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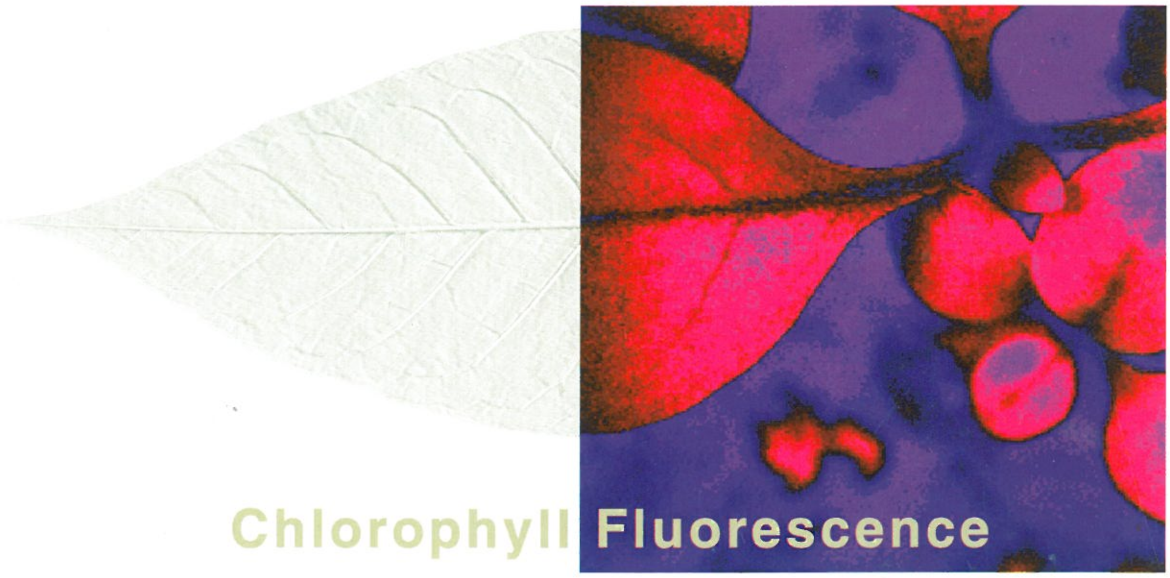

Chlorophyll Fluorescence

The Research School of Biological Sciences is one of Australia's leading centres for basic biological research and graduate training. Since its inception in 1967, it has focussed on three domains: plant science, genetics, and neuroscience. This work is carried out in 11 research groups, organised as follows.

Visual Sciences aims to better understand how the optical image captured by the eye is analysed by the visual system to produce perception. To maximise the chances of success in this enterprise, research has concentrated on simple visual systems such as those of insects. Group leader: Dr Srini Srinivasan.

Plant Cell Biology aims to explain the cellular basis of plant development and how it is regulated. The group also investigates cell division in plants using the latest techniques of molecular biology and molecular genetics. Group leader: Professor Brian Gunning.

Molecular and Population Genetics examines biological problems with genetic and molecular techniques using two organisms — yeast and the fruit fly (*Drosophila*). Because yeasts can be grown and manipulated like bacteria, they are ideal model organisms for experiments in cell biology. The research on *Drosophila* concerns the micro-evolutionary features that affect genetic variation in natural populations. Group leader: Professor John Gibson.

Plant Microbe Interaction studies plant pathology, resistance, symbiosis and defence systems.
A particular focus of the group is the engineering of nitrogen-fixing bacteria for more effective nodulation of legumes. Group leader: Professor Barry Rolfe.

Molecular Evolution and Systematics applies the latest techniques in molucular and information science to study the evolution, systematics, identification and functional biology of selected organisms important to Australia. The work includes study of viruses, their vectors and hosts, as well as the molecular cytology and development of insects and their populations. Group leader: Professor Adrian Gibbs.

Ecosystem Dynamics seeks to understand the structure and function of plant and animal communities. It develops theories of complex community dynamics, and how communities respond to environmental variations. The aim is to formulate models that can predict how communities will respond to different environments. Group leader: Dr Ian Noble.

Plant Molecular Physiology conducts research into how physiological, biochemical and molecular attributes of individual plants contribute to their fitness in agricultural and natural ecosystems. A long-term goal is the identification of genetic elements that could be incorporated into commercially important agricultural species. Group leader: Dr John Andrews.

Environmental Biology conducts research into how ecological, physiological, and biochemical attributes of individual plants contribute to their fitness in agriculture and natural ecosystems. A key theme is photosynthetic carbon gain in relation to growth, respiration and decomposition, and water use, all in the general context of global change. Group leader: Professor Graham Farquhar.

Molecular Structure and Function employs molecular genetics to study the development of brain and muscle as well as the genes that control growth and differentiation of cell types. Group leader: Dr George Miklos.

Director's Research Unit researches fundamental aspects of photosynthesis using novel techniques in biophysical chemistry, biochemistry and cell physiology, and explores their application to ecophysiological problems. Understanding photoinhibition — the process that reduces the efficiency of photosynthesis at high light levels — is pivotal in learning how plants respond to environmental and biological stresses. Group leader: Professor Barry Osmond.

Developmental Neurobiology studies the development and activity of nervous systems. Most work involves the sensory systems of hearing, vision and touch, using the sensors of mammals, birds and crabs, insects and spiders. Much of the research makes use of an extensive wallaby colony, a facility that allows study of the coordinated development of vision and hearing in pouch young. Group leader: Professor Richard Mark.

If you would like to know more about any of the research activities at RSBS, you are welcome to contact the principal researchers involved. The address is: Research School of Biological Sciences, GPO Box 475, Canberra, ACT 2601 Phone: (06) 249 2999 Fax: (06) 249 4891

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Manganese: the magic heart of photosynthesis

Plants perform one of the toughest feats in nature's chemistry book, creating oxygen by splitting that ubiquitous substance, water, in a process we call photosynthesis.

Think of the tremendous energy released when a rocket blasts off into space; that masterly feat of pyrotechnics is powered by the chemical combination of hydrogen and oxygen to form water, the exact reverse process occurring in photosynthesis. Undoing the energy producing reaction of water formation requires real chemical muscle.

In fact, the stability of the hydrogen-oxygen combination accounts for the great abundance of water. When combined with oxygen in this way, hydrogen occupies a very low energy state. This 'run down' state is typical of all oxides, and thus we find that most of the earth's crust is either water (the oxide of hydrogen), or rocks (the oxides of silicon). Life-forms which can liberate hydrogen from its oxide (water), can generate energy to drive life-sustaining processes or, to continue the analogy, a sort of rocket fuel equivalent.

Life on this planet has, so far, not devised a way to disengage oxygen from rocks, but about 3 billion years ago plants managed the comparable feat of disengaging oxygen from water and thereby liberating hydrogen. For plants, oxygen is a gaseous by product. The other product generated from this reaction, hydrogen, is utilised to fuel the production of carbohydrates by the plant — a process known as photosynthesis.

The increasing levels of oxygen, created by photosynthesis, dramatically changed the makeup of the earth's atmosphere. The presence of oxygen brought about the evolution of animals, creatures which use oxygen to power their metabolism, which is effectively the reverse of plant metabolism. Animals are indebted to plants and their photosynthetic efforts for both their oxygen and their fuel (carbohydrates).

In this article we want to examine the heart of photosynthesis by describing recent studies at the Research School of Biological Sciences investigating the molecular mechanism by which plants perform this intricate, and — if we did not know otherwise — seemingly impossible chemical reaction.

It has long been known that photosynthesis is dependent upon manganese (Mn), a trace element constituting less than 0.1% of the earth's crust. Dr Tom Wydrzynski of RSBS and his colleagues are exploring the unique properties of this element which facilitate the transformation of light energy into chemical energy.

From bacteria to higher plants

Single-celled purple bacteria, the ancestors of plants, capture light energy to build complex molecules and run their metabolic machinery. Unlike higher plants, however, these anaerobic bacteria don't produce oxygen (oxygen is toxic to them).

The molecular structure of photosystem II (PS II) in plants (a complex of proteins and pigments at the starting point in the photosynthetic factory) has been derived largely by analogy with crystallographic studies of the anaerobic bacteria's photosystem. This work, which won its three discoverers a Nobel prize for chemistry in 1988, relied on isolating the hydrophobic bacterial material and forming it into crystals, allowing structural relationships to be decoded by X-ray crystallography.

A major impediment to fully understanding PS II, unlike the bacterial photosystem, is that no-one has yet been able to perform X-ray

"Photosynthesis is an exquisite amalgam of physics, chemistry, and biology."

crystallography for this structure, although many are keenly trying. The difficulty is that PS II is also hydrophobic — as it lies within the thylakoid membrane in the chloroplast, and a suitable method for preparing crystals of PS II has not been developed.

