that vitamin K_1 is a typical genuine plant product particularly in green leaves, it was later termed phylloquinone. Since it contains a phytyl side chain, as do the two chlorophylls *a* and *b*, our assumption in 1958 was that it could be associated together with the chlorophylls in chloroplasts and play a role in their photosynthetic process. Upon column chromatography of leaf pigment extracts with the then applied sucrose columns, K_1 showed up in the β-carotene fraction. By a repetition of the column chromatography of the β-carotene fraction with a slowed down elution time I could partially separate K_1 from β-carotene. Via a subsequent paper chromatography the existence of vitamin K_1 in all green leaf tissues was proven, whereas only trace amounts were found in white plant tissues (leucoplasts) and in orange fruit tissue (chromoplasts). K_1 could be well located on the chromatograms because it emits an intense light green color upon illumination with UV light, which is specific for phylloquinone K_1. In contrast, in yeast and various edible fungi I could not detect any K_1. First approaches towards a quantitative determination of K_1 in green leaf extracts of different plants (applying column chromatography followed by a chemical reduction to its hydroquinone) revealed that K_1 was present in leaves at a low concentration of only about one to two molecules per 100 molecules of chlorophyll (Lichtenthaler 1962).

Laboratory research in botanical institutions in Germany and other European countries in the 1950s was rather strenuous and difficult. In those postwar times modern instruments for experimental research were still lacking. The powerful techniques of thin layer chromatography that allows the separation of minor plant lipids (e.g., phylloquinone K_1) from major plant lipids, such as carotenoids or chlorophylls, had not yet been developed. Moreover, recording spectrophotometers were not yet available. In fact, one had to determine the absorbance spectrum of an isolated pigment by measuring the absorbance step by step at each wavelength, first for the blind and then for the sample in order to finally obtain an approximate spectrum of a carotenoid or a chlorophyll in the visible region or of a lipid fraction enriched with phylloquinone in the UV region.

In contrast to Europe, the working conditions for scientific research were completely different in the USA at that time. Thus, in 1962 when I joined, as a postdoctoral research associate, Melvin Calvin's laboratory at the University of California in Berkeley for 2 years, I found there excellent scientific equipment and modern instruments which simplified and advanced scientific research. There I quickly proved that phylloquinone K_1 was, indeed, located in isolated chloroplasts and also in the smallest thylakoid fragments isolated from sonicated chloroplasts, then termed "quantasome aggregates". When I presented M. Calvin a short note to publish these results, he had just received a manuscript in print by F.L. Crane. Already in 1959 the latter had discovered plastoquinone-9 in chloroplasts (Crane 1959), and now also described the location of K_1 in chloroplasts (Kegel and Crane 1962). For this reason, my K_1 results were only published later, together with other

observations on the prenylquinone and carotenoid content of thylakoids (Lichtenthaler and Calvin 1964). This information is also found in the paper published with Rod Park where we had summarized the basic lipid and protein composition of thylakoids (Lichtenthaler and Park 1963). This paper in Nature being the first description of the complete lipid composition of a biomembrane strongly stimulated research in other laboratories. Thus, within about 2 years the lipid composition of various other biomembranes in plants and animals was published.

Via further analysis of isolated chloroplasts I detected that their thylakoid membranes also contained α-tocopherol and α-tocoquinone and that plastoquinone-9 was present in its reduced form plastoquinol-9. In addition, I could prove that the thylakoid-free 145,000 × *g* supernatant contained a layer of osmiophilic globuli with high amounts of plastoquinone-9, including its reduced form plastoquinol-9, as well as α-tocopherol (Lichtenthaler and Calvin 1964). For more details on osmiophilic globuli, see below paragraph 3.

After my return to Germany these studies were continued and showed that phylloquinone K_1 was enriched in the photosynthetic photosystem I (PSI), whereas the major part of plastoquinone-9 was bound to photosystem II (PSII) (Lichtenthaler 1969a). α-tocopherol and α-tocoquinone were not specifically bound to one photosystem but were found in both PSI and PSII particle fractions. In further studies on the partition of phylloquinone K_1 between digitonin particles and chlorophyll carotenoid proteins of tobacco we could prove that phylloquinone K_1 is, in fact, exclusively bound to the photosystem I particles (Interschick-Niebler and Lichtenthaler 1981) where it has a function in photosynthetic electron transport (Golbeck 1987). Another observation emphasized the essential requirement of phylloquinone K_1 and β-carotene for a functional photosynthetic apparatus. Etiolated leaf tissue already contained plastoquinone-9 and lutein, yet phylloquinone K_1 and β-carotene in trace amounts only. However, during the first hours of illumination etiolated leaf tissues synthesize and accumulate phylloquinone K_1 and β-carotene at high rates parallel to the formation of the first thylakoids, whereas the de novo accumulation of lutein and plastoquinone-9 that had been formed before in the dark started much later (Lichtenthaler 1969b). Concerning the question which light was responsible for the light-induced biosynthesis of thylakoids and their carotenoids as well as prenylquinones we could show that active phytochrome, P730, is required (Lichtenthaler and Becker 1972) and that this process proceeds also in blue light and red light.

It was also an essential finding that plastoquinone-9 and α-tocopherol are present in the chloroplast envelope membranes and in low levels also phylloquinone K_1 which seem to reflect biosynthetic pool sizes (Lichtenthaler et al. 1981b). Moreover, we addressed the question on the occurrence and concentration of ubiquinone homologues in plants and their mitochondria and found that these contained ubiquinone-9 and ubiquinone-10, also known as coenzyme Q-9 and Q-10 (Schindler 1984; Schindler and Lichtenthaler 1984).

3 Osmiophilic Plastoglobuli: Structure, Composition, and Function

A major part of my early research in the 1960s until the mid-1970s was to establish the fact that osmiophilic plastoglobuli are genuine morphological structures of chloroplasts and other plastid forms and that they function primarily as a lipid store for excess lipids, such as α-tocopherol and plastoquinone-9 + plastoquinol-9, as well as in some plastid stages for glycerolipids and in chromoplasts also for carotenoids. Examples of chloroplasts with many plastoglobuli are shown in Fig. 2. Plastoglobuli are particularly frequent in sun chloroplasts as shown in the scheme of Fig. 1.

In the early 1960s several authors had described the principal structure of chloroplasts as revealed by studies with the electron microscope. Besides the structures of biomembranes partially stapled to grana stacks, which later were termed thylakoids by Menke (1962), usually rather small osmiophilic globuli were found but only after the fixation of leaf tissues with osmium tetroxide. Other authors who had applied $KMnO_4$ as a fixation medium detected only "star-shaped bodies" in the chloroplast stroma. At that time "osmiophilic globuli" and "star-shaped bodies" were regarded as fixation artifacts. Already in 1962 I had analyzed from sonicated chloroplasts a thylakoid-free supernatant of the centrifugation tube (145,000 × *g* supernatant) that contained a yellowish lipid layer

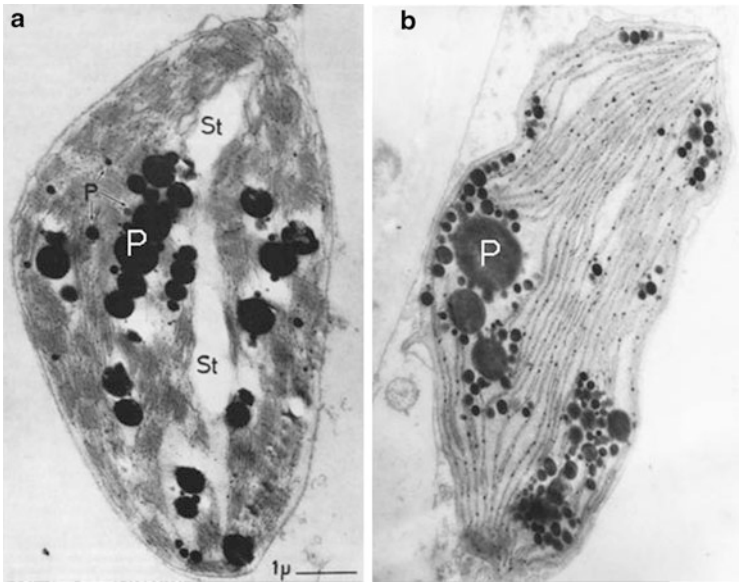


Fig. 2 Chloroplasts with numerous osmiophilic plastoglobuli in green perennial plant tissue. (**a**) From leaves of *Hoya carnosa* R.Br. and (**b**) from the green stem of *Cereus peruvianus* (L.) Mill. *P* plastoglobuli, *St* starch. Fixation of tissues with OsO_4. (Based on Lichtenthaler and Peveling (1966, 1967) and also presented in Lichtenthaler (2013)

consisting of relatively small osmiophilic globuli, first seen by Park and Pon (1961). This layer contained high amounts of α-tocopherol and plastoquinone-9 + plastoquinol-9, apparently excess amounts that were not bound to the photochemically active thylakoids (e.g., Lichtenthaler 1964). This globuli fraction was free of chlorophylls and contained only traces of xanthophylls. Later I showed in a detailed investigation that the "osmiophilic globuli" are regular structural components of the chloroplast stroma and are present in practically all differentiation stages of plastids (see Lichtenthaler 1968). Thus, we termed them "*osmiophilic plastoglobuli*" (Lichtenthaler and Sprey 1966). Since α-tocopherol and plastoquinol-9 are strong reducing agents, the plastoglobuli readily reduce OsO_4 and thus appear osmiophilic. Moreover, we isolated them from several plants, e.g., *Billbergia, Eucharis, Ficus, Spinacia*, and *Tradescantia*, and showed that in chloroplasts of older green leaves the osmiophilic plastoglobuli possess considerably larger diameters than in young spinach leaves (Lichtenthaler and Sprey 1966; Grumbach and Lichtenthaler 1974).

Plastoglobuli predominately function as an extra-thylakoidal store for plant lipids and in particular for excess α-tocopherol and plastoquinone-9 and plastoquinol-9 which are accumulated in high amounts in sun leaves (see review Lichtenthaler 2007) and older green leaves of perennial plant tissues e.g., *Ficus* (Lichtenthaler and Weinert 1970). In chromoplasts plastoglobuli contain also carotenoids and secondary carotenoids that are accumulated together with α-tocopherol and plastoquinone-9 as well as plastoquinol-9 during chromoplast formation. Further, in etioplasts of dark-grown plant seedlings plastoglobuli contain, besides α-tocopherol and plastoquinone-9 + plastoquinol-9, also xanthophylls and possibly also glycerolipids, lipids that are used for the light-induced thylakoid formation. Thus, young chloroplasts are free of osmiophilic plastoglobuli. During chloroplast degeneration and thylakoid breakdown plastoglobuli become more numerous, and usually only a few rather large plastoglobuli remain in the final gerontoplast. Observations by other authors indicate that at a disturbance of normal thylakoid biosynthesis, e.g., by herbicides or other treatments, even triacylglycerides may accumulate and be deposited in plastoglobuli as well, whereby also translucent plastoglobuli can be formed because such lipids are less osmiophilic or not at all [for details and the original references, see the recent plastoglobuli review by Lichtenthaler (2013)]. All data available so far indicate that the interior of osmiophilic plastoglobuli is of pure lipid nature. Due to their more aqueous character proteins cannot be stored inside the plastoglobuli.

