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PERSONAL VIEW

Germination, dormancy and red tape

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Introduction

My curriculum vitae

Seed science has dominated my life for 30 years. It started in 1963 when I was 26 years of age and entered the Botanical Laboratory of Utrecht University as a junior lecturer and chose seed germination and dormancy as the subject for my PhD thesis. In 1969, after graduation, I moved to the Department of Plant Physiology of Wageningen Agricultural University (now Wageningen University), where John Bruinsma composed a new group with a main emphasis on the hormonal regulation of generative plant development. While I moved through the ranks, to become a full professor in 1984, several colleagues and a large group of students joined me and helped me to establish a diversified research programme on different aspects of seed science.

Seeds lost their dominant position in my academic life in 1993 when I was appointed as Rector Magnificus of Wageningen Agricultural University, a position that left hardly any time for an active involvement in research. I retired from the rector's position in late 2000. I continue, at the moment, with a few activities from my life in administration, in particular the stimulation of co-operation between European agricultural universities, colleges and faculties. However, in this more relaxed period of my life, I have noticed that my interest in the secret life of seeds survived the period of scientific dormancy. Therefore, the invitation to write a Personal View in *Seed Science Research* offered a most welcome opportunity to revive my memory and to stimulate my appetite for research.

Born on the fourth of July

I was born on the sunny afternoon of Sunday, July the 4th, 1937, 161 years after the birth of the United States of America. The late thirties of the 20th century seemed, at first glance, a rather quiet and prosperous period in world history, certainly because the world was reviving from the great economic depression of the years before. However, the signs of approaching disaster were already seen and heard. A newspaper, published on the day after my birth, reported on the Civil War in Spain, the war between Japan and China

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and on the arrest of the German reverend Martin Niemöller, who openly defended Christianity against the '*Blut und Boden*' philosophy of the Nazis.

I was the fourth child and second son in a family that finally grew to six children. My father was a bookseller in Bodegraven, a small village in the centre of Holland, the central-western province of The Netherlands. A bookshop is a wonderful environment to grow up in. It is certainly good for the development of one's cultural interest.

My grandfather started the bookshop in 1899, and I am proud that, under the leadership of my elder and younger brothers, the business has extended to a prosperous chain of bookshops in the rural heart of Holland. It is now in the hands of the fourth generation, two of my nephews, and is doing so well that it was awarded at its 100th anniversary as a 'purveyor to the Royal Household of Her Majesty Queen Beatrix of the Netherlands'.

I was raised in a rather strict Calvinistic tradition. My parents and grandparents belonged to the Dutch Reformed Church. That Church did not preach isolation from the sinful world around, as so many Christian groups tended, and tend, to do. On the contrary, my forefathers and -mothers were driven by their Christian beliefs to take an active part in politics and other movements in society. Christian leadership of society was their ideal. Both my parents took initiatives and were very active in the Church, local politics, youth movements and women's clubs, to mention a few.

The members of that Church continued that attitude during the Second World War. As a consequence, they belonged to the first and most active part of resistance against the German occupation. My father was arrested twice and barely escaped execution during the final days of the war. As a little child I lived quite happily during those years. Actual fighting did not happen in our area, and my parents kept the tension they lived under far from their children.

I left the safe environment of my parents' home in 1955 at the age of 18 to go to university. As a consequence my life took its own course, certainly in the liberal sixties and seventies. Science changed my attitude towards religion. However, when I look back now at my life at the contemplative age of retirement, I see the strong effects of my genetic background. It is, as a matter of fact, phenotypically modified, but can still be recognized. My constant itch for leadership was simply in my genes and any attempt to resist it failed.

Biology

An academic study of biology was not in my genes. There had never been a biologist in my family. In fact, I was the first university student. At the beginning, my choice for a university education was a negative one: the bookshop of my father had at that time no economic support for another son and, since I did well at secondary school, study at university was an obvious choice. The choice of biology was not inspired by a passion for field biology, as with so many colleagues. My biology teacher at the secondary school stimulated it. He aroused my curiosity for the secrets of the living organism.

My first years at Utrecht University were tough. Biology teaching was still dominated by descriptive courses in plant and animal anatomy and morphology. Ecology, biochemistry and statistics were missing. The first chair in genetics was just introduced and molecular biology was still in its infancy. To be honest, I passed through several periods of serious doubt whether biology had been the right choice. However, I had been educated to persist when you had made a choice, so I kept going. I compensated for the stuffiness of my early years in biology with an active involvement in every other aspect of student life. It allowed the first expression of the leadership gene.

Biology became really interesting during my Masters degree, when I learned to design and perform experiments, the art of looking around many corners without ever seeing the real event. Physiology came closest to my wish to unravel regulatory mechanisms in living creatures, and in plants in particular. It is said that a choice for plants instead of animals is a soft one. If that is true, I like to be a softie.

The start at Utrecht

In the first part of the 20th century, the Botanical Laboratory of Utrecht University had been one of the world-famous cradles of hormone research in plants. In the basement of this laboratory, Frits Went did his famous experiments with *Avena* coleoptiles. He proved the existence in plants of a transportable compound (baptized auxin and later shown to be indole-3-acetic acid) that was responsible for the regulation of elongation growth. Several important theses followed Went's primary results. Unfortunately, the Second World War finished abruptly that prosperous line of research. After the war it turned out that the core of hormone research had moved to the USA.

When I entered the Laboratory in 1963, only scattered remains of the former programme were left. A new programme had to be developed. My supervisor, the late Professor Ruud van der Veen, suggested beginning a study of seed dormancy, even though neither he nor any other member of the staff was familiar with this area. The adventure of exploring a totally unknown field of research attracted me and so started my life with seeds.

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Like a dry sponge, I absorbed the 'water' from literature and from the experience of scholars with a good record in seed science. In that respect I profited immensely from my attendance at the Botanical Congress at Edinburgh in 1964, where I could discuss my preliminary ideas with some of the foremost seed scientists of the time and could attend their lectures. Since that first experience, I have always been convinced how important it is for young scientists to attend international conferences, even when they have no results to show.

