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TRIBUTE

Hartmut Lichtenthaler: an authority on chloroplast structure and isoprenoid biochemistry

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Abstract We pay tribute to Hartmut Lichtenthaler for making important contributions to the field of photosynthesis research. He was recently recognized for groundbreaking discoveries in chloroplast structure and isoprenoid biochemistry by the Rebeiz Foundation for Basic Research (RFBR; http://vlpbp.org/), receiving a 2014 Lifetime Achievement Award for Photosynthesis. The ceremony, held in Champaign, Illinois, was attended by many prominent researchers in the photosynthesis field. We provide below a brief note on his education, and then describe some of the areas in which Hartmut Lichtenthaler has been a pioneer.

Keywords Melvin Calvin · Isoprenoids · Phylloquinone · Plastoglobuli · MEP pathway

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Education

Hartmut Lichtenthaler, born in 1934, studied Pharmacy, Botany and Chemistry at the Universities of Karlsruhe and Heidelberg, obtaining his Ph.D. in Plant Physiology at the University of Heidelberg in 1961. From 1962 to 1964, he performed research on photosynthesis at the University of California Berkeley with the 1961 Nobel-laureate in Chemistry, Melvin Calvin. [Lichtenthaler et al. (2015) have written about Andy Benson, a close associate of Calvin.] Upon his return to Germany, his research focused on the structure, function and biosynthesis of the photosynthetic apparatus with particular emphasis on the isoprenoid pigments and quinones of chloroplasts. After his move to Karlsruhe in 1970, he extended his research to plant lipids and the mode of herbicide action in chloroplasts (see Fig. 1 for a 1997 portrait).

Figure 2 shows two photographs of Hartmut at the time of the Lifetime Award for Excellence in Photosynthesis: the top one is with Pierre Joliot, the second awardee, and Tino Rebeiz, who had presented these awards; the bottom photograph is with several researchers in photosynthesis, including the authors of this tribute.

Research

Hartmut Lichtenthaler has made numerous original contributions to the understanding of photosynthesis, many of them involving isoprenoids[1] During his PhD work with

[1] Polymers whose carbon skeletons consist in whole or in large part of isoprene units joined end to end. These include β-carotene, lycopene, vitamin A, plastoquinone-9 and the phytyl tails of chlorophyll, vitamin K_1 (phylloquinone), and vitamin E (tocopherols).

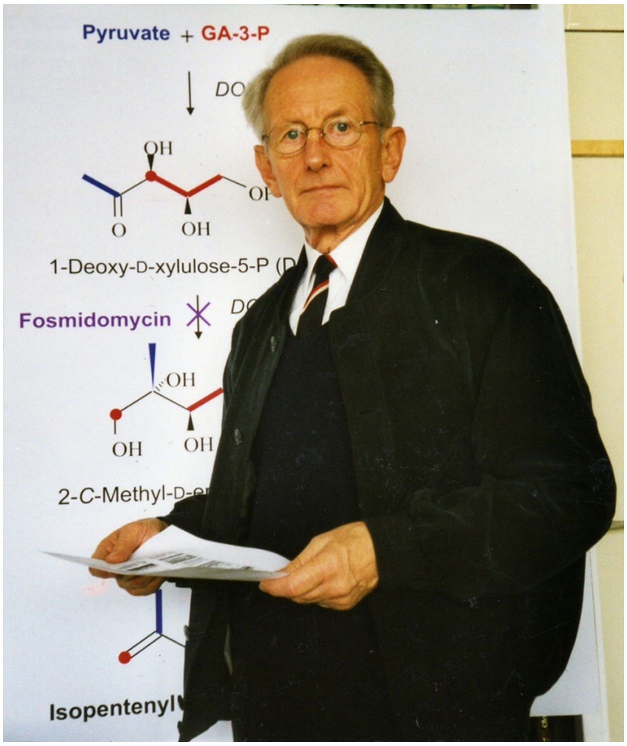

Fig. 1 A 1997-portrait of Hartmut Lichtenthaler. *Source* Lichtenthaler family archives