Concerning plastoglobuli function, in 1974 we made the highly interesting observation in several plants that the plastoquinol-9 pool in plastoglobuli becomes partially photo-oxidized during the first minutes of the light-induced onset of photosynthetic processes (Grumbach and Lichtenthaler 1974). This photo-oxidation of the plastoquinol-9 pool proceeded in parallel to the photoreduction of violaxanthin to zeaxanthin in the xanthophyll cycle, a process that was reversible in darkness. Thus, it appears that during illumination there occurs an electron flow from plastoglobuli to thylakoids, a process that is partially or fully reversed in the dark (Grumbach and Lichtenthaler 1974). This indicates an interesting regulatory function of the plastoquinol-9 pool of plastoglobuli in the photosynthetic light

reactions and the performance of the xanthophyll cycle. More recent observations indicate that in chloroplasts plastoglobuli may contain on their outer surface certain functional chloroplast proteins, which may be coupled to thylakoids and could function in the biosynthesis of chloroplast lipids and possibly also in an active channeling of lipid molecules and lipid breakdown products (Austin et al. 2006; Bréhélin et al. 2007; Bréhélin and Kessler 2008). This opens up an interesting additional aspect concerning plastoglobuli function but requires much further research. More literature and many further details on our research on osmiophilic plastoglobuli as well as references to the parallel observations of other laboratories are found in a recent comprehensive plastoglobuli review (Lichtenthaler 2013).

4 Composition, Structure, and Function of the Photosynthetic Apparatus of Sun and Shade Chloroplasts

An essential part of the research in my group over the last 50 years was to investigate the adaptation of the photosynthetic apparatus and to establish the irradiance-induced differences in pigment composition, photosynthetic quantum conversion, and CO_2 fixation rates of sun and shade chloroplasts of trees. This also included a detailed investigation of the fine structure and in particular of the differential arrangement and stacking of thylakoids in sun and shade chloroplasts as well as in high-light and low-light chloroplasts from leaves of plants grown under high-light and low-light growth conditions (cf. Fig. 1). This research also contained a detailed investigation of the light-induced biosynthesis of the photosynthetic apparatus in dark-grown etiolated leaf tissues. In this very broad field of photosynthesis and plant physiology very little was known in the mid-1960s. We made large progress in our knowledge particularly by applying in parallel various different techniques, including electron microscopy, spectroscopy, and fluorescence analysis, and also by developing new methods, such as reversed phase high-performance liquid chromatography (HPLC) for separation of leaf pigments, gel electrophoresis (PAGE) for the separation of chlorophyll–carotenoid protein complexes of whole chloroplasts, and the superb technique of chlorophyll fluorescence imaging of the photosynthetic quantum conversion of intact leaves. In addition, I redetermined the absorption coefficients of chlorophylls and all individual carotenoids in different solvents and established new equations for the quantitative determination of chlorophyll *a* and *b* and the sum of leaf carotenoids ($x + c$) next to each other in one leaf extract solution (Lichtenthaler 1987). This allows an easy determination of the pigment levels per leaf area or leaf weight unit, including the pigment ratios Chl *a*/*b* and total chlorophylls to total carotenoids $(a + b)/(x + c)$. Today this method is applied in most laboratories of photosynthesis and plant physiology.

4.1 Differences in Chlorophyll and Carotenoid Composition

The differentiation between sun and shade leaves as well as sun and shade plants was already made by August Seybold in the 1930s when he analyzed the chlorophyll and carotenoid composition of sun and shade leaves of trees by chromatography of leaf pigment extracts using sugar powder columns. Although he could not yet separate the different xanthophylls from each other, he already demonstrated that sun leaves had higher values for the ratio Chl a/b, and considerably lower values for the ratio xanthophylls to β-carotene, x/c, and also for the ratio of total chlorophylls to total carotenoids, $(a+b)/(x+c)$ (Seybold and Egle 1937). With the establishment of thin layer chromatographic (TLC) techniques for the separation of chlorophylls and individual carotenoids in the 1960s (e.g., Hager and Bertenrath 1962), I reinvestigated the pigment composition of plant leaves in dependence of the incident light and confirmed these particular pigment ratios that are quite different for sun leaves as compared to shade leaves. In addition, the individual levels of the different xanthophylls in sun and shade leaves were determined for the first time. Moreover, we could demonstrate that the same differences in pigment ratios and xanthophyll levels as for sun and shade leaves also existed for leaves of high-light and low-light seedlings that were grown at either high or low irradiances, respectively. Major parts of these results are briefly summarized in the review of Lichtenthaler (2007) and Lichtenthaler and Babani (2004). Reversed phase TLC allowed a distinct separation of zeaxanthin from lutein. Thus, we could clearly demonstrate that sun leaves and leaves of high-light plants had much higher levels of xanthophyll cycle carotenoids (zeaxanthin + antheraxanthin + violaxanthin) as compared to shade leaves or leaves of low-light plants, both on a leaf area as well as on a total carotenoid or on a total chlorophyll $a+b$ level.

After having established a high-performance liquid chromatography (HPLC) technique for fast chlorophyll and carotenoid separation within 20 min (e.g., Schindler et al. 1992, 1994), this strict irradiance dependence of the photosynthetic pigment ratios and the level of xanthophyll cycle carotenoids of chloroplasts during leaf and chloroplast development was further accentuated. With this powerful HPLC method we also determined the kinetics of the light-triggered photoreduction of violaxanthin to zeaxanthin in field-grown maple trees during the course of a sunny and a cloudy day (Schindler and Lichtenthaler 1996) which showed the spontaneous response of the redox state of the xanthophyll cycle carotenoids to transient changes in the irradiance of leaves. Moreover, we could demonstrate in leaves of the tobacco "aurea" mutant Su/su grown at medium irradiance that at high irradiance stress zeaxanthin accumulated in a dynamic biphasic process, i.e., not only via a fast transformation of violaxanthin to zeaxanthin, but by doubling the level of xanthophyll cycle carotenoids within 5 h of high irradiance exposure by de novo biosynthesis and accumulation of new zeaxanthin (Schindler et al. 1992; see also review Lichtenthaler 2007). In the same time period also the β-carotene pool increased by one-third via de novo biosynthesis. These results, which were supplemented by parallel chlorophyll fluorescence measurements and determination of characteristic

fluorescence ratios, such as ratios F_v/F_m, dF/F_m' as well as photochemical quenching q_P and non-photochemical quenching q_N (see also Schindler and Lichtenthaler 1996), demonstrated the high flexibility and adaptation capacity of chloroplasts and their photosynthetic pigment apparatus against high-light stress to avoid photo-inhibition and photo-degradation (Lichtenthaler and Schindler 1992). Moreover, additional investigations showed that in fully developed and differentiated leaves the complete photosynthetic pigment apparatus of shade chloroplasts can successively be changed by partial pigment breakdown and de novo pigment accumulation to that of sun chloroplasts and vice versa within a few days.

4.2 *Differences in Photosynthetic Rates of Sun and Shade Leaves*

That sun and shade leaves may have different rates and capacities in photosynthetic quantum conversion had long been assumed, e.g., by Seybold in the 1930s. In the late 1960s and early 1970s it had been shown by some authors that sun plants have higher photosynthetic CO_2 fixation rates than shade plants. Yet, a detailed analysis of sun and shade leaves of the same tree or of plant species grown at different incident light conditions had not yet been performed in a direct comparative way. Some of the early general knowledge of that time was later summarized by Boardman 1977. When we were able to buy one of the (back then still complex) infrared CO_2 gas analyzer systems, I specifically addressed this topic. The small and very handy CO_2 measuring systems (CO_2/H_2O porometers) of today did not yet exist. As expected, our measurements showed that the net CO_2 fixation rates P_N were considerably higher in sun and high-light leaves as compared to shade and low-light leaves of the same plants (Lichtenthaler 1981; Lichtenthaler et al. 1981a), which was correlated with a higher stomata density of the leaves. Further, we could show that sun and high-light leaves exhibited a higher level of soluble sugars. The highly significant differences in photosynthetic quantum conversion between sun and shade leaves are found in different reference systems, not only on a leaf area basis, but also on a total chlorophyll basis. In addition, these differences also showed up in the Hill activity of isolated chloroplasts, which proved to be significantly higher in sun and high-light chloroplasts as compared to shade and low-light chloroplasts.

Furthermore, the same differences were found in the values of the variable chlorophyll fluorescence decrease ratio R_{Fd} that was measured, in parallel, of intact sun and high-light leaves as well as shade and low-light leaves. In the mid-1970s we had established this chlorophyll fluorescence decrease ratio R_{Fd} (originally addressed by us as vF) being based on the measurement of the light-induced slow Chl fluorescence decline during 5 min (slow component of the Kautsky Chl fluorescence induction kinetics) as a valuable indirect measure of the net photosynthetic rates [e.g., Lichtenthaler et al. (1981a, 1984), see also review Lichtenthaler and Babani (2004)]. For details see below paragraph 7.2. The method

is much faster than measurements of the photosynthetic CO_2 fixation rates, it can easily be applied in outdoor measurements and was successfully applied as a stress and damage indicator in our forest decline research in the Black Forest between 1983 and 1990 [e.g., Lichtenthaler (1988a, b), Lichtenthaler and Rinderle (1988a)]. The fact that there is a direct correlation between the R_{Fd} values and the photosynthetic net CO_2 fixation rates of sun and shade leaves of trees has been confirmed more recently for various trees at several locations applying the new technique of Chl fluorescence imaging where several ten thousands R_{Fd} values are simultaneously determined for all parts of one leaf (Lichtenthaler et al. 2000a, 2005b, 2007). It has been demonstrated very recently (Lichtenthaler et al. 2013a) that, with respect to their chlorophyll–carotenoid composition and their photosynthetic activity (P_N rates, R_{Fd} values), *blue-shade* and *half-shade leaves* possess an intermediate position between sun and shade leaves.

4.3 Differences in Chloroplast Ultrastructure and Thylakoid Arrangement

Based on the large differences in the chlorophyll–carotenoid composition and photosynthetic activity between sun and shade leaves and the leaves of high-light and low-light plants one could anticipate considerable differences in the fine structure of sun and shade chloroplasts as well as high-light and low-light chloroplasts. In fact, our electron microscopical investigations revealed that the chloroplast ultrastructure of shade and low-light chloroplasts is characterized by a much higher number of thylakoids per granum stack and a significantly higher stacking degree of thylakoids, but also by a significantly broader width of grana thylakoids and grana stacks than in sun and high-light chloroplasts (Lichtenthaler et al. 1981a; Meier and Lichtenthaler 1981) as summarized in Fig. 1 and Table 1. In addition, sun chloroplasts exhibit large starch grains (Fig. 3) which are usually missing in shade and low-light chloroplasts. Moreover, sun and high-light chloroplasts contain more and larger osmiophilic plastoglobuli and consequently higher levels of excess α-tocopherol and plastoquinone-9 that are located in the plastoglobuli as compared to shade and low-light chloroplasts [see the review Lichtenthaler (2007)].

