PERSONAL VIEW

Learning from seeds

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Carl Leopold

As a professional plant physiologist for the past 50 years, I have worked on many aspects of plant growth and development. I started working with plant hormones and regulatory systems, and moved gradually toward developmental processes. These were certainly interesting areas for study, but perhaps the most productive events occurred following my transition to the study of seeds. For the past 25 years, I have been learning from seeds.

To start from the beginning, I was born in New Mexico in 1919, and grew up in Madison, Wisconsin, until the start of World War II. My father, Aldo Leopold, was a Wisconsin professor, and a major figure in forestry, ecology and environmental ethics. Mother was from a Spanish family that owned a major sheep ranch in New Mexico. It is remarkable that, of the five offspring from the Leopold family, every single one of us became professional biologists/ environmentalists.

After 4 years attending the University of Wisconsin, I started graduate study at the University of Missouri, but before the first semester was completed, progress was interrupted when President Franklin Roosevelt made his famous 'Day of Infamy' speech on 8 December 1941; thereupon the entire graduate student body enlisted in the military services. I enlisted in the Marine Corps and served 4 years in the Pacific War. I spent those 4 years on various Pacific islands; intellectually speaking the time was spent majoring in beer drinking. After that, it was a huge transformation moving to Cambridge, Massachusetts, and majoring in plant physiology at Harvard. But with great effort and some luck, I was able to recover enough student skills to survive. I completed my PhD under the inspiring tutelage of Kenneth V. Thimann. My thesis was on the physiology of flowering.

Professor Thimann was clearly disappointed when I then accepted an applied science position with the Hawaiian Pineapple Company, where I was to work on the physiology of flowering in pineapples. But within a year, there was a major upheaval amongst the Directors of the Pineapple Company, and the Vice-President for Research was fired, along with his entire crew of young researchers. So I had to leave my lovely house on Kahala Beach, to return to the mainland. It was my good fortune that, at that time, positions for new PhDs were abundant, and I accepted a position as Assistant Professor of Horticulture at Purdue University. This was a time and place of many great advantages, including abundant laboratory and greenhouse space, growth chambers and even abundant research dollars. Grant proposals were almost unheard of at that time, and I wrote my first grant proposal after I had become a full professor. The situation at Purdue was very supportive and

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presented the unusual opportunity to combine basic plant research with an awareness of realistic field horticulture. I remained there for 25 years. I then spent a year working with the Science and Technology Policy Office in Washington, DC. In addition to hobnobbing around with Congressional committees and power politics, I served as Program Director for a major study of the world food situation (NAS, 1977). This was a time of important growth for me, with truly remarkable opportunities to expand my knowledge of art, music and theatre, as well as learning about administration and governmental politics. A year later I accepted a post as Dean of the Graduate College at the University of Nebraska, thinking that it would be exciting to deal with a wide range of graduate programmes in science, humanities and agriculture. However, the mid 1970s were stressful years at Nebraska, and I became eager to get back to experimental science. I subsequently was happy to accept a position as Distinguished Scientist with the Boyce Thompson Institute at Cornell University.

A New Start

While I was at Purdue, approximately in 1973, I was serving as a consultant to a large agro-chemical firm that was considering a reorganization of its plant research programme. The company asked me to prepare a recommendation to help them identify the most promising area of future research in the plant sciences. I spent a substantial amount of time thinking and reading about this charge. And I ultimately prepared a report in which I suggested that seeds offered the most promising area for plant research in the future.

My reasoning was that seeds are crucial for all crop production, and in spite of the fact that they commonly limit agricultural production, they were very poorly understood. The numerous papers that were being published on seeds each year were mostly empirical, and generally lacked a focus on underlying biochemical/biophysical or genetic bases of good vs. poor quality. Nor had contemporary studies even attempted to find the basis for the amazing ability of seeds to withstand drying, though drying is an integral part of most plant life cycles, as well as a pivotal part of seed technology. I pointed out that whereas many new techniques were becoming available for analysis of biochemical components and biophysical features, few had as yet been applied to studies of seeds, with detailed knowledge of seed composition and metabolism limited to only a few special sectors. In addition, seeds have a particularly appealing characteristic of convenience for both laboratory and field work, and treatments applied to seeds can alter the entire life cycle of the consequent plant. Thus, the prospect for new and useful information on seeds seemed particularly good.