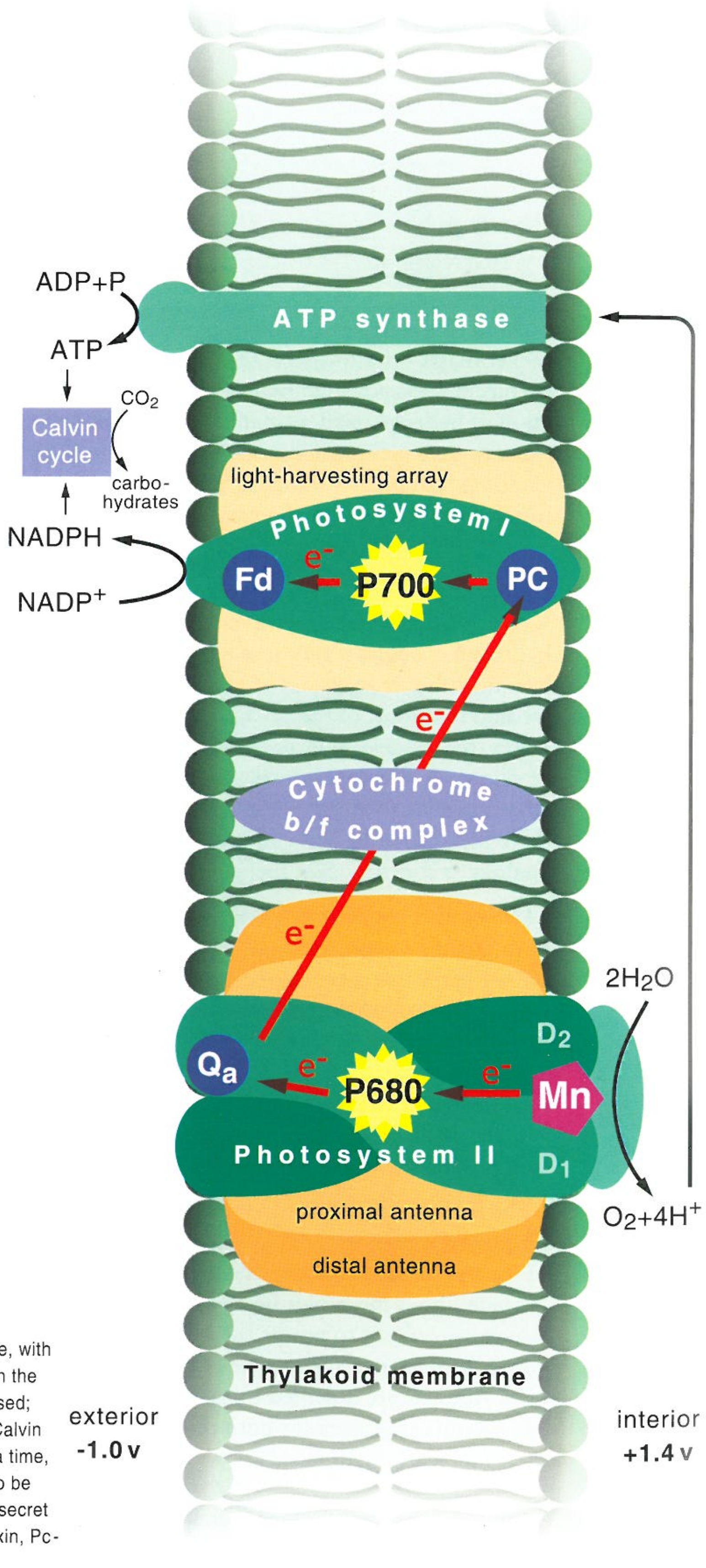
Nevertheless, careful analysis of many experiments, in conjunction with the purple bacteria data, has given insight into the structure of PS II with manganese at its heart.

PS II, amongst other molecules, comprises more than 20 large polypeptide molecules — linear polymers of amino acids, typically hundreds of units long, arranged in a fixed sequence. The whole complicated entity can be divided into a few major parts.

Vital light-harvesting chlorophyll pigments collect photons from sunlight and funnel their energy toward the inner sanctum — the so-called reaction core where the chain of chemical reactions transforming light energy into chemical energy begins. The chlorophylls, which are blue light- and red light-absorbing molecules with a molecular weight of about 900 (containing magnesium not manganese), are arranged in two circles, an outer circle — or distal antenna, containing about 200 chlorophylls and an inner circle — or proximal antenna, containing about 60 chlorophylls.

The centre-piece of the entire PS II assembly, the reaction core, comprises two entwined proteins called D1 and D2, and it is within this protective environment that the scission of water takes place. Two water molecules are introduced, and stripped of four electrons to produce four protons and an oxygen molecule. The oxygen is released as a gas into the atmosphere, the electrons are eventually used to reduce carbon dioxide for incorporation into carbohydrates, and the protons are finally used to form the energy-rich molecule ATP.

The primary electron donor in the reaction core is a pair of special chlorophyll molecules known as P680 (because they absorb light most strongly



Photosynthesis can be likened to a one-electron (e^-) pin-ball machine, with two photon collectors, one in the initial Photosystem II and another in the following Photosystem I. At one end of a long chain, oxygen is released; at the other carbon dioxide is assimilated into carbohydrates in the Calvin cycle. The entire sequence involves the shuffling of one electron at a time, except for the oxygen-liberating step, which calls for four electrons to be simultaneously stripped from two water molecules — herein lies the secret of the manganese clock. (Mn-manganese, Q_a-quinnone, Fd-ferredoxin, Pc-plastocyanin, D1 & D2-reaction core proteins) at a wavelength of 680 nm), and they pass on the donated electron in a complex chain of reactions known as the Z-scheme. This chain covers 20 or more steps from P680 to ATP. On the way electrons move step by step through the chemical entities illustrated in the diagram on page 4.

Like a flipper in a pin-ball machine, photons boost the electrons in photosystem II to higher states, via the primary acceptor quinone (Q_a). Then they fall back down via intermediate states until they are held by plastocyanin (PC). Here they enter another pin-ball machine known as photosystem I (because it was discovered first), and are kicked into higher energy states, via the terminal acceptor ferredoxin (Fd), the process then culminates in the production of reducing compounds ($NADPH_2$).

Effectively, then, photosynthesis is a scheme for mining photons from sunlight to use for stripping electrons from water. Plants, by exploiting this energy source, have spread into nearly every environment. The wonder is that they can perform such an energetic feat using a fragile protein structure — chemists are at a loss to explain why the electrons don't get stripped from the protein rather than from the electron-begrudging water. Dr Ron Pace, a chemist colleague of Dr Wydrzynski, likens this feat to trying to light a fire in a wicker basket without setting alight the basket. Only further research will solve this puzzle, and Dr Pace believes that a new sophisticated ESR spectrometer, described later, will help provide an answer.

The clock

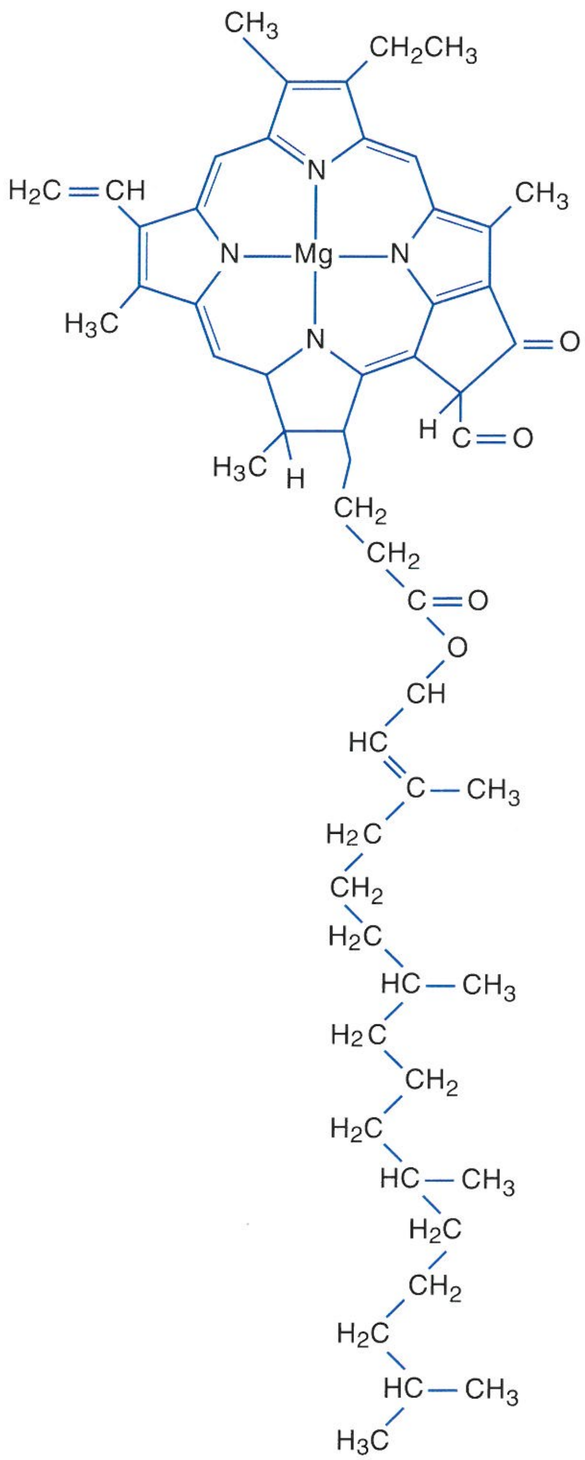
Manganese is at the heart of a 'biological clock' that, with every four ticks, enables plants to transform two captive water molecules into a molecule of oxygen and the equivalent of two molecules of hydrogen.

We now come to the heart of photosynthesis. Dr Wydrzynski points out that splitting two molecules of water and liberating oxygen calls for stripping away four electrons and the investment of a hefty amount of energy — about 5 electron-volts. A fundamental limitation in photosynthesis is that the individual photons captured by chlorophyll molecules carry not more than 1.8 eV.

To compound this difficulty, the photosynthetic pathway can only deal with one electron at a time.

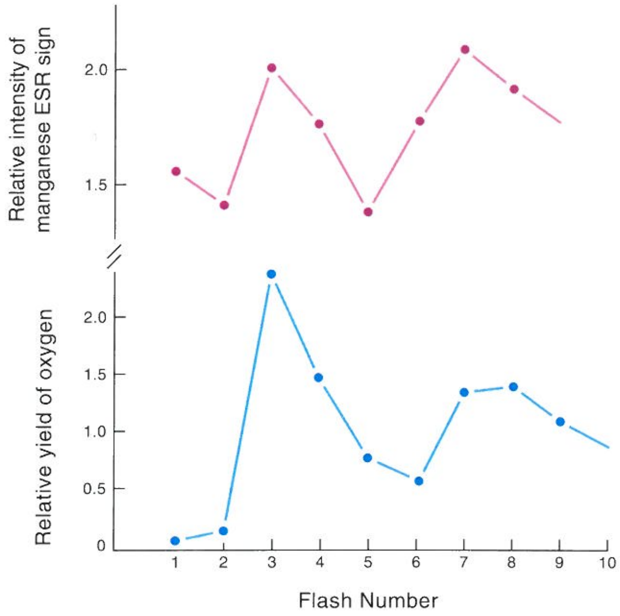
Plants have solved this monumental problem by devising a scheme in which the charge and energy

Chlorophyll-*a* — the light-harvesting molecule



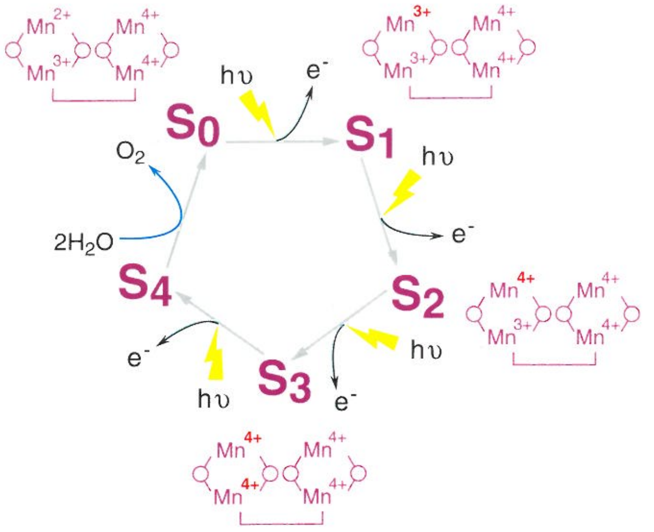
With their magnesium-centred head and carbon-chain tail, chlorophyll molecules are designed to capture light energy. Each photosynthetic unit takes in energy from about 200 chlorophyll molecules.

