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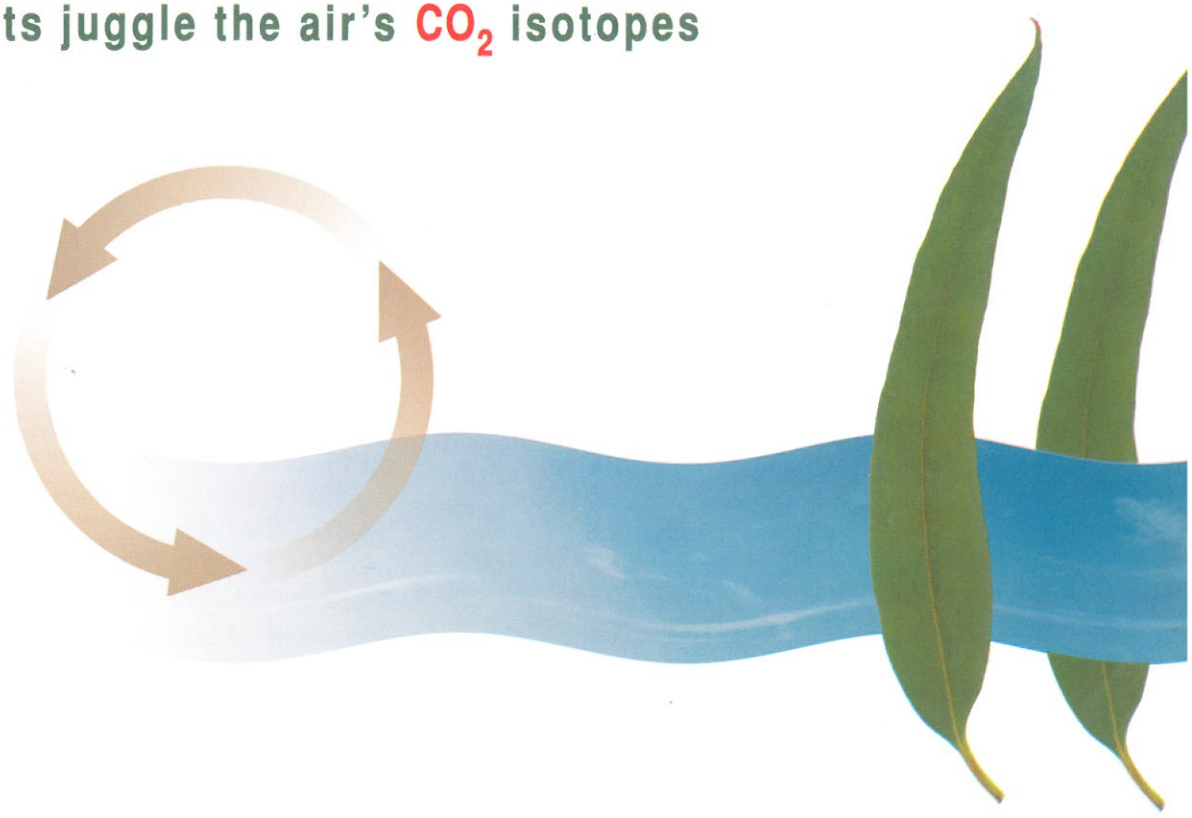
How plants juggle the air's CO_2 isotopes



Inside

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- **neurobiology**, especially the development and function of the visual system in insects and mammals, and its relation to hearing
- **genetics and molecular biology** of viruses, bacteria, fungi, *Drosophila* and other insects, and research on plant–microbe interactions

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- Research that sets the agenda, rather than that which follows the fashions
- Research that changes the way we think about biology, as judged by
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  - publication in leading review series
  - recognition of research leadership of RSBS faculty, as judged by election to learned societies, award of prizes, and other honours
  - success in competition for external funding in our chosen areas of research

