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Torn Between Nature and Lab: A Dying Breed of Plant Scientists?

E.H. Beck

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Abstract In his life as a scientist, E. Beck addressed questions, concerning a very broad spectrum of disciplines in plant science. He likes research in the laboratory as well as in the field. Admitting that specialization is inevitable given the complexity of the systems we deal with, he is not flagging to encourage his students and colleagues to go back and consider the entire system once in a while for verification of the reasonability of their research questions and approaches.

1 Introduction: Studying Biology, Chemistry, and Geography

I grew up in an atmosphere created for generations by teachers. No wonder that I, too, aimed at becoming a teacher with a special interest in biology kindled by my mother. Due to the chaos of world-war II and the post-war period, biology was taught in school either not at all or as a minor subject not every year to the classes. Apart from the Mendelian laws I never received a lesson in botany during my entire school days. Starting from the scratch, lectures in botany at the University of Munich opened a new world for me when I learned that algae, herbs, shrubs, and trees, which I already knew by name, belong to the same kingdom, unified by a common type of metabolism, termed photosynthesis. In 1956 when I started my teacher's studies in biology, chemistry, and geoscience, vitaminized by educational

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Preamble Having been asked to report on 55 years of my life as plant scientist is a great honor for which I am very grateful to Ulrich Lüttge, and which I would like to pass on to the numerous friends, colleagues, and students to whom I feel deeply indebted for their contributions to my projects and ideas, and *vice versa* for sharing their projects with me. It is a pity that space limitation constrains my presentation to the more complex subjects and to leave out many projects and experiences which, while challenging my skills, enriched my life as human being and as scientist as well. Unforgettable are, e.g., the three expeditions with the group of soil scientists led by my friend Wolfgang Zech to the Himalaya where we tried to reconstruct landscape history around the Annapurna massif from soil cores and pollen diagrams (Zech et al. 2001a, b). Thinking of Nepal and the Tribhuvan University in Kathmandu is also not possible without mentioning the fruitful cooperation in plant molecular biology with my friend Tribikram Battarai (Beck et al. 2007) and his doctoral fellow Deepak Pant. I apologize for not expanding to the fruitful and long-standing collaboration with Prof. J. C. Onyango PhD (e.g., Netondo et al. 2004a, b), and his students from Maseno University (Kenya) and to the fantastic revival of my experiences on Mt. Kilimanjaro with my friends Andi and Claudia Hemp. These names stand for many others whom I could not mention for shortage of space and I hope that they would forgive me. To illustrate the environments of my professional life and its relationship with the time spirit and with academic and social events, I include a few snapshots, termed "interludes" in the text. As this contribution is dedicated to research, I will abstain from expanding my experience in academic teaching which in the retrospective was inherently positive, whenever I had to do with students. Special highlights were the botanical fieldtrips to many exciting regions in Europe, Africa, and Australia. Representatively for such fieldwork, I refer to the outstanding Summer Schools of the German Studienstiftung in the Alps where teaming up with my friend Ulrich Lüttge, we could introduce highly interested students to the exciting life of plants in the stressful alpine environment.

theory, philosophy and psychology, the path of carbon in photosynthesis had just been published with many open questions and the term “Calvin Cycle” had not yet been coined. The required sources of reducing equivalents and ATP were still unclear, as neither the pathway of the photosynthetic electron transport, nor the chemi-osmotic ATP-synthesis were yet known. Later, while doing advanced practical in botany, dedicated to details of plant structure, our supervisor, Meinhart Zenk, who just had returned from Purdue University fascinated us with the latest news in plant biochemistry, such as the Krebs cycle and Daniel Arnon’s idea of the *zig-zag*-photosynthetic electron transport. In the late 1950s, neither microbiology nor genetics was represented in the Biological Faculty by a professorship in Munich and therefore also not included in the curricula. Microbiology was an excursus of the lecture “General Botany” and genetics likewise of “General Zoology”. Studying also chemistry promoted my interest in biochemistry and I was happy to attend a lecture by the later Nobel Prize laureate Feodor Lynen.

2 Chemotaxonomy

At that time, a few groups, in particular Hans Reznik in Muenster had introduced secondary plant constituents as characters in plant taxonomy, especially the recently discovered “nitrogen-containing anthocyanins” (now known as betacyanins) as a unique character of the entire group of the Centrospermae. Interested equally in plant taxonomy and plant biochemistry I asked the plant systematist,[1] Prof. Herrmann Merxmüller for a problem that could be solved by chemotaxonomy. This was pioneer work in Munich, and since there was no lab in the Institute of Systematic Botany I got a working place in the new Institute of Pharmacy of the University of Munich where paper chromatography had just been introduced as a method of analysis. My question was, whether the Centrospermae form a consistent group with only one exception, the carnations (Silenoideae) or whether there are more families in this group which share the chemical marker “true anthocyanins.” With a thesis in which I could show that also the Molluginaceae, Plumbaginaceae, and the Alsinoideae contain anthocyanins instead of betacyanins and thus together with the Silenoideae form a separate branch (Caryophyllinae) within the Centrospermae (Beck et al. 1962), I finished my teacher’s study in 1961 with the state examination. As a trainee teacher in a secondary high school in Munich I had the opportunity to continue my studies in plant chemotaxonomy for a doctoral thesis on more Centrospermae as well as on the Primulaceae for which a separation of the genera *Lysimachia*, *Anagallis*, and *Cyclamen* from the Primulaceae *sensu stricto* had been proposed (Lys 1956). In addition to confirming my earlier results on the Caryophyllinae I could show that a separation of the

[1] At that time plant taxonomy (without emphasis in phylogenetic relationship) was differentiated from plant systematics, aiming at elucidating phylogenetic relations.

Primulaceae into the proposed branches could not be supported by chemical characters (Beck 1963).