Professor August Seybold, Hartmut discovered phylloquinone (also known as vitamin K_1) to be abundant in green leaves. It required significant work to separate phylloquinone from β-carotene. Lichtenthaler recognized that phylloquinone is connected with photosynthesis because it was specifically associated with chloroplasts, not leucoplasts or chromoplasts, and because of its isoprenoid (phytyl) side chain. Although this work started in Germany, it continued in the laboratory of Melvin Calvin and ultimately resulted in a paper in *Nature* (Lichtenthaler and Park 1963). Further, Lichtenthaler showed that phylloquinone is associated with photosystem (PS) I while plastoquinone-9 is associated with PS II. These associations are now so well accepted that it is sometimes hard to remember that this information was not always known.

Characterizing osmiophilic plastoglobules

The distinctive dark spots in electron micrographs of chloroplasts were dismissed as unimportant lipids by most, but not by Hartmut (see Lichtenthaler 2013). In the 1970s, he had shown that these dynamic structures were repositories for plastoquinone and various tocopherols (vitamin E). He showed that plastoglobules, or plastoglobuli (used interchangeably), were dynamic, increasing in high light and in aging leaves. One of the authors of this tribute (TDS) has also seen that they swell at high temperature and contract when leaves are returned to non-stressful temperature for just a few minutes (Zhang et al. 2010). The full story of plastoglobules is yet to be told, and Lichtenthaler's work will play an important role as the function of plastoglobules become better known.

We note that the main reason for the very dark staining of plastoglobuli in osmium-tetroxide-stained sections is that plastoglobuli—due to their high plastoquinol content—are highly reducing and thus reduce osmium, causing it to accumulate (Lichtenthaler 2013). Plastoglobules have proteins on their surface and there is currently an interest in attaching enzymes to the surface of the plastoglobuli to make desirable products that might accumulate in the plastoglobuli.

Sun and shade leaves

Throughout his career, Hartmut Lichtenthaler has investigated differences in chloroplast structure between sun and shade leaves (Lichtenthaler et al. 1981). He noted that sun leaves are often smaller but thicker than shade leaves. He reported large thylakoid stacks and high amounts of light-harvesting chlorophyll proteins in shade plants, while confirming and extending our knowledge of the high chlorophyll a/b ratio in sun leaves, the high levels of carotenes and xanthophylls in sun leaves, and the rapid interconversion of violaxanthin and zeaxanthin in response to high or low light conditions (Lichtenthaler 2007). Along the way, Hartmut developed methods for accurately identifying and quantifying photosynthetic pigments in leaves, including equations for spectrophotometrically determining chlorophyll levels (Lichtenthaler 1987), that are utilized by many researchers in this area. He also showed remarkable differences in chlorophyll *a* fluorescence between sun and shade leaves, and was able to track how long it takes for the herbicide *Diuron* to enter leaves through changes in fluorescence emission (see, e.g., Lichtenthaler et al. 2005; Fig. 3).

Forest decline

In tune with many of the problems facing our World, Lichtenthaler used his intimate knowledge of photosynthesis to study the very immediate problem of forest decline (Lichtenthaler and Buschmann 1984). Trees in the Black Forest were dying back, especially in the crown where the growing meristems should be healthiest. Lichtenthaler was able to show that this was caused by air pollutants, the most detrimental of which was ozone

Fig. 2 (*Top*) from *left* to *right*: Hartmut Lichtenthaler, Tino Rebeiz, and Pierre Joliot. (*Bottom*) Front row (*left* to *right*): Steve Long, Pierre Joliot, Hartmut Lichtenthaler and Tony Crofts. *Back row* (*left* to *right*): Christoph Benning, Tom Sharkey, and Govindjee, all at the Rebeiz Foundation for Basic Research ceremony honoring the lifetime achievements of Hartmut Lichtenthaler and Pierre Joliot (September 12, 2015). Photographs are by Laurent Gasquet

generated in the atmosphere when sulfur dioxide, nitrogen oxide, and hydrocarbons were acted on by sunlight. The resulting ozone was killing trees, to say nothing of the effects on human health (Schulze 1989; Wilkins et al. 2001).