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- RSBS does collaborative research with half of Australia's other universities (that is, 19 universities in 1992)
- RSBS collaborates in research and teaching with The Faculties of the Australian National University
- RSBS collaborates in research with CSIRO and other institutes

Internationally, RSBS serves as a major conduit to leading universities and research institutions

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Plants and CO_2 swap isotopic visiting cards

When wandering carbon dioxide molecules drift through stomata and into a leaf, most of the time they diffuse out again without being captured by the plant's photosynthetic machinery. And yet we now know that this seemingly casual encounter involves the exchange of isotopic visiting cards, and creates a tell-tale signature that should allow us to track down elusive CO_2 repositories.

Each year we burn 6 billion tonnes of carbon, accumulated from billions of years of photosynthesis, and liberate the resulting carbon dioxide into the air. Together with deforestation, the result is a fast rise in the concentration of the gas in the atmosphere and, worryingly, an impending global warming from the greenhouse effect.

Thankfully, there are one or more 'sinks' slowing down the rise. Careful measurements tell us that only $^1/_3$–$^1/_2$ of the carbon dioxide liberated accumulates in the atmosphere; the other half must be secreted away somewhere else. Most scientists accept that the oceans absorb some of the CO_2; the politically sensitive question is the role of the biosphere: are certain plant communities — tropical rainforests are often mentioned — playing a major part in soaking up the excess?

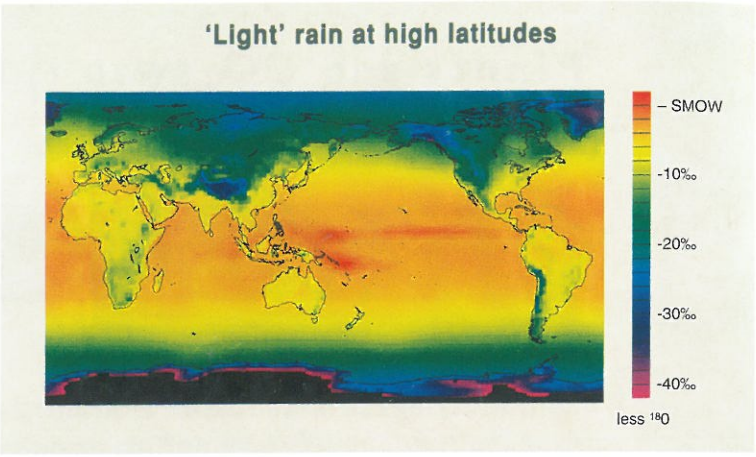
Clearly, it is important to identify the size and nature of these sponges, which buy us time as we work out how to cut back on fossil fuel combustion. Professor Graham Farquhar of the Research School of Biological Sciences and his collaborators believe they can pin-point these sinks by using measurements of the global distribution of a particular isotope — oxygen-18 — in carbon dioxide.

In a paper published in a recent issue of *Nature* (3 June), they present a precise mathematical model of how plants enrich (or deplete) this isotope, and back it up with experimental measurements. Unexpectedly, plants exert enormous power in modifying the oxygen-18 content of atmospheric carbon dioxide, and we should be able to use this fact — in addition to existing data on carbon isotopes — to locate regions where carbon is accumulating or dissipating.

The *Nature* paper provides an explanation for a startling anomaly first documented by atmospheric scientists in 1987: for every 1000 molecules of carbon dioxide loaded with an oxygen-18 atom which are detectable in the Southern Hemisphere, we find only about 998 in the Northern. At the time, the scientists — Francey of CSIRO, and Tans of the University of Colorado — suggested it could be associated with an asymmetry in Hemispheric plant cover, and speculated that leaves and atmospheric carbon dioxide exchange isotopes. The latest paper strongly confirms this idea and, in supplying a comprehensive set of equations, it allows us to calculate the size of regional effects and global balances.