Ticking of the photosynthetic clock



When a spinach sample is illuminated with brief flashes of light, oxygen yield shows a peak every fourth flash starting with the third flash in the sequence (lower). The oscillations die out due to 'double hits' and 'misses'. Strongly implicating manganese in the cycle, ESR spectroscopy shows that the characteristic manganese II signal follows a parallel course (top).

The manganese clock



One by one, photons progressively build up a charge within the oxygen-evolving centre of Photosystem II until there is enough energy to strip, in one fell swoop, four electrons from two water molecules to produce an oxygen molecule. The five-step process is known as the 'water-oxidising clock' or 'Kok's clock' after its initial propounder.

supplied by photons is progressively stored until enough has assimilated to do the job. Photosystem II therefore centres around a kind of tiny battery, which stores energy by separating positive and negative charges on either side of the thylakoid membrane.

Indeed, we find that plants draw on the energy of four successive photons in a remarkable process involving a 'manganese clock', often called 'Kok's clock' after the researcher who first proposed it in 1970. Although Kok didn't realise it at the time, the mainspring of this life-bestowing clock is a certain aggregation — where arrangement is still uncertain — of four manganese atoms.

Mangania is the Greek word for magic, an appropriate name for the 'biggest black box in photosynthesis', as Dr Wydrzynski describes the manganese connection. Somehow, the four manganese atoms at the very heart of the catalytic reaction are able to consecutively store one, two, three, and finally four electrons generated by chlorophyll-captured photons and then, like a miniature lightning bolt, zap two captive water molecules to free an oxygen molecule. The clock is then reset to zero, and the process starts over again.

Clues to the existence of the clock date back a number of decades. The best evidence for it are measurements made of oxygen yield from leaves in response to very brief flashes of light. Experiments show that no oxygen is released after the first flash, and very little after the second. Most oxygen is evolved with the third, and thereafter the yield oscillates with a period of four as shown in the bottom half of the above graph, with peaks on flashes seven, eleven, etc., until the oscillations die out.

To account for this periodic behaviour, Kok assumed that each 'oxygen-evolving centre' cycles through five different states, S_0 to S_4, as shown in the manganese clock diagram (above). To explain the peak on the third flash, Kok postulated that the most stable state, the one that predominates in the dark, is the S_1 state.

The damping out of the oscillations, an ever-present feature, can be attributed to the occurrence of 'double hits' (when a flash contains two effective photons) and 'misses' (when it contains none), processes which together desynchronise the 100 000 clocks typically present in a square millimetre of leaf.

In 1980, Dr Wydrzynski was able to strongly implicate manganese as the key cogwheel in the clockwork. Manganese had long been known as an essential trace element for plant's photosynthetic activity — in the same way as we need traces of it to maintain our health. He and Dr Kenneth Sauer were the first to show that manganese chemically extracted from chloroplast samples (as measured by ESR spectroscopy at room temperature) oscillated under illumination with brief flashes from a xenon flash lamp. In the same way as with oxygen yield measurements, a peak occurred on every fourth flash, and this suggested that the oxidation state of manganese was going through a four-phase cycle.

One of the exceptional properties of manganese is that it can assume a wide range of oxidation states, so manganese compounds exist in which anything from Mn^{2+} to Mn^{7+} can be found. This makes the element perfect as a charge accumulator, or 'battery'.

Chemical analysis shows that there are actually four manganese atoms for every reaction centre, and a loss of 25% of the manganese will stop oxygen evolution. Much work and speculation has been devoted to finding out the specific arrangement of the four manganese atoms. For example, it could be a four-atom complex (a tetramer) or two two-atom complexes (a dimer of dimers), or perhaps some other structure.

Dr Wydrzynski has been chasing the magic of manganese for nearly 20 years. Recently, he has teamed up with Dr Pace of the Department of Chemistry at ANU, another researcher devoted to elucidating the mystery of photosynthesis, and together they have turned a newly acquired tool — a Bruker ESP 300E electron spin resonance spectrometer — to the task of unravelling the behaviour of manganese in photosynthesis.

'Photosynthesis and ESR spectroscopy are simply made for each other,' says Dr Wydrzynski. 'ESR detects the energy states associated with unpaired electrons, just the situation you find in photosynthesis which is distinctively a one-electron process. This situation is in marked contrast to most chemical reactions, where paired electrons shuttle around.'

'Of the 20 or more sequential steps between manganese and NADP, most can be followed using ESR spectroscopy.'

When a leaf sample is placed between the poles of this instrument's magnets, and microwave energy is applied, the chemical environment of each of the unpaired electrons can be probed. By applying brief light flashes, different S-states can be produced, and when the sample's temperature is lowered to liquid-helium temperatures (-269°C), particular states can be frozen in and examined in detail.

The new ESR spectrometer, with its 10-fold improvement in sensitivity over the ANU's existing ESR apparatus, has recently provided evidence that the charge accumulator is actually a single pair of manganese atoms, just as the earliest models proposed. Apparently, the other two manganese atoms perform a lesser role, more as spectators than as active participants. This is indicated in the manganese clock model on the previous page.

The ESR spectrometer is proving its mettle, giving the researchers detailed insights into other aspects of photosynthetic mechanisms.

'Photosynthesis is an exquisite amalgam of physics, chemistry, and biology,' says Dr Wydrzynski, 'With the right tools — and a little insight — we are getting closer to understanding this fundamental process, which underlies nearly all the life on this planet.'

Andrew Bell

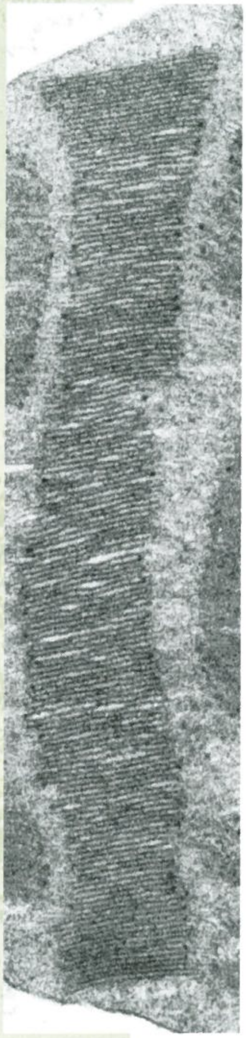
If you want to know more

- Current perceptions of Photosystem II. Ö. Hansson and T. Wydrzynski. *Photosynthesis Research*, 1990, **23**, 131–162.
- How plants make oxygen. Govindjee and W.J. Coleman. *Scientific American* 1990, **262**, 49–58.
- You are welcome to contact Dr Wydrzynski via the address and phone numbers on page 2.

Dr Tom Wydrzynski.

Photoinhibition

In the early days of spring, most of us will have been tempted to give the house plants a good dose of sunshine and fresh air following the dull days of winter indoors. Most of us do it only once, however, because our good intentions usually mean we end up having to buy another plant.



A massive thylakiod stack – or grana – in an *Alocasia* chloroplast. Plants' light harvesting machinery, concentrated in this stack, maximises photosynthetic capacity on the shady forest floor but is a crippling disability in brighter light. The molecular composition of a thylakiod membrane is shown on page 4.

Early researchers referred to the yellowing and death of leaves following exposure to too much light as solarisation. Today the term photoinhibition is used to describe this phenomenon, this term is usually attributed to Bessel Kok, a pioneer in the study of photosynthesis, who was born in Holland in 1893. Photoinhibition means the light-dependent inhibition of photosynthesis — in other words, too much light can actually cause the efficiency of photosynthesis to fall.

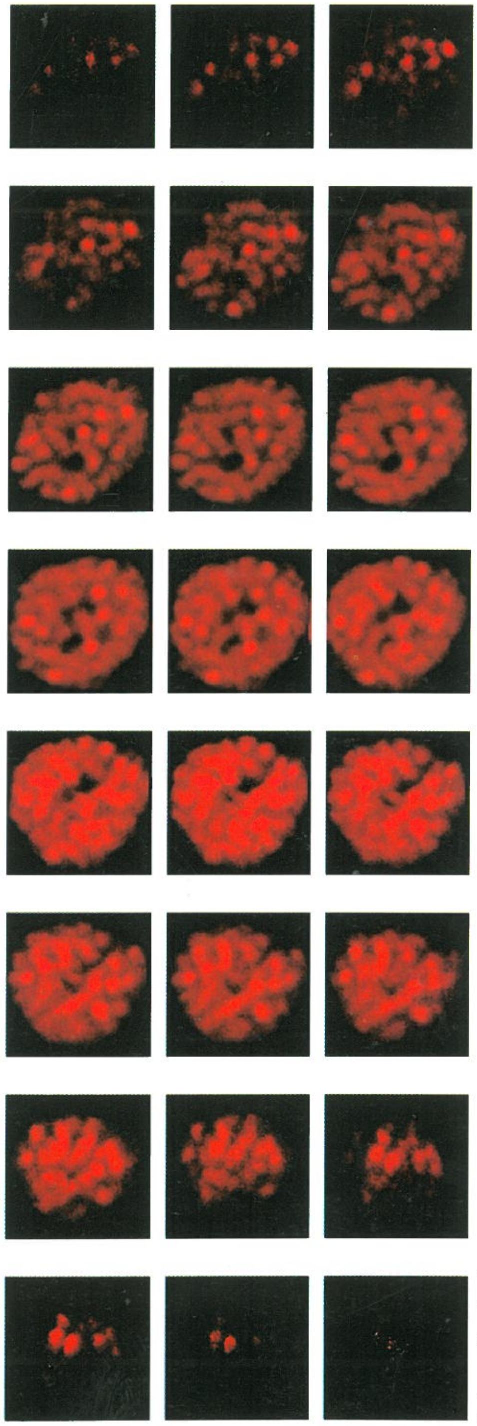
The leaves of a house plant grow slowly because they spend most of their life in weak light, barely able to make enough sugar during photosynthesis to balance the respiration that takes place to keep the plant alive. The leaves of the house plant, living in fairly dim light, adjust (or acclimate) their photosynthetic apparatus to harvest the maximum amount of light possible, at the expense of their capacity to utilise bright light. We do not do them a favour by giving them a good dose of sunshine. Indeed, more than a few hours can be deadly, and will literally fry their photosynthetic apparatus.