When it had been shown in 1975 that chlorophylls and carotenoids within the photosynthetic membrane are bound to the different chlorophyll–carotenoid proteins CPa, CPI, CPIa and the light-harvesting chlorophyll–xanthophyll proteins LHCPs (Thornber 1975), we adopted the gel electrophoresis techniques (PAGE) for isolated whole chloroplasts and in a quantitative way we studied the presence of the chlorophyll–carotenoid proteins in sun and shade chloroplasts. This way we could show that the higher stacking degree of thylakoids in shade and low-light chloroplasts is, in fact, associated with a significantly higher level of the light-harvesting chlorophyll–carotenoid proteins LHCPs (Lichtenthaler et al. 1982a, b) that are known to be responsible for thylakoid stacking.

In summary, our comparative investigations revealed that leaves and their chloroplasts are highly reactive, adaptive, morphological, and biochemical systems

that specifically adapt to the prevailing incident light conditions by forming either sun and high-light chloroplasts or shade and low-light chloroplasts. Thus, sun and high-light leaves with their chloroplasts are adapted for high rates of photosynthetic quantum conversion and CO_2 fixation and contain high amounts of xanthophyll cycle carotenoids to avoid photo-inhibition, whereas the photosynthetic apparatus of shade and low-light leaves primarily "invests" into increasing the light-absorbing pigment cross section in order to catch enough light for performance of

Table 1 Major differences in ultrastructure, thylakoid arrangement, pigment composition, and photosynthetic function of sun and shade chloroplasts

Sun chloroplasts	Shade chloroplasts
Low thylakoid amounts (per chloroplast section)	**High thylakoid amounts** (per chloroplast section)
Narrow grana stacks (width: 0.20–0.26 μm)	**Broad grana stacks** (width: 0.33–0.50 μm)
Few thylakoids per granum	**High grana stacks**
Lower stacking degree (%)	**High stacking degree** (%)
Fagus: 57 ± 6	**82** ± 6
Raphanus: 55 ± 5	**64** ± 4
Triticum: 54 ± 5	**73** ± 3
Zea mays: 55 ± 3	**77** ± 3
Appressed thylakoids: low level	**Appressed thylakoids**: high level
Appressed/exposed thylakoids	**Appressed/exposed thylakoids**
Fagus: 1.3	**4.7**
Raphanus: 1.2	**1.8**
Triticum: 1.2	**2.7**
Zea mays: 1.2	**3.3**
Low levels of LHCPs	**High levels of LHCPs**
Numerous and large plastoglobuli	Few small plastoglobuli
Large starch grains	No starch
High values for Chl a/b	Low values for Chl a/b
3.0–4.3	2.3–2.7
Pigment ratio *x*/*c*	**Pigment ratio *x*/*c***
Low values: 1.7–2.3	High values: **2.6–4.0**
Pigment ratio $(a+b)/(x+c)$	**Pigment ratio $(a+b)/(x+c)$**
Low values: 3.8–4.9	High values: **5.1–6.5**
Xanthophyll cycle carotenoids	Xanthophyll cycle carotenoids
High levels	Low levels
High amounts of excess α-T	Low α-T levels
High level of excess plastoquinone-9 (PQ-9 + PQ-9•H_2)	No excess plastoquinone-9 (PQ-9 + PQ-9•H_2)
High R_{Fd} values	Low R_{Fd} values
3.5–5.5	1.8–2.7
High photosynthetic rates P_N	Low photosynthetic rates P_N
4.6–11.5 μmol CO_2 m^{-2} s^{-1}	2.6–3.8 μmol CO_2 m^{-2} s^{-1}

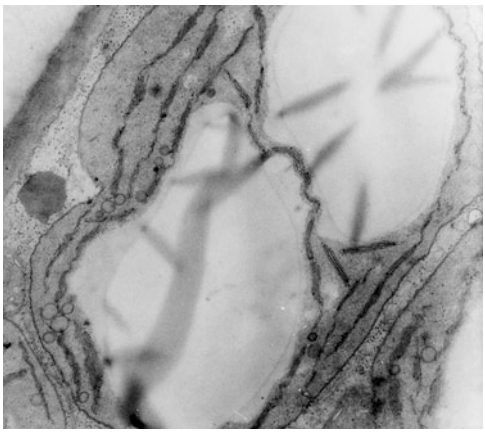


Fig. 3 Sun chloroplast of beech (*Fagus sylvatica*) with large starch grains and several translucent plastoglobuli at the *lower left side* within the chloroplast

photosynthesis. We also checked which other factors besides irradiance control the formation of sun and shade chloroplasts. We could show that the formation of sun chloroplasts is induced by blue light (Lichtenthaler et al. 1980) and enhanced by the phytohormone kinetin (Lichtenthaler and Buschmann 1978), whereas shade chloroplast formation and increased stacking of thylakoids are caused by red light illumination and is further promoted by the application of photosystem 2 herbicides, such as bentazon (Meier and Lichtenthaler 1981). This dependence of the formation of sun and shade chloroplasts on either blue or red light indicates that the phytochrome system—specifically the ratio of red/far-red light—is involved in this adaptation response of chloroplasts. In fact, the incident light in the shade of trees

Table 1 (continued)

Presented are the differential frequency, width, and stacking degree of thylakoids and the level of light-harvesting Chl *a*/*b* proteins LHCPs, which is also documented in the differential ratios of appressed to exposed thylakoid biomembranes. In addition, the size and frequency of osmiophilic plastoglobuli and the differences in the level of total plastoquinone-9 (oxidized and reduced form: PQ-9 and PQ-9•H_2) and α-tocopherol (α-T). Significant differences also exist in the pigment ratios Chl *a*/*b*, xanthophylls to carotenes, *x*/*c*, as well as total chlorophylls to total carotenoids $(a+b)/(x+c)$ that are presented. Moreover, the differences in photosynthetic quantum conversion, i.e., the variable Chl fluorescence decrease ratio R_{Fd} and the net photosynthetic rates P_N yielding high values in sun leaves as compared to shade leaves, are presented

Higher values of individual parameters either present in sun or in shade chloroplasts are shown in bold print. Concerning the stacking degree of thylakoids and width of grana stacks electromicrographs of *Fagus* chloroplasts of sun and shade leaves were investigated and in the case of *Raphanus, Triticum, Zea mays* chloroplast electromicrographs of seedlings grown at high-light and low-light conditions. The ultrastructural and thylakoid arrangement data of chloroplasts in this table are primarily based on Lichtenthaler (1981) and Lichtenthaler et al. (1981a, 1982a, 1984), whereas the pigment ratio data, the level of α-tocopherol and plastoquinone-9, and the differences in R_{Fd} values and photosynthetic CO_2 fixation rates are based on our earlier data reviewed in Lichtenthaler (2007) and Lichtenthaler and Babani (2004); see also Sarijeva et al. (2007) and Lichtenthaler et al. (2013a). The significance levels for the differences between sun and shade leaves and sun and shade chloroplasts in the indicated parameters are ranging from $p<0.05$ to $p<0.001$ as indicated in the original publications cited above.

and forests is enriched with far-red light, whereas sun light and blue skylight only contain relatively low amounts of far-red light. Thus, in sun light and blue skylight the ratio red/far-red light amounts to values of 1.56 and 1.52, respectively, whereas in the shade the red/far-red ratio exhibits a value of 0.21 and in the half-shade of 0.42. The values of the red/far-red ratio presented here were calculated from those of the reverse ratio given by Lichtenthaler et al. (2013a).

5 Forest Decline Research

In the summer of 1982 I became aware of the reports of several German foresters who complained about the unusual decline of spruce (*Picea abies* (L.) H. Karst.) and fir trees (*Abies alba* Mill.) at several locations in the Northern Black Forest, e.g., on the Mauzenberg (altitude 755 m) near the town of Herrenalb. Together with those foresters I examined this decline and the particular damage symptoms. Apparently I was one of the first German plant physiologists who took this threat to our forest trees seriously. The decline started with a considerable loss of the older 3- to 6-year-old needles, a yellowing and bleaching of chlorophyll in younger needles as well as a reduced accumulation of chlorophylls and carotenoids in current and first-year needles, combined with a diminished formation and growth of needles, to just name a few major damage symptoms. In addition, the tree crowns of spruces having shorter branches and fewer side branches became fairly open, whereas the tree tops of firs exhibited a strongly reduced growth of length resulting in compressed tree tops that were termed "stork's nests" and were easily visible from the long distance. In each case tree stands on western exposed slopes and hilltops above 600–1,000 m were affected. The foresters also showed me several unusual damage symptoms on older beech trees (*Fagus sylvatica* L.). In the spring of 1983 more forest sites were affected and the initially more isolated stands were increasing and extended further. In fact, in some places the development was dramatic; thus, from mid-June to the beginning of October 1983 all spruces and firs of a whole mountain top, the Katzenkopf (altitude 900–1,100 m), in the Black Forest had died off.

The causes for such a fast progressing damage and tree decline in the Black Forest initially remained mysterious, although one began to discuss the possible effects of air pollutants, in particular sulfur dioxide, being transported by the predominantly western winds to the western exposed upper tree stands of the Black Forest. At that time high sulfur dioxide levels had already been recognized as the major cause for the forest decline in many mountain areas in Czechoslovakia. The fact that I had early access to the results of fumigation experiments with greenhouse plants of English colleagues (see below) allowed me to be among the first to point out that, in addition to sulfur dioxide, nitrogen oxides—which also provoke the light-induced formation of ozone—were essential causes for the large-scale tree and forest decline.

During a sabbatical in 1981, which I spent at the University of Lancaster, I learned there about the essential research results of Alan Wellburn and Terry Mansfield showing that low atmospheric levels of sulfur dioxide plus nitrogen dioxide had more than additive inhibitory effects on plants cultivated in growth chambers (Wellburn et al. 1981; Mansfield et al. 1982). The cause for this was the fact that sulfur dioxide inactivates the plants' nitrite reductase, which reduces nitrite and starts its successive transformation into ammonia that is incorporated into amino acids. When in 1982 and 1983 I checked the measured levels of nitrogen oxides (NO and NO_2) and sulfur dioxide in the Karlsruhe area and in the Black Forest nearby, it was clear that on a large number of days the levels of these air pollution gases were much higher than the levels used by Mansfield and Wellburn in their growth chamber experiments. Therefore, it was evident that in the Black Forest not only sulfur dioxide but also nitrogen oxides were essentially responsible for the decline of forest trees. In addition, those high levels of nitrogen oxides caused the irradiance-induced formation of ozone which, during sunny and hot summer days, rose to extremely high levels at the Rhine river valley in Karlsruhe and the nearby Black Forest, levels that were not only dangerous to humans but also caused considerable damage to plants and their photosynthetic apparatus. We summarized this information, together with the different damage symptoms of forest trees presented in photos, in a short review "The forest decline: progression, causes and consequences" (Lichtenthaler and Buschmann 1983). This review was sent out to colleagues, to politicians, to people in private industry, as well as to interested laymen. It received great resonance among the public, politicians, and also foresters, and it triggered the general discussions in the public. Yet, at that time many of my German colleagues in plant physiology refused to accept the fact that, except for the particular situation in the Czech mountains, air pollutants would or could cause tree damage and forest decline. In fact, several colleagues postulated infections by unknown fungi and microbes as a primary cause for this large-scale forest decline.

