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REVIEW

PAVEL ŠIFFEL (1954-2003) or Life full of chlorophyll

J. KVĚTOŇ[*,+], M. DURCHAN[**,***], K. ROHÁČEK[**,***], J. ŠANTRŮČEK[*,**], F. VÁCHA[**,***], and Z. ŠESTÁK[++]

Faculty of Biological Sciences, University of South Bohemia, Branišovská 31, CZ-370 05 České Budějovice, Czech Republic[*]
Institute of Plant Molecular Biology, Academy of Sciences of the Czech Republic, Branišovská 31, CZ-370 05 České Budějovice, Czech Republic[**]
Institute of Physical Biology, University of South Bohemia, Zámek 136, CZ-373 33 Nové Hrady, Czech Republic[***]
Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Na Karlovce 1a, CZ-160 00 Praha 6, Czech Republic[++]

Abstract

Life and research results of Pavel Šiffel, a talented but untimely deceased Czech scientist in photosynthesis, are reviewed. He studied biophysics and physiology of chlorophyll, its complexes with proteins, their absorption and fluorescence spectra, activities in mutants and transformants, dealt with chlorophyll biosynthesis and protochlorophyllide photoreduction, pigments in plants grown at CO_2 deficiency and under simulated acid rain, with changes accompanying leaf and plant development, photobleaching, *etc*. He participated in construction of specialised spectrofluorometers, finally he built the kinetic spectrophotometer *SpeKin*.

Additional key words: absorption and excitation fluorescence spectra; chlorophyll biosynthesis; chlorophyll-protein complexes; CO_2 deficiency; kinetic spectrophotometer; leaf age; mutants and transformants; photobleaching; photosystem 2; simulated acid rain.

Pavel's youth and university studies

Pavel Šiffel was born on November 23, 1954, in the north-east part of Bohemia. He spent his childhood and youth in Žamberk, a small town in the scenic foothills of the Eagle Mountains. Pavel's life was intimately connected with a deep interest and respect for nature. He liked to roam in hills and woods surrounding his hometown, watch the behaviour of plants and animals in their environment, and find the beauty in the everyday demonstrations of life. He was enchanted by the river Divoká Orlice passing through Žamberk. His lifelong hobby became fishing which helped him both to relax and concentrate effectively on solving problems in his personal and, more later, scientific life.

In the time of his studies at the Grammar School (1971–1974), Pavel was very good in mathematics and physics. He was successful in the students' Science Olympiads, constructed many helpful or simply amusing electronic appliances with his father and brother. His schoolmates and friends often felt his specific and likeable sense of humour. Because his favourite subject became the physics, Pavel decided, after successful passing the school-leaving examination, to enter the Faculty of Mathematics and Physics (FMP) of the Charles University in Prague.

The period of university studies (1974–1979) was very inspiring for him and stimulated his mind and knowledge. Since 1978, he started working as a student assistant at the Department of Chemical Physics of FMP. Here Pavel graduated with a first class honour degree in June 1979 and also wrote his first article dealing with an excitation energy transfer between chlorophyll *b* and *a* molecules in an artificial system (Šiffel and Vavřinec 1980). During his university studies, Pavel displayed excellent experimental craftsmanship and invention. Acquired experiences significantly helped him in his later scientific work at the Czechoslovak Academy of Sciences (CAS)

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[+]Author for correspondence; fax: +420 387 772 371, e-mail: queton@bf.jcu.cz

where he devised and constructed new sophisticated devices and accessories. In this respect, Pavel was very imaginative and tenacious. His capability to analyse a given problem and an admirable intuition allowed him to successfully solve many technical tasks and come up with new ideas. Due to his clear forming of hypotheses and straightforward thinking, his university teachers predicted Pavel's future career as one of theoretical physicists.

Beginning of scientific career

Pavel was very devoted to the field of spectroscopic methods and their application in photosynthesis. He decided to continue the work he started at the Charles University, despite an offer to work at *Meopta*, the former Czech company producing optical instruments. He started his scientific career as a PhD student at the Institute of Experimental Botany (IEB) of CAS in Prague in autumn 1979. His first contact with academic science lasted only one month because he had to experience, like other young university-trained men of that time, one year of military service. After his return to IEB, a period of building an appropriate laboratory in the limited spaces of the Institute started. First in the large building at Fleming Place, occupied by many institutes of CAS, and later in a rather remote place, far from the Prague centre of science, in a small brick house formerly used by some artisans and recently abandoned by another research group. The small house was situated in the backyard of a block of flats at Kladenská street belonging to the district Prague 6. The rooms were without furniture and equipment. For Pavel, it was time of perusing catalogues, writing orders, and drawing plans of his "own" lab. He mastered this job and learned to like it and, as far as we remember, he repeated these activities at least four times in the following 20 years.