Interlude 1: Attaining Dr. Rer. Nat. in the Sixties *It might be of interest to our junior staff that at that time the realization of a doctoral study was quite different from to-day's mode: The subject was usually proposed by the student after a thorough discussion with the supervisor and an approximate date of submission of the thesis in 2 or 3 years was agreed upon; regular reports about the progress of the work were not expected and the costs for equipment and consumables had to be defrayed by the candidate, as well as the costs for using lab facilities. Doctoral students were not payed, except a small compensation, if supervising students in a practical. Of course the subjects of the doctoral theses were less comprehensive and the volumes of the scripts were smaller, usually not exceeding 100 pages. There was no pressure on publishing the work, as a doctoral thesis was acknowledged as a publication providing more details than a publication in a journal. External funding of projects was an exception, as doctoral students did not participate in the departments' budget. Also the final exam, termed "Rigorosum" was more comprehensive: Mine consisted of two orals (1 h each) in botany (systematics and physiology), one in zoology and one in inorganic chemistry. The system clearly promoted a broad knowledge in natural sciences including biology. With that knowledge and skills postdocs could enter a university career or could join a research team, mostly in the chemical/pharmaceutical industry. This system changed, triggered by the students' riots in the late 1960s and the advancing specialization that was promoted by the upcoming technical revolution in laboratory equipment and methodology, concomitant with a dramatic rise in the costs of research. As a consequence external project funding by the government, the German Research Foundation or the industry became more important including salaries for the doctoral candidates.*

My training in plant taxonomy was and still is a fruitful basis of my research, as well as of my teaching botany in lectures, practicals, and fieldtrips. Even when investigating chemical markers, I had to know about all other types of characters and their importance for taxonomy, had to know about the phylogeny of my groups in particular and of the plant kingdom in general, had to know details of their way to propagate, about their ecology and geographical distribution. Having the entire plant in mind when following a particular research question helps to place this problem into a wider context, to comprehend the experimental plants as individuals with a certain life history and in a given environment. This has now been termed "Systems Biology."

Interlude 2: A Plea for Systems Biology *To illustrate the value of that kind of systems biology, let me briefly tell a story which is only partly publishable (nevertheless, we can learn a lot from experiments which do not meet our expectations): A doctoral student tried to improve the yield of a wheat cultivar from his country Argentina by enhancing the cytokinin content especially of the flag leaf which is the main carbohydrate provider for the grains. The idea was to extend the lifetime of this*

leaf by a higher cytokinin content. From his study we learned three lessons. First, it is possible to increase the cytokinin content and also lifetime of the flag leaf a little by introducing an additional gene for cytokinin synthesis, the isopentenyltransferase gene from Agrobacterium tumefaciens under the control of the senescence-specific promotor HvS40. But expression of the gene was not consistent and increase of the grain yield was either negligible or sporadic (Souza-Canada 2012*). Second, not only a prolonged carbohydrate input is crucial for an enhanced yield, but also the supply of the seeds with other nutrients. Grain development and filling is a multi-faceted complex process, encompassing the entire plant. It cannot be over-run by fortifying only one single reaction, even if this controls one of the major processes. Third, and this tells us about the importance of the life history of our experimental plant material: Bread–wheat as a hexaploid species comprises the genomes of 3 other grass species, one of which is Aegilops tauschii (genome DD). When growing the T2 generation of our transformed wheat, several plants emerged which clearly showed the characters of Aegilops; we interpreted this as an effect of the changed hormonal pattern; however the effect was not reproducible and finally it turned out that the overly expression of the Aegilops genome must have resulted from an infection of the plants by the barley midge known as Hessian fly.*

3 Plant Biochemistry

Soon after receiving my doctoral degree in 1963, Prof. Dr. Otto Kandler offered me a fellowship (later a staff position) in the Department of Applied Botany at the Technical University of Munich. So I left school teaching and started for a career as plant scientist. Kandler is a plant physiologist and a microbiologist as well. I decided to stay with plants moving from taxonomy to plant biochemistry. But at the same time I learned the basics of working with bacteria of which I could make good use in my later work. During his postdoc period in the USA Kandler had worked with Melvin Calvin and Martin Gibbs joining their research in photosynthetic carbon metabolism. In the context of the Calvin cycle were a lot of open questions and Kandler was not absolutely convinced of the proposed mechanism of CO_2 fixation resulting in two molecules of 3-phosphoglycerate. He favored the idea that the CO_2-fixation product could be immediately reduced to yield a branched-chain hexose. The respective monosaccharide D-hamamelose was known as glycosidic moiety of a secondary constituent of the bark of witch hazel (*Hamamelis virginiana*). He suggested to investigate the biosynthesis of this sugar. To that end he offered me a working place in his isotope lab in the dairy institute of the Technical University in Freising which was the only laboratory for studies with radioactive tracers in plant science in Munich at that time.

3.1 Plant Branched-Chain Monosaccharides

To achieve the lecturer's degree (Habilitation) I had to deliver a thesis to the faculty, and I decided to address the biosynthesis of the two branched-chain monosaccharides of higher plants: the pentose D-apiose and the hexose D-hamamelose. D-apiose was well known as the glycosidic component of the flavone apiin, but its occurrence also in cell walls of a few plant species had been suggested. I succeeded in isolating a D-apiose containing pectin form the cell wall of duckweed and could establish its structure (Beck and Kandler 1965; Beck 1967). With sterile cultures of another duckweed species (*Lemna gibba*) I could elucidate the entire biosynthetic route from glucose via D-glucuronic acid, decarboxylation of carbon-6 to yield 4-keto-D-xylose from which D-xylose results upon reduction, and in a parallel step, D-apiose by intramolecular rearrangement and, again, reduction (Fig. 1). Since glucuronic acid, xylose and apiose are incorporated into cell wall polysaccharides we concluded that the entire pathway takes place in the nucleotide-activated state of the sugars (Beck and Kandler 1966).

3.2 The Hamamelose Story, Part I

Because of Otto Kandler's interest I further concentrated on the biosynthesis of D-hamamelose. A doctoral student of Kandler, Josef Sellmair at the same time investigated the occurrence of hamamelose in the plant kingdom establishing a kind of "chemical herbarium." To that end some 600 plant species were exposed to air containing 1% $^{14}CO_2$ under high light and the labeled compounds were separated by 2-dim paper chromatography and identified to the extent possible. This "^{14}C-herbarium" was the basis for many theses in plant carbohydrate metabolism in the Kandler group. Surprisingly, D-hamamelose could be demonstrated in almost all investigated species except for the Leguminosae. Highest concentrations were in the primrose family, where also the corresponding polyol hamamelitol (Sellmair et al. 1968) and a hamamelitol-containing disaccharide clusianose (Beck 1969) could be identified. While also participating in these studies my group focused on the biosynthesis of hamamelose as a potential early product of photosynthetic CO_2

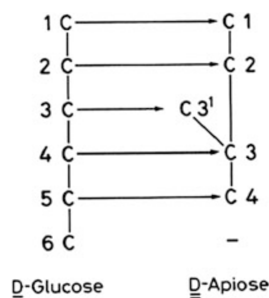


Fig. 1 Biogenetic relations between the carbon atoms of D-glucose (respective D-glucuronic acid) and of D-apiose (from Beck 1982)