Moreover, we demonstrated via chlorophyll (Chl) fluorescence measurements and also by determining the net CO_2 fixation rates that the photosynthetic quantum conversion of the needles of damaged trees was declining and that the Chl and carotenoid levels of needles declined as well. In addition, together with foresters and the tree physiologist Donald Pigott of the University of Cambridge, England, whom I had invited to Karlsruhe, we checked in the Black Forest the different types of damage symptoms of conifer and broadleaf trees to separate them from symptoms caused by natural stressors, such as heat, cold, or water stress. Then, we reported our findings on the relationship between photosynthesis and tree decline (Lichtenthaler and Buschmann 1984a) as well as on air pollutants as a trigger of the forest decline (Lichtenthaler 1984). For more detailed information, we additionally published a booklet "The Forest decline from a botanical point of view" in German language (Lichtenthaler and Buschmann 1984b). Fortunately, we had the chance of sending several hundred free copies of that booklet to interested colleagues and laymen in the German Democratic Republic, GDR, where any discussion on forest decline was officially forbidden.

With the financial support of the W. & E. Heraeus foundation in Hanau, Germany, I was able to invite, in 1984, 44 foresters and plant physiologists from six European countries to a small workshop in Bad Honnef, Germany. There, German, Swiss, and Austrian foresters involved in forest decline research met for the first time, exchanged their individual observations on forest decline and tree damage, and discussed the causes and consequences with plant physiologists. This workshop strongly stimulated future exchange and research cooperations. In a cooperation with Barry Rock of the NASA, USA, and his team we compared in 1984 and 1985 by means of in situ spectral measurements the forest decline symptoms in Vermont, USA, and the Black Forest, Germany, and found that the symptoms were the same in both locations (Rock et al. 1986). In addition, in cooperation with the German space research center, the Deutsches Zentrum für Luft- und Raumfahrt (DLR) in Oberpfaffenhofen, Germany, and the NASA, USA, we classified the trees of damaged spruce stands in the Northern Black Forest by airborne reflectance and terrestrial Chl fluorescence measurements (Schmuck et al. 1987; Rinderle and Lichtenthaler 1989). At the Mauzenberg forest location (altitude 650–755 m) we analyzed the seasonal variation in photosynthetic activity of healthy and damaged spruce trees over 2 consecutive years, whereby 3 needles years were studied in parallel. In damaged spruce trees we found not only a reduced photosynthetic quantum conversion as detected via Chl fluorescence measurements, but even more reduced rates of net CO_2 fixation P_N, both on a chlorophyll and on a needle area basis, as well as reduced rates of transpiration and stomatal conductivity (e.g., Lichtenthaler et al. 1989). Moreover, needles of damaged spruces (damage class 3–4) could no longer regulate and fully close their stomata, which caused a fast desiccation and dropping down of needles.

At that time the state of Baden-Württemberg started and supported the interdisciplinary European research project PEF (Projekt Europäisches Forschungszentrum), where various research groups studied different aspects of the forest decline in the Black Forest at the same locations, with the Schöllkopf (altitude 840 m) near the town of Freudenstadt being the most important location. Within this program we analyzed the performance and pigment composition of the photosynthetic apparatus of different needle ages of healthy and damaged spruce trees (e.g., Lichtenthaler et al. 1985, 1989; Zimmer-Rinderle and Lichtenthaler 1995). The results of all those investigations (major parts were later published in the book by Bittlingmeier et al. 1995) demonstrated that the large-scale forest decline was caused by a combination of natural environmental stress with air pollution stress (e.g., SO_2, NO_x, ozone), whereby the latter considerably enhanced the natural stress, led to potassium and magnesium deficiency of the soil and trees, caused bleaching of photosynthetic pigments, and reduced the vitality of trees predominantly by an early decline of the photosynthetic function and a progressing damage to the photosynthetic apparatus.

6 Mode of Action of Herbicides in Photosynthesis, Chloroplasts, and the Apicoplast

Besides the Calvin–Benson cycle of CO_2 assimilation and the pigment apparatus catalyzing the photosynthetic light and associated electron transport reactions, chloroplasts possess various other biosynthetic pathways that are potential targets for herbicides and natural antibiotics and inhibitors. The goal of our research was to apply inhibitors and herbicides in order to find out more about the photosynthetic electron transport reactions, to learn more about the special metabolic pathways of chloroplasts, and also to detect the mode of action of new herbicides.

Thus, we clarified that the herbicide bentazon blocks the photosynthetic electron transport by specifically binding to the Q_B protein of the photosystem 2 reaction center (Pfister et al. 1974). In addition, we developed isolated chloroplasts and etioplasts as test systems for inhibitors against de novo fatty acid biosynthesis and proved that the herbicides diclofop and other aryloxy-phenoxy-propionic acids as well as sethoxydim, cycloxydim, and other cyclohexane-1,3-diones, all of them specific graminicides, inhibit the plastidic fatty acid biosynthesis by specifically blocking the acetyl-CoA carboxylase (Kobek et al. 1988a, b; Lichtenthaler 1989). They also block the development and replication of chloroplasts (Lichtenthaler and Meier 1984). Moreover, we detected that the two natural antibiotics cerulenin and thiolactomycin are also inhibitors of de novo fatty acid biosynthesis in chloroplasts (Feld et al. 1989; Golz et al. 1994) where they block the β-ketoacyl-ACP synthases KAS I (cerulenin, thiolactomycin) and KAS III (thiolactomycin). We also showed that ethyl-adenylates inhibit the acetyl-CoA synthetase and acetylmethyl-phosphinates the plastidic pyruvate dehydrogenase complex as shown in Fig. 4 (Golz et al. 1994). Such inhibitors are essential tools for the clarification of the metabolite flow from either acetate or pyruvate into de novo fatty acid biosynthesis and into isoprenoids or branched-chain plastidic amino acids. Later we demonstrated that 6-ketoclomazone is a specific inhibitor of the DOXP synthase (1-deoxy-D-xylulose-5-phosphate synthase) and fosmidomycin a specific inhibitor of the DOXP reductase (1-deoxy-D-xylulose-5-phosphate reductase), i.e., the first and the second enzyme of the plastidic DOXP/MEP pathway of isoprenoid biosynthesis, inhibitors that essentially helped to establish this newly detected non-mevalonate pathway of isoprenoid biosynthesis (see Lichtenthaler 2000a). This plastidic DOXP/MEP pathway for isoprenoid biosynthesis is named after its first and second intermediates: 1-deoxy-D-xylulose-5-phosphate (DOXP) and 2-C-methyl-D-erythritol-4-phosphate (MEP). Details of the DOXP/MEP pathway are found below in paragraph 8.

Another observation of particular interest was the finding by several authors that the malaria inducing parasite *Plasmodium falciparum* had a nongreen, plastid-type cell organelle, the apicoplast that, during evolution, was taken up from either a green or a red alga. Since the malaria parasite is dependent on the metabolic activities of its apicoplast we cooperated with physicians and proved that the apicoplast possesses the DOXP/MEP pathway of isoprenoid biosynthesis which can be blocked by the herbicide fosmidomycin. In fact, in our joint efforts we could show that malaria-

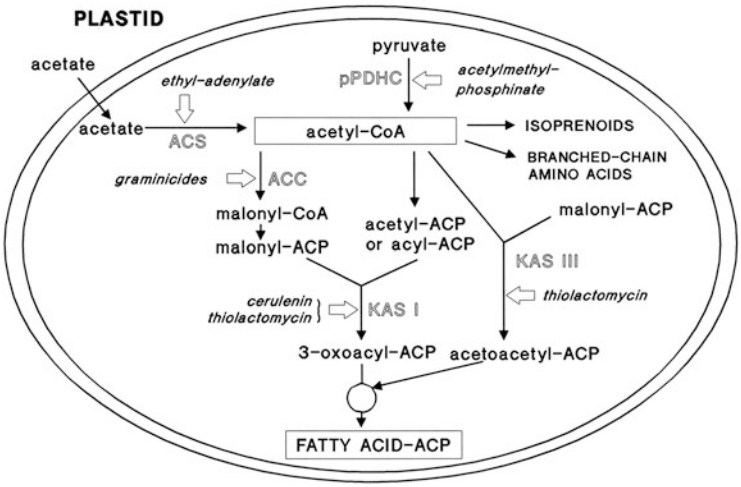


Fig. 4 Scheme of de novo fatty acid biosynthesis in chloroplasts starting from acetate and pyruvate. The enzymes and their specific inhibition by active ingredients and herbicides are indicated (Based on Lichtenthaler 1989, 2000c). *ACC* acetyl-CoA carboxylase, *ACP* acyl carrier protein, *ACS* acetyl-CoA synthetase, *KAS I and KAS III* β-ketoacyl-ACP synthase, *Malonyl-ACP* malonyl-acid carrier protein, *pPDHC* plastidic Pyruvate Dehydrogenase Complex

infected mice were cured by fosmidomycin treatment (Jomaa et al. 1999). Since the DOXP/MEP pathway of isopentenyl diphosphate (IPP) biosynthesis also occurs in pathogenic eubacteria, such as *Mycobacterium tuberculosis* and *Helicobacter pylori* [for a complete list see Lichtenthaler (2000a)], plants with their easy-to-handle DOXP/MEP pathway are very suitable test systems for new drugs against pathogenic bacteria and the malaria parasite (Lichtenthaler et al. 2000b).

7 Chlorophyll Fluorescence and Fluorescence Imaging of Photosynthetic Activity and Plant Stress

Essential progress in our understanding of photosynthetic processes came from the application of chlorophyll fluorescence induction kinetics which several decades later were further promoted by the introduction of laser-induced fluorescence imaging of plant leaves and their photosynthetic activity.