At Edinburgh I had, among others, my first inspiring talks with Michael Black; they formed the start of a long-standing friendship. I also met the late Michael Evenari, the founder and head of the very active and successful school on seed science at the Hebrew University at Jerusalem and one of the giants of seed biology. He planted the idea in my mind that for the study of dormancy in seeds two different approaches are available. The majority of authors study ways to break dormancy in *mature seeds* under the influence of various physical and chemical agents. The second approach – which is also applied nowadays only in a very small number of studies – investigates the conditions that induce dormancy during *the development of seeds* on the mother plant. I have taken that lesson to heart, and as a consequence studies on seed development became a steady component of my programme.

Outline of this paper

It is in the character of these Personal Views that the author concentrates mainly on his or her own results. However, it is neither my intention, nor that of the editors, to give this paper the status of a review. I will concentrate my view in particular on the circumstances and the people that made me choose certain research topics.

During all my years in research, I enjoyed considerable freedom of choice. As a consequence I was able to maintain the fundamental character of my research programme. It may be a surprise that such a programme could also be continued at Wageningen University, which is known for its applied character. My university, however, has always maintained its fundamental core. It is realized that applied research is absolutely dependent on a constant input of new fundamental knowledge. Nevertheless, society rightly asks that a university with a mission like ours explains what the long-term benefits of its research activities are for agriculture, nature management and environment. I have never had any problem in explaining that seed science has such a potential.

Seed development, control by photoperiod

At the Edinburgh conference I also met Francesco Lona from Parma, Italy, a pioneer in seed science who is often forgotten. He was not only the first author to report the stimulating effect of gibberellic acid on seed germination (Lona, 1956), but he also demonstrated for the first time an effect of day length on the induction of seed dormancy during seed development (Lona, 1947). In short days (6–8 h), plants of *Chenopodium amaranticolor* produced a small percentage of dormant seeds (10–40%), while in long days (16–18 h) nearly all seeds became dormant.

The contacts during the Edinburgh conference were decisive for the research programme for my PhD thesis (Karssen, 1970a). Inspired by Lona's results and with Evenari's lessons in mind, I decided to study the light control of dormancy induction and germination of *Chenopodium album* seeds. This choice of a wild species, unknown in seed science, was rather adventurous and risky, but it had the advantage that I created my own safe haven apart from the competitive research world of lettuce seed, the dominant model species of those days. My results have shown that I had made a right decision.

The classical way to prove that effects of short and long days are an effect of photoperiodic timing, and not an effect of differences in total light energy, is the introduction of a third light regime, whereby the long night is interrupted by a short (1 h) red illumination. The application of such an approach to developing seeds of *C. album* showed that two different types of dormancy were induced (Karssen, 1970b). Shortly after harvest, seeds that were raised in long days (LD; 18 h light) or SDR (8 h light + 1 h red light in the middle of the dark period) were dormant (type 1), whereas short-day (SD) seeds showed hardly any inhibition of germination. After 3 months of dry storage at room temperature, only the LD seeds were still dormant (type 2), but SD and SDR seeds germinated 100% in both light and darkness.

I concluded that the similarity between LD and SDR seeds shortly after harvest indicated – according to the classical theory of photoperiodism – that the level of Pfr most probably regulated this first form of dormancy. After dry storage, dormancy correlated with clear anatomical differences. LD seeds were rather small with a thick, black, outer seed-coat layer, whereas SD and SDR seeds were larger, with a much thinner, brownish, seed-coat layer. The similarity between SD and SDR seeds suggested that the total light energy received by the plants is decisive for the second type of dormancy.

A thesis has to contain some risky speculation. Mine did so on the nature of the two forms of

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dormancy. The only concrete evidence I had was that the degree of the second type of dormancy was clearly related to the thickness of the outer seed coat layer. I suggested a mechanical function of the testa. In the discussion about the first form, I cited speculations from literature about a possible role of an inhibitor. It was suggested that photoperiod could influence the level of the so-called inhibitor-β complex, a zone on paper chromatograms that was inhibitory in different bioassays. Some years later, abscisic acid (ABA) was recognized as the main inhibitory compound in that zone. In the sixties I had no opportunity to test the hypothesis. The available methods were still very rough, and certainly not suitable for extraction of hormonal compounds from small seeds such as *C. album*. Fortunately, some years later, I could definitely prove that ABA plays a dominant role in dormancy induction (see below).

Thirty years after the publication of my thesis, Yitzchak Gutterman, from the Ben-Gurion University of the Negev, published a review about the maternal effects on seeds during development (Gutterman, 2000). He describes in his paper a vast number of scattered observations on maternal effects. Unfortunately, he had to conclude that the physiology of all these effects is still unknown. It is clear that an open field is waiting for exploration.

How to open the black box of germination?

Time-sequence studies

The long duration of the experiments on seed development in *C. album* (about 5 months) gave me ample opportunity to study other aspects of seed physiology. I noticed in the literature of those days that most germination experiments hardly revealed any information about the nature of the sub-reactions that occur between the start of imbibition and the emergence of the radicle. One reason was that inhibitors and stimulators were present from the start of imbibition until the end of the experiment. I attempted to open the black box of germination by a series of time-sequence studies. It became a second long-standing component of my research programme.

Seeds of *C. album* are of great use for such experiments because they show signs of internal radicle growth before final radicle protrusion (Fig. 1). The seeds (diameter 1–2 mm) contain a peripheral embryo that forms a ring around the central perisperm. The tip of the radicle is surrounded, like the tip of a glove, by one cell layer of endosperm. The testa consists of two layers. The inner testa layer is thin and brownish; the outer layer is dark and much thicker (10–50 µm). The first visible sign of germination is the splitting of the outer testa (stage 1). It is followed by an extension of the radicle, which occurs first within the inner testa and endosperm layer (stage 2), and finally leads to the protrusion of these layers (stage 3). The whole sequence of events takes about 28 h at 23°C. Interestingly, seeds in stages 1 and 2 are still resistant to dehydration. This clear morphological distinction, between the induction and progress of growth inside the covering structures, made it possible to better locate the sites of action of different stimulatory and inhibitory factors.