With considerable pride then, I turned in my report to the company. Months passed without a response and it gradually became clear that my recommendation had changed nothing in the company's research programme. However, in contrast to the company's reticence, I took my recommendation seriously. I changed the research orientation of my laboratory, and I have been working actively on seed physiology for the subsequent 25 years.

The Course of Time

Over this period of time, my learning about seed physiology has expanded, enhanced by an array of highly talented colleagues. Together we began by looking for clues about the nature of seed deterioration (Bernal-Lugo and Leopold, 1998; Leopold, 1990; Leopold *et al*., 1992, 1994; Leopold and Vertucci, 1986, 1989; Vertucci and Leopold, 1986).

We spent considerable effort studying the dramatic leakage of solutes associated with seed ageing and the associated changes in metabolic and enzymic activities. Possible involvement of oxidative activities such as polyphenol oxidase, alternative oxidase, lipase and non-enzymic oxidations were pointed to in work carried out by David Parrish, William Bramlage, Yohji Esashi, Mary Musgrave and David Priestley.

From the leakage studies, it became increasingly evident that seed ageing involved changes in membranes. Pursuing the membrane involvement, we found that we could detect substantial losses in certain major phospholipids occurring with ageing, and there was an associated production and oxidation of fatty acids. And ultimately it appeared that phase changes within the membranes contributed to the leakage associated with ageing. These were studied in experiments by David Priestley, Martin Caffrey, and later by Wendell Sun.

Then there was this remarkable ability of seeds to withstand drying. Knowing that most membranes and many proteins are destroyed by desiccation, we diverted our attention to the biophysics of seed dehydration and examined how adaptation to desiccation stress might relate to the state of water. The experiments of Christina Walters Vertucci and Fabio Bruni revealed a series of three water binding sites in dry seeds, to which pools of water were bound with increasing enthalpy. As water was removed from the weakest bound pool, metabolic activity was stopped; and as drying proceeded into the intermediate pool of bound water, there was a lowering of germinability in some seed species. The

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most severe damage, however, was done when water was removed from the most tightly bound pool of water. It was relevant to find that these thermodynamic pools of water binding were not detectable in recalcitrant tissues. This suggested that water-binding sites played a role in desiccation tolerance. The damage done by removal of bound water and the relevance to optimal storage conditions was made more evident in subsequent work by Christina Walters Vertucci (1993) and Vertucci and Roos (1992).

The desiccated state severely restricts the types of reactions that can occur in dry seeds. Electron transport was immobilized by removal of even the first shell of bound water, which resulted in respiration being shut down. The fluorescence yield of chlorophyll in response to light – an indicator of integrity of the photosynthetic system – was suppressed when the first two shells of bound water were removed. The phytochrome shift to Pfr was also shut down when the first two shells were removed, but the reverse reaction forming Pr was able to occur even in the lowest levels of water. Levels of desiccation that were too low for respiration still allowed for reactions which would break dormancy and cause afterripening; likewise, some oxidative activities could occur. The analyses of reactions in the anhydrous state were done by Christina Walters Vertucci, Marc Cohn, and Yohji Esashi.

As the issue of desiccation tolerance in seeds came into focus, we learned from the work of John and Lois Crowe and D. Chapman (1984) in California about the importance of sugars in desiccation tolerance in animals. Their work stimulated us to study the relation of sugars to desiccation tolerance and seed quality. Protective effects of soluble sugars in dry seeds are due in part to the binding of the hydrophilic sugars to proteins and membranes, as had been shown by the Crowes. The binding by sugars resulted in a stabilization of membranes and a prevention of denaturation of proteins. Further work, carried out by Karen Koster, Sheila Backman, Irma Bernal-Lugo and Wendell Sun, established a correlation between the species of sugars contributing to seed quality and storage stability. Stabilizing effects of sugars in dry food systems had been described by food technologists such as Marcus Karel, at M.I.T. and Rutgers. An intriguing suggestion made by Michael Burke (1986) led us to realize that the glassy state is a major component of stabilizing dry seeds in storage. As polyols such as soluble sugars are dried, they may form an unstructured solid – a glassy state. Work by Robert Williams, Fabio Bruni, Irma Bernal-Lugo and Wendell Sun confirmed the suggestion of Burke and developed evidence for the contribution of the glassy state in seed physiology. In fact, the glassy state is a major factor in the storabiltiy and ultimate storage quality of dry seeds. It has two relevant qualities: it forms a solid state which can suppress reactions in biological materials, and its hydrophilic quality prevents structural damage to macromolecules and membranes through the drying process. In addition to the beneficial role of soluble sugars, work by Sheila Blackman and Wendell Sun revealed that desiccation tolerance also involves the presence of some small hydrophilic stress proteins.