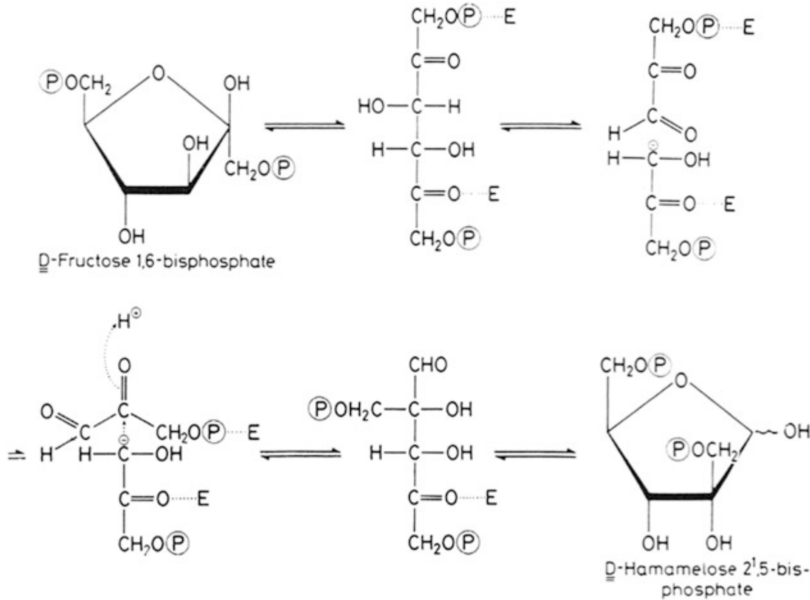


Fig. 2 Interconversion of D-fructose 1,6-bisphosphate and D-hamamelose 2¹,5-bisphosphate in chloroplasts. E… suggests fixation to the enzyme (from Beck 1982)

fixation. Therefore we incubated photosynthetically active intact spinach chloroplasts in bright light with ^{14}C-bicarbonate for a short-time span and investigated the labeled products. In place of free hamamelose in the chloroplast extract we could identify labeled D-hamamelose2¹-5-bisphosphate (Beck et al. 1971). The urgent question now was, as to whether carbon2¹ which could have derived directly from CO_2 was the "hottest" carbon after short-time photosynthesis in $H^{14}CO_3^-$.

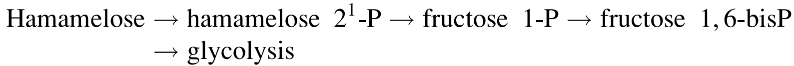
With primrose leaves (*Primula clusiana*) we had already elucidated the biosynthetic relations between the carbon atoms of glucose and hamamelose and proposed an intramolecular rearrangement by which all carbons of glucose are incorporated into hamamelose (Beck et al. 1968). To that end I developed a procedure of chemical degradation of hamamelose and isolation of the individual carbons. Similarly the hydrogens of hamamelose could be traced to those of ^{3}H-position-labeled glucoses. Using these methods we could show that the immediate precursor of hamamelose2¹ ,5-bisphosphate is fructose 1,6-bisphosphate and that the carbon2¹ is not the carbon immediately derived from CO_2 (Gilck and Beck 1973, 1974). We could further show that free hamamelose results from a stepwise and presumably unspecific dephosphorylation (Beck and Knaupp 1974) in the cytosol where free hamamelose accumulates. However we could not show any kind of metabolism apart from reduction to hamamelitol, mainly in the primroses (Fig. 2). Hamamelose-bisphosphate in contrast is metabolized, at least by reconversion into fructose-bisphosphate. The enzyme that catalyzes the interconversion appears to follow an aldolase mechanism. Unfortunately we were not able to isolate and investigate this enzyme in detail, we only found it with the thylakoid fraction. At that state we finished our work on hamamelose.

3.3 Out of Hibernation

The issue slept for 15 years when a potent inhibitor of Ribulose-bisphosphate carboxylase/oxygenase (RUBISCO) was found (Gutteridge et al. 1986). It was described as 2-carboxyarabinitol-1-P. By several methods we could show that this compound is D-hamamelonic acid2^1-P (Beck et al. 1989b). By infiltration of bean leaves with ^{14}C-labeled hamamelose in the dark, Andralojc et al. (1996) showed the formation of ^{14}C-labeled 2-carboxy-D-arabinitol (CA, D-hamamelonic acid) and 2-carboxy-D-arabinitol 1-phosphate (CA1P, hamamelonic acid2^1-P). The crucial step filling the gap between our studies and those on the inhibitor is the oxidation of hamamelose to hamamelonic acid (CA). It escaped our attention because it is formed in the dark or under very low light, whereas our experiments were performed in highlight. Andraloic et al. (2002) showed the entire pathway to CA1P: Dephosphorylation of HDP to free H, NADP-dependent oxidation to CA, and rephosphorylation to CAP1, which as an analogue to the transition product of CO_2 fixation binds to RUBISCO. Removal of the inhibitor is either by a specific phosphatase producing free CA or by the light-activated RUBISCO activase. The additional regulation of the RUBISCO activity is neither ubiquitous in the plant kingdom, nor a feature of special plant families.

3.4 The Hamamelose Story, Part II

In the leaves of *P. clusiana,* D-hamamelose and in particular hamamelitol (and to a lower extent clusianose) accumulate to considerable amounts especially under low temperatures. Even prolonged cultivation of the plants did not result in a measurable decrease and senescent leaves still contained substantial amounts of these carbohydrates. We therefore tried to find microorganisms that grow on D-hamamelose and used soil from underneath rosettes of the primrose for our search. We found a strain of *Pseudomonas* which could use hamamelose as a hydrogen donor, producing hamamelonic acid (Thanbichler et al. 1971), however, no degradation of the carbon skeleton could be observed. Therefore we collected bacteria from the surface of the leaves and isolated a strain identified as *Kluyvera citrophila*, from the Enterobacteriaceae. It differs from the American Type-Culture Collection strains only by the capability to grow on hamamelose as the only carbon source and was given the strain number 627. Using uniformly ^{14}C-labeled D-hamamelose in a time kinetics experiment we could show its fermentative dissimilation and compare it to the dissimilation of uniformly and position-labeled ^{14}C-glucose (Thanbichler and Beck 1974). The sequence also comprises an intramolecular rearrangement from the branched-chain sugar to a straight-chain hexose which takes place at the stage of monophosphates:



$$\text{Hamamelose} \rightarrow \text{hamamelose } 2^1\text{-P} \rightarrow \text{fructose } 1\text{-P} \rightarrow \text{fructose } 1,6\text{-bisP} \rightarrow \text{glycolysis}$$