Chlorophyll *a* fluorescence

Hartmut Lichtenthaler recognized the rich source of information present in measurements of chlorophyll *a* fluorescence from leaves (see Papageorgiou and Govindjee 2004; Demmig-Adams et al. 2014 for further details). Hartmut made contributions both to the detection of stress using chlorophyll *a* fluorescence (Schweiger et al. 1996; Lichtenthaler et al. 1998) and detecting variations in photosynthetic rate across leaves and canopies using chlorophyll fluorescence imaging (Fig. 3; Lang et al. 1996; Lichtenthaler et al. 1996; Lichtenthaler and Miehé 1997).

The methyl erythritol 4-phosphate (MEP)/deoxyxylulose 5-phosphate (DOXP) pathway

After a lifetime of achievements that constituted a worthy body of contributions, Hartmut Lichtenthaler began work on a new metabolic pathway in chloroplasts. The papers from this work are some of his best known, and heavily cited, even though they are the most recent. According to the Thompson/Reuters ISI Web of Science, five of

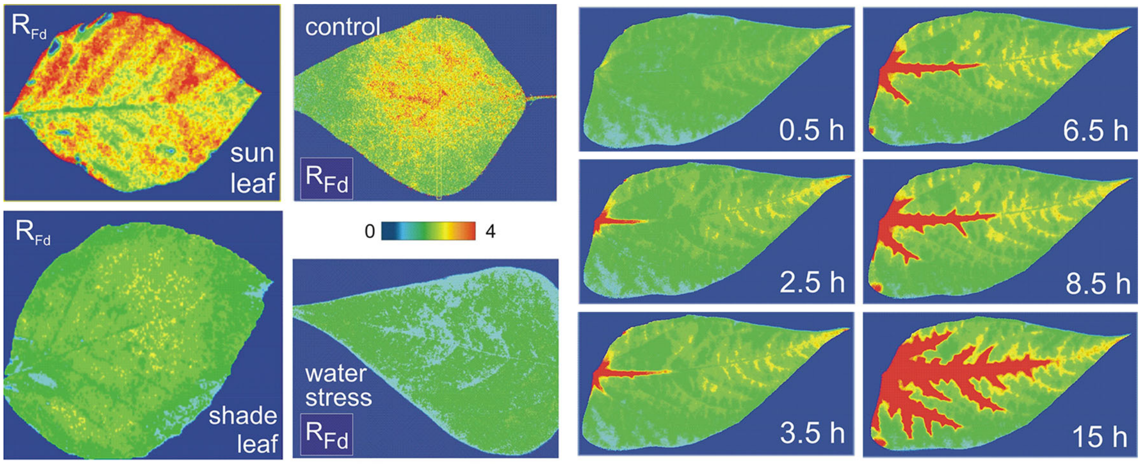

Fig. 3 (*Left panel*) fluorescence imaging (shown in *false color*) of sun and shade leaves of beech (*Fagus sylvatica*; *left*), and common bean (*Phaseolus vulgaris*; *right*), well-hydrated versus water-stressed. R_{Fd} stands for the ratio of maximum fluorescence (F_m) minus steady state fluorescence (Fs) divided by Fs and is an indicator of the photosynthetic activity of leaves. (*Right panel*) Fluorescence images of bean leaves at different times following watering of the plant with 10 mM herbicide Diuron. From Lichtenthaler et al. (2005), with permission of Springer

Lichtenthaler's seven most highly cited papers are on the topic of this newly described metabolic pathway. This pathway is the source of carotenoid precursors, the phytyl tail of chlorophyll, phylloquinone, tocopherols and the nonaprenyl chain of plastoquinone, and thus connects back to Lichtenthaler's earliest work.