The red light stimulus

Red light is the natural inducer of germination in *C. album* seeds. Pfr had to be present only during a rather short period of time to induce germination. After the escape from short far-red irradiation a prolonged far-red treatment, which established a Pfr/P ratio of 0.02, inhibited the progress of germination, even during the stages of visible growth inside the testa (stages 1 and 2). Induction of growth and growth itself had different Pfr requirements.

Two sites of hormonal action

Apart from light, germination could also be induced by a mixture of the gibberellins (GA) 4 and 7 and by ethylene (Karssen, 1976b). There was not a constant need for the presence of the hormones. When the compounds were applied rather late during dark incubation, the responsiveness of the seeds decreased, as happened with a short red-light stimulus. Simultaneous application of GA and ABA did not

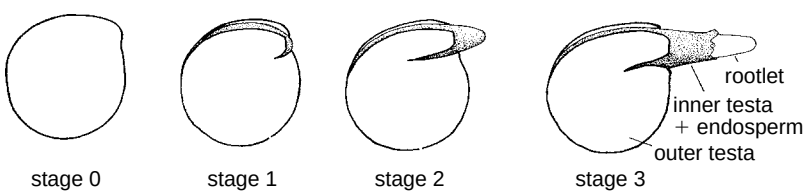

Figure 1. The stages of visible growth in seeds of *Chenopodium album*. Stage 0, seed before growth; stage 1, the outer testa layer splits near the radicle; stage 2, the radicle extends, still enclosed by the inner testa and the endosperm; stage 3, the radicle protrudes through all inner layers. (From Karssen, 1976b.)

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hinder the induction of growth. All seeds entered into stage 2 but, to our surprise, none entered stage 3.

I detected the location of the inhibitory action of ABA on *C. album* seeds in my first experiments with the newly isolated hormone (Karssen, 1968). ABA had been isolated and characterized from the inhibitor-β complex and very recently synthesized. The first indications for the existence of a general inhibitory plant hormone had arisen, on the one hand, from studies on bud dormancy and, on the other hand, from studies on abscission. Confusion arose about the name of the compound. After some discussion, the abscission lobby swayed the decision for the name of the compound in its favour; dormin lost the first round. I will show later in this paper that, in the light of present data, this decision was wrong.

Due to connections of my supervisor with the agrochemical industry, I was among the first to receive a small sample (2 mg) of synthetic ABA. As expected, ABA inhibited germination of *C. album* seeds, but with the aforementioned surprise. After a few basic experiments, my small stock of ABA had run out. I dare to confess now that I then violated all rules of careful scientific behaviour by publishing these preliminary results without any checking or double-checking. Fortunately, after my move to Wageningen, I could, with a much larger stock of ABA, fully repeat my original results (Karssen, 1976a).

The time-sequence studies showed that applied hormones were active at two sites: the first one at the induction of growth and the second one at some process regulating the protrusion of the rootlet through the surrounding endosperm (Fig. 2). It was one of the first of this kind of analyses in literature. Many followed, and continued to follow until the present day. Evidently, the black box of germination showed its first cracks.

How to crack it further? An urgent question that awaited an answer was whether all these experiments with applied regulators represented in any way the endogenous regulation of germination by hormones. Unfortunately, reliable methods to determine endogenous hormone levels in plants in general, and certainly in such small objects as seeds, were still missing. When they became available, the result was, at most, correlative evidence for the involvement of a regulator in the response to environmental perturbation.

The blessings of hormone mutants

The situation improved dramatically in the early eighties when Maarten Koornneef, of the Department of Genetics of Wageningen University, isolated and characterized a new set of hormone mutants in *Arabidiopsis thaliana* and tomato. After 2 years of employment as a plant breeder at a horticultural company, Maarten had been called back to the university in 1976 by Professor Jaap van der Veen. He started his PhD study with the assumption by his supervisor that two non-germinating *Arabidopsis* mutants might be hormone mutants. Carel Spruit, of our plant morphogenesis group, and myself were in a privileged position as his close neighbours. Spruit, soon joined by Dick Kendrick, became Maarten's partners in the analysis of the different photoreceptor mutants, and I joined him in the analysis of hormone

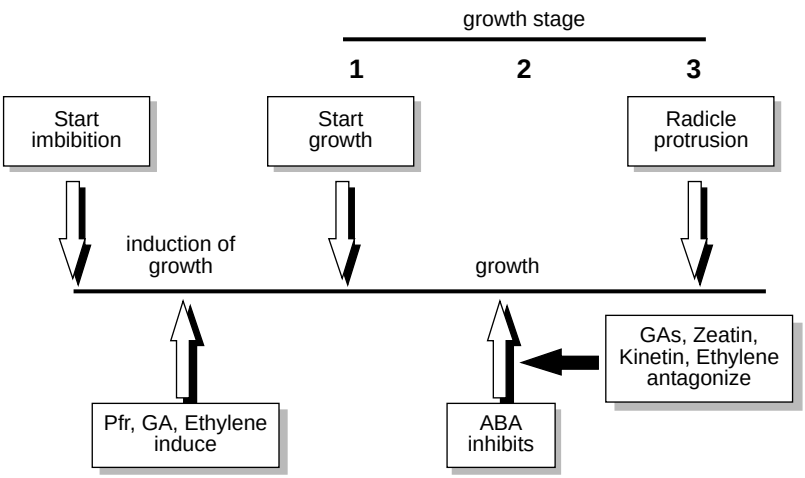

Figure 2. Schematic presentation of the two sites of hormone action during the germination of *Chenopodium album*. Growth is induced by the far-red absorbing form of phytochrome (Pfr), a mixture of the gibberellins 4 and 7 (GA) and ethylene; growth within the surrounding endosperm and inner testa layer is inhibited by abscisic acid (ABA), the effect of ABA is antagonized by GA_{4+7}, GA_3, ethylene and the cytokinins, zeatin and kinetin. (Adapted from Karssen, 1976b.)