The first enzyme, ATP: hamamelose2[1] phosphotransferase, is specific for hamamelose and different from hexokinase or glucokinase from *Kluyvera* (Beck et al. 1980). The second enzyme requires free hamamelose for activation and catalyzes the intramolecular rearrangement without liberation of triose or triose-P (Wieczorek 1976). The third enzyme is complementary to phosphofructokinase. Under aerobic conditions, only $^{14}CO_2$ was found as degradation product of hamamelose by *K. citrophila 627*. So, at least a microbial pathway for degradation of D-hamamelose could be shown. However, the question of a physiological or ecological function of the high concentrations of hamamelose and its derivatives in the primrose leaves still remained open. As mentioned above, these sugars accumulate especially in the cold and their content in leaves of same age fluctuates during the course of the year, being highest in winter. Polyols are known as compatible solutes improving drought and cold resistance. Compounds which resist metabolic degradation are in particular suitable for that function and the members of the hamamelose family appear to be striking examples.

Interlude 3: Experimental Skills and Efforts *At this point I would like to briefly mention how we measured radioactivity (^{14}C, ^3H) in the 1960s. In 1968 Otto Kandler's group bought the first liquid scintillation counter in Munich. The technicians of the company and we were similarly pioneering with the new method which all of us had to explore from the scratches. Before we got this advanced tool, ^{14}C and ^3H had to be measured in the gas phase. The crystalline and dry compounds were combusted in a special oven and $^{14}CO_2$ and ^3H (as water) were separated as gases by their different freezing points in high vacuum, purified and frozen at −196°C into the so-called counter tubes which were backfilled with an inert gas (mostly helium) before attaching to a counter. The character line of the filled tube had to be determined for each sample to find the "plateau," where an increase of the voltage did not further enhance the count rate. The plateau represented the amount of radioactivity as "counts per unit time." Measuring a plateau took between two and several hours depending on the amount of radioactivity. At that time I was not yet married and had an air mattress in my office and an alarm, working day and night for counting my radioactive samples in due time.*

3.5 From Postdoc to Lecturer

In October 1967, Otto Kandler encouraged me to combine the pieces of my work on branched-chain carbohydrates in a thesis ("Isotopenstudien zur Biosynthese der verzweigt-kettigen Monosaccharide der Höheren Pflanzen") to be submitted to the Faculty of Natural Sciences of the Technical University Munich for achieving the lecturer's degree (Habilitation). The following examination was in January 1968.

At that time, the candidates in addition to delivering a lecture on a subject selected by the faculty (without connection to the research of the applicant) had to undergo an interview in a faculty assembly where members could ask any kind of questions. Preparation for that event gave me a tough time due to the breadth of the faculty's disciplines, including mathematics, geometry, physics, chemistry, biology, earth sciences, ergonomics, and photography – in total there were 32 members who might ask questions. It was a check of the candidate's general scientific standing.

As a lecturer I could supervise diploma and doctoral students in my group that grew since 1968. I had so many ideas – but now I had to raise the funds. In the 1970s the competition for funds was not as strong as today, and I received funds from the Bavarian Ministry of Research, from the Federal Ministry of Education and Research, from the VW-Foundation, and in particular from the German Research Foundation. In 1969 I accepted an associated professorship at the Botany Department of the University of Munich with a small budget, ample laboratory space, and a considerable teaching load.

My particular task, the establishment of an advanced practical course and a lecture on plant ecology, was the trigger of another shift in my interests in plant science. The exciting field work in the scope of the practical course urged me to also enter basics of geobotany, phytosociology, and soil science which partly complied with my training as plant taxonomist. While still working in the lab, I got interested in ecophysiological problems, such as frost resistance of perennial plants and the association between nitrogen metabolism and the competitive strength of weeds.

4 Plant Physiology: Isolated Chloroplasts as a Powerful Tool

Between 1969 and 1975 (when I followed a call to a full professorship for plant physiology at the University of Bayreuth), we worked mainly with photosynthetically competent isolated chloroplasts. After the move to Bayreuth, we continued these studies but started additional projects, which I will report on later.

With isolated chloroplasts we worked on five major topics: The formation of glycolate during photosynthesis (J. Eickenbusch), the role of oxygen in photosynthesis (H.M. Steiger, D. Groden), formation and degradation of assimilatory starch (P. Pongratz), light activation and inactivation of chloroplastic enzymes (R. Scheibe), and frost hardening and dehardening of spruce and its chloroplasts (M. Senser). The available space does not allow me to report many details about this fascinating work with intact and "broken" chloroplasts (thylakoids) striving for the "world record" in chloroplastic photosynthetic CO_2 assimilation; unfortunately, we were consistently second to David Walker (Sheffield) who used pea chloroplasts.

Interlude 4: Safety Inspectors Not Allowed *Preparing such chloroplasts was an art by itself, starting from the optimal growth of the spinach, the time of leaf harvesting and then, most important the speed of the preparation. Less than 10 minutes were allowed for processing from the leaves to the purified intact*

chloroplasts, which was only possible by braking the centrifuges by hand. Richard Jensen (Phoenix Arizona), Erwin Latzko (TU Munich), and Ulrich Heber (at that time University of Düsseldorf) were my taskmasters in chloroplast preparation and David Walker introduced us in using his clark electrode for oxygen measurements.

4.1 The Chloroplast's Handling of Oxygen

With our chloroplasts we could show that a minor amount of the early photosynthetic product glycolate resulted from the oxidation of "activated," i.e., transketolase-bound glycolaldehyde by an unknown oxidant, whereas the bulk of glycolate originates in an oxygen dependent reaction from Ribulose-bisphosphate (Eickenbusch and Beck 1973; Eickenbusch et al. 1975; Beck et al. 1974). This coincided with Tolbert's group (Lorimer et al. 1973) publishing the oxygenase reaction of RUBISCO. During the course of our studies on glycolate formation, we became interested in the role of oxygen in the chloroplast metabolism. When searching for the unknown oxidant of activated glycolaldehyde we came across H_2O_2 and its formation and biochemical destruction by the chloroplast. In the scope of these studies we measured the oxygen concentration in photosynthesizing intact chloroplasts (Steiger et al. 1977) and found that H_2O_2 is formed especially if the natural electron acceptor NADP falls short. Continuous removal of oxygen from the chloroplasts inhibits photosynthesis by over-reduction of compounds of the linear electron flow (Steiger and Beck 1981). Although initially considered without special function we investigated the role of ascorbate in photosynthetic CO_2 assimilation. In these studies we found the membrane-bound ascorbate peroxidase, showed its substrate H_2O_2, and characterized its properties (Groden and Beck 1979). Now, we could put together the reactions of the so-called pseudocyclic photosynthetic electron transport, now known also as the "water-water cycle" and explained the function of oxygen for the poising of photosynthetic NADP reduction and ATP-formation (Steiger and Beck 1981). In that context we could also demonstrate the presence of a specific ascorbate transporter in the chloroplast envelope which facilitates equilibrium between the stromal and the cytosolic ascorbate concentrations or the exchange of didehydroascorbate for ascorbate (Beck et al. 1983).