Some of the changes that accompany acclimation to weak light can be seen in the membranes of the chloroplast when examined with an electron microscope. The membranes that contain the light harvesting and energy transducing machinery of photosynthesis (the antennae, photosystems I and II and the electron transport pathway between the photosystems) are called thylakoids (see diagram page 4). In shade-acclimated house plants, thylakoids maximise their light harvesting capacity by stacking, like a pile of pancakes, in huge assemblies called grana. In the Queensland rainforest shade plant *Alocasia,* a hundred or more thylakoids may stack on top of each other. We are not entirely sure how this stacking increases the efficiency of light capture, but it is clear that strong light quickly fries the granal stacks. The D1 protein, one of the proteins that hold together the core chlorophylls of the photosystem II reaction centre, is especially vulnerable to excess light. Research by the RSBS team in collaboration with Dr. Jan Anderson and Dr. Fred Chow at CSIRO and Dr Christa Critchley at the University of Queensland, showed that D1 is metabolised more rapidly in strong light, and is destroyed in severe photoinhibition. Photosystem II is restricted to the granal stacks, and its proteins, including D1, can only be repaired when exposed to soluble enzymes in the thin connecting thylakoids between the granal stacks. We think that the huge granal stacks might make it difficult for the D1 protein to have access to repair mechanisms.

With limited capacity to use the excess energy in strong light, as well as limited capacity to repair the damage done by the excess energy, shade plants can quickly lose all their photosynthetic functions. The photosystems are the first to be inactivated, and ultimately all light harvesting ceases. Fortunately, this chronic photoinhibition is not a common event, except among domesticated house plants. Research by Jenny Watling, a collaborative research scholar from James Cook University working at RSBS, shows that the normal sunflecks which move across the rainforest floor do not cause photoinhibition. They seem to be neither sufficiently intense, nor prolonged to cause damage. About the only equivalents in nature to our common abuse of house plants are found in tree-fall gaps in rainforests where many forest floor species succumb to photoinhibition.

Plants acclimated to growth in bright light invest less in the light-harvesting parts of the photosynthetic apparatus, and more in the energy-utilising reaction. Because of this they have small granal stacks, and can switch on rapid repair processes which minimise chronic photoinhibition.

Roger Beckman



Professor Brian Gunning

Sections down through a chloroplast of *Alocasia* imaged by the fluorescence of chlorophyll in grana, as revealed by confocal microscopy. The piles of granal membranes in the stacks can be traced from one frame to the next.

SLIP, SLOP, SLAP

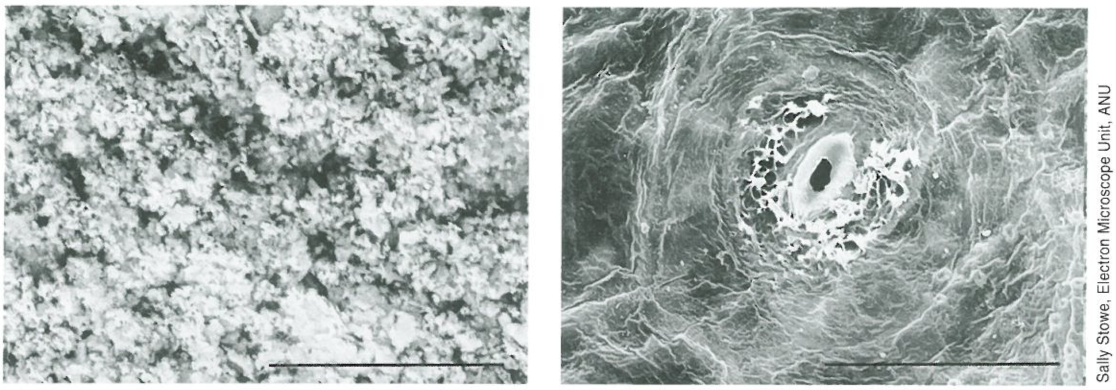
On a sunny day outside, we can slip into the shade or, failing that, we can don sunglasses, suncream and hat. At first sight, it seems that plants can use none of these options, but closer study shows that plants are more able to protect themselves than it first appears.

Consider this: the angle of a leaf or an entire frond can slowly change, so that it hangs vertically rather than horizontally and thereby intercepts less light (this accounts for the rather thin shade offered by many eucalypts). In some plants, the leaves can roll up so as to cut down the amount of light intercepted. The chloroplasts (light absorbing structures) within a cell are mobile, and may move to the less exposed part of the cell, so reducing their exposure to light. The number of chloroplasts within cells can also change with time, increasing or decreasing in response to light levels. Biologists call such mechanisms photoprotection, as they protect plants against too much light. Other photoprotective mechanisms are less obvious, and involve biochemistry within the photosynthetic cells (this is called internal photoprotection).

But a more obvious way of protecting the photosynthetic apparatus against the damaging effects of too much light is simply to put some protection on the leaf, for example, a reflective coating. A shiny surface, a covering of fine hairs, or crusts of salt exuded by the leaf may all help to reduce the amount of light reaching the photosynthetic tissues. Dr Sharon Robinson and Dr Cath Lovelock, plant physiologists at RSBS, wondered whether the shiny wax on the leaves of a desert-dwelling plant was the plant's equivalent of total-block zinc cream. If the wax were removed, what would happen? Would the plant suffer from photoinhibition — the inhibition of photosynthesis as a result of too much light? In other words, was the plant adapted to tolerate only as much light as could penetrate its coating?

Cotyledon orbiculata, is a succulent plant from Africa which has thick, fleshy leaves. This property makes biochemical analysis of different layers of the leaf much easier. The plant is adapted to life in a sunny, fairly dry environment and, like the prickly pear cactus described in the last issue of *Biologic*, uses the water-conserving CAM pathway of photosynthesis. The greyish-green leaves of *C. orbiculata* have a reflectivity of about 60% — that is, they reflect 60% of the light falling on them, absorbing only 40%. All leaves reflect some light, but the high reflectance of *C. orbiculata* is due to its shiny wax coating. It's quite straight-forward to remove the wax without damaging the leaf — you simply brush the stuff off — and that's just what Dr Robinson and her colleagues did. They wanted to find out if the wax was protecting the plant from photoinhibition and to discover what effect exposure to extra light would have on leaves normally protected. A related species called

Cotyledon orbiculata grown at 50% and 9% of normal light. The plant on the left produced copious amounts of wax while the plant on the right – grown at 9% light – produced almost none.



Electron micrograph of the waxy *C. orbiculata* leaf surface (top) and of a brushed *C. orbiculata* leaf surface. New wax seems to be secreted from pores around the stomata.

Cotyledon paniculata does not form wax. It, therefore, acted as a useful comparison. Presumably, it had other ways of dealing with excess light — internal ways, as we will see.

Sun and shade

Plant physiologists have long known that certain plants are better adapted to bright sunshine while others are more successful in the shade. This difference comes about from differing biochemistry within the leaf. The rate of photosynthesis is often limited by insufficient light, however, beyond a certain point, more light will not increase photosynthesis. This saturation point is higher in sun plants than in shade plants. Sun plants have internal ways of protecting themselves against damage caused by photoinhibition — when the light gets too bright. One such mechanism is the xanthophyll cycle, where the yellow pigments zeaxanthin and possibly antheraxanthin are thought to short-circuit light absorption and dissipate excess light energy as heat (we can think of the pigments' protection as being like reactolite sunglasses — those glasses that change their darkness according to the brightness of the light, and thus match their protection to the exposure level). Analysis by Sharon Robinson and her colleagues showed that *C. paniculata* (the succulent without wax), whose leaves have low reflectivity, has a higher concentration of xanthophyll cycle pigments than its more waxy relative, and that it concentrates these pigments towards the exposed surfaces of the leaf. *C. paniculata* is definitely a sun-loving plant — it has to be, living without 'zinc cream' in a sunny environment.

C. orbiculata was found to be different. With the wax gone, and exposed to the same light intensity as *C. paniculata*, the fluorescence characteristics of the de-waxed leaves changed (fluorescence from chlorophyll, described on the next page, is a simple and non-destructive way to gauge the general 'state of health' of a plant's photosynthetic apparatus). The chlorophyll fluorescence data indicated that the plant was suffering a reduction in the efficiency of photosynthesis as a result of too much light. Analysis of de-waxed leaves after 12 days showed that the pool of xanthophyll cycle pigments, particularly zeaxanthin, had increased. Deprived of zinc cream, the plants were making use of their sunglasses.

The thickness of the succulent's leaves allowed the scientists to examine the possibility of pigment gradients within the leaf. As expected, the photoprotective xanthophyll pigments were at their lowest concentration in the middle layers, but after removal of the wax, concentration of pigment increased here. In other words, the plant already used some of this photoprotection near the surface, but after losing its wax, it proceeded to increase its internal protection throughout the leaf, although the middle layers remained less stressed than the exposed outer layers. The leaves were clearly adapting to the loss of its external waxy protection from the sun.

Other biochemical measurements confirmed the idea that C. *orbiculata* was essentially a shade plant, able to grow in sunny environments mainly because of its external protection.

continued on page 14

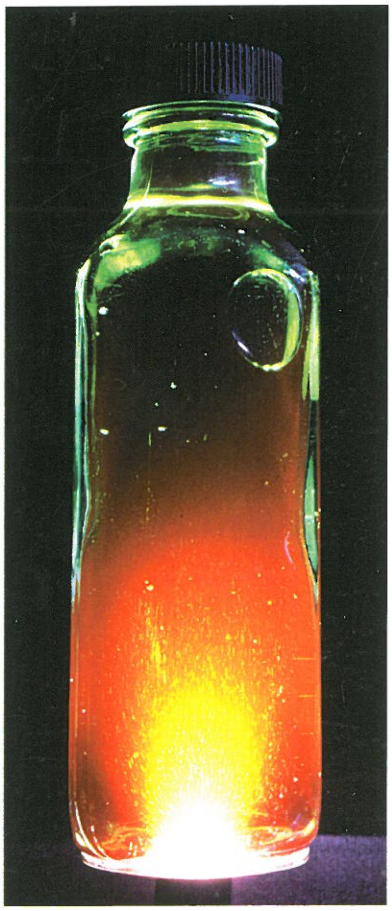
Finding out: The far-red glow of chlorophyll fluorescence

Absorption of blue and red light gives chlorophyll its characteristically brilliant green colour. Under different conditions, chlorophyll can also produce a deep red glow (see the photo on the right). In this instance the red light is fluorescence and its measurement from chlorophyll is rapidly becoming one of the most essential and widely used techniques for the study of photosynthesis.