Prof. Farquhar explains that plants don't initiate isotope-altering nuclear reactions; rather, they neatly perform a chemical sleight of hand. When plants open their stomata, molecules of carbon dioxide diffuse in. Some CO_2 molecules are heavier than others, in particular those containing the naturally occurring non-radioactive oxygen isotope, ^{18}O, which makes up only 0·2% of the total oxygen (most of the remainder comprises the common ^{16}O). Significantly, CO_2 molecules burdened with the heavier oxygen-18 diffuse more slowly into the leaf than do those containing oxygen-16.

And once inside the leaf, more isotope discrimination is in store. The CO_2 molecules dissolve in the leaf water, exchange oxygen atoms with those in the water, and, as likely as not, diffuse back out again. Now because the leaf transpires, leaf water must be slightly enriched in oxygen-18 (because lighter water molecules evaporate more readily than heavier ones). So when the atomic juggling is over, the exiting CO_2 molecule is likely to be heavier than when it wandered in.



How the oxygen-18 content of rain varies over the globe. Light water molecules (containing ^{16}O) in the oceans evaporate faster than heavy ones (with ^{18}O), resulting in rain depleted in ^{18}O. The bias largely depends on temperature — and hence latitude. Note that the well-mixed oceans have a nearly constant ^{18}O-content, which is taken as the reference point (Standard Mean Ocean Water = 0‰). Significantly, most of the green–blue regions are occupied by vegetated land in the Northern Hemisphere, but not so in the Southern.

A surprising fact is that this 'stomatal visiting' is frequent enough to support a two-in-a-thousand (2‰) deficit in the Northern Hemisphere despite vigorous atmospheric mixing. Prof. Farquhar estimates that each year approximately 300 000 million tonnes of carbon, as carbon dioxide, must fleetingly visit plants in this way.

What makes this huge figure possible is a ubiquitous plant enzyme called carbonic anhydrase, which speeds up the exchange process a million-fold. Without the enzyme, it takes about 20 seconds for carbon dioxide to exchange oxygen atoms with water. But with carbonic anhydrase powerfully catalysing the formation of carbonic acid, it permits incoming CO_2 molecules to dissolve, exchange oxygen atoms, and diffuse back out all in about a tenth of a second.

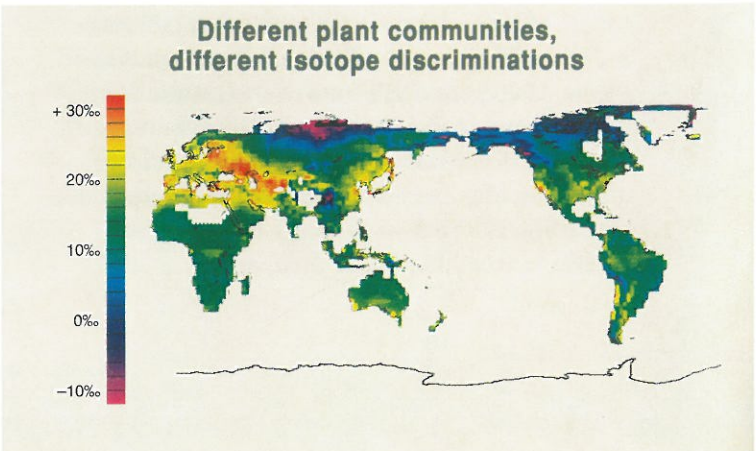
The ratio of $^{18}O/^{16}O$ in atmospheric carbon dioxide should reflect the relative abundance of these isotopes in the liquid water reservoirs with which the atmosphere makes most frequent and prolonged contact. The ratios found in oceans, cloud droplets, rain, and soil water vary, depending largely on the temperature at which evaporation and condensation take place.

Clearly, this means latitude will be a major factor, and comparing precipitation at the Equator with that at the Polar Circles, we see about a 20‰ enrichment in oxygen-18. Plants will take up this enriched water through their roots, following which more enrichment will take place at the leaves.