Although it was known that the mevalonate pathway makes the precursors for cholesterol in animals and sterols in plants, it was difficult to prove that carotenoids were also products of the mevalonate pathway, despite the fact that there was no alternative explanation. In the "Preliminary Ph.D exam" of the author TDS in 1977, Jan Zeevaart asked about the issues and problems with the idea of acetyl CoA and the mevalonate pathway as the source of plastid isoprenoids (the correct answer was that the mevalonic acid pathway does not explain carotenoid synthesis). This problem had also worried Hartmut, and in the 1990s, together with Michel Rohmer, he showed that plastids have a different pathway, the MEP pathway, for the synthesis of isoprenoid precursors (Lichtenthaler 1999, 2000; see also the background in Fig. 1). Hartmut also proved that TDS' favorite molecule, isoprene, is made via pathway in plants (Zeidler et al. 1997). This MEP pathway appears to be associated with photosynthesis and "armchair" biochemistry indicates that it is especially efficient when photosynthesis is also occurring. However, the energetics are not very different from the mevalonate pathway in heterotrophic tissues despite the fact that the two pathways have no reactions in common. Hartmut showed the phylogenetic distribution of this pathway and that it is common to all photosynthetic organisms (Schwender et al. 2001), but also occurs in certain pathogenic bacteria and in the malaria disease agent *Plasmodium falciparum* (Lichtenthaler 2004).

The discovery that isoprene and monoterpenes are made by the MEP pathway meant that the majority of hydrocarbons entering the atmosphere come from the MEP pathway. This is important for understanding how ozone, that was causing forest decline, is made.

The MEP pathway is also a target for antibiotics and, with any luck, a new class of antimalarial drugs (Zeidler et al. 2000). The identity of the second enzyme in the pathway was discovered by its sensitivity to an antibiotic called fosmidomycin (Kuzuyama et al. 1998; Zeidler et al. 1998). The pathway is also the target of herbicides. The mode of action of the herbicide chlomazone (Command®) had been unknown until Hartmut showed that a metabolite, 5-keto-chlomazone, inhibits the first enzyme of the MEP pathway, opening the door to the discovery of new herbicides (Lichtenthaler et al. 2000).

An inspiration to scientists

Hartmut has worked with the leading figures in photosynthesis and received numerous awards (Fig. 4, including Hartmut's photograph with several pioneers in photosynthesis: Bob Buchanan, James Al Bassham, Andy Benson, Roland Douce, and Laurie Bogorad; further, in the bottom right, Hartmut is shown receiving a honorary doctorate degree in 1997 in Budapest, Hungary).

Fig. 4 Hartmut Lichtenthaler with Bob Buchanan (*top left*) and with James Al Bassham and his wife Leslie Bassham (*top right*). The photograph in the middle is with Andrew Benson, Roland Douce, Gérard Milhaud, and Bob Buchanan (from Lichtenthaler et al. 2008). The photographs on the bottom row show Hartmut Lichtenthaler with Laurie Bogorad (*left*) and receiving an honorary doctorate and professorship from the ELTE University, Budapest, Hungary, in 1997 (*bottom right*); with him are Rector Miklos Szabo (*left*) and Dean Andras Benczur. *Source* Lichtenthaler family archives

Hartmut also has inspired younger scientists. *Thomas Bach* (Institute de Biologie Moléculaire des Plantes, Université de Strasbourg) recalls his experience with Professor Lichtenthaler this way:

> *"After three semesters I was ready to give up "biology" and to exclusively continue with chemistry, being completely frustrated. And then a newly nominated and young full professor started with a lecture course in plant physiology, and for the first time there was a chemical formula on the blackboard, this in "biology", a true revelation! This lecture course was filled with the "latest news" from research on photosynthesis and aspects around, and finally led me to become his Ph.D. student later on, starting with enzymology, at that time a rather new field in Prof. Lichtenthaler's laboratory."* (Communication received from Thomas Bach, by Govindjee, via Tino Rebeiz on the occasion of the presentation of the Rebeiz Foundation Lifetime Achievement Award, 2015)