Fluorescence is the re-emission of light, at a longer wavelength, from an illuminated substance. Not all substances fluoresce, chlorophyll however, does and this characteristic of chlorophyll allows us to calculate the state of a plant's photosynthesis. One of the advantages of this technique is that it is non-intrusive, consequently, scientists can now measure how light is being used by plants in a variety of situations.

When chlorophyll, isolated from leaves, is placed into solution and examined, it is possible to observe a deep red fluorescence. Isolated chlorophyll has a high level of fluorescence, as much as 30% of the light absorbed is re-emitted as far-red fluorescence. This is the result of damage to the complex apparatus of photosynthesis during the purification of chlorophyll from the leaf. Normally, that is when still in the leaf, chlorophyll makes use of the light, rather than re-emitting it as fluorescence.

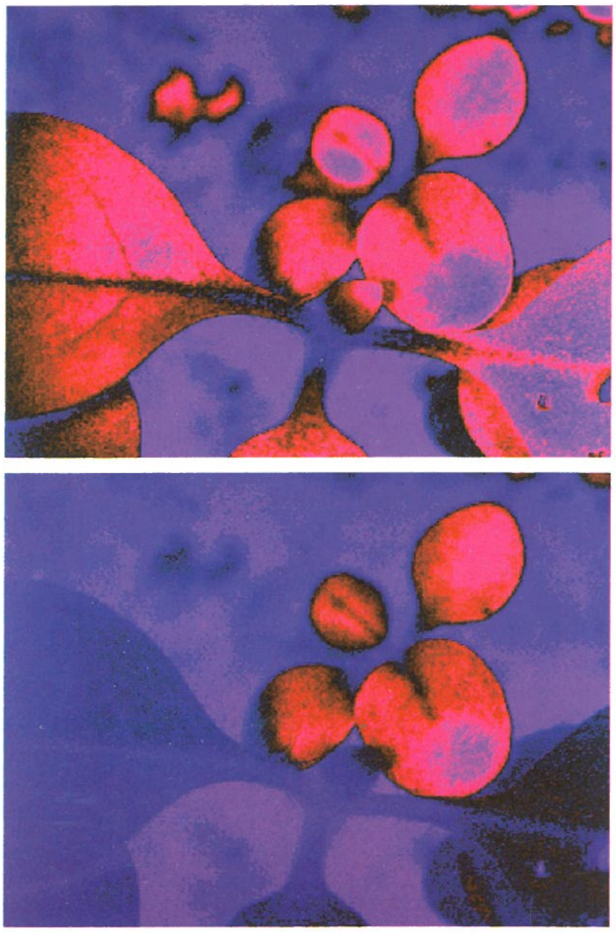
In a functioning leaf, chlorophyll complexes with many different proteins. These complexes are located within thylakoid membranes. The function of this intricate structure is to allow a greater efficiency of light harvesting and energy transfer. In this natural state, as little as about 1% of absorbed light energy escapes as fluorescence. In general terms, the yield of fluorescence is high when photosynthesis is low. There are a variety of instances when scientists may want to measure chlorophyll fluorescence. For example, some potent herbicides rapidly impair photosynthesis, and leaves sprayed with these herbicides show sustained high levels of fluorescence.



The fluorescent red glow of chlorophyll

Gathering of the Gurus

Chlorophyll Fluorescence at work.



In these video based images, chlorophyll fluorescence (red) is seen to reduce quickly in healthy plants. In genitically engineered plants with reduced electron transport, a high level of chlorophyll fluorescence is maintained.

Four experts on chlorophyll fluorescence; left to right: Professors Govindgee (USA), Schreiber (Germany) and Doctors Björkman and Falkowski (USA).

The Research School plays a major role in keeping Australian research in the front line, internationally, and in serving as a conduit between researchers overseas and those in Australia. The experts above were among more than a dozen research leaders from overseas brought to Canberra for the recent Robertson Symposium on "Chlorophyll Fluorescence: Origins, Measurement, Interpretation and Applications". The Symposium was attended by over 100 other researchers from around Australia.

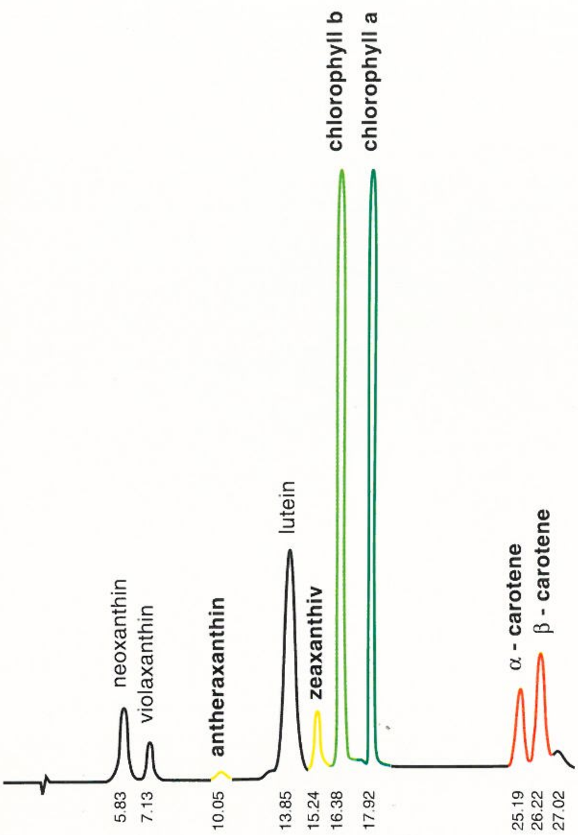
Researchers in the Molecular Plant Physiology Group in RSBS are specifically reducing levels of some of the electron transport proteins in chloroplast membranes by genetic engineering. They then screen trays of transformed plants with a video camera to observe fluorescence. In healthy plants chlorophyll fluorescence declines quickly as electron transport begins. In the successfully engineered plants, fluorescence stays high.

These days researchers routinely use chlorophyll fluorescence to diagnose how plants, such as Cotyledon, suffer after removal of reflective waxes or whether Eucalyptus seedlings will survive in bright light after cold nights (see article on page 15). We look forward to the day when this wonderful method of remotely sensing the photosynthetic wellbeing of plants may be used to estimate productivity in field crops, to give early warning of virus infections in a plant nursery, or perhaps even allow us to integrate ecosystem productivity from space.

Kautsky, a German researcher, was among the first to use fluorescence as a quantitative measurement, in the 1930's. With the advent of new equipment the technique has been further developed using sensitive photomultipliers, photodiodes and video cameras, fitted with the appropriate filters. This allows scientists to measure fluorescence more precisely. Most recently, state of the art equipment for measuring fluorescent emissions from leaves has arrived from Germany. The PAM 2000 is a lightweight and portable instrument, which allows large scale sampling of plants in the field.

Interpretation of details remains a controversial business and experts from all over the world gathered at RSBS at the end of May to debate these issues. Several experts and instrument makers attended a practical workshop, with much interest displayed in the development of simple instruments for classroom use.

Finding out:
Looking at plant pigments



Plant pigments can be extracted with acetone and analysed by high pressure liquid chromatography (HPLC). The above HPLC chromatogram shows the pigments found in a typical shade plant, *Tradescantia*. Chlorophyll a and b are the most abundant pigments and are responsible for the green colour of leaves. The other pigments are called carotenoids and are mainly yellow or orange, but in leaves their colour is masked by chlorophyll. When chlorophyll is absent, for instance, in some variegated leaves and in senescing autumnal leaves we can see the orange and yellow colour of the carotenoids. Some of the carotenoids, in particular antheraxanthin and zeaxanthin (highlighted in yellow), are thought to be involved in photoprotection. The most polar carotenoids are α-carotene and β-carotene, these are orange in colour and are found in carrots, hence the name.

continued from page 11

Slapping on the reflective wax in *C. orbiculata* is a slow process, which has not yet been fully investigated. What we do know is that leaves developing under low light intensity produce much less wax than those growing under bright light. Once leaves are mature, however, they cannot change their wax production schedule. It seems that there is a 'window' during development — a critical period during which the leaf becomes committed to producing a lot or a little wax, depending on the light, and thereafter its fate is . . . well, sealed.

Leaves of plants grown in high light for 20 weeks produced plenty of wax and had a high reflectance of 52%. Those grown in low light produced very little wax; their leaf reflectance was only 9%.

Electron micrographs show that the wax seems to be secreted from pores surrounding the guard cells of the stomata, as the picture on page 11 shows. Analysis of collected wax failed to find any specific UV-screening compounds. So the plant wasn't wearing light-absorbing 'sunscreen', but just a sun 'blocker' (like zinc cream), which reflects light across the board.

But the idea of actual UV protection (as opposed to general photoprotection) is worth considering. Like visible light, intense UV can harm plants' photosynthetic apparatus and, as with animals, can damage their DNA too. Some plants and marine algae do appear to produce UV-absorbing compounds. Terrestrial species growing at high altitude, especially in tropical and mid-temperate latitudes, will usually have to endure greater UV exposure. Such plants may use external protection as well as UV-blocking chemicals.

Rodger Beckman

If you want to know more

- Wax as a Mechanism for Protection against Photoinhibition – A Study of *Cotyledon orbiculata*. S.A. Robinson, C.E. Lovelock and C.B. Osmond. *Botanica Acta*. 1993, **106** (4), 307–312.
- Internal Gradients of Chlorophyll and Carotenoid Pigments in Relation to Photoprotection in Thick Leaves of Plants with Crassulacean Acid Metabolism. S.A. Robinson and C.B. Osmond. *Australian Journal of Plant Physiology*, 1994, **21**.