7.1 Chlorophyll Fluorescence

Already in 1931 Hans Kautsky (Fig. 5) had measured in pre-darkened green leaves that upon illumination there is a red Chl fluorescence that initially rises within a few seconds to a maximum and then slowly decreases within a few minutes to a considerably lower steady level (Kautsky and Hirsch 1931). In more than

Fig. 5 Hans Kautsky in Marburg, around 1950

14 subsequent papers [reviewed in Lichtenthaler (1992)] he analyzed and characterized this Chl fluorescence induction kinetics and its dependence on chemicals and environmental factors. Back then already Kautsky had concluded that the photosynthetic light process consisted of two light reactions, one that reduces a substance (e.g., an electron acceptor) and a second light reaction that oxidizes this reduced substance. Therefore, it was Kautsky who first detected that the photosynthetic apparatus consisted of two photosystems cooperating with each other. This knowledge became evident in the photosynthetic community only in the early 1960s when other groups, in particular those of Duysens, Govindjee, and Butler, repeated and advanced Kautsky's Chl fluorescence measurements [for references see Lichtenthaler (1992)]. This was the beginning of the evaluation of the two photosystems and the photosynthetic electron transport chain and the search for its components, whereby plastoquinone-9 was detected by Crane in 1959 and phylloquinone K_1 independently of each other by Crane and by Lichtenthaler in 1962 as mentioned above (see paragraph 2). In subsequent years Chl fluorescence induction kinetics developed to a routine method of photosynthesis research, various Chl fluorescence parameters, ratios, and coefficients were established, e.g., the ratio Fv/Fm and the photochemical and non-photochemical quenching coefficients q_P and q_N. Much of this research on the role of Chl fluorescence in the detection of stress conditions in plants was summarized in the comprehensive review by Lichtenthaler and Rinderle (1988b). Further information is found in the articles by various authors in the two books on Chl fluorescence by Lichtenthaler (1988a) and Papageorgiou and Govindjee (2004). An exact guide of how to measure and correctly apply these Chl fluorescence parameters and ratios was given more recently by Lichtenthaler et al. (2005a).

We also showed that one should be very cautious with the interpretation of such Chl fluorescence parameters when they are solely measured at the upper, i.e., the adaxial, leaf-side. In fact, the values of the Chl fluorescence ratios and quenching coefficients obtained in that way only reflect the responses and reactivity or inhibition of the chloroplasts of the upper leaf-half. The chloroplasts of the lower leaf-half, which are

accessible only via Chl fluorescence measurements at the lower leaf-side, may still be fully functional even if the chloroplasts of the upper leaf-half are fully inhibited. Thus, in maple leaves exposed to full sunlight on a hot sunny day the Chl fluorescence ratios F_v/F_m, dF/F_m' as well as photochemical quenching q_P and non-photochemical quenching coefficients q_N, measured at the upper leaf-side, indicated a complete photo-inhibition of the photosynthetic apparatus, yet the CO_2 fixation measurements clearly proved that the leaves still exhibited about 78 % of their maximum CO_2 fixation rates (Schindler and Lichtenthaler 1996). In this respect see also the corresponding results described by Lichtenthaler et al. (2005a). Thus, Chl fluorescence measurements should always be performed on both leaf-sides and be complemented by net CO_2 fixation measurements with a CO_2/H_2O porometer in order to clarify to which degree a presumed photo-inhibition really exists at the whole leaf level.

7.2 *The Chlorophyll Fluorescence Ratios* R_{Fd} *and* F690/F730

Concerning Chl fluorescence I introduced in my research two other Chl fluorescence ratios which are excellent parameters to determine photosynthetic activity and stress effects in plants. Moreover, based on Hans Selye's stress concept for humans I established a general stress concept of plants, a list of stressors and stress responses in order to simplify the discussion on plant stress (Lichtenthaler 1996).

Fluorescence Ratio R_{Fd} The Chl fluorescence decrease ratio R_{Fd}, i.e., the ratio of the slow fluorescence decrease F_d to the steady-state fluorescence F_s reached after 5 min of illumination, ratio F_d/F_s, proved to be an exact indirect indicator of the net photosynthetic CO_2 fixation rates P_N as reviewed in Lichtenthaler and Babani (2004). In the years from 1983 through 1990 we successively applied this Chl fluorescence decrease ratio R_{Fd} in our forest decline research in order to determine the decline in photosynthetic activity and the damage degree of spruces, firs, and deciduous forest trees (e.g., Lichtenthaler 1988b). The ratio R_{Fd} is also a very suitable parameter to sense a decline in photosynthetic quantum conversion due to water stress or other stress events, such as nitrogen deficiency, and also to describe the differential activities of sun and shade leaves of trees as shown in a recent original paper (Lichtenthaler et al. 2013a) and reviewed in Lichtenthaler and Babani (2004).

Fluorescence Ratio *F690/F730* We also introduced another Chl fluorescence ratio, i.e., the ratio of the fluorescence yield in the red (near 690 nm) and far-red (near 730–740 nm) maxima of the Chl fluorescence emission spectra, i.e., the ratio *F*690/*F*730 also known as ratio *F*690/*F*735. With increasing chlorophyll $a+b$ content of leaves the *F*690 maximum decreases, whereas that of *F*730 is almost unaffected. Thus, the ratio *F*690/*F*730 is an inverse indicator of the Chl $a+b$ content of leaves. Hence, its increase with decreasing Chl content from low regular values of 0.4–0.6 for green leaves to considerably higher values is an excellent

stress indicator (Rinderle and Lichtenthaler 1988; Hák et al. 1990; see also the review of Buschmann 2007). The inclusion of the ratio $F690/F730$ opens new possibilities for remote sensing of terrestrial vegetation by a combination of laser-induced Chl fluorescence and reflectance measurements (Lichtenthaler 1989). In further investigations we could retrieve the actually emitted Chl fluorescence emission spectrum as compared to the measurable spectrum of green leaves by evaluating the degree of reabsorption of the emitted red Chl fluorescence by means of absorption and reflectance measurements (Gitelson et al. 1998).

7.3 *Fluorescence Imaging of Plants*

We also investigated in detail the blue and green fluorescence emission of green leaves and their spectral characteristics together with the red and far-red Chl fluorescence of leaves (Stober and Lichtenthaler 1992; Stober et al. 1994). In contrast to the red and far-red Chl fluorescence, the blue and green fluorescence of plant leaves are also emitted by nongreen plant leaves and they remain constant during the Chl fluorescence induction kinetics known as Kautsky effect (Stober and Lichtenthaler 1993). Thus, the blue fluorescence can be taken as a standard when the red and far-red Chl fluorescence are decreasing due to stress events. In fact, we detected that the ratio of blue to red fluorescence can be taken as stress indicator. The blue fluorescence of plant leaves shows a maximum near 440 nm ($F440$) and the green fluorescence mostly a shoulder (sometimes also a maximum) near 520 nm ($F520$). As the major substance of the blue-green fluorescence emission of plants we identified cell wall bound ferulic acid (Lichtenthaler and Schweiger 1998). All this fluorescence information came from measurements at small individual spots of a leaf. In order to obtain reliable information for the whole leaf several measurements had to be performed at different spots across the leaf surface.

In cooperation with physicists from the CNRS in Cronenbourg near Strasbourg we checked the possibilities for laser-induced imaging of the plants' blue and green fluorescence together with the red and far-red Chl fluorescence. The advantage of fluorescence imaging is that one image contains the information of several 10,000 pixels per leaf, and this is of high statistical significance and reliability. The first fluorescence images were taken of green tobacco leaves (Lang et al. 1994) as shown in Fig. 6, whereby the fluorescence intensity is indicated in false colors. The images clearly indicate that the four fluorescence bands are not homogeneously distributed across the leaf area. The highest blue ($F440$) and green ($F520$) fluorescence emanate from the leaf veins where the chlorophyll content is low. The image also demonstrates that the blue fluorescence is higher than the green fluorescence. In contrast, the red ($F690$) and far-red ($F740$) chlorophyll fluorescence primarily come from the vein-free leaf regions where the Chl density is high. In addition, it can be noticed that the red fluorescence is higher than the far-red fluorescence, and both Chl fluorescences are higher than the blue and green fluorescence. By a pixel-to-pixel division one obtains the corresponding fluorescence ratio images blue/green,

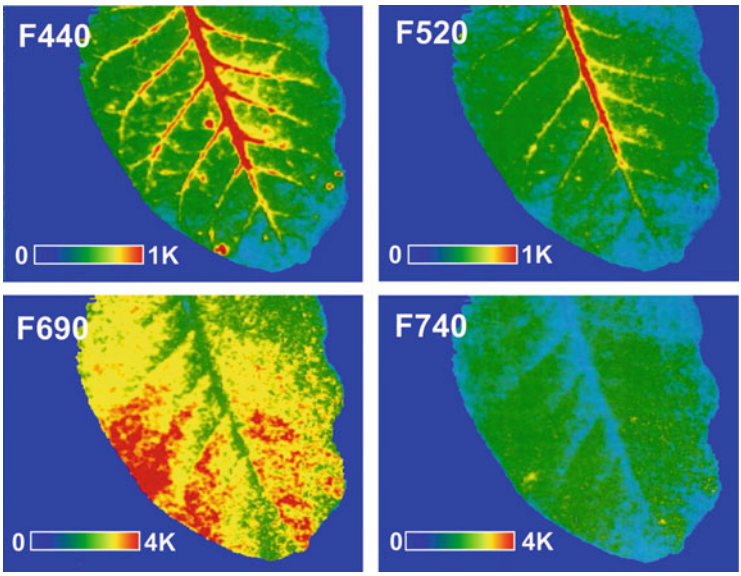


Fig. 6 Fluorescence images of the *upper side* of a green tobacco leaf. The intensity of the blue (*F*440) and green (*F*520) fluorescence as well as the red (*F*690) and far-red (*F*740) chlorophyll fluorescence is shown in false colors, whereby the fluorescence yield in the images increases from blue (no fluorescence) via green and yellow to red as the highest fluorescence. The highest blue and green fluorescence are emitted by the leaf veins, whereas the highest chlorophyll fluorescence comes from the intercostal fields, i.e., the vein-free leaf regions. Note that the scales for the red and far-red chlorophyll fluorescence are different from those of the blue and green chlorophyll fluorescence. K in the scales means kilo (=1,000) counts. [Based on Lang et al. (1994), Lichtenthaler et al. (1996) modified]. Each image consists of several ten thousand pixels over the leaf surface

blue/red, and blue/far-red and red/far-red as indicated in detail in Lang et al. (1996) and Lichtenthaler et al. (1996).

Both fluorescence images and fluorescence ratio images allow the detection of spatial heterogeneities and small local disturbances in fluorescence yield over the leaf surface and also in the values of the individual fluorescence ratios, which are early stress and damage indicators long before a damage can visually be detected. Thus, a high-resolution fluorescence imaging system allows an early detection of vegetation stress (Lichtenthaler et al. 1996). We performed fluorescence imaging of water and temperature stress (Lang et al. 1996); we applied laser-induced fluorescence imaging for monitoring a nitrogen fertilizing treatment (Heisel et al. 1997). This multicolor fluorescence imaging is an excellent diagnostic tool for the detection of plant stress and changes in photosynthetic quantum conversion (Lichtenthaler and Miehé 1997). Its principles and characteristics as well as stress-induced changes of the fluorescence ratios and the application possibilities of this powerful investigation method were summarized by Buschmann and Lichtenthaler (1998) and Buschmann et al. (2000).