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mutants. Each of us benefited enormously from this co-operation.

Mutants in plants that are impaired in hormone synthesis were not new in those days. In a number of species it had already been shown that adding GAs to dwarf mutants restored their growth pattern to that of the wild type. GA synthesis was blocked in the mutants. ABA-deficient mutants had been recognized in some species by their increased tendency to wilt on mild exposure to water stress. These GA- and ABA-deficient mutants had been instrumental to the analysis of the biosynthetic pathways of GAs and ABA, respectively, and to the identification of the active GAs. Moreover, they proved an involvement of GAs in elongation growth and of ABA in stomatal closure.

Koornneef and van der Veen isolated the *ga* mutants of *Arabidopsis*, not on the basis of dwarfism, but on their inability to germinate under conditions that were suitable for the germination of wild-type (*Ler*) seeds. Addition of GA to the germination medium induced germination (Koornneef and van der Veen, 1980). It was later proven that all these mutants are GA deficient (Zeevaart and Talon, 1992). By selecting for germinating revertants in the progeny of the *ga-1* mutants, Koornneef isolated mutants that alleviated the GA requirement by a second-site suppressor mutation, which was a gene (*aba*) that controls a step in ABA synthesis (Koornneef *et al*., 1982; Rock and Zeevaart 1991). Other mutants were isolated by their ability to germinate at ABA concentrations that inhibit wild-type seeds. In these lines, mutated at the *abi1*, *abi2* and *abi3* loci, the sensitivity to ABA is reduced (Koornneef *et al*., 1984). So we now had at our disposal *Arabidopsis* seeds of two non-dormant lines (*aba* and *abi*) and one non-germinating (*ga*) line. We could also use tomato mutants: two GA-deficient non-germinating lines (*ga-1* and *ga-2*) and an ABA non-dormant line (*sitiens*), which were all isolated in a background of the cultivar Moneymaker (Koornneef *et al*., 1985).

Apart from Maarten Koornneef and his co-workers in the Department of Genetics, many people joined me in these fascinating studies. At the start of the *Arabidopsis* programme, I was joined by a Masters student, Dorien Brinkhorst-van der Swan, and later by two Polish students, Eva Laçkka and Slavik Zagorski. In a second phase, the PhD students Steef de Bruyn and Jaap Ooms, were partners in research. Steven Groot took a major role in the study of the tomato mutants.

Seed development

Having Evenari's lessons still in mind, I realized that we had to involve seed development in our experiments, because the origin of dormancy had to be found in that phase of seed life. Moreover, immature seeds are one of the richest sources of ABA. Testing the germination of seeds excised at a certain time-intervals after pollination can monitor the development of dormancy. Dormancy set in during maturation of the wild type, but not in the seeds of the ABA-deficient mutant (*aba*). Dormancy is also very much reduced in seeds from ABA-insensitive mutants (*abi1* and *abi3*) (Karssen *et al*., 1983; Koornneef *et al*., 1984). Interestingly, *abi* seeds contained significantly higher amounts of ABA throughout seed development than wild-type seeds. Reciprocal crosses between *aba* and wild-type plants showed that dormancy is initiated only when the embryo itself produces ABA. Neither maternal nor applied ABA is able to induce dormancy. So, it was finally proven that ABA plays a crucial role in dormancy induction. In retrospect, it seemed that 'dormin' would have been a better name for the compound than 'abscisic acid'.

In tomato, crosses between the ABA-deficient *sit* mutant and wild-type plants indicated a similar distinction between maternal and embryonic ABA. Also, in this species, embryonic ABA is required for dormancy to set in, but, in contrast to *Arabidopsis*, maternal ABA also contributes to a small extent (Groot and Karssen, 1992).

Apart from the reduction of dormancy, seeds of the single mutants *aba*, *abi* and *sit* developed normally. In contrast, seeds of the *aba abi3* double mutants in *Arabidopsis* did show incomplete seed development. The double mutants stayed green, retained a higher water content and showed a reduced accumulation of storage polypeptides and Lea proteins. The seeds were viable, and in a humid atmosphere they germinated viviparously in the siliques. However, the seeds died when dried rapidly. Desiccation tolerance could be induced in these seeds by slow drying, by osmotic stress or by induction in 100 μM ABA (Ooms *et al*., 1994).

In his PhD thesis, Steef de Bruyn proved that ABA has no major influence on the long-distance transport of assimilates in *Arabidopsis* and *Pisum* (de Bruyn, 1993). However, ABA appeared to be involved in the distribution of assimilates for various types of storage compounds during seed development. In particular, the hormone is involved in the regulation of elongation of fatty acids.

Like many other species, developing seeds of *Arabidopsis* and tomato are both rich sources of GAs. Nevertheless, mutation of the GA synthesis capacity did not interact with the roles of ABA in developing seeds. However, it interfered with the development of siliques and fruits (Groot *et al*., 1987).

Germination

The countless examples of GA-promoted germination favoured the hypothesis that endogenous GA

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regulates the breaking of dormancy, but definite proof was still missing. Our studies with GA-deficient seeds of *Arabidopsis* and tomato also presented that proof for the first time.

Interestingly, red light could not replace the absence of GA biosynthesis, indicating that light might be involved in GA biosynthesis (see also below). The observation that mutants without dormancy, such as the *aba* and the extreme *abi3–3* mutants, germinate in a GA-deficient background (Koornneef *et al.*, 1982) showed that GA has a function in the alleviation of the ABA-induced dormancy. We wondered whether the opposite was also true: did ABA still influence the action of GA during germination? The essential question was whether sufficient ABA was still left in the mature seeds. We concluded that residual ABA was insufficient to inhibit germination (Groot and Karssen, 1992).