Dr Sharon Robinson

Protection against sunburn for Australian trees — an important step when regreening Australia

Jenny Butterworth

Even Snow Gums suffer sunburn

The idea that sunlight is detrimental to the growth of seedlings may seem absurd — especially to the sunbronzed Aussies populating this sunburnt county. However, basic research led by ecologist/ecophysiologist Dr Marilyn Ball, has shown that prolonged exposure to winter sunshine is damaging for snow gum seedlings.

Too much light can hinder photosynthesis in stressed plants, a phenomenon known as photoinhibition. Many different environmental factors may stress plants, too much salt, lack of nutrients and, in the case of the snow gum *Eucalyptus pauciflora*, very cold conditions. Dr Ball and her colleagues have found that when a *Eucalyptus pauciflora* seedling suffers from cold-induced photoinhibition during winter, its growth remains stunted during the warmer spring months. The findings from this basic research are important for efforts to regenerate areas of Australia and show that such efforts could be more successful if steps were taken to ensure that the seedlings were not at risk of photoinhibition.

Photoinhibition — when light stunts growth

Photosynthesis has been studied at the RSBS for over 20 years and the phenomenon of photoinhibition has been well described by biochemists within the school. Briefly, photosynthesis is comprised of two sets of reactions: 1) the light reactions where photons are harvested in the chloroplast to generate energy for electron transfer; and 2) the dark reactions where this energy is used for carbon fixation (for more detail on photosynthesis please see the previous articles).

Cold conditions and bright sunlight are the ingredients for cold-induced photoinhibition. In cold conditions the dark reactions slow down, as do most biochemical reactions. On a frosty morning, as long as the chloroplasts have not been damaged by frost, the light reactions proceed as normal to harvest photons. On the other hand, under these conditions, the dark reactions proceed too slowly to make use of all the harvested energy from the light reactions. Thus, in this situation, where disparity exists between the efficiencies of the light and dark reactions, there are too many excited electrons to be channelled into the slower carbon fixation reactions. This extra energy is dissipated from the leaf as heat, resulting in a net decrease in carbon fixation. In other words the quantum yield of photoinhibited plants is less than for plants grown under the same amount of light in warmer conditions. Chronic cold-induced photoinhibition will cause a reduction in net carbon gain.

Dr Ball was aware of cold-induced photoinhibition as it had been described by biochemists. Physiological studies of photoinhibition had suggested that species should be most vulnerable to photoinhibition at the limits of their distribution along climatic gradients and that seedlings should be more susceptible than established plants (since they do not have large carbon reservoirs). Thus, Dr Ball hypothesised, cold-induced photoinhibition may affect regeneration along climatic boundaries and timber lines.

A serendipitous finding

A series of experiments were conducted by Dr Ball, Honours student Ms Vicki Hodges and Dr Greg Laughlin (Geography Department, ANU) to investigate the natural regeneration of the snow gum *Eucalyptus pauciflora*, in the Orroral Valley, Namadji National Park, New South Wales. The snow gum is an evergreen, broad-leaved species which inhabits southeastern Australia across a wide range of elevations, from sea level to 1900m, but occurs mainly in areas of high rainfall where the mean temperature ranges from 5 to 8°C. Ms Hodges had noticed an unusual, asymmetrical pattern of seedling growth, where *E. pauciflora* seedlings grew best when located under the canopy and on the southern side of an established tree. Seedlings which were scattered outside these areas were not as healthy or as advanced in growth. Was this asymmetrical pattern of regeneration due to cold-induced photoinhibition?

Predicting the effects of cold-induced photoinhibition on the growth of snow gum seedlings requires an understanding of microclimate. Leaves in forest canopies experience a very different microclimate to leaves near the forest floor. The microclimate of the canopy correlates well with conditions in the surrounding atmosphere. The canopy acts as a blanket, protecting the forest floor from heat loss by radiation. Thus, on a clear

Figure 1a: The microclimate beneath a canopy is warmer than in the surrounding exposed areas, due to protection from heat loss by radiation.

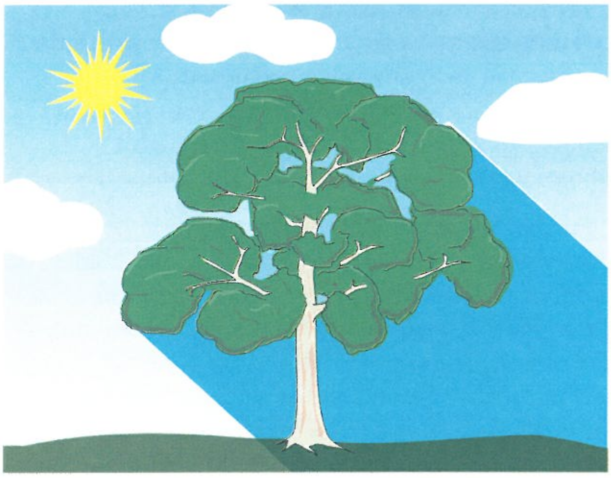


Figure 1b: The canopy provides protection from intense exposure to winter sunlight on the southern side of adult trees.

Figure 1c: The combination of these two factors creates a regeneration niche for seedlings and is consistent with the asymmetry of seedling distribution found around adult trees in the field.

winter night, the canopy may be exposed to freezing conditions, whereas air temperatures near the forest floor can remains above zero. Once a forest is cleared, seedlings are no longer protected from combinations of low temperatures and high irradiance.

By monitoring leaf temperatures during winter Ms Hodges found that exposed seedlings experienced freezing night time temperatures even when the air temperature remained above freezing. As successful seedlings in the Orroral Valley were found mostly under the canopy of mature trees, it follows that warmer conditions under the canopy are conducive to seedling establishment.

So far in this discussion of the regeneration of snow gum seedling in the Orroral Valley, we have neglected the second ingredient of cold-induced photoinhibition — light. If exposure to extreme cold were the single cause of the lack of regeneration of seedlings in exposed areas, we would expect seedlings to be distributed randomly beneath the canopy. Instead the healthy seedlings occur in an asymmetrical pattern, clustered beneath the southern side of the mature tree's canopy.

During winter months, the southern side of a mature tree is sheltered from morning sunlight (see figure 1). Seedlings growing in the open experience full sunlight irradiance all day long. We may think that full sunlight would be ideal for seedling growth, but that is not the 'natural' condition. In a forest, a seedling usually establishes itself in a small clearing. Such sites are protected from morning irradiance by the surrounding vegetation. Thus, seedlings in this natural setting are protected from photoinhibition by being shielded from prolonged exposure to sunlight, especially in the morning, when they are most vulnerable.

Another striking example of the way in which seedling distribution patterns are in accordance with cold-induced photoinhibition, was found by Dr Ball, Mr Jack Egerton and Dr Matt McGlone (Landcare Research New Zealand) in New Zealand (see figure 2). On the Waimakariri floodplain, two thirds of the seedlings of mountain beech trees are situated within a tight south-west segment of the parent tree's canopy (165°S to 285°W), despite the prevailing winds blowing in a direction likely to distribute the fewest seedlings to that region!

Figure 2a: Distribution of mountain beech tree seedlings around an adult tree in the Waimakariri floodplain, New Zealand.

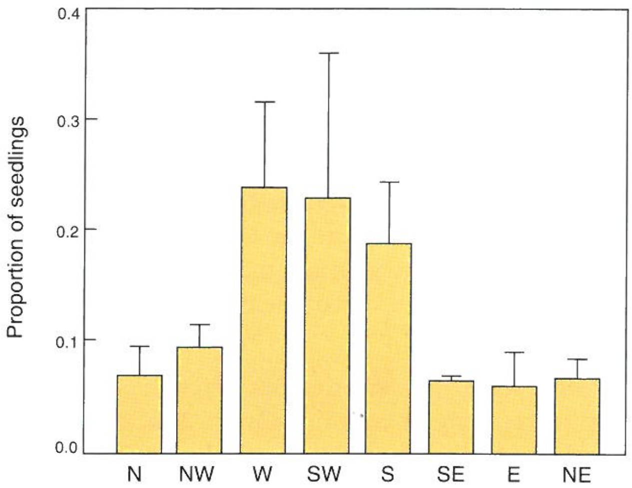


Figure 2b: Relative distribution of seedlings in relation to geographical bearing around adult trees in the Waimakariri floodplain.

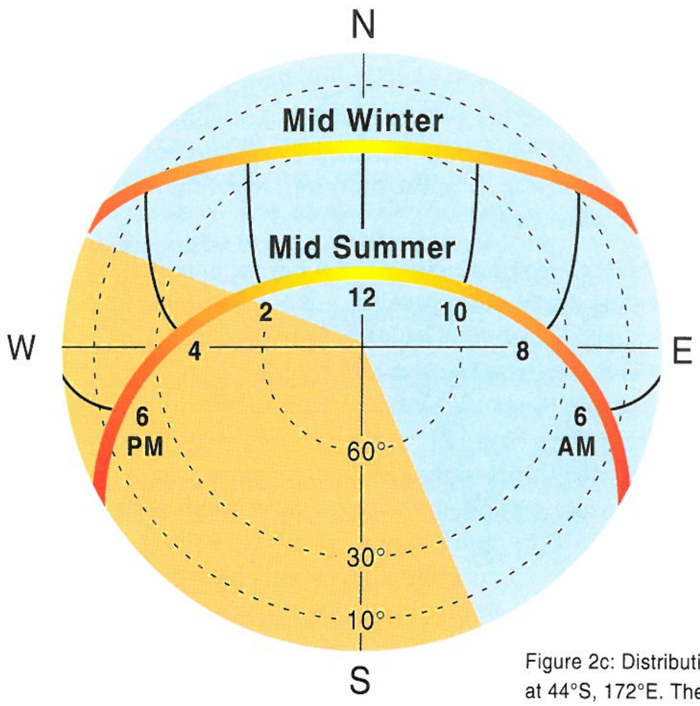


Figure 2c: Distribution of seedlings in relation to the sun path in mid-summer and mid-winter at 44°S, 172°E. The concentric circles indicate the angle of the sun above the horizon. Summer and winter sun paths are indicated by bold lines running east-west. These two sun paths are connected by lines to show the seasonal changes in the sun's position during the day. The shaded area from 165°S to 285°W is the regeneration niche where 65% of seedlings in the Waimakariri floodplain occur.