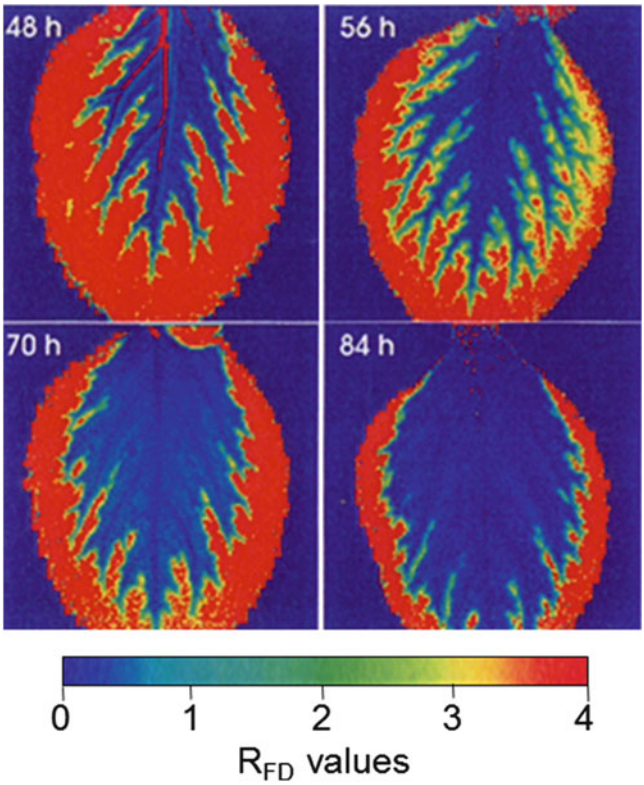


Fig. 7 Successive loss of the photosynthetic activity of intact green leaves of foxglove (*Digitalis purpurea* L.) leaves after the uptake of the photosystem II herbicide diuron as visualized here via images of the Chl fluorescence ratio R_{Fd} that decreases with increasing herbicide uptake. Images of the Chl fluorescence decrease ratio R_{Fd} were taken at different times after application of the herbicide diuron (10^{-5} M) via the root of a young plant. The values of the Chl fluorescence decrease ratio R_{Fd} are given in false colors in absolute values with decreasing values from red (highest R_{Fd} value of 4) via yellow and green to light-blue (low intensity) to dark-blue (zero). (Based on Lichtenthaler and Miehé 1997 and Lichtenthaler et al. 2013b, modified)

Replacing the expensive HeNe laser by a flash lamp UV excitation we developed the much smaller Karlsruhe fluorescence image system which was successfully applied in multicolor fluorescence imaging of sugar beet leaves with different N-status (Langsdorf et al. 2000) and also for imaging the photosynthetic activity of leaves. It was already mentioned above that the Chl fluorescence decrease ratio R_{Fd} (the ratio F_d/F_s) is linearly correlated with the photosynthetic net CO_2 fixation P_N of leaves (e.g., Lichtenthaler and Babani 2004). By imaging the Chl fluorescence (a) in pre-darkened leaves at the fluorescence maximum Fm reached after an illumination time period of ca. 1 s and (b) again at the steady level F_s reached after 5 min of illumination, one can form the R_{Fd} ratio images of leaves providing ample information, e.g., on the differences in the photosynthetic activity between sun and shade leaves of trees including needle twigs of conifers (Lichtenthaler and Babani

2000; Lichtenthaler et al. 2000a, 2005b, 2007). This R_{Fd} imaging technique also allows studying the uptake of the herbicide diuron into green leaves by a progressing decrease of the R_{Fd} values (Lichtenthaler et al. 2013b) as shown in Fig. 7.

The efficient multicolor fluorescence imaging technique is presently the best and superior fluorescence method for plant tissue. In the future it may become an essential method in agriculture, horticulture, silviculture, plant food production, and agro-forestry. It also allows to track the ripening of fruits as shown for the ripening of apples during storage (Lichtenthaler et al. 2012).

8 The Non-mevalonate Chloroplast Pathway for Isopentenyl Diphosphate and Isoprenoid Biosynthesis, the DOXP/MEP Pathway[1]

In the early 1950s it had been shown by the groups of Konrad Bloch and Fjodor Lynen that acetate and acetyl-CoA were the precursors of cholesterol biosynthesis, in 1956 mevalonic acid (MVA) was detected as an intermediate, and in 1958 isopentenyl diphosphate (IPP) and farnesyl diphosphate. In 1958 the well-known plant biochemist and carotenoid specialist T.W. Goodwin was the first to study and to prove the incorporation of ^{14}C-acetate and ^{14}C-MVA yet at low rates, into carotenoids and sterols of higher plants (e.g., Goodwin 1958). Since the typical labeling pattern of the acetate/MVA pathway was found by means of the chemical degradation of ^{14}C-labeled *Euglena* β-carotene (Steele and Gurin 1960), it was generally accepted that the isoprenoids of plants, such as sterols as well as the plastidic carotenoids and chlorophylls (phytol side chain), are all synthesized via the acetate/MVA pathway as is cholesterol in fungi and animals [for original literature of the cited authors see the reviews Lichtenthaler (1999), (2000b)].

There remained, however, doubts and several inconsistencies concerning the biosynthesis of plastidic isoprenoids via the acetate/MVA pathway. Thus, photosynthetically fixed $^{14}CO_2$ was readily incorporated into carotenoids, phytol, and cytosolic sterols, whereas ^{14}C-labeled acetate and MVA were readily incorporated into cytosolic sterols, yet only at very low rates into chloroplast isoprenoids, an observation made by many authors and first by Goodwin (1958). In addition, we found that mevinolin, a specific inhibitor of the acetate/MVA pathway, efficiently blocked the biosynthesis of sterols and the mitochondrial ubiquinones, whereas the accumulation of chlorophylls (phytol side chain), carotenoids, and other plastidic isoprenoids was not affected (Bach and Lichtenthaler 1983). Our attempts to detect a separate plastidic HMG-CoA reductase, which is the key enzyme of the acetate/

[1] The plastidic DOXP/MEP pathway for isoprenoid biosynthesis is named after its first and second intermediates: 1-deoxy-D-xylulose-5-phosphate (DOXP) and 2-C-methyl-D-erythritol-4-phosphate (MEP).

MVA pathway, were negative. These findings suggested that chloroplasts might have their own biosynthesis system for IPP and isoprenoid biosynthesis which should be different from and independent of the cytosolic acetate/MVA pathway.

New progress in this field of chloroplast isoprenoid biosynthesis came from the application of the ^{13}C-labeling technique combined with high-resolution NMR spectroscopy that allows an exact location of the ^{13}C-atoms within the carbon skeleton of carotenoids or any other plant isoprenoid. Using this new evolving technique my chemist colleague Michel Rohmer in Mulhouse, France, had found in 1988 an unusual labeling of hopanoids in two eubacteria. At the beginning of 1993 we started a very close cooperation of our laboratories using this new technique, whereby my group grew the sterile algae and plant cultures on ^{13}C-labeled glucose and Rohmer's group performed the NMR spectroscopy of the ^{13}C- labeled isoprenoid compounds. This way we detected in 1995 the existence of the non-mevalonate plastidic pathway for IPP formation, first in green algae (Lichtenthaler et al. 1995; Schwender et al. 1995, 1996) and later also in higher plants (Lichtenthaler et al. 1997a, b). This pathway starting from pyruvate and glycerinaldehyde-3-phosphate has been termed DOXP/MEP pathway of plastidic IPP and isoprenoid biosynthesis after the first (DOXP, 1-deoxy-D-xylulose-5-phosphate) and second intermediate (MEP, 2-C-methyl-D-erythritol-4-phosphate).

In my group we cloned the genes of the first two enzymes of the DOXP/MEP pathway and found two specific inhibitors: 5-ketoclomazone and fosmidomycin for these two enzymes (cf. Fig. 8). Later I also cooperated with my brother Frieder Lichtenthaler, Darmstadt, a sugar chemist, who provided us with ^{13}C-labeled 1-deoxy-D-xylulose (DOX) that was readily incorporated into phytol, carotenoids, isoprene, and other plastidic isoprenoids by higher plants and algae (Schwender et al. 1997; Zeidler et al. 1997). There exists a cooperation, a cross talk, between both cellular isoprenoid biosynthesis pathways which, at photosynthesis conditions, primarily works via an export of active C_5 units from chloroplasts to the cytosol that are used predominantly for sterol biosynthesis (cf. Fig. 8). An import of short isoprenoid chains from the cytosol into the plastid may occur as well, however, only at extremely low rates if at all. In fact, our investigations with inhibitors demonstrated that the cytosolic acetate/MVA biosynthesis cannot provide the IPP or short chain isoprenyl phosphates required for carotenoid, chlorophyll, and prenylquinone biosynthesis in chloroplast when the plastidic DOXP/MEP pathway has been blocked by fosmidomycin. Once we had detected the plastidic DOXP/MEP pathway of IPP biosynthesis, various other groups jumped into this new research field and detected the following enzymes 3–7 of this pathway. Additional literature references on the detection, establishment and significance of the DOXP/MEP pathway including contributions of other laboratories are found in the extended review articles by Lichtenthaler (1999, 2010).

In cooperation with Rohmers group we also checked evolutionary aspects of the distribution of the DOXP/MEP pathway, e.g., its presence in different algae groups.

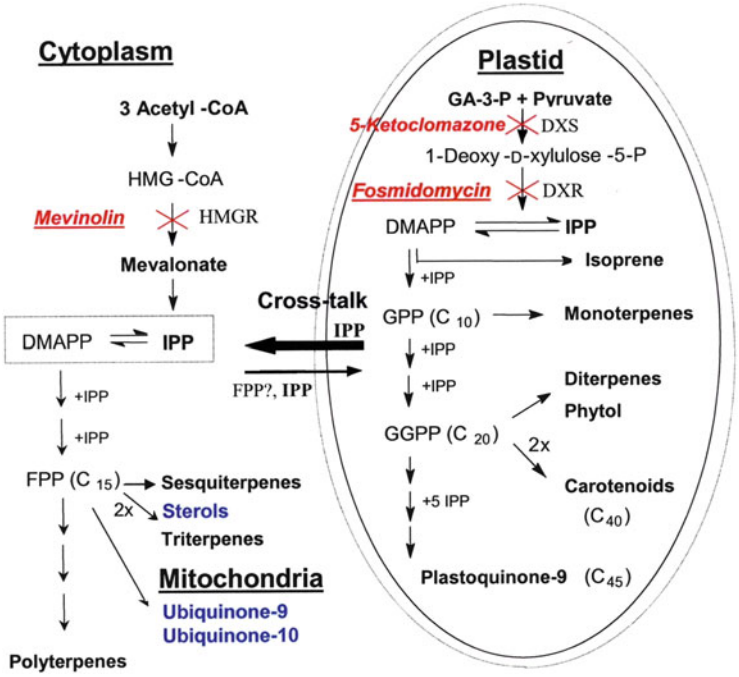


Fig. 8 Scheme showing the two independent isoprenoid biosynthesis pathways in plant cells: (1) the chloroplastidic DOXP/MEP pathway and (2) the cytosolic acetate/mevalonate pathway. The DOXP/MEP pathway provides the active isoprenic C_5 units (IPP, DMAPP) for the biosynthesis of carotenoids, chlorophylls (phytyl side chain), and prenylquinones (phytyl and nonaprenyl side chains). The acetate/mevalonate pathway delivers the isoprenic C_5 units for the biosynthesis of sterols and the prenyl side chain of the mitochondrial ubiquinones. The specific inhibition of the DOXP/MEP pathway by *5-ketoclomazone* (target DOXP synthase, DXS) and *fosmidomycin* (target: DOXP reductase, DXR) and of the acetate/mevalonate pathway by *mevinolin* (target: HMG-CoA reductase = HMGR) is indicated. The indicated "cross talk" between the two cellular biosynthetic isoprenoid pathways primarily consists of an export of IPP from chloroplasts to the cytosol for sterol biosynthesis. Scheme based on Lichtenthaler et al. (1997a) and Lichtenthaler (1999, 2010). *DMAPP* dimethylallyl diphosphate, *DOXP* 1-deoxy-D-xylulose-5-phosphate, *DXR* DOXP reductase, *DXS* DOXP synthase, *IPP* isopentenyl diphosphate, *FPP* farnesyl diphosphate, *GPP* geranyl diphosphate, *GGPP* geranylgeranyl diphosphate, *HMG* 3-hydroxy-3-methyl-glutaryl-CoA, *HMGR* 3-hydroxy-3-methyl-glutaryl-CoA reductase, *MEP* 2-C-methyl-D-erythritol-4-phosphate