Interlude 5: A High-Ranked Guest *Let me, for a moment jump ten years ahead. Having been competitors in the early days of glycolate research, I was surprised to receive a telephone call from Ed Tolbert, in which he asked me, whether he (and his wife) could come to my department for half a year with money from a Humboldt award. I agreed and we had a very pleasant and interesting time together, and the former competitors became friends. Our idea was to check, whether in analogy to the photosynthetic CO_2 compensation point, C_3-plants would also show an oxygen compensation point (Γ_{O2}), i.e., an atmospheric oxygen level with a given CO_2 level and temperature, at which net oxygen exchange is zero. For tobacco at 220 ppm CO_2, Γ_{O2} was at 23% O_2, increasing to 27% at 350 ppm CO_2 and 35% at 700 ppm*

CO_2 (Tolbert et al. 1995). Unfortunately, Ed died 10 months after his return to East Lansing, and since I was his last "coworker," I was invited for an opening lecture of a Gordon Conference that was dedicated to his memory. Later, we continued that research growing tobacco plants at different oxygen concentrations. Expectedly plants showed reduced growth at an atmosphere with a higher oxygen concentration, however, the data are still sitting in my desk, waiting for publication.

4.2 Poising in Photosynthesis

It opened another branch of our studies with intact photosynthesizing chloroplasts. The Calvin cycle alone consumes more ATP as the linear electron transport can produce. Support by the cyclic electron transport could take up the slack but obviously cannot completely prevent over-reduction of the NADP and associated pools of electron acceptors, leading to H_2O_2 formation. In the mid-1970s the question of **light activation of chloroplastic enzymes** in particular those of the Calvin cycle came up. Interestingly, a non-photosynthetic enzyme, NADP-dependent malate dehydrogenase showed strong activation by light (Scheibe and Beck 1979). It could prevent over-reduction of NADP by consuming reduction equivalents in the presence of oxaloacetate and was therefore termed "malate valve" (see also Scheibe and Beck 1994). An activator protein, whose nature was unknown (now known as the thioredoxin system, see Schürmann and Buchanan 2008), was concluded to effect light activation of chloroplastic enzymes. In 1979 Renate Scheibe went for a postdoc stay to Louise Anderson (University of Illinois, Chicago) to work on that light effector mediator (LEM) which controls the activity of the enzymes catalyzing the irreversible reactions of the Calvin cycle (as well as the inactivation of glucose-6-P-dehydrogenase in the light). It was shown that the LEM was identical with the thioredoxin system.

Interlude 6: The Origin of the Wallenfels Meetings *With respect to the light activator, Louise Anderson and Bob Buchanan were strong opponents. For the 6th International Congress in Photosynthesis in Brussels (1984), Renate Scheibe, after her return to Bayreuth, had proposed a satellite meeting on light activation of enzymes. We organized this meeting in the ecological field station of the University of Bayreuth in the little city of Wallenfels north of Bayreuth, and hired a bus to bring the 33 participants in 2 days from Brussels to Wallenfels. As this meeting was not only very successful but also highly delightful, e.g., with a ride on a raft, it was decided, that such a meeting on photosynthesis should be held every spring in Wallenfels. This was the beginning of the almost legendary Wallenfels meetings, meanwhile 32 by number, which are always overbooked, although they are not externally funded. Illustrious names garnish the list of the organizers: Hans Heldt, Ulrich Heber, Erwin Latzko, Renate Scheibe, Stephan Clemens, and Ekkehard Neuhaus. In the meanwhile the topic of the meetings has been widened from photosynthesis to plant biochemistry. Many professors of plant physiology and*

biochemistry had entered the Wallenfels community as junior staff, selected by their professors to present their work.

Two more topics merit mentioning when reflecting on our work with chloroplasts.

4.3 Starch Metabolism and Frost Hardening

Preparation of photosynthetically competent chloroplasts depends on the integrity of the thylakoids as well as the envelope. Therefore it is necessary to harvest the leaves at the end of the night when the granules of assimilatory starch have disappeared and would not disrupt the chloroplast upon centrifugation. On this background we became interested in the starch metabolism of chloroplasts (Beck and Ziegler 1989), especially in the degradation of the starch granules, their chemical composition and changing appearance. We found an oscillating amylolytic activity (endo-, exo-amylase, and a debranching enzyme) in isolated chloroplasts which is partly due to the diurnal change of the stromal pH that strongly curtails the activity of the system during the light phase, while inhibiting it only slightly in the dark (Pongratz and Beck 1978; Beck et al. 1981). We could show that the major end-product of the nocturnal starch degradation is maltose, which is exported by a maltose-specific transporter in the chloroplast envelope (Rost et al. 1996). It took a while, until this contribution to the chloroplastic starch metabolism was recognized and acknowledged, but meanwhile the transporter MEX1 (maltose export 1) has been studied in detail (Niittylä et al. 2004). We further concentrated on the debranching enzyme as the pacemaker of the degradation of the starch granules. The enzyme which consists of a single polypeptide chain showed up to seven interconvertible forms upon gel electrophoresis and isoelectric focusing which all exhibited debranching activity (Ludwig et al. 1984; Henker et al. 1998). Since this was a strange behavior of a protein, we isolated and sequenced its DNA and expressed it in *Escherichia coli* (Renz et al. 1998). The heterologous protein showed the same interconvertible forms which had different specific activities. Interconversion and change of the overall specific activity is triggered by a pH–shift, by addition of a substrate (e.g., amylopectin), or by reduction/oxidation. A shift in this microheterogeneity could serve the regulation of the enzyme activity (Schindler et al. 2001) in a way that could be considered a primitive form of allosteric regulation.