Hormone balance

Based on these results, I took the courage to propose a revision of the hormone balance theory (Karssen and Laçka, 1986). The classical concept of dormancy correlated the degree of dormancy with a balance between simultaneously present inhibitory and stimulating compounds. I concluded from our results that the actions of ABA and GA were separated in time: ABA was the essential factor for dormancy induction during seed development and, in that way, indirectly influenced germination of the mature seeds via the state of dormancy. GAs were inactive during seed development but were the essential factor for breaking dormancy in the mature seed.

Some years later, my colleague Henk Hilhorst corrected one aspect of our arguments (Hilhorst, 1995). He showed that the germination of mature *Arabidopsis* and tomato seeds from various harvests and sources negatively correlated with the ABA content of the mature seed. Recently, it was observed that inhibitors of ABA biosynthesis promote germination of *Arabidopsis*, indicating that the maintenance of dormancy in imbibed seeds is an active process involving *de novo* ABA synthesis (Debeaujon and Koornneef, 2000). Looking back, it seems that I revised the theory a bit too drastically.

The tomato model

Over the past years *Arabidopsis* has rightly earned the position of a model plant for genetic and molecular studies. Unfortunately, the very small size of its seeds makes it not very suitable for the physiological analysis of hormonal action during germination. Tomato seeds are much easier to handle and to analyse biochemically.

Steven Groot measured the puncture force that is required to disrupt the endosperm layers that surround the embryo tip. He proved that GA is needed for the mechanical weakening of those layers. GA-deficient (*ga-1*) seeds only germinated in the presence of GA or after removal of the layers (endosperm and testa) that oppose the radicle (detipping) (Groot and Karssen, 1987). Isolated endosperms from wild-type seeds also required exogenous GA to reduce their mechanical resistance. It was concluded that GAs were synthesized in the embryo and transported to the endosperm, where a process was initiated that altered the mechanical properties of the endosperm layers.

Biochemical analysis showed that GA induced the enzymatic hydrolysis of the galacto-mannan-rich endosperm cell walls. Especially, the enzyme endo-β-mannanase was under complete GA control (Groot e*t al*., 1988). Endosperm weakening is not the only factor involved in protrusion. The growth potential of the embryonic axis also plays a role, and hormones also control that factor. Wild-type seeds have a lower resistance to osmotic stress than *sit* seeds. GA deficiency enlarged that trend (Groot and Karssen, 1992).

In a second PhD thesis on this model system, Peter Toorop slightly corrected the results obtained by Steven Groot (Toorop, 1998). He concluded, for instance, that endo-β-mannanase is a prerequisite for the completion of germination, but not the sole factor. Other putative cell wall hydrolases, probably GA- and ABA-regulated, are involved as well.

It is satisfying that our results have stimulated a number of groups to adopt the tomato seed as a model for the physiological and molecular analysis of germination and coat-imposed dormancy. During the recent Seventh International Workshop on Seed Biology, held at Salamanca, Spain, keynote addresses by Derek Bewley (Guelph), Kent Bradford (UC-Davis) and Henk Hilhorst (Wageningen) reported the substantial progress that has been made in the biochemical and molecular study of this biological system. It is understandable that the original conclusions of Groot's studies need some modification, which is inherent to progress.

Dormancy cycles

During my years at Utrecht and the early years at Wageningen, my experiments were purely restricted to laboratory studies. I became interested in seed ecology during a stay in Israel. I was invited by the well-known seed biologist Dov Koller to teach for one semester (academic year 1974–1975) at the Faculty of Agriculture of the Hebrew University at Rehovot. In his laboratory I co-operated closely with Moshe Negbi, another representative of the Evenari School.

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My stay in Israel had, in several ways, a great impact on me. I arrived in the country 1 year after the third war between Israel and the neighbouring Arab countries. The country was still in turmoil, and attacks by terrorists were a regular phenomenon. It is sad that the situation since then has not improved, and has even deteriorated dramatically in recent years. The prospects for people on both sides of the conflict are still dark. My view on the political situation in the country is still influenced by that stay.

Also, my ideas on seed biology were strongly influenced by this visit. In a semi-arid climate zone, survival seems of much higher significance for seeds and plants, in general, than in the evergreen, temperate Dutch climate. The sudden explosion of seedlings on the desert floor after the first autumn rain struck me as an ecological shock. The control of germination in the field has, since then, always been on my research agenda. During my stay I discussed several ecophysiological questions with my hosts and with other Israeli colleagues, such as Yitzchak Gutterman. I also had my first stimulating encounter with Alfred Mayer and Alex Poljakoff-Mayber.

Back in Wageningen I started – quite strongly on impulse – my first outdoor experiments, with the help of undergraduate students. We collected seeds of six different annuals, often with a weedy character, and buried them in soil at a depth of 10 cm. At regular intervals over 2 years, samples were exhumed and tested for germination in the laboratory in light and darkness at a fixed temperature. In all species we observed some form of an annual cycle. Freshly harvested seeds were more or less dormant at the start of the experiment; dormancy was relieved during winter and re-induced during summer. The cycle appeared again the second year, in essentially the same form. The observation was certainly not new. Cyclic changes had been observed before and in parallel. Later, I found out that Harold Roberts at Wellesbourne and Carol and Jerry Baskin at the University of Kentucky had started similar experiments during the same period of time. Was it coincidence or a common source of inspiration? The three of us discussed it later but did not have a clue.