Even as the protective effects of sugars were established for dry seeds, we found that some sugars produced negative effects on storage stability. As we learned from food technologists, reducing sugars can cause a loss of quality in dry systems. Specifically, they are a component of seed deterioration reactions. Even in dry systems, reducing sugars can facilitiate non-enzymatic oxidative processes such as the Maillard reactions, as has been established by work of Scott Wettlaufer and especially by Wendell Sun.

There is a further complexity about soluble sugars: many sugars tend to crystallize in the dry state, and the crystals can be very disruptive to macromolecules or to membranes. Thus, a seed containing only the sugar sucrose gradually loses its vigour and onion seeds which rely on sucrose alone are well known to be short-lived in storage. Most crops with better storage stability contain a mixture of sucrose with other soluble sugars which can suppress crystallization. These beneficial effects of mixtures of soluble sugars are illustrated by the long-lived seeds of maize. Sugar mixtures in seeds often include sucrose plus galactosyl oligosaccharides such as raffinose or stachyose. The remarkably beneficial effect of mixtures of sugars has become evident from the work of Martin Caffrey, Irma Bernal-Lugo, Sheila Blackman and Wendell Sun.

We can learn about seed deterioration by considering the dynamics of mortality curves. Many seeds of good storage capabilities show a distinctive mortality curve; loss of germination proceeds initially at a very retarded rate, before advancing into a sigmoid progression of deterioration. On the other hand, seeds with poor storage characteristics are liable to lack such a retarded period and proceed to lose germinability in the usual sigmoid fashion. The accumulated evidence indicates that the initial period of retarded deterioration may be related to the occurrence of the glassy state in the seeds. Evidence for this contribution of the glassy state to longer-term seed viability was worked out by Irma Bernal-Lugo. So the soluble sugars play multiple roles in the quality and longevity of seeds in the dry state. They act partly through their ability to protect membranes, partly through holding proteins in their native folded state, partly through the formation of the stabilizing glassy state and partly through their mutual inhibitions of crystallization. And conversely, the

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reducing sugars pose a threat because of their potential for oxidative reactions. Hence we learn to define components of seed quality through the combined functions of simple biochemical constitutents, and their biophysical states.

A pleasing dividend from these studies has been the finding that the glassy state, which forms as sugars are drying, has practical applications. After we found evidence for the glassy state contributing to stability of dry seeds, we set up model systems in which we could study the behaviour of individual enzymes in the glassy state. Using the combination of sucrose and raffinose, a mix which dominates in good-storing maize seeds, we found that we could store isolated enzymes in the dry (glassy) state for periods of years and recover full activity by simply adding water. In this fashion, by following the lead of good-storing maize seeds, we could store biological materials with sugars in the dry state for long periods of time without refrigeration. The rather obvious applicability to storage of enzymes and pharmaceuticals in the glassy state was worthwhile patenting and the first licensing of the sugar-glass patent was made to the Inhale Company in San Carlos, California, in 1998, which is preparing to market insulin in a sugar-glass. The insulin can be taken up by diabetics by simple inhalation of the dispersed glassy particles instead of by hypodermic injection. This is an example of the occasional cases in which basic research findings can be applied to an unexpected technology.

Progress in these various aspects of seed physiology was greatly facilitated by continuing intellectual help from friends and colleagues, including Robert Williams, Daphne Osborne, Randy Wayne and Irma Bernal-Lugo. Likewise, David Priestley's splendid book *Seed Aging* (1986) was a continuing and salient guide for all of us.

Visual Aids

Work in the research laboratory has left some delightful mental images. A few of my favourite recollections are:

> Christina Walters Vertucci moving around in the lab with her baby in her back-pack, the baby's head bobbing up and down as her mom moves about collecting the incredible amounts of data on seed hydration that she habitually assembled.

> Fabio Bruni poring over his magnificent Thermal Stimulated Currents apparatus which he built himself, watching the computer trace out the various peaks of bound-water pools and glass transitions in seed samples, with liquid nitrogen emitting mysterious steamy vapours from the apparatus.