Finally, the two-room laboratory was simply but fairly well and sensibly equipped. It was a good fit for Pavel's lifestyle in those years. Pavel spent weekends with his family in Žamberk but in the weekdays he used to work in Prague till late night and it was clear that he was not only working but also living in the laboratory (there was a couch in one of the rooms). When Pavel was entirely electrified by some matter, he was able to fully forget the surrounding world. The deeply burned-in circle on the Pavel's worktable was the clear evidence of a forgotten immersion heater in a beaker of water when making tea at lunchtime. Displayed oil sketches made by Pavel during his military service surprised many visitors of the laboratory. When painting a human figure the most difficult parts are ears and hands, and Pavel's paintings of hands were perfect!

Absorption, excitation, and emission spectroscopy

In the first half of the 1980s, Pavel dealt mainly with low-temperature spectroscopy. The papers that emerged in this period arose from a fruitful collaboration with scientists from the A.N. Bakh Institute of Biochemistry (INBI) of the USSR Academy of Sciences in Moscow. At that time one of the world best research laboratories in biophysics of photosynthesis was in Moscow headed by a famous scientist, a member of the Academy of Sciences of the USSR, Professor A.A. Krasnovskiĭ. Pavel's supervisor was in good personal relationships with Prof. Krasnovskiĭ and hence it was not difficult to arrange a two-year stay in this lab for Pavel. In INBI Pavel worked mainly with Nikolai Lebedev (Lebedev *et al*. 1985a,b,c, 1986a,b,c, 1992a,b, Šiffel *et al*. 1985, 1987, Shiffel and Lebedev 1988) who soon became his both professional and personal friend. In Moscow, Pavel became acquainted with Gulya, a talented biochemist and his future wife.

Pavel's invention and skilfulness manifested itself in constructing devices for measuring excitation and emission fluorescence spectra. For example, commercially available fluorometers were not very good at that time, and Pavel had to develop his own fluorometer for low temperature differential and derivative excitation and emission spectroscopy. His home-made spectrofluorometer was assembled with a powerful 1 000 W xenon arc placed in a home-made housing as a radiation source, a home-made Dewar flask with a home-made holder for flat samples, two (for excitation and emission spectroscopy) Russian *LOMO* monochromators with an extremely wide aperture that were initially constructed for space research, a Russian IR photomultiplier with such good parameters that it can operate at room temperature without any cooling, and a two-coordinate recorder. Soon after his arrival Pavel improved the detection limit of the fluorometer with a *Unipan* (Poland) lock-in amplifier, full of American electronic "bugs".

Based on these sophisticated techniques and creative thinking about the design of experiments Pavel and Nikolai made two fundamental discoveries that formed a background of the modern view on chlorophyll biosynthesis in higher plants: the discovery of a new mechanism of protochlorophyllide photoreduction that operates in light-adapted plants (Lebedev *et al.* 1985b,c) and the discovery of a new, so-called water-soluble (or monomeric) chlorophyll-protein (Šiffel *et al.* 1987, Shiffel and Lebedev 1988, Lebedev *et al.* 1992,a,b).

After returning to Prague and for some time after the defence of his PhD thesis entitled "Spectral Characteristics of Higher Plants During Leaf Ontogeny", Pavel was rambling around plant physiology, photosynthesis, chlorophyll biosynthesis, leaf age and senescence, *etc*., searching for interesting topics. For example, he measured chlorophyll *a* emission in clear epidermis of a white mistletoe berry, in dead leaves in the course of autumn, in white parts of tobacco callus cultivated on agar medium *in vitro*, in *xantha* and *albino* mutants of barley, plant transformants, and so on. These and other

results gave birth to publications Šiffel *et al*. (1986, 1988, 1991), Šesták and Šiffel (1988), and Šiffel and Šesták (1988, 1989). Unfortunately, many results of these interesting experiments have never been published. They ended somewhere in Pavel's "drawer for results". Even if in those days of "deep socialism", the team did not have much trouble with scientific literature (maybe a little bit with book monographs) thanks to Drs. Šesták and Čatský and their volumes of "Photosynthesis Bibliography". However, Pavel was the only one who had no need to read much or even at all what was done in the field of his interest before starting his own experiments. His supervisor spent some time to persuade Pavel that results published more than five years ago may still be important for science and that he has to learn the history of his favourite research fields. Pavel liked going his own way and owing to his intuition, original thinking, and ideas he mostly reached new interesting results.