4.4 Frost Hardening of Plants, Part I

Reversible deposition of starch in chloroplasts associated with their change in function was the starting point of our investigations on frost hardening and

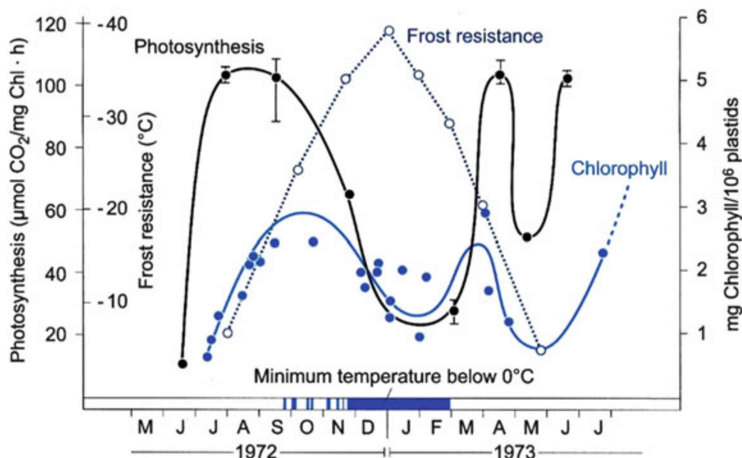


Fig. 3 Photosynthetic capacity (1% CO_2), chlorophyll content, and frost-resistance of one generation of spruce (*Picea abies*) needles measured under identical conditions in the course of the year (from Senser and Beck 1979)

dehardening of spruce needles which we later extended also to Scotch pine needles. We started with an EM study of the shape, thylakoid system, and starch deposition in spruce chloroplasts during the annual course. Evergreen conifers do not need to accumulate storage material in autumn for sprouting in spring, as, e.g., apple trees, because they can produce it by photosynthesis when needed. Shortly before sprouting of the new needle generation the chloroplasts of the older needles accumulate large amounts of starch, mostly from ongoing photosynthesis and thus become amyloplasts with only a few thylakoids remaining. After consumption of the starch for the formation of the new needles, the chloroplasts rebuild their thylakoid system and act as normal chloroplasts accumulating starch during day-time and degrading it during night. Upon frost hardening in autumn the chloroplasts swell, become amoeboid, and partly disintegrate the thylakoid system which recovers at the end of the winter to photosynthesize and deposit the big amount of starch for sprouting. Degradation of the thylakoid system, either during frost hardening or upon the functional change into amyloplasts is mimicked by the chlorophyll content of the plastids (Senser et al. 1975). Division of chloroplasts in the overwintering needles was observed very rarely and thus the entire annual change of form and function of the plastids is understood to take place in the extant organelles. Subsequently, we focused on the annual fluctuations of frost hardiness and sensitivity, measuring the photosynthetic capacity of needles and chloroplasts. Frost resistance is lowest in June and July when minus 10°C kills the needles while in winter temperatures as low as minus 40°C and even lower can be survived. Photosynthetic capacity in winter is about 25% of that achieved in summer. In the amyloplast stage of the plastids, a residual photosynthetic activity of around 40% remains (Senser and Beck 1977, 1979). Frost hardiness and photosynthetic capacity of the chloroplasts are countercurrent (Fig. 3; for physiological interpretation see

Sect. 4.6). Changes of frost hardiness in spruce chloroplasts are accompanied by changes of the lipid: protein ratio in favor of the lipids and by a desaturation of the lipid fatty acids resulting in a lower viscosity of the membrane system and the envelope (Senser and Beck 1982a). With small spruce trees which we cultivated for at least 3 years under different combinations of day length and temperatures we showed that the changes in membrane lipids could never be separated from the degree of frost resistance and that short-day conditions as well as subzero temperatures induced these changes (Senser and Beck 1982b). The changes in lipid composition could also be related to the photosynthetic capacity, as indicated by an Arrhenius plot of the Hill reaction.

Interlude 7: Gaining Momentum in Bayreuth *This might be the right place to change the topic for a while and report about my move to the University of Bayreuth in 1975, where the first building was just brought up and the offices of the few professors were put together in a wooden barrack, while their labs were still at their home universities. As ecology has been put up as one main focus of this university, each professor should dedicate part of his research to an ecological problem. One of my ecological interests was frost resistance of plants. While still working on that topic in Munich (see above), I also tried to broaden my view having come across another type of frost resistance which lasts all year round: The frost tolerance of tropical alpine plants. After his retirement from the University of Tübingen my former academic teacher in cryptogam botany in Munich, Karl Mägdefrau, inspired me with his reports about the afroalpine vegetation and the giant rosette Senecios. After a student's fieldtrip to Mt. Kenya in 1974 I burnt for research on these plants. At the same time, our proposal for a botanical garden of the University of Bayreuth was accepted by the Government of Bavaria under the condition, to create a novel type of garden which at the same time serves research, academic teaching, and the public as an area of education and recreation. The plan was an ecological botanical garden with several stations for demonstration purposes and ecological experimentation, also showing typical vegetation of selected areas. Instead of an alpine garden which is of the common jewelry of most botanical gardens we proposed a greenhouse for tropical alpine vegetation which would be unique worldwide in cultivating the giant rosette plants.*

4.5 Facing Mt. Kenya[2]

For collecting these plants and to investigate and document their environmental conditions, I organized an expedition with my entire group to Mt. Kenya in 1979. At that time export of living plants and seeds was easy, as the Convention on Biological Diversity was not yet on the horizon. That expedition was supported by

[2] Title of a book by Yomo Kenyatta, the first President of Kenya after its liberation.