> Martin Caffrey crouched in front of the Cornell High Energy X-ray apparatus in the Cornell Synchrotron, watching the monitors as they measured the transitions of membranes influenced by temperatures, sugars and hydration. This meant working through the night in the cavernous basement of the Synchrotron, where flashing red lights and loud beepers announced the times when the electron beam was acting up, periodically requiring that everybody vacate the lab and go drink coffee until the electrons behaved properly again.

Five Lessons

While scientists each pursue diverse sectors of scientific knowledge, we share the opportunity to learn about the overall nature of science. Different researchers will emphasize different points about its nature, but my list of primary lessons about science is as follows:

1. **Working with intelligent colleagues** is rewarding, often fun and even compelling. Progress is marked by great moments. The fun and reward of a research career – finding answers to questions of your own choice – are highly satisfying. For me, sharing in experimental work in keen partnership with bright colleagues has been far more fun than a career that pursues power in the science hierarchy, or in circles of academic management.
2. A major factor in research success is **the selection of promising subjects**. It is crucial to choose a subject which deals with really important issues. One that also shows possibilities for substantial progress will maximize your chance of making interesting contributions. And, conversely, it is usually non-constructive to pursue a subject in which progress has become stagnant and the prospect for substantial progress has become dim – unless you can find a truly new approach. Lacking that, such a subject should be avoided.
3. Progress in **research thrives on good communication** with other researchers. Sharing ideas with your peers is not only a major source of excitement, it is also a basic ingredient of progress.
4. Use of excessively **complex technologies can be a serious trap**. Simple experiments focused on novel questions are often more fruitful than experiments with complex technologies. It may be flashy to use new, popular reductionist techniques, but the outcome can be superfluous unless it provides creative new information about the organism you are studying.

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5. I am seriously worried about the **research community becoming increasingly the handmaiden of corporations**. As research becomes increasingly expensive, and the federal support system is increasingly limited in support funds, our faculties are turning increasingly toward corporate funding. In spite of the alleged goals of academic research to provide intellectual information to the community that supports it, academic research is instead becoming increasingly the handmaiden of corporate interests. The situation in 1998 is markedly more serious in this regard than it was when Martha Crouch (1990) published her important caution about the inappropriateness of the academic science community serving the needs of corporations instead of serving the people.

Examination

In retrospect, as I have grown older, I feel that my research work has become progressively more interesting and relevant. I may be wrong, but I would like to think that such a progression represents at least in part, some increase in skill at selecting promising avenues of pursuit. It is my perception that a large proportion of my most interesting publications have been produced in the 9 years since my retirement. Now, even after all research support has expired and I am alone in the laboratory, the tiny experiments I am doing to study seed physiology continue to be interesting and fun. Collecting the data at the end of each experiment still brings that wonderful increase in heartbeat that comes with testing your own hypotheses, and with the expectancy of finding new and relevant information.

As a less optimistic postscript, I must add that rewards from the profession are fickle. Credits for contributions that you have made will quickly lose their connection with you. Is it not remarkable, for instance, that people's role in research so quickly fades from view? Consider an impressive example: most of the Cornell students who study plant molecular biology today are unaware of the research contributions of Frederic C. Steward. This man made giant contributions to plant physiology, including the discovery of totipotency of individual plant cells and their consequent capability for regenerating whole plants – an essential component of most research in contemporary plant molecular biology. The disappearance of appreciation is also remarkable in that F.C. Steward's work was done here in this same Cornell Plant Science Building a mere generation ago. It illustrates that fame from contributions to science is generally ephemeral.

And so, in the pursuit of seed physiology, or of plant biological research in general, one needs to realize that rewards are mainly personal. Making contributions to your science may provide benefits to both science and agriculture, but your principal reward will be your own personal gladdening. Among the most important of these personal rewards are (i) the joy of widening your intellectual abilities; and (ii) the happy confirmation that good mental processes combined with good experiments can do wonders for your understanding of nature. The former might be compared to the pleasure you get from learning how to play a musical instrument well; the latter may be analagous to the gladness from applying your skill, then, to search out the beauty and subtleties of fine music.

I want to express my indebtedness to the late Kenneth V. Thimann, who inspired me from the start of my graduate studies, and whose elegant enthusiasm and probity have been a glowing example for me through my entire professional life.

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