In retrospect, I believe that the start of this programme is an example of acting first and thinking afterwards. It happens even in science. Fortunately, some years later, I was invited to carry out the urgently needed analysis of the cyclic dormancy pattern of buried seeds. I had two opportunities. First, in 1980 I was invited by Alfred Mayer to attend a workshop in Jerusalem on control of seed germination. It later turned out to be the cradle of more meetings of this kind. The first one was a small gathering of about 20 specialists and lasted 2 weeks. So there was ample time for every participant to present both a research report and a review. I delivered a review on the annual patterns, and of secondary dormancy in particular (Karssen, 1980/1981a, b). During the workshop my good friend Anwar A. Khan from Geneva, New York, invited me to write a chapter on seasonal patterns of dormancy for the second edition of his book on the physiology and biochemistry of seed development, dormancy and germination (Karssen, 1982). Anwar had, during a sabbatical leave at Wageningen in 1978, himself contributed to our studies on secondary dormancy (Khan and Karssen, 1980).

I based my analysis on an observation by Vegis (1964). He considered that changes in the dormancy of seeds involve changes in their temperature requirements for germination. The papers published by Jerry and Carol Baskin contain several excellent illustrations of that theory. In the 1982 review I schematically summarized their data on *Ambrosia artemisiifolia* seeds (Baskin and Baskin, 1980; Fig. 3). These data show that, in the fall, the fully dormant seeds do not germinate in light at any given temperature. Dormancy breaking is essentially a widening of the temperature window, while induction of secondary dormancy is the opposite. The conclusion is that seasonal periodicity in field-emergence is the combined result of seasonal periodicity in the field temperature and seasonal

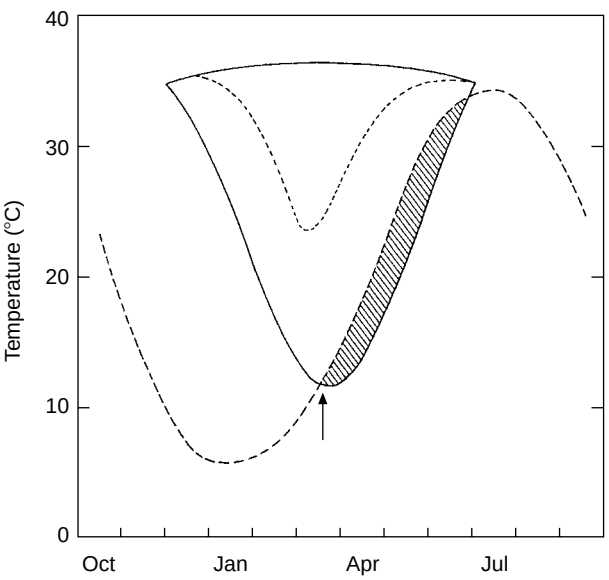

Figure 3. Widening and narrowing of the temperature range of germination in light (solid line) and darkness (dotted line) of a summer annual in relation to the temperature in the habitat during the seasons (broken line); in the hatched area the actual and the required temperatures overlap. The arrow indicates the threshold temperature for germination in spring. Data obtained from a study with *Ambrosia artemisiifolia* by Baskin and Baskin (1980). (Adapted from Karssen, 1982.)

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periodicity in the width of the range of temperatures suited for germination. Germination in the field is restricted to the period when the field temperature and the temperature range for germination overlap. Another condition is that the seeds are exposed to light, for instance after soil disturbance. For dark germination the window is much smaller.

This analysis urges a redefinition of dormancy. I propose: dormancy is a seed characteristic, regulated by external conditions, the degree of which defines what conditions should be met to make the seed germinate (slightly modified from Vleeshouwers *et al*., 1995). Temperature, for instance, has a dual role in seedling emergence: (1) in a seasonal fashion it influences the state of dormancy; and (2) it is a regulatory element in the germination process. Optimum temperatures for dormancy release and germination are not necessarily similar.

My analysis acted as the input to four PhD studies in my group. Harro Bouwmeester studied the particular factors that regulate the dormancy pattern in the field (Bouwmeester, 1990). In a simulation study he needed only soil temperature as the explanatory factor to simulate the seasonal changes in the width of the temperature range over which exhumed seeds germinate (Bouwmeester and Karssen, 1996). The changes in dormancy did not correlate with seasonal changes in soil moisture or soil nitrate content. Since the seeds were buried in continuous darkness at 10 cm depth, the conclusion can be extended to rule out light as a factor.

Henk Hilhorst and Ria Derkx analysed in detail the relationship between the degree of dormancy and the fluctuations in germination factors other than temperature. Henk Hilhorst joined my group in 1983 as a technician. I soon found that he was a good and independent researcher. I suggested to him that he should go for a PhD and he agreed happily. After graduation he joined the academic staff of our department.

Both studies used seeds of *Sisymbrium officinale* and *Arabidopsis thaliana*. *S. officinale* (hedge mustard) was an interesting new species in our collection, because its germination was absolutely dependent on the simultaneous presence of light and nitrate. Based on circumstantial evidence from experiments with inhibitors of GA biosynthesis, it was concluded that Pfr in the presence of nitrate leads to synthesis of GAs (Hilhorst and Karssen, 1988). Interestingly, *S. officinale* seeds without nitrate behaved similarly to GA-deficient *ga-1* mutants of *Arabidopsis*. In both cases GA was the limiting factor.

By means of extensive dose–response experiments under conditions that were limiting for one particular factor, the changes in sensitivity were further analysed. Henk Hilhorst started with laboratory experiments. He showed that germination of both species requires an increasing dose of light, and, for *S. officinale*, nitrate during dormancy induction at constant temperature (Hilhorst, 1990a, b).

To test the ecological relevance of these observations, Ria Derkx repeated the experiments with *S. officinale* seeds buried in the field during two successive years. Sensitivity to light and nitrate showed remarkably similar reversible changes over the seasons. Changes occurred in the maximum and minimum responses and in the dose required for half-maximum response (Fig. 4). Sensitivity to GAs gradually increased from burial onwards and was not particularly related to changes in dormancy. Thus, GA sensitivity cannot be regarded as a limiting factor in regulating the dormancy of this species (Derkx and Karssen, 1993).