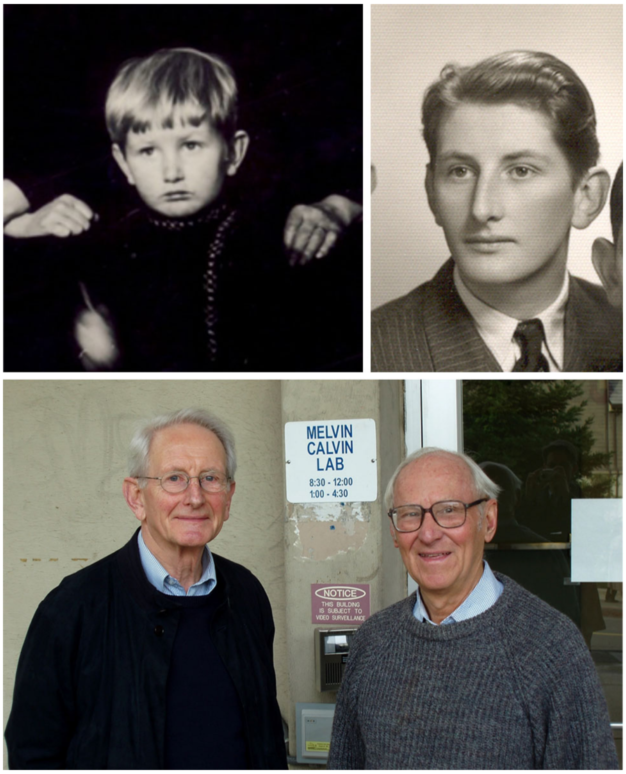

Fig. 5 Hartmut Lichtenthaler (*top*) as a child (1936) and as a young man (1951) and (*bottom*) with Ken Sauer visiting the Berkeley lab again in 2006, nearly 50 years after working with Melvin Calvin on his photosynthesis endeavors. *Source* Lichtenthaler family archives

Moreover, the author TDS recalls with great fondness visiting Hartmut in Karlsruhe and discussing at length the isoprenoid MEP pathway in chloroplasts as well as a walk in the Black Forest to see the forest decline first hand.

Finally, Hartmut deserves the community's gratitude for his many efforts at organizing societies to promote biological, and especially plant, research. He played a key role in the establishment of the Federation of European Societies of Plant Physiology (founded in 1978) and the International Symposia on Plant Lipids, which has met every other year since 1974.

We end this Tribute by showing several photographs of Hartmut throughout his life. Figure 5 shows his photograph when he was a child, when he was 17 years old, and when he was visiting the University of California Berkeley in 2006.

Conclusion

Hartmut has contributed in many ways to photosynthesis as it is now taught (for his own account of many of the topics covered here, see Lichtenthaler 2015). In addition to our better understanding of the process of photosynthesis that is essential for life as we know it, Hartmut has addressed important applied questions. From vitamin K_1 to reversing forest decline to the MEP pathway and antimalarial drugs, the spinoffs from Hartmut's long fascination with photosynthesis has helped make the world a better place. For this, the Rebeiz Foundation for Basic Research presented a Lifetime Achievement Award to Hartmut Lichtenthaler.

Acknowledgments We are highly thankful to Constantin (Tino) and Carole Rebeiz for their outstanding hospitality during the celebration at the Rebeiz Foundation on September 12, 2015. We are grateful to Tino for inviting both authors of this tribute (G and TDS) to prepare a presentation honoring Hartmut. The current text and photographs are based on this presentation, and some of this material can also be found at the Foundation web side (http://vlpbp.org/). We are grateful to the generosity of the Lichtenthaler family for a number of photographs reproduced here. We thank Laurent Gasquet for Fig. 1; additional photographs by Gasquet are available via one of us (G).

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