Protection against photoinhibition

There is a clear need to replicate natural conditions in reafforestation and commercial planting to maximise seedling growth. Work by another Honours student Mr Chris Holly, investigated the use of sun shelters to mimic the microenvironment experienced by a tree growing in a gap in the forest. Use of an open topped cylinder, constructed from shade-cloth allowed natural irradiance conditions for seedlings. In winter the shelters protected the seedlings from high irradiance in the morning.

In a telling experiment, Mr Holly planted *Eucalyptus polyanthemos* seedlings in an open pasture where they were covered by shelters constructed of one of five materials — translucent white plastic, shadecloth that transmitted 30, 50 or 70% incident sunlight, open wire or nothing. In this experiment the major difference between treatments was the level of irradiance to which the seedlings in each group were exposed. All of the seedlings grew slowly over winter, with the least amount of growth occurring in chronically photoinhibited seedlings.

Further studies by PhD student Jenny Butterworth, isolated the effects of winter cold-induced photoinhibition upon spring growth. *Eucalyptus pauciflora* seedlings were grown under different light regimes (100 and 50% sunlight) during winter. The shelters were then removed from all the seedling groups in spring. She found that the seedling exposed to 100% sunlight over winter had 50% less growth in spring than the sheltered seedlings.

Grass makes seedlings cold

The establishment of many species of tree, including the snow gum *E. pauciflora*, is strongly retarded if the seedlings are surrounded by grass. This was previously thought to be due to direct competition between the grasses and seedlings. Dr Ball together with Mr Jack Egerton and Dr Ray Leuning (CSIRO Centre for Environmental Mechanics) have revealed that grasses make exposed areas an even more hostile environment for seedlings by increasing their susceptibility to cold-induced photoinhibition.

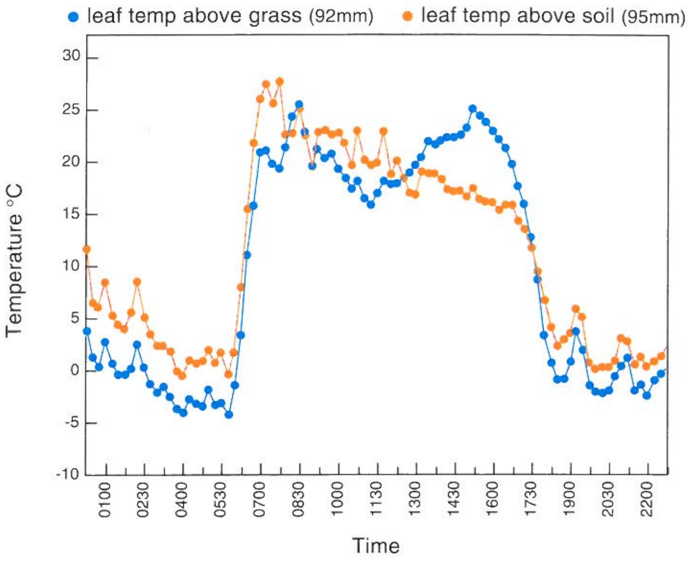


Figure 3: Leaves above grass experience more frequent and more severe frosts than leaves above soil as shown in this example of leaf temperature during two consecutive nights in early spring.

Grass changes the microclimate around a seedling. Leaves of a seedling, pushing up above the grassline can become much colder on a winter night than leaves of a seedling growing in bare ground. Grass has a very low thermal mass, so at night grass loses heat more rapidly than soil, thus, lowering the temperature and gathering frost. Therefore, seedlings growing in grass experience lower temperatures and are more prone to frost damage and subsequent photoinhibition.

Dr Ball and her colleagues have tested this theory in the field, at a farm in Bungendore, New South Wales. Here, *E. pauciflora* seedlings were grown in bare ground patches of various diameters (see figure 3). The seedlings in the largest patch of bare ground benefited as the mean minimum temperatures were up to 2°C warmer than in the smallest patches. Furthermore, the seedlings surrounded by grass were found to have less photosynthetic potential (Fv/Fm) over winter and showed less growth in early spring. The warmer plants had a 17% increase in growth in spring.

These studies show that reducing light in winter, in combination with warmer temperatures, yields the biggest improvement in growth in spring.

Future research

Dr Ball and her colleagues are excited by the prospect of collecting more data about photoinhibition, using a new state of the art instrument — the PAM 2000. This portable device measures fluorescent emission from a leaf. These measurements show how light is being used by a leaf, for example, whether it has a high light efficiency or if it is photoinhibited. That may not sound different from earlier experiments, but the PAM 2000 provides a multitude of advantages. It may be used in the field non-destructively whereas in earlier research, specimens — healthy looking leaves — were removed and their photosynthetic capacity was measured back at the laboratory. There the procedure, which was performed in a dark room, took many hours. As fluorescence measurements can now be made in a few seconds, a greater number and variety of leaves may be sampled.

The information from this new research will help to understand how the rate of photosynthesis is affected by different environmental conditions. Additionally, Dr Ball says that this work will provide insights into 'How functioning of leaves affects the organisation and growth of plants and ultimately the structure of vegetation'.

This basic research has been the result of a continuum of effort at RSBS, from the biochemists — who first described photoinhibition, to the physiologists — who characterised photoinhibition in intact leaves, to the ecologists — who are using this knowledge to interpret their field studies.

As Dr Ball points out, the asymmetrical patterns of seedling growth were there all the time; she explained quite rightly 'we see what we are ready to see.'

Sarah Vandermark

If you want to know more

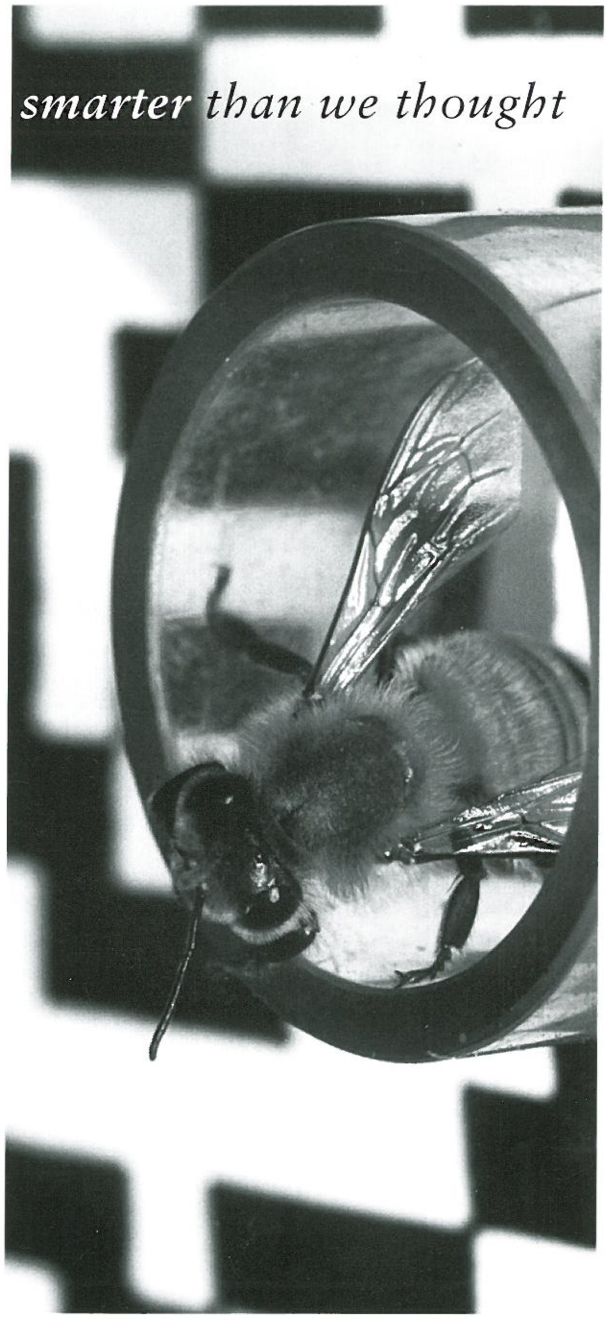
• The role of photoinhibition during tree seedling establishment at low temperatures. In: NR Barker and JR Bowyer, (1994). *Photoinhibition of Photosynthesis: from molecular mechanisms to the field.* Bios Scientific Publishers, Oxford UK.

• Cold-induced photoinhibition limits regeneration of snow gum at tree line. M.C. Ball, V.S. Hodges and G.P. Laughlin. *Functional Ecology,* 1991, **5**, 663–668.

Dr M. Ball

• You are welcome to contact Dr Ball via the address and phone numbers on page 2.

Bees:



Cunning experiments calling for bees to fly through a maze in search of a sweet reward have demonstrated that the humble bee, in its own way, possesses noteworthy astuteness

It seems as though these social creatures possess considerable powers of memory, intelligence, and — perhaps — even consciousness.

Experiments, conducted at the Research School of Biological Sciences and reported recently in *Nature* (24 March 1994), showed that bees can be taught to uncover camouflaged patterns just like we can. That is, bees can learn to recognise features of uncamouflaged objects — such as their shape — which later enables them find a camouflaged reward.

Unless they are first taught the correct shape of the object, bees fail to recognise it in its disguised form — just as we find it very difficult to find the Dalmatian in the accompanying figure (page 23) if we've never seen it before, but once we've found him it makes subsequent recognition easy. Somehow, our brains help our eyes pick out the lurking shapes.

According to the scientists who did the experiments, Dr Shao-wu Zhang and Professor Mandyam Srinivasan, bees' ability to interpret what they see in the light of prior experience suggestes that they possess a trait previously reserved for humans and other animals credited with 'higher' intelligence.

'Bees are smarter than we previously supposed' says Dr Zhang. 'They are capable of learning in more flexible and sophisticated ways than we thought.'

'Our earlier work has demonstrated that bees see in much the same way as humans. They even see the same visual illusions as we do (see *Biologic*, July 1993). Our new findings suggest that bees also interpret the world in a similarly intelligent way.'

The *Nature* paper shows that when bees fly through a maze in search of a reward (sugar water), they can learn 'sign-posts' offered to them by the experimenters to successfully navigate the maze. Later, even when the sign-posts are camouflaged, and invisible to naive bees, the experienced bees will recognise them and take the correct path.

'When bees use their prior experience from previous maze negotiation to help them solve fresh mazes, they are demonstrating an intelligent 'top down' approach to vision' says Dr Zhang.