the Bavarian Ministry of Research and Arts and we worked for 6 weeks in an absolutely fantastic landscape between 4,000 and 5,000 m. With the produced data, we could not only feed the engineers who worked on the construction of the above-mentioned special greenhouse, but together with my colleague Detlef Schulze could also prepare a concept for ecophysiological research on the tropical alpine plants, to be submitted to the DFG for funding. We succeeded and went several times to the East African high mountains including also vegetation analysis initiated by my former colleague Helmut Rehder from the Technical University in Munich. The scientific return of these expeditions was remarkable. Among others we now understand the type of frost hardiness which is required by plants living in a climate of "everyday summer, every night winter" (Hedberg 1964), which follows several mechanisms: Extracellular freezing of the cell water creating an extremely negative water potential at which water vapor bubbles form in the vacuole. This contrasts with supercooling, where crystallization of cellular and tissue water is avoided. Other mechanisms are delaying of freezing by accumulation of large volumes of liquid, or insulation, either permanent or temporarily during the cold night by nyctinastic leaf movement, forming a closed "night bud" which protects the central leaf bud from radiation emission and freezing (Beck 1982, 1984). We included also photosynthesis in our program, showing that the leaves are photosynthetically fully competent immediately after thawing, but that too high intensities of radiation result in photoinhibition. The plants avoid this stress by an almost vertical position of the leaves during hours of bright sunshine (Schulze et al. 1985; Bodner and Beck 1987). Within the scope of the project several other studies could be placed, the most important of which is the result, that fire might be more selective for the composition of the afroalpine vegetation than the nocturnal frost all year round (Beck et al. 1986a; Hemp and Beck 2001). Together with my Kenyan friend Prof. O. Kokwaro (Nairobi) we produced a vegetation map of the alpine zone of Mt. Kenya and described the afroalpine plant communities (Rehder et al. 1988a, b, 1989). It was a special honor for me, to publish a paper on the biology of the unique globular mosses together with my former academic teacher Karl Mägdefrau (Beck et al. 1986a, b). And we described a new species of the giant rosette groundsels *Senecio x saundersii* Sauer & Beck and investigated the population biology of this hybrid and of its parents (Beck et al. 1992). Two doctoral students did their geobotanical theses on the vegetation of the East African high mountains (Klaus Schmitt on the Ruwenzori: Schmitt and Beck 1992, and on the Aberdare Mountains, Schmitt 1991), and Rainer Bussmann on the forests and forest turnover on Mt. Kenya (Bussmann and Beck 1995a, 1995b, 1995c; Lange et al. 1998).

4.6 Frost Hardening of Plants, Part II

After this short excursion to the tropics I will return to our further work on cold hardening and frost resistance of higher plants. Related to the freezing behavior of

the afroalpine plants we started to investigate the freezing process and metabolism in our local overwintering plants. With respect to water relations we worked with ivy, winter barley, and the ornamental plant *Pachysandra terminalis* (Buxaceae). At subzero temperature the leaves of these plants freeze, adopting a glassy appearance. The question was how much of the cellular water crystallized in the intracellular spaces and which kind of damage may still occur in frost-hardened plant organs. The idea was that cellular water moves out of the cell and crystallizes until a physicochemical equilibrium (i.e., of the water potentials) between the concentrating cellular solutions and the extracellular ice is reached. This process is termed equilibrium freezing and the portion of the cellular water that freezes depends on the subzero temperature and the original osmolality of the cellular solutions. We elaborated on the theory of extracellular freezing (Hansen and Beck 1988) and showed equilibrium freezing for ivy leaves down to about −10°C where some 80% of the leaf water has solidified as ice. However, in the rosette leaves of winter-barley less water froze than calculated from the water potentials and we termed this non-ideal equilibrium freezing. A doctoral student from China who traveled 6 weeks by train from Shanghai to Bayreuth, arriving on Christmas Eve 1987, investigated the phenomenon of non-equilibrium freezing with leaves of *Pachysandra* in detail. Inspecting the leaves at −20°C by light microscopy he could for the first time produce photographs of extracellular ice and show that upon dehydration of cells during extracellular freezing a negative turgor develops that counteracts further freeze dehydration (Zhu et al. 1989; Zhu and Beck 1991). The negative turgor results from the fact that air in contrast to water cannot penetrate imbibed cell walls which are therefore sucked inwards. This negative turgor saved the cell about 10% liquid water giving rise to the phenomenon of non-equilibrium freezing. Nevertheless, damage by frost of frost-hardened leaves could still occur, mainly by a too high concentration of ionic solutes upon cell dehydration and a too low concentration of osmolytes (cryoprotectants) or membrane protecting proteins (dehydrins). Such a situation could result in a breakdown of the membrane potential or disintegration of membranes. Within the scope of the EUROSILVA-EUREKA program established under the debates about the Central European forest dieback in the 1980s and 1990s and needs for clarification, we investigated the frost hardening process in *Pinus sylvestris* as affecting chloroplast ultrastructure and photosynthetic activity. Like in spruce the amounts of the light harvesting complexes as well as the photosynthetic activity decreased significantly during frost hardening. Reduction of light harvesting complexes was interpreted as a protective measure to mitigate overexcitation of the photochemical machinery and its destruction by radicals when the biochemical reactions of the chloroplast are blocked by the low temperatures (Vogg et al. 1998a). Incorporating a spin label in the thylakoid membranes, we could show that the reduction of the protein content of the thylakoid membranes maintains the fluidity of the membranes at subzero temperatures (Vogg et al. 1998b). By applying artificial light regimes to young trees of Scots pine we could demonstrate that day length measured via the phytochrome system triggers the onset of frost hardening whereby a low level of phytochrome$_{fr}$ promotes hardening and a high level dehardening. The highest rate of frost hardening

achievable with short-day conditions was −0.7°C per day. The first incidence of a subzero temperature triggers a second hardening process with a daily rate of −0.9°C. Both processes are additive resulting in maximum hardening of −1.5°C per day (Beck et al. 2004).

5 Nitrogen, Cytokinins, and Plant Growth: A New Era and New Friends

Already 5 years after the opening of the University of Bayreuth, biologists and soil scientists succeeded in implementing a Collaborative Research Center (CRC, Sonderforschungsbereich 137) on “Regulation and Flux Control in Ecological Systems” which was funded for 12 years (1981–1993) by the German Research Foundation (DFG). The contribution of my coworker Inge Rosnitschek to that program aimed at understanding the mechanisms of growth regulation of plants by the nitrogen supply. Already in Munich, I had started a pot experiment with stinging nettles (*Urtica dioica* and *U. urens*) to investigate the dependence of their competitive strength on the nitrogen supply to the soil. In Bayreuth the first greenhouses of the University were finished in the early 1980s which allowed to continue the experiments on the nettles under controlled conditions. We grew the plants from the seeds in washed quartz sand which was continuously percolated with nutrient solution of different nitrogen content. Biometric data showed the influence of the nitrogen supply on the total biomass, on the root to shoot ratio of biomass and on the leaf area (Rosnitschek-Schimmel 1982). Other traits were determined as well, such as the light response curve of the net CO_2 uptake rates of the individual leaves and nocturnal losses of carbon by respiration. Combining an infrared gas analyzer with a setup for $^{14}CO_2$ application allowed assessment of the photosynthetic performance of the individual leaves on the nodes of the experimental plants and the carbon export and distribution over the entire plants. In that way the leaves could be characterized as source or sink leaves and the plastic response of the plant to the nitrogen supply could be traced to their ratios of carbon import: carbon export. Especially under low nitrogen supply we could also show sink limitation of photosynthetic net CO_2 uptake, by removing all except one source leaves what tripled the rate of CO_2 uptake by the remaining source leaf (Fetene et al. 1993). At that time, the cytokinin concentration in the plant, especially in the root has been proposed to mediate the growth response of *Plantago* to the nutrient supply (Kuiper 1988). With our experimental setup and a split-root system allowing application of cytokinin (zeatin-riboside) to part of the root system, we could show a change of the carbon export from the source leaves in favor of the shoot apex, enhanced CO_2 net uptake and growth of the shoot, and concomitant reduction of carbon import into the root (Fetene and Beck 1993). Supervised by my coworker Anton Fußeder my doctoral student Bernd Wagner elaborated a method for comprehensive analysis of cytokinins in plant tissues allowing the quantification of