In effect, plants use their leaves to expose a very large surface area of water, derived from the soil, to carbon dioxide. Carbonic anhydrase so efficiently amplifies the exchange process that 1 sq. m of leaf can be as effective as hundreds of sq. m of open water. Due to the combined effects of fractionation from evaporation, and differential diffusion of light and heavy CO_2 molecules, atmospheric carbon dioxide differs isotopically from what it would be without the presence of plants. (To simplify matters, we ignore a constant difference in the proportion of oxygen-18 that always occurs in water and carbon dioxide, even after equilibrium has been reached. That is, ^{18}O is more attracted to CO_2 than to H_2O.)

In fact, the modellers' calculations tell us that plants must dominate the exchange process. Whereas the exchange time for carbon dioxide with the oceans is 8·3 years, the maintenance (against rapid atmospheric mixing) of the 2‰ deficit in the Northern Hemisphere means that the comparable figure for plants must be only 2·2 years.

Plants' biassing of isotope ratios in atmospheric carbon dioxide is not just a curiosity. Importantly, it lets us figure out, by working backwards from isotope imbalances, the power of the collective green machinery. And, since soil water in each latitude band has its own isotopic signature, we should be able to work out where the most plant activity is occurring. Is it the tropical rainforest, temperate forests, grasslands, or tropical savannas?



The effects of stomatal visiting on the isotope balance of CO_2 vary from one region to another. The highest depletions in ^{18}O (blue–magenta areas) occur near the Northern Polar Circle, due mainly to similarly depleted rain there. To obtain the total effect of plants on atmospheric CO_2, we need to add one other influence: that of plant respiration (the combination is shown in the diagram at the bottom of page 7).

Easier said than done, of course. We need a complete, accurate, mathematical model that takes us from soil water ratios, via physiology of leaves, to global balances. The *Nature* paper tackles this tall order, with expertise from Prof. Farquhar, Dr Jon Lloyd, Dr Chin Wong, and Dr Kerry Hubick, all from RSBS; Dr John Taylor, from the Centre for Resource and Environmental Studies at ANU; Assistant Prof. Lawrence Flanagan, from Carleton University, Ottawa; Prof. James Syvertsen, from the University of Florida; and Prof. James Ehleringer from the University of Utah.

This wide-ranging yet compact paper covers six major steps.

1. Soil water composition

We need to know the isotopic composition of water taken up through plant roots, and to this end the authors provide a map of how the isotopic composition of precipitation varies over the globe. This map, shown on page 4, was derived empirically by fitting observed data to an equation (labelled A in the box) involving mean annual temperature, precipitation rate, and elevation — all factors which, on theoretical grounds, control isotope fractionation.

As you can see, over most of the world's land mass, rain falls in the yellow zone, meaning that it lacks about 7‰ of oxygen-18 compared to the norm (scientists use standard mean ocean water — SMOW). The exception is the severely depleted green-to-blue area (15–30‰ shortfall), occupied mainly by vegetation from high Northern latitudes. Here lies the beginnings of why oxygen-18 is depleted by 2‰ in the Northern Hemisphere compared to the South (where green areas over land are nearly non-existent).

2. Enrichment of leaf water due to evapotranspiration

Water sucked up by leaves through the roots and stems will be lost to the air by evapotranspiration. Most of the sun's rays are absorbed in chloroplasts (where photosynthesis takes place), and evaporation will, accordingly, take place at the nearby air–water surfaces. The researchers present an equation (labelled 1 in the box) that expresses — purely in terms of a leaf's physical environment — how much the water at the evaporating site inside the leaf will be enriched in oxygen-18 (relative to water in the soil).

In essence, this simple equation reflects the build-up of oxygen-18 in the leaf due to faster evaporation, and faster diffusion, of the lighter molecules. Relevant variables here are vapour pressure levels inside and outside the leaf, and relative diffusion rates through the stomata.