It was essential that we could prove the presence of the DOXP/MEP pathway in cyanobacteria, since cyanobacteria-like organisms are regarded as the ancestors of chloroplasts. Like higher plants, Rhodophyta and Heterokontophyta possess both pathways for IPP biosynthesis, whereas Chlorophyta have lost their cytosolic acetate/MVA pathway during the evolution and they not only synthesize plastidic isoprenoids but also cytosolic sterols via the DOXP/MEP pathway (e.g., Lichtenthaler 2004c).

So far *Euglena* (Euglenophyta) is the only exception among all algae groups which, during evolution, has lost its DOXP/MEP pathway of isoprenoid

biosynthesis and therefore is dependent on the acetate/MVA pathway for the synthesis of all its isoprenoids including carotenoids and chlorophyll (phytol side chain) [see the reviews Lichtenthaler (2004c) and (2010)]. After Steele and Gurin (1960) had unequivocally shown via the chemical degradation of ^{14}C-labeled *Euglena* β-carotene that it was labeled via the acetate/MVA pathway, nobody doubted anymore that all photosynthetic organisms made their carotenoids via the acetate/MVA pathway. If they had taken a different alga instead of *Euglena* for their ^{14}C-labeling experiments of β-carotene, they and/or Goodwin and his group could have detected already then that chloroplasts possess their own pathway for IPP and isoprenoid biosynthesis.

9 Support by Elder Colleagues

During the start of my research in plant science in 1958 I was essentially supported by professor *August Seybold*, Heidelberg, who accepted me as a Ph.D. student in plant physiology although I had studied pharmacy. He taught me to perform scientific research always on a broad, comparative level. Seybold who since the late 1920s had extensively worked on the transpiration of plants, the photosynthetic pigments in sun and shade plants, and the light perception of plants and algae, also shaped me for ecophysiological research, in particular for questions on the influence of high and low quanta fluence rates on growth and the photosynthetic function of plants. I am grateful to him for his inspiring support.

After my Ph.D. I had the chance, in 1961, to work in the laboratory of *Paul Ozenda* at the Centre d'Etudes Nucleaire, Grenoble, France, where I learned the application of radioisotopes in plant physiology research and studied the kinetics of ion absorption (e.g., ^{32}P) by plant roots. He was very supportive and strongly encouraged the French-German cooperation.

From 1962 to 1964, I had the great privilege to work in the laboratory of *Melvin Calvin* (Nobel laureate 1961) in Berkeley, California (see Fig. 9). This was an exciting time and an atmosphere of departure in plant sciences and photosynthesis research. Melvin Calvin showed much interest in my research on the types of prenylquinones, carotenoids, and lipids in the photosynthetic membrane. He started his day very early, and discussions with him often took place at 7:00 a.m. He was an extremely fast thinker and quick to evaluate consequences of a scientific observation and immediately came up with ideas on the essential steps that should follow. Calvin knew how to stimulate young scientists and gave me the valuable advice to always concentrate fully on new promising research topics and to avoid performing parallel research. The whole scientific environment of Calvin's group was extremely stimulating; he usually had more than 70 individuals (staff members, graduate students and many foreign postdocs) in his laboratories. Among them were chemists, physicists, and plant physiologists, and in the regular Friday morning seminars we had excellent interdisciplinary discussions that often led to scientific cooperation. The scientific exchange with Melvin Calvin continued after

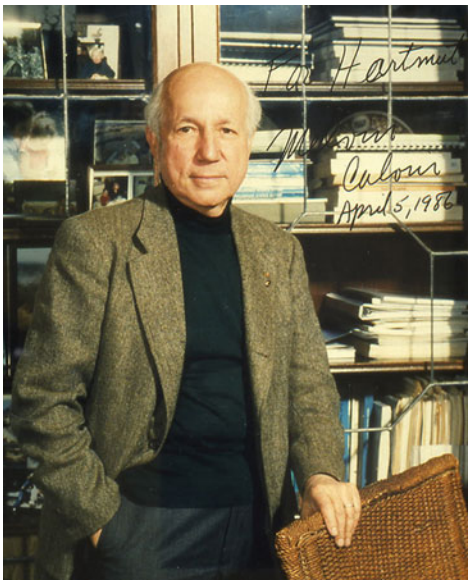


Fig. 9 Melvin Calvin, Berkeley, in his office in 1986

my return to Germany until his passing. He was, indeed, a great and inspiring scientist and academic teacher.

In Berkeley I also had extensive and very stimulating discussions with *Daniel Arnon* on the function of vitamin K_1 in the photosynthetic membrane. He had just shown that vitamin K_3, a methyl-naphthoquinone, catalyzed cyclic photophosphorylation in isolated spinach chloroplasts and wanted to learn more on the genuine substance phylloquinone K_1. At that time the idea of two photosynthetic photosystems in series came up, a concept that was based on the early observations of Kautsky (Kautsky and Hirsch 1931). This concept of two photosystems was intensively discussed by Arnon as well as in all the other photosynthesis research groups in Berkeley until it was finally established.

In 1963 I became acquainted with *Andy Benson* who had essentially contributed to the detection of the photosynthetic carbon reduction cycle, today known as Calvin–Benson cycle. Back then he worked in La Jolla, California, on the glycerolipids and the sulfolipid of the photosynthetic membrane. He had invited me to give a lecture on my paper on the total lipid and protein composition of the photosynthetic membrane that had just appeared in Nature (Lichtenthaler and Park 1963). We extensively discussed various possibilities how the photosynthetic pigments and glycero-lipids were arranged in the membrane and finally came up with the conclusion that the sulfo-, galacto-, and phospholipids were arranged in the membrane in a double-layer structure into which the pigments and proteins were integrated or attached to. We developed this concept clearly before the lipid double-layer structure of biomembranes had been established. Various aspects of our discussion together with my just published thylakoid lipid table became an essential

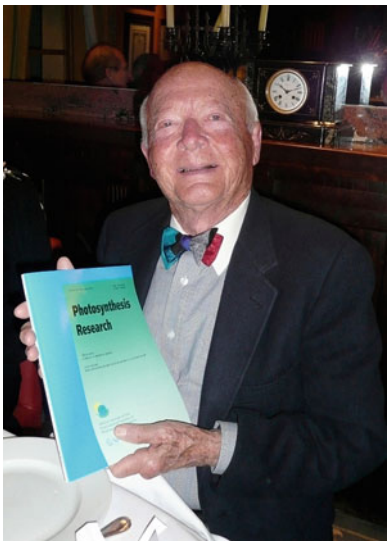


Fig. 10 Andy Benson, La Jolla, here shortly after his 90th birthday in Paris, 2007

part of Andy Benson's review paper "Plant Lipid Membranes" (Benson 1964). This inspiring discussion, followed by many others on the international photosynthesis congresses, was the starting point of a lifelong friendship and scientific exchange. In fact, in 2007 three of us from the photosynthetic community (Bob Buchanan, Roland Douce and myself) celebrated and honored Andy Benson on the occasion of his 90th birthday in a famous restaurant in Paris presenting a special issue of the journal Photosynthesis Research (see Fig. 10) with papers dedicated to him (see Lichtenthaler et al. 2008).

In 1964, shortly after my return to Germany, *Wilhelm Menke* who in the 1930, was the first to isolate chloroplasts from spinach and in 1962 had created the term "thylakoids" invited me to Köln for a lecture and shortly afterwards *André Pirson* invited me to Göttingen. Both photosynthesis researchers accompanied and promoted my further research. I am also grateful to *Hans Reznik*, then at the University of Münster, Westphalia, for his offer to continue my photosynthesis research in Germany and for his promotional support. My research was also inspired by continuous discussions with *Kazuo Shibata*, Tokyo, *Hemming Virgin*, Göteborg, *Kurt Mühlethaler* and *Albert Frey-Wyssling*, Zürich, *Cyrille Sironval*, Liège, and particularly accentuated in repeated discussions, over many years, with *Trevor W. Goodwin*, Liverpool (see Fig. 11), the pioneer and expert of carotenoid and isoprenoid research in plants, whom I first met in 1968 on the first international photosynthesis congress in Freudenstadt, Black Forest, Germany.

Fig. 11 Trevor Goodwin, Liverpool (*right*), with Paul Mazliak, Paris, and Hartmut Lichtenthaler (*left*) on a European plant lipid meeting in September 1993 in Karlsruhe

10 Cooperations with Scientific Colleagues

Extremely essential impulses for photosynthesis research as well as European and international scientific cooperation in plant science came from the first international photosynthesis congress held by *Helmut Metzner* (Tübingen) in Freudenstadt, Black Forest, Germany, in 1968. There, many young as well as older colleagues from Eastern and Western European countries met for the first time after World War II and exchanged their ideas on all aspects of photosynthesis. Among them were physicists, biochemists, classical botanists, plant physiologists, and cytologists. In fact, that congress was the starting point of a broad and interdisciplinary scientific cooperation on a European and worldwide level, which was further promoted by the subsequent international photosynthesis congresses that have been held every 3 years. In this respect I need to emphasize that from the 1960s through the 1990s a much higher percentage of plant scientists worked on particular aspects of photosynthesis than today.