Based on a detailed analysis of the dose–response curves, a receptor-regulated dormancy cycling was proposed, whereby regulation occurs at the level of perception of the primary stimulatory factors, such as Pfr and nitrate. Such a regulation is also most favourable from a viewpoint of energy expenditure (Hilhorst *et al*., 1996).

Applied research

The agricultural environment of Wageningen University opened up interesting possibilities for translating my research outcomes to certain areas of application. I mention in particular: weed management and seed technology.

Weed management

Most of the species that were studied in our dormancy work had a weedy character. It was hoped that our results might be a first step towards a predictive model of the time of emergence and the number of seedlings emerging after soil cultivation. Such a model should be a very useful tool in the design of integrated weed management. In his PhD thesis, Leo Vleeshouwers developed such a model based on the aforementioned model developed by Henk Hilhorst. Unfortunately, he had to conclude that the quantitative prediction of seasonal changes in dormancy and germination were not accurate enough to predict field emergence, and the model also appeared to be weak in predicting weed emergence patterns (Vleeshouwers, 1997).

Seed technology

The high degree of mechanization in modern plant cultivation systems demands fast, uniform and full germination. This agricultural demand is the extreme opposite of the evolutionary adaptations that ensure

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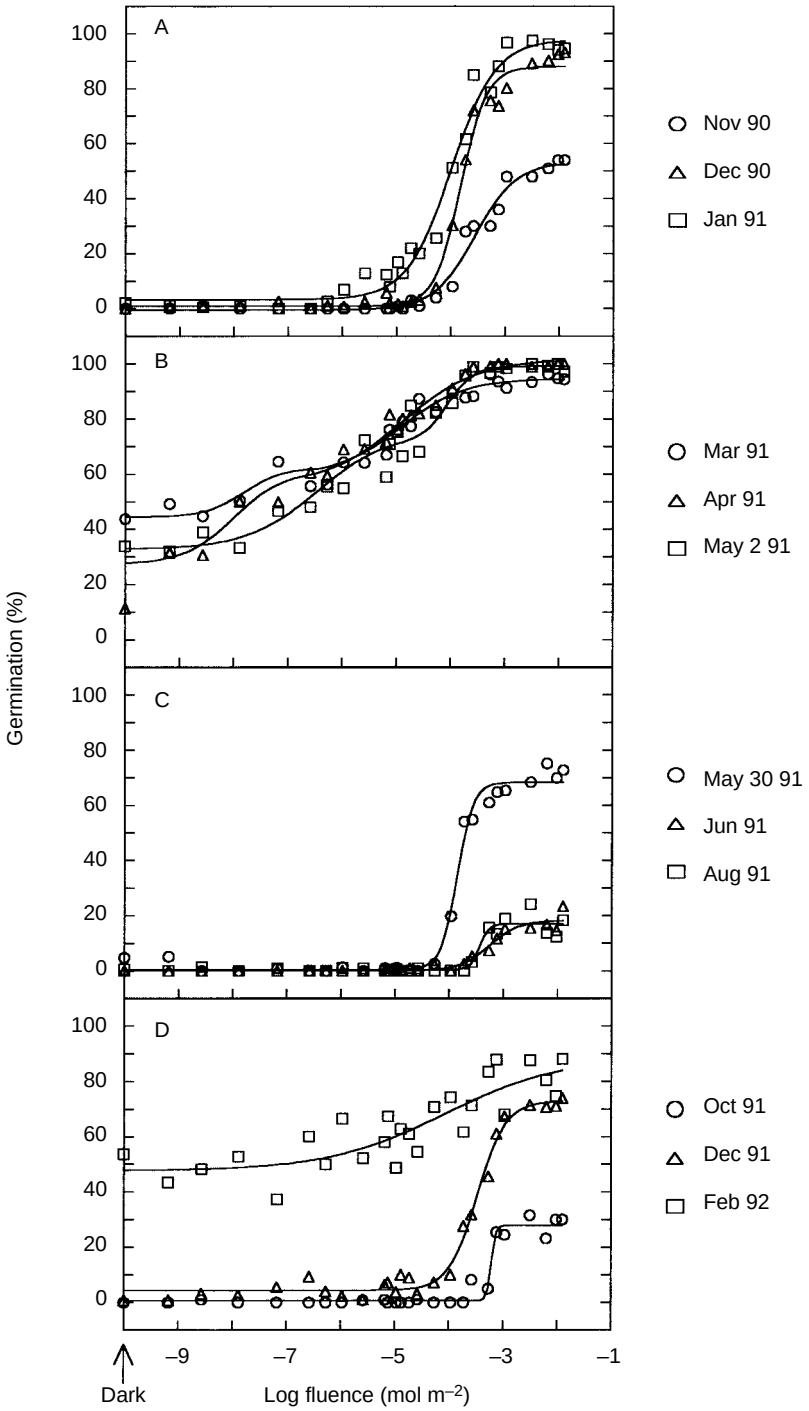

Figure 4. Fluence-response curves of *Sisymbrium officinale* seeds. Seeds were buried in soil at 10 cm depth in November 1990 at Wageningen. After exhumation at the indicated dates, the germination was determined at 15°C in 25 mM KNO_3 at a range of fluence values. Germination data were fitted as logistic dose–response curves. The first point of each curve represents germination in darkness. (From Derkx and Karssen, 1993.)

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the survival of wild plants in their natural environment. Therefore, it was very stimulating to combine my group's fundamental (eco)physiological studies with efforts to unravel the physiology of seed priming. All priming treatments have in common the visible prevention of germination, either by inhibition of germination or by prevention of water uptake needed for actual radicle growth. Osmopriming has been one of the most suitable priming methods. We studied priming in celery, tomato and lettuce seeds.