15 species of cytokinins and their derivatives, by column chromatography, HPLC, deglycosylation, and ELISA. Using this method they could address the missing link between the nitrogen supply to the plant and the cytokinin-mediated modulation of growth (Fußeder et al. 1988b; Wagner and Beck 1993; Beck and Wagner 1994) showing that the cytokinin content of the root and the export to the shoot are proportional to the nitrogen content of the root (Beck 1994). This work brought us in contact with Miroslav Strnad and his coworker Karel Doležal from the Czech Academy and University of Olomouc, who spent several months in our department. They had specific antibodies for cytokinins and auxins which were very welcome in order to broaden our spectrum of quantifiable phytohormones. We had a successful Conference in Liblice (1991) on all aspects of plant hormones known at that time, from which we could produce a well-received book (Strnad et al. 1999). The connections to this very successful group in Olomouc remained vivid (e.g., Strnad et al. 1992, 1994; Doležal et al. 2002) and I was lucky to meet the grand-seigneur of cytokinin research in the Czech Republic Miroslav Kaminek several times.

Interlude 8: Ethiopian Skills and Consequences *Although the program and the experimental setup appear straight forward and not exceptionally complicated, one of the major difficulties was the appropriate growth of the nettles: Stinging by the nettles, pests (pathogenic fungi, aphids), controlling and adjusting of the continuous flow of the nutrient solution simultaneously through many pots, the necessity to change the position of the pots for equal illumination gave us a lot of problems and several students gave up finally. At that moment I received a message by my friend Ulrich Lüttge from the TU Darmstadt, that a student from Ethiopia, who had just finished the doctoral exam was interested to spend the rest of his DAAD stipend in another group to widen his physiological scope. This was Masresha Fetene from Addis Ababa University (AAU). We combined Masresha's remaining and our money and could employ him as a postdoc for more than a year. He was keen to work in our CRC, meeting other physiologists, ecologists, and soil scientists, and agreed to work in the nettle project. Thanks to his skills and the dedicated assistance by his wife Selome Bekele, we finally succeeded in the proper growth of the nettles. He performed the above-described experiments and participated in a study unveiling the pathway architecture of the vascular system of the nettles by autoradiography of the carbon flow from given source into particular sink leaves (Fetene et al. 1997). With a series of well-placed publications and continuously increasing his international connections Masresha made a steep career as professor of plant ecology and physiology of AAU, which he finally served as Deputy Vice President and Head of the publication office for years, before being appointed the Executive Secretary of the Ethiopian Academy of Science. While associate professor in the AAU, Masresha later received a fellowship of the Georg Forster Program of the Alexander von Humboldt foundation and is a regular visitor of Germany in official functions. There was never any doubt that he would bring back the knowledge achieved in Germany into his country and would encourage his students, who came to Germany or to other European countries and to the USA to follow his example. Since 1994, at the end of the last civil war in Ethiopia I have regularly*

visited Ethiopia and several times served as co-supervisor or external examiner of his masters and doctoral students. Together with his doctoral supervisor Ulrich Lüttge (TU Darmstadt) we succeeded in raising funds for an Ethiopian-German student's fieldwork and later achieved funding by the Volkswagen-Foundation and for several years also by the DFG. More than ten further papers on afroalpine plants of Ethiopia, especially on the giant Lobelias,[3] *but more recently on the trees in the remnants of the montane forests have meanwhile been jointly published. Our families have become close friends and it is a great honor for me having been elected associated member of the Ethiopian Academy of Science.*

After this excursus to Ethiopia, let me finish the nitrogen story. The investigations with the nettles were complemented by studies on the carbon sinks in the growing leaves. We investigated the invertases as potential mediators of phloem unloading, but could rule out a function at least of the apoplastic invertases in that process (Fahrendorf and Beck 1990; Möller and Beck 1992). Therefore symplastic unloading and splitting of sucrose by non-cell wall invertases appears likely. The entire story, from the nutrient supply to the cytokinins and to the growth response of the whole plant, was summarized by Beck (1999).

6 Photoautotrophic and Other Cell Suspension Cultures

Nitrogen supply to or the C/N ratio of a plant tissue reveals still an undifferentiated picture of the fate of nitrate or ammonium in a photosynthesizing cell. Therefore we started to work with a suspension culture of photoautotrophic *Chenopodium rubrum* cells (new name: *Oxybasis rubra*). Special growth conditions allowed the use of this cell culture as a model for the regulation of the intracellular metabolism, being aware of the fact of neglecting the exchange of metabolites and signals with the natural neighborhood in a plant tissue. Nevertheless this cell suspension culture turned out as a very potent tool and was used for various investigations. One topic was nitrogen metabolism, e.g., nitrate and ammonium uptake, regulation of nitrate reductase (NR) by nitrate, light and cytokinins, and determination of intracellular fluxes of nitrogenous compounds (Renner and Beck 1988; Beck and Renner 1989, 1990). I will not go into detail because several of our questions, e.g., the regulation of NR by 14-3-3 proteins, have been solved in the meantime in great detail by others (e.g., Lambeck et al. 2012). However, the cell culture brought us back to the cytokinins in the context of ageing and senescence of photosynthesizing cells. From a not successful endeavor to implement a collaborative research center on the cellular processes associated with ageing of plants, bacteria, humans, and animals, a few promising projects remained which we combined in a research

[3] The up to 6 m tall *Lobelia rhynchpetalum* from Ethiopia was the first of the giant Lobelias which could be brought to blossom in our tropical alpine greenhouse of the University of Bayreuth. Now, it flowers every year, mostly in winter and serves as object for further ecophysiological studies.