With *Hubert Ziegler* (München) as an essential supporter, *Peter Böger* (Konstanz), *Ulrich Lüttge* (Darmstadt), and other German colleagues I had a close cooperation on the establishment of the "Section Plant Physiology" within the German Botanical Society DBG. On a European level discussions went on with *Paul-Emil Pilet* (Lausanne), *John Dale* (Edinburgh), *Laszlo Erdei* (Szeged), *Anders Kylin* (Lund), *Valentin Kefeli* (Moscow), *Stanislav Procházka* (Brno), *Miloje Saric* (Belgrade), *Ernesto Vieitez* (Santiago de Compostela), *Charles Wittigham* (London), *Zdenek Sestak* (Prague), and many others on a cooperation of European plant physiologists. In 1975 on the XII. International Botanical Congress in Leningrad (today St. Petersburg) we had an informal discussion meeting with the Russian and East European plant physiologists. These discussions, essentially

initiated and supported by Hubert Ziegler (München), led to the formation of a Federation of European Societies of Plant Physiology, FESPP, which could finally be founded in 1978 with the participation of several East European countries (for details see the report of Lichtenthaler 2004a). With other colleagues, such as *Paul Stumpf* (Davis, California), *Peter Biacs* (Budapest), *Trevor Goodwin* (Liverpool), *Ernst Heinz* (Köln), *John Harwood* (Cardiff), *Conny Liljenberg* (Göteborg), *Paul Mazliak* (Paris), *Paul-André Siegenthaler* (Neuchatel), *Joseph Wintermans* (Nijmegen), and *Norio Murata* (Japan), I cooperated to establish the International Symposia on Plant Lipids, ISPL which, since 1974, have been held every 2 years all over the world (details are given by Lichtenthaler 2004b). The large and fast progress in photosynthesis, biosynthesis, metabolism, and function of plants lipids as well as in many other topical fields of plant science since the 1970s was, indeed, possible due to the fact that colleagues of different countries got to know each other on such regular international conferences and workshops. They started their scientific cooperation and then an exchange of their graduate students and postdocs. In addition, in 1980 I started with *Peter Böger* (Konstanz), *Aloys Wild* (Mainz), *Manfred Kluge* (Darmstadt), and *Heinrich Fock* (Kaiserslautern) annual photosynthesis workshops where our graduate and Ph.D. students as well as our young scientific staff members could present and discuss their scientific results. Also these workshops provided distinct impulses for progressing with our photosynthesis research towards new horizons.

In 1962 my own scientific cooperation had already started with *Roderic Park* in Berkeley on the lipids and proteins of the photosynthetic unit in thylakoids. Later, this was continued with many colleagues mentioned below in a chronological order, such as *Benno Sprey* and *E. Peveling*, Münster, on osmiophilic plastoglobuli, with *Günter Retzlaff*, BASF, on the mode of action of herbicides in photosynthesis, with *Conny Lilienberg*, Göteborg, on the separation of prenols on TLC plates, with *Alan Wellburn*, Lancaster, on cytosolic and plastidic isoprenoids and their labeling from ^{14}C-mevalonate, with *Pierre Dizengremel*, then Paris, on occurrence and function of different ubiquinone homologues in plants, with *Peter Biacs*, TU Budapest, on saponins in plants, with the biochemist *Janos Retey*, Karlsruhe, on HMG-CoA reductase in plants, with *Roland Douce*, Grenoble, on the localization of prenylquinones and carotenoids in the chloroplast envelope, with *Karl Erismann*, Bern, on ^{14}C-labeling kinetics of prenylquinones in *Chlorella*, with *Barry Rock*, NASA, Pasadena, USA, on forest decline in Germany and the USA including remote sensing and airborne classification of forests, with physicists *Laslo Koscany* and *Peter Richter*, TU Budapest, on creating new instruments for spectroscopy of plant leaves as well as for indoor and outdoor Chl fluorescence measurements, with biochemist *Wilhelm Boland,* Karlsruhe, on the inhibition of fatty acid biosynthesis by cerulenin derivatives, with *Vladimir Saakov*, St. Petersburg, on the effect of gamma ray irradiation on the photosynthetic apparatus, with *Jiri Santrucek* and *Pavel Siffel*, České Budějovice, on photosynthetic activity of green tobacco and aurea mutants, with *Nicola D'Ambrosio*, Napels, on the carotenoid composition of leaf and stem tissue of the CAM plant Cissus, with *Anatoly Gitelson*, Lincoln, Nebraska, on retrieving the actual Chl fluorescence spectra and emissions by plant leaves via simultaneous

absorbance and reflectance measurements, with *Fatbardha Babani*, Tirana, Albania, on fluorescence imaging of photosynthetic activity, with *Zoltan Tuba*, Gödöllö, Hungary, on the photosynthetic apparatus of homoio- and poikilochlorophyllous desiccation-tolerant plants, as well as with *Otmar Urban*, Brno, on Chl fluorescence imaging of sun and shade leaves of trees in the Beskydy Mountains. I would also like to mention here the long-term scientific exchange with *Bob Buchanan*, Berkeley, on photosynthetic topics and historical aspects of photosynthetic carbon fixation and the close cooperation with *Tino A. Rebeiz*, Champaign, USA, regarding the organization of the First International Symposium on Chloroplast Bioengineering held at the University of Illinois, Urbana-Champaign in May 2005 and in editing the book "The Chloroplast, Basics and Applications" (Rebeiz et al. 2010).

Particularly close and intensive was the cooperation with physicist *Joseph Miehé* and coworkers, CRNS, Cronenbourg near Strasbourg, from 1994 through 1998, on the development and application of laser-induced fluorescence imaging of plants in the four plant fluorescence emission bands blue, green, red, and far-red as well as with chemist *Michel Rohmer* and his coworkers, University of Strasbourg, in the detection and establishment of the novel DOXP/MEP pathway of chloroplast IPP and isoprenoid biosynthesis from 1993 through 1999. In fact, such international cooperations as mentioned here were, for all those active in plant physiology research, the essential basis for the large progress made in so many fields of plant science in the last five decades. It was a pleasure that I had the chance of contributing to this enormous development.

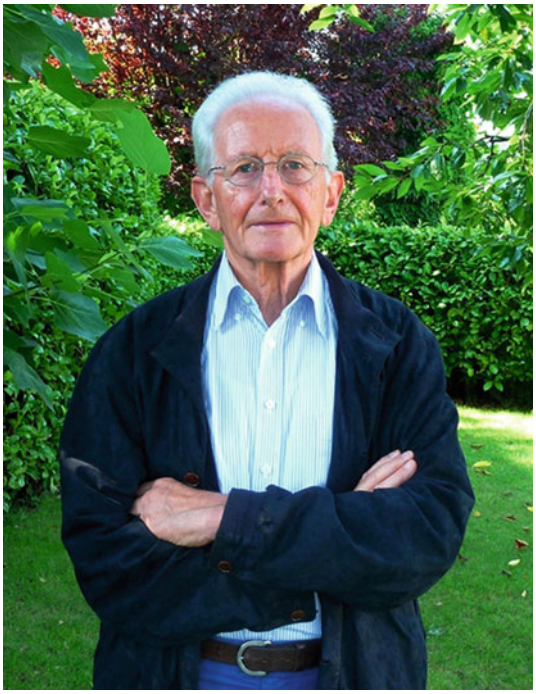
11 Epilogue

The large progress made in photosynthesis and plant science over the past 55 years was essentially a result of the increasing work with isolated cell organelles and the continuous invention and application of new and advanced instruments as well as investigation techniques that allowed the studies and revelations of details of plant structures, metabolic reactions, and their responses to the environment. Such novel and superior techniques and approaches were the application of electron microscopy, the labeling of cellular metabolites with radioisotopes (e.g., ^{14}C), the application of ^{13}C labeling in combination with high-resolution NMR spectroscopy, the introduction of PAGE and HPLC techniques, the availability of CO_2/H_2O porometer systems, the measurement of laser-induced chlorophyll fluorescence kinetics, the powerful technique of fluorescence imaging of plants and their stress responses as well as the application of inhibitors to specifically block enzymes in metabolic pathways, and of course the use of molecular biology, to just name a few major ones. Thus, progress in science is and has always been dependent on the development of new investigation techniques. In addition to these new techniques there has been the progressing international scientific cooperation and exchange of scientists that have essentially been enhanced and promoted by regular international scientific meetings and workshops, such as the triennial international congresses on Photosynthesis, the biannual meetings

of the European plant physiologists, FESPP, or the biannual international symposia on plant lipids, ISPL, which are mentioned above. I hope that also in the future the long-standing, successful international cooperation will continue on a worldwide level and include many more countries. This international cooperation that is so essential for the progress in science is based on the mutual understanding of and the respect for people.

12 Curriculum Vitae of Hartmut K. Lichtenthaler

Hartmut Lichtenthaler was born on June 20, 1934 in Weinheim, Baden, Germany



Education and Professional Experience

1953–1958: Study of pharmacy in Heidelberg and at the University of Karlsruhe

1958: Masters degree (Staatsexamen) in Pharmacy

Spring 1961: Ph.D. in Botany at the University of Heidelberg with August Seybold

1961: Euratom Research Fellow at the Centre d'Etude Nucléaires and the University of Grenoble/France with Paul Ozenda

1962–1964: Research fellow at the University of California, Berkeley, with Melvin Calvin

1964–1970: Botanical Institute, University of Münster/Westphalia, scientific assistant, 1967 Habilitation in Botany, then Dozent and Associate Professor

1970–2001: Full professor in Plant physiology, Pharmaceutical Biology and Plant Biochemistry at the University of Karlsruhe (now: Karlsruhe Institute of Technology, KIT)

since 2001: Professor emeritus

Other Activities

1980–1986: Chairman of the Section Plant Physiology of the German Botanical Society DBG; 1978 Founding member of the Federation of the European Societies of Plant Physiology (FESPP) and FESPP President 1984–1986; Founding Member of the International Symposia on Plant Lipids ISPL 1974–1976. Coordinator and participant in several European research programs, such as OECD, LASFLEUR, QAAFFI, INTERREG, PEF.

1973: Visiting professor at the University of California, Berkeley with Melvin Calvin

1975: Guest professor at the University of Gothenburg, Sweden with Hemming Virgin

1981: Guest professor at the University of Lancaster, England with Alan Wellburn

Honors

1992: Honorary member of the Hungarian Society of Plant Physiology.

Honorary doctoral degrees: 1996 Mendel University of Brno, Czech Republic; 1997 ELTE University, Budapest; and in 2001 St. Istvan University, Gödöllö, Hungary. In 2001 "Bundesverdienstkreuz am Bande" (Cross of Merits) of the Federal Republic of Germany. 2003 Gregor Mendel Medal of the Czech Academy of Sciences; in 2004 Terry Galliard Medal and in 2010 Corresponding Membership Award, American Society for Plant Biology ASPB

Acknowledgements I am very grateful to my entire team of former graduate and Ph.D. students as well as scientific and technical group members who actively participated, over the past 50 years, in performing some truly pioneering research in photosynthesis and plant science. Without their continuous effort, enthusiasm and understanding we could not have made such an enormous progress in our plant science research spanning from biophysics and organic chemistry via pure physiological and structural investigations using the electron microscope to pure biochemical topics including some molecular biology. This interdisciplinary research was also made possible by multiple German and international cooperation partners at universities, research institutions, and the chemical industry and—last but not least—by the continuous support of granting agencies, such as the German Research Council DFG, the German Ministry of Research, the European Community, and various others, all of which is gratefully acknowledged. In addition, I wish to thank Ms Gabrielle Johnson for her long-term English language assistance with many of my scientific publications. Last but not least, my special gratitude goes to my wife for her kind understanding, her patience, and her continuous support of my scientific research over almost five decades now.

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