New outset in České Budějovice

In the 1980s, part of IEB was relocated from Prague to České Budějovice, a town situated in South Bohemia. One day in 1987, Pavel was proposed by his employer to take a position in the newly established workplace in České Budějovice. Pavel hesitated. He considered a range of pros and cons. There was a balance of at least two main things: A wish of the family for their own flat, which was offered in České Budějovice on the one hand, and Pavel's fear that he will be pushed to enter the Communistic Party on the other. Luckily, this fear was not real at that time, and when Pavel in addition received a promise of big money for purchasing a new spectrofluorometer, he, together with Gulya and their one-year-old son Erik, decided to leave for České Budějovice. Soon after, Pavel spent time looking over catalogues and manuals of *Aminco* and *Spex* models. A year after the family moved to South Bohemia, a daughter Kamila was born. In the newly formed Institute of Plant Molecular Biology (IPMB) of CAS in České Budějovice, Pavel started building another optical laboratory, forming his team, and looking for interesting subjects for investigation. His astonishment with a "white" photobleached plantlets grown *in vitro* and suffering CO_2 deficiency resulted in a series of papers in the next years: Šantrůček *et al*. (1991), Šiffel *et al*. (1992, 1998), Šiffel and Vácha (1995, 1998), Šiffel and Braunová (1999). Photosynthetic properties of *aurea* tobacco were the topic of collaboration with the laboratory of Prof. H.K. Lichtenthaler in Karlsruhe, Germany (Lang *et al*. 1992, Šantrůček *et al*. 1992, Šiffel *et al*. 1993). Since 1991, Pavel also collaborated with the Laboratory of Forest Ecology of the Institute of Landscape Ecology of CAS on a somewhat new matter for him, namely on the investigation of the effect of simulated acid rain on the source-sink relationship in Norway spruce seedlings (Roháček and Šiffel 1995, Šiffel *et al*. 1996).

Soon after the branch of biophysics at the Faculty of Biology of the University of South Bohemia enrolled its first students, Pavel started preparing and teaching courses in quantum physics. He discovered a New World for himself, enjoyed the job and, perhaps, the teaching partly helped him to overcome his specific speech handicap. Pavel liked to save money but never asked for help even if he would deserve it. It is our shame that he was never properly granted or acknowledged for the teaching activities at the Faculty of Biology.

Dedication to fluorescence quenching and kinetic absorption spectroscopy

When working with *in vivo* chlorophyll fluorescence induction as a tool detecting and quantifying abiotic stress in plants, Pavel abandoned eco-physiological research and went back to his scientific roots as a physicist. Thus he started research on basic principles of fluorescence quenching during the fluorescence induction curve. This was initiated mainly by the work on aggregation of light-harvesting complexes (LHC) of photosystem 2 in stressed plants at low air concentration of CO_2 (Šiffel and Vácha 1998, Šiffel and Braunová 1999). A study on the relation between changes in non-photochemical quenching and artificial stacking of thylakoid membrane and aggregation of LHC2 was simply a reflection of previous debates (Štys *et al*. 1999). The deeper Pavel was thinking about the fluorescence quenching, the closer to the molecular level he went. He started to study the cause of the fluorescence quenching down from leaves to chloroplasts and isolated photosystem 2 (PS2) particles. His conclusion was that the irradiation-induced fluorescence decline at 77 K is related to formation of a charge on a non-pigment molecule of the PS2 complex (Šiffel *et al*. 2000). Further study on the fluorescence quenching in isolated reaction centres of PS2 was presented at the XIIth International Congress on Photosynthesis in Brisbane, Australia (Šiffel and Vácha 2001). This work argues against the common concept of pheophytin as the fluorescence quencher in the PS2 reaction centre. Instead, the previous observations of a charge on a non-pigment molecule of PS2 complex, being the fluorescence quencher, were confirmed localising the charge within the complex of PS2 reaction centre (Litvín *et al*. 2005).