Celery 'seeds' (morphologically a schizocarpic fruit) consist largely of endosperm, surrounded by a thin testa and relatively thick pericarp. The small linear embryo has to grow to at least twice its size before visible germination occurs. Peter van der Toorn, who was on the staff of a seed firm, showed in his PhD thesis that the embryo grows at the expense of the endosperm during osmopriming. Primed seeds germinated much quicker and were better synchronized (van der Toorn, 1989).

In tomato seeds, priming also causes a strong reduction of the germination lag-time. The rapid germination of primed and redried seeds was the result of changes in imbibition rate, radicle cell wall loosening and endosperm weakening (Karssen *et al.*, 1989).

In lettuce seeds, germination is often limited to maximum temperatures of around 20–25°C, which is undesirable for most habitats. This phenomenon is due to the common dormancy mechanism in wild plants that I have explained above. Priming of lettuce seeds is a form of dormancy breaking. In his PhD thesis, Roelf Weges studied methods to relieve dormancy in lettuce seeds (Weges, 1987). During a pretreatment in darkness at 15°C, lettuce seeds developed the capacity to germinate at much higher temperatures and at much more negative osmotic potentials. Both changes were linearly connected. Therefore dormancy breaking seemed somehow related to changes in the water relations of the seeds. Psychrometric measurements revealed that the effect of temperature on germination correlates with the yield threshold of turgor pressure for cell expansion (Y) and, therefore, with cell wall extensibility. Relief of dormancy correlates with a decrease of Y, and induction with an increase. A comparison of the three studies shows that priming permits a quick and uniform germination by stimulating cell wall extensibility in the radicle and specific weakening of essential endosperm cell walls in all three species.

Based on the results that we obtained with the ABA mutants of *Arabidopsis*, Frans Tetteroo developed a protocol for the induction of carrot embryoids, which might be a step towards the development of 'artificial seeds' (Tetteroo *et al.*, 1995). ABA and slow drying were essential conditions during embryoid culture to develop tolerance to drying. Prehydration in water vapour was needed to prepare the embryoids for full imbibition.

Red tape

In 1993, after a very productive research period, I became Rector Magnificus of Wageningen Agricultural University. At our university, as in many other universities around the globe, the central administration and the academic staff are separate worlds that often are on a war footing. In the eyes of some of my colleagues, I 'defected to the enemy'. Dutch universities are governed by an executive council of three persons: a president, a vice-president and the rector. The first two governors are responsible for the different aspects of management and political relations; they often have a background outside the university. However, it is laid down in Dutch law that the rector has to be chosen from the sitting faculty. It is a good rule because it assures that at least one university governor is experienced in the academic profession. As a consequence, the rector is responsible for the primary tasks of the university: teaching, research and, for the most part, the international relations that are connected to it. The position of a Dutch rector is comparable to the provost in the American system, but differs strongly from the Continental European system, where the rector is the boss of the university. In The Netherlands – as always – we chose a compromise, we mixed the Anglo-Saxon and the European systems of university governance. The adverb 'magnificus' is certainly from a European background.

It has always been my approach to leadership to build bridges between opposing opinions or factions within an organization. In my new position I fully needed that approach. In the academic community of a university, every member is, by definition, intelligent and highly educated, but also very independent and stubborn. Such are excellent qualities in research; they are often used as selection criteria. Unfortunately, it strongly hinders the formulation of a common strategy. Moreover, every faculty member regards his or her research programme as the best and, therefore, needing more money.

Why did I accept the invitation to become rector of such a beehive? To be honest, vanity was among the reasons. Moreover, as I wrote in the introduction, it was in my genes and in my education. Leadership in my Calvinistic family is seen as a duty to society. But I also accepted this challenge because I liked the change. I found it attractive to do something different during the last part of my career. And I do not regret my decision at all, in spite of the fact that the task became much more arduous than I ever expected.

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A few weeks after the start of my new job, the Dutch government issued its first cut of our budget; two additional cuts followed in the next 7 years. As a consequence, we had to lead the university through a series of reorganizations, which included serious staff reductions. However, it was not all sadness, as new perspectives came into view. The government decided, with our support, that the Wageningen Agricultural University had to be united with the public organization, the Agricultural Research Institutes (DLO), which were also housed primarily at Wageningen. The process started in 1997 with the appointment of one Executive Board for both organizations. I retained my position. We named the new organization Wageningen University and Research Centre (Wageningen UR) and renamed the university, Wageningen University. The term 'Agriculture' was regarded as too limiting to the much broader mission of the new organization. In the new organization all elements of the knowledge chain are under one roof: fundamental, strategic and applied research and higher education. It enlarges the critical mass and opens possibilities for sharing facilities. And above all, it strengthens our national and international position as a leading institution in our field.

It took more than 4 years to execute the change. It functions now, and the support among the staff is still growing. You can imagine that many bridges had to be built, certainly the ones between fundamental and applied research. But, we succeeded.

Wageningen Seed Centre

Fortunately, my move to the position of rector did not mark the end of seed science at Wageningen. I was in the fortunate position that Henk Hilhorst could take over my heritage. He did not stand alone. Together with Raoul Bino and Steven Groot of the DLO organization (in particular, Plant Research International), he took the initiative for the Wageningen Seed Centre. They were real pioneers because they did so in the days before Wageningen UR was started. The Centre has built up strong international links, both in teaching and research. It gives me the pleasant feeling that seed science has survived and will survive the total changeover at Wageningen.

Epilogue

I look back with great satisfaction at my career in science and leadership. I am of the generation that lived in an ever-expanding world with unlimited possibilities in the post-war period. We were 'born with golden spoons in our mouths'; trees were growing into heaven. Time has changed, certainly in science. It is worrying that the interest of young people has shifted so much in many countries to social and economic sciences at the expense of hard science and technology. However, I am not pessimistic. I cannot imagine that the curiosity, which is the driving force for an interest in science, will ever disappear among young people. It never left me; it even survived my side step into management.

I will not return to an active role in science, but hope to follow the new developments through my many former students who are still active in seed science and technology; and that is perhaps the greatest satisfaction of all.

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