In computer vision circles, 'top down' is used to describe the way in which a high-level device (like a brain or computer) exerts a downward influence so as to affect the way a low-level sensor (such as an eye or camera) processes information.

Normally, efforts in artificial vision have focussed on 'bottom up' processing, in which the influence percolates the other way. Many models of insect vision have used the bottom up approach, which sees insects as miniature robots responding automatically and inflexibly to their environment.

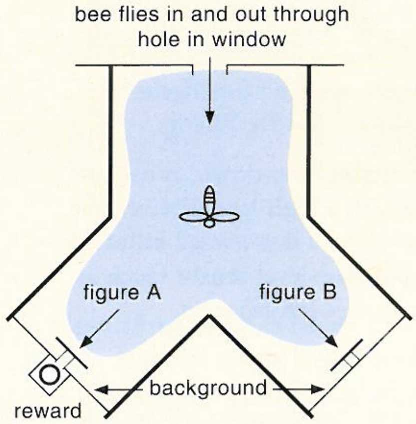
The latest work turns that belief on its head, and shows that bees possess something 'up there' in their 1 milligram brain that affects the way they see and respond to the world. The research has implications for understanding insect vision, a major interest of the Research School's Centre for Visual Sciences, which studies the way people, animals, and machines see.

A good way to get a feel for what the bee experiments entail is to consider what happens when we first encounter a pair of stereoscopic images, particularly the currently popular type that are computer-generated and hidden within a random swirling pattern.

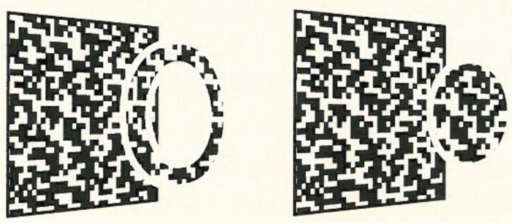
Looking intently at the patterns, we at first can't see a thing (apart from the pattern). This is akin to the situation the bees initially find themselves in when they are presented with camouflaged rings and circles — it's all a random checkerboard.

Finding out: What the experiments with bees involved

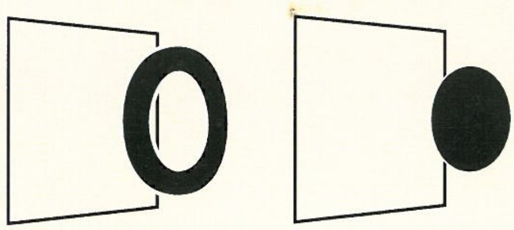
The experiments reported in the *Nature* paper involved observing which way bees flew into a Y-shaped maze. At the end of each arm of the of the maze was a figure, and behind one figure was a reward of sugar water. By learning the correct shape, the bees could fly straight to the reward.



When the figures in the two arms were camouflaged with a random checkerboard pattern, the bees failed to see the difference, and on repeated fly-ins, flew to the left and right arm equally often.



However, if the bees were first shown what the disguised figure looked like (with a reward only behind a plain uncamouflaged ring), then subsequently they could easily tell apart the camouflaged ring and circle (which they had previously failed to distinguish).



But, by applying ourselves to the task, and given a few tricks and hints, we are at last able to fuse the random pattern into a startling 3-D image. Our brain has instructed our eyes to work in a new way. We are not quite sure how we did it, but once we've achieved it, subsequent pictures can be fused quite easily.

The important point is that we use a top-down strategy, in which we consciously force lower processing stages to operate in different ways. It's extremely unlikely that if we just happened to come across the images, and we had no incentive, our eyes would automatically feed us the intended image — which is the bottom-up approach to vision.

On the one hand, the latest results could be explained by supposing that bees subconsciously memorise an image, or way of doing something, and somehow respond automatically and unconsciously to it in just the right way — that is, they use a middle-down approach.

A more interesting, and provocative, interpretation is that bees know what they are doing. That is, they consciously understand what they have learnt and so can readily apply it to novel situations. They employ a 'top-down' analysis in which the 'top' is a conscious awareness; furthermore they deliberately make an effort to direct that awareness so as to reinterpret the messages they are getting from their eyes.

Awareness, or consciousness is a faculty that many scientists are reluctant to attribute to animals, let alone insects. They are apt to view consciousness as a 'ghost in the machine', an entity that doesn't fit comfortably with mechanistic explanations of brain function.

And yet other scientists, a minority, see consciousness as essential in explaining how animals can execute complicated behavioural patterns.

For example, Konrad Lorenz relates how he observed a male jewel fish perform an 'absolutely astonishing' feat in which it had to decide what to do when, on its way back to the nest with a rounded-up stray young in its mouth, it couldn't resist taking a morsel of food into its mouth as well. 'If ever I have seen a fish think, it was in that moment!' Lorenz says. After seconds of hesitation, the fish resolved the conflict by spitting everything out, eating the morsel, and then retrieving the erring off-spring.

A foremost proponent of animal consciousness is Dr Donald Griffin, whose recent book (*Animal Minds*, Chicago University Press, 1992) makes a strong case for animals sharing this mysterious faculty that we tend to think is exclusively ours. A major thrust of Griffin's argument is that if we find consciousness of great survival value, then so too would animals. Like the outmoded concept that the earth is the centre of the universe, Griffin thinks we are being excessively anthropocentric thinking that humans possess a monopoly on consciousness.

Although Prof. Srinivasan is not inclined to enter debate on this issue, he acknowledges that consciousness cannot be excluded as an explanation of the results of his recent experiments. He doesn't want to attribute full-blown consciousness to bees (in the way children's story books do to every animal), but the latest experiments give a hint that bees may possess more than we thought. In any case, it is clear that bees are able to learn to use new ways to distinguish patterns. That is, they can learn to look at the world in new ways.

'Perhaps consciousness is only a matter of degree,' he says, 'and that there is a continuum from higher animals to the lowliest creatures, including bees.'

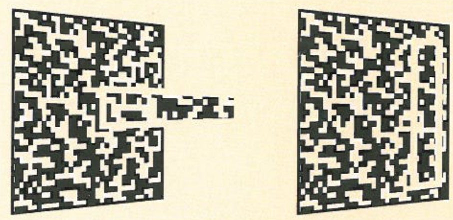
Andrew Bell

If you want to know more

Dr S.W. Zhang

- Prior experience enhances pattern discrimination in insect vision. S.W. Zhang and M.V. Srinivasan. *Nature*, 1994, **368**, 330–332.
- The world through the eyes of an insect. *Biologic*, July 1993.
- You are welcome to contact Dr Zhang via the address and phone numbers on page 2.

Moreover, once trained to distinguish ring and circle, they could also successfully tell apart other camouflaged shapes, like horizontal and vertical bars — a feat that bees not given the big hint could never get the hang of.



In other words, bees learn to break camouflage using prior experience — the same way we do. For example, the first time we see this picture we find it difficult to see the camouflaged dog

However, once we have found the Dalmatian, or he is pointed out to us, it becomes very easy for us to find him next time we see the picture. (If you are having trouble finding him, try turning the picture upsidedown.)

RESEARCH, NATIONAL PROSPERITY AND THE QUALITY OF LIFE

The Industry Commission is currently inquiring into research and development in Australia, including the role of the higher education sector. A key question it addresses is 'How should governments attempt to influence the amount and pattern of R&D innovation in Australia?'

That's a very pertinent and sensitive issue, given the widely acknowledged failure of Australian corporations to exploit our knowledge base.

In my submission to the inquiry, I argued that the present level of public investment in research, in generation of new knowledge in the higher education sector is about right, in relation to our expenditure in tertiary education. This is also true for the level of investment in the vital knowledge-transfer to industry that occurs through the Cooperative Research Centres (which embrace universities, CSIRO, and industry).

Sadly, however, the 1993 Science and Technology Budget Statement revealed that Australian business invests, proportionally four to five times less in R&D than do our principal competitors in Asia, America, and Europe. Here lies the nub. Business itself must hugely expand investment in R&D. We must resist the typical Australian responses of calling for more public investment (to take the risks on behalf of business), and demanding more management of public investment in research.

Australian business has to develop its own culture of R&D, of managed research for utilitarian objectives. In day to day terms, this is likely to be a very different culture from the traditional curiosity driven research that Australians do so well. For example, whereas careful management, and even secrecy, is essential to the development of a knowledge-based industry and its commercialisation, such tight controls are inimical to the process of generating and acquiring new knowledge — without which there would be no knowledge-based industry. As a science journalist recently commented ' we must be sure not to constrain basic science too much, or we will cease to discover anything we don't already know.' Furthermore, a large part of new knowledge has to remain in the public domain and be used by governments to regulate over-exuberant exploitation of other ideas by industry.

The essential dichotomy between basic research and R&D was recently made abundantly plain by the Royal Society of London in its response to the U.K. government's White Paper on funding of science and technology. It expressed the difference thus: 'In the final stages of a development project, major surprises are a problem; in basic research they are an exciting opportunity.'

It goes on to observe that 'If the White Paper turns out in practice to be saying that basic research should be managed like industrial development, the outcome will be not 'relevant' basic research but second-rate basic research. This would do nothing for national prosperity or the quality of life . . .'

'What the wealth-creating sector needs most [from the higher education institutions] are skilled people, advances in fundamental knowledge, and access to fundamental advances made elsewhere in the world. Attempts systematically to extract more immediate fruits [from them] in any quantity will imperil these other, unique, contributions.'

In a nut-shell, the Royal Society advises that we should 'avoid pointing universities towards short-term research into yesterday's problems.'

Professor David Craig, past-President of the Australian Academy of Science and former director of the Research School of Chemistry here at the ANU, has commented on the Australian R&D scene in the following evocative terms. 'Our masters want the golden egg, but prefer to do away with the goose.

'At best it must be a small, cheap and obedient goose, laying market-oriented eggs, which hatch into consumer goods.'

'You and I know that discoveries are not made to order. Luck, chance, accident, come into it all the time.'

'Officials try to squeeze out personal and institutional independence. That independence is what gives the 'climate' and the 'headroom' in which creativity appears.'

So the bottom line is that it makes no sense at all for economic planners to impose managerial principles on the conduct of university research. It makes a great deal of sense to build a culture of business R&D that can feed on the knowledge generated by university research. It makes no sense at all to build this culture at the expense of university research funding. This task is the business of business.

If business fails to build a culture of business R&D it may well, as P. P. McGuiness said earlier this year, 'undermine Australia's economic and intellectual independence in the next century'.

Barry Osmond