By that time Pavel realised that the equipment and instrumentation available limited him. With the progress in technique, access to the latest technology, and also with more money going now into the science, Pavel got a chance to realise his ideas about a simple but very powerful kinetic absorption spectrophotometer based on the "pump and probe" principle with multi-channel detection. He used xenon (Xe) flash lamps as the sources of radiation for both the pump (a high power Xe flash lamp) and the probe beam paths. A beam splitter divides the probe beam path into two paths: one for sample and the

other for reference. Both signals are then directed by light guides into an imaging spectrograph and, above each other, they are focused on an imaging plane. Both signals are further dispersed on a grating of the spectrograph and directed to an output plane. Two arrays of photodiodes are attached to the output of the spectrograph detecting the spectra of the sample and the reference. Using one imaging spectrograph and the sample and reference paths, correction of the instability of Xe in the spectra and intensity flashes can get to a signal to noise ratio of 10^4. The duration of Xe flashes limits the time resolution of the spectrophotometer to the range of microseconds. The sequences of the pump and probe flashes, other optional radiation sources, and various accessories were fully programmable using a commercial driving unit (*PSI*, Brno, Czech Republic). Development of such a unique kinetic spectrophotometer, named by Pavel as *SpeKin*, saturated him fully and therefore first publications on this apparatus originated mainly from collaboration (Bertrand *et al*. 2001, Zehetner *et al*. 2002). Later, most of the Pavel's work on the problem of fluorescence quenching was based on the measurements with *SpeKin*. The instrument was also intensively used to study the multimeric excitonic interaction of pigments in PS2 (Vácha *et al*. 2002) and the localisation of an accumulated positive charge in a reaction centre of PS2 after charge separation (Vácha *et al*. 2003, 2005).

Because the time resolution of the original *SpeKin* was limiting its application in research, Pavel planned to acquire a better radiation source. The chance appeared with new Research Centre projects announced by the Czech government. Pavel has fully immersed himself in development of a new femtosecond laboratory, searched the market, consulted with the experts, travelled and visited laboratories, discussed problems, and put the ideas together. His ideas have crystallised into the high power system enabling to pump and probe single femtosecond shot experiments with multi-channel detection. Unfortunately, he passed away right before the final decision regarding the types of the fs lasers and the accessories. The decision was made by his closest co-workers, who have completed the work commenced by him, and the laboratory is now equipped with operating fs system and ready to work.

Besides the development of new spectroscopic tools, Pavel never forgot his basics, fluorescence emission and excitation spectroscopy, and contributed to several studies with his skills and knowledge (Koblížek *et al*. 1999, Synková *et al*. 2005a,b). One of Pavel's latest ideas is now realised by his former colleagues at the IPMB. It deals with an application of interference of coherent radiation to measurement of photosynthetic activity *in vivo*.

One of last research activities of Pavel was again connected with plant physiology and his feeling for nature. J. Šantrůček spent a one-month winter stay with Pavel in the central Siberia forest – a place isolated from civilisation. In a friendly atmosphere, they studied winter adaptation of photosynthetic apparatus in Siberian Scots pine (Šiffel and Šantrůček 2005).

Tribute

We pay homage to Pavel Šiffel not only to adore a man devoted to science. We also want to remember a special person to those who knew him as a positive, remarkable man. We wish to emphasise his extraordinary features that are following worth to those who did not have the chance to meet him and work with him. The features, which formed a curious and strange person, that was kind to most and tough to only a few. He was difficult to understand for some and admirably clear for others, a creative thinker always ready for experimental proofs, a man who died before he reached the top of his power. Those who contributed to the "review of Pavel's personality" are his friends and, of course, the review keeps the bias of friendship: the faithful hand sorting chaff and grain among the words and thoughts, keeping what is worth and blowing away the rest.

Pavel was not a success hunter, rather a humble man gifted with stamina, driven by thirst for knowledge, and dedicated to thorough research. He liked simple things (in both life and science) and used rather creative than memorised thinking.

Pavel passed away, together with his wife Gulya, unexpectedly during a tragic traffic accident on July 12, 2003. We are still missing him, his smiling face clouded within a blue-grey smoke from his almost ever-burning cigarette…

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