The maths of isotope discrimination

A few basic equations govern the enrichment (or depletion) of the air's CO_2 in oxygen-18.

- First, the isotopic composition of precipitation (δ_P) is given by:

$$1000\,\delta_P = 0{\cdot}579\,T - 0{\cdot}0114\,T^2 - 1{\cdot}35\,P_a + 4{\cdot}47\,P_a^{\,2} - 0{\cdot}147\sqrt{E_V} - 9.80 \qquad (A)$$

where T is the mean annual temperature (°C); P_a is the annual precipitation in m; and E_V is the elevation in m.

- The next equation gives the isotopic composition of water (δ_E) at the evaporating sites in the leaf by:

$$\delta_E = \delta_S + \varepsilon_k + \varepsilon^* + (\delta_V - \delta_S - \varepsilon_k)\cdot(e_a/e_i) \qquad (1)$$

where δ_S is the isotopic composition of the source water; ε_k is a fractionation factor to account for differing diffusion speeds (26‰); ε^* is 9‰; δ_V is the isotopic composition of water vapour in the air; δ_S is the isotopic composition of source water; and e_a and e_i are the vapour pressures in the atmosphere and intercellular spaces. At equilibrium, $\delta_c = \delta_E + 41‰$ (when δ_E and δ_c are measured on the same scale), where δ_c is the isotopic composition of CO_2 in the chloroplast.

- Then we express the discrimination (Δ_A) due to diffusion of CO_2 away from the chloroplast (before fixation) — essentially the effect of stomatal visiting:

$$\Delta_A = a + C_c(\delta_c - \delta_a)/(C_a - C_c) \qquad (2)$$

where a is 7‰; C_c and C_a are partial pressures of CO_2 in the chloroplast and ambient air; and δ_c and δ_a are the isotopic compositions of CO_2 in the chloroplast and ambient air.

- Finally, it can be shown that the the isotopic composition of respired CO_2 (δ_r) is well represented by

$$\delta_r = \delta_P - b \qquad (3)$$

where b is a constant (8‰ when δ_r is measured on its usual PDB scale and δ_P is measured on the relevant SMOW scale).

Putting the appropriate figures into these equations allows the separate and combined effect of plants on atmospheric CO_2 — the effects of respiration, assimilation, and stomatal visiting — to be calculated. The graph at the bottom of page 7 shows how successfully this can be done.

Global and Climate Change research group

The Research School of Biological Sciences is setting up a Global and Climate Change Group to study the key problems to do with the greenhouse effect. One of its activities will be detailing the oxygen isotope effect discussed here, and seeing how much it can tell us about global balances and trends.

The group will be set up using a grant from the ANU's Strategic Development Fund. Staff are presently being selected, and work will commence immediately appointments are made.

We should note that enrichment at the evaporating surface will differ from that in stem water. Oxygen-18 enrichment will be greatest at the evaporating surface, near the chloroplasts, and it will tend to diffuse away from this surface; however this tendency will be opposed by the flow of water replacing, by convection, that lost by evaporation.

However, Prof. Farquhar maintains that, since the chloroplasts (where carbonic anhydrase lurks) are close to the evaporating surface, the isotope enhancement will effectively be that of the evaporating surface, not that of the stem water. Evidence for this is shown in the graph on page 7, and is discussed later.

3. Passing on of enrichment from water to CO_2

Once the chloroplast water is enriched, the inevitable next step is for the incoming carbon dioxide, with the help of carbonic anhydrase, to receive a corresponding dose of enrichment. If CO_2 reaches equilibrium with water, then its isotopic ratio will be similarly enriched, so equation 1 gives us the needed figure. (But because the enrichment standard for CO_2 — the gas derived from a certain carbonate deposit known as PDB — is some 41‰ higher than for SMOW, we have to be careful not to be confused by this change of scale. This scale change largely accommodates the preferred binding of ^{18}O to CO_2 in preference to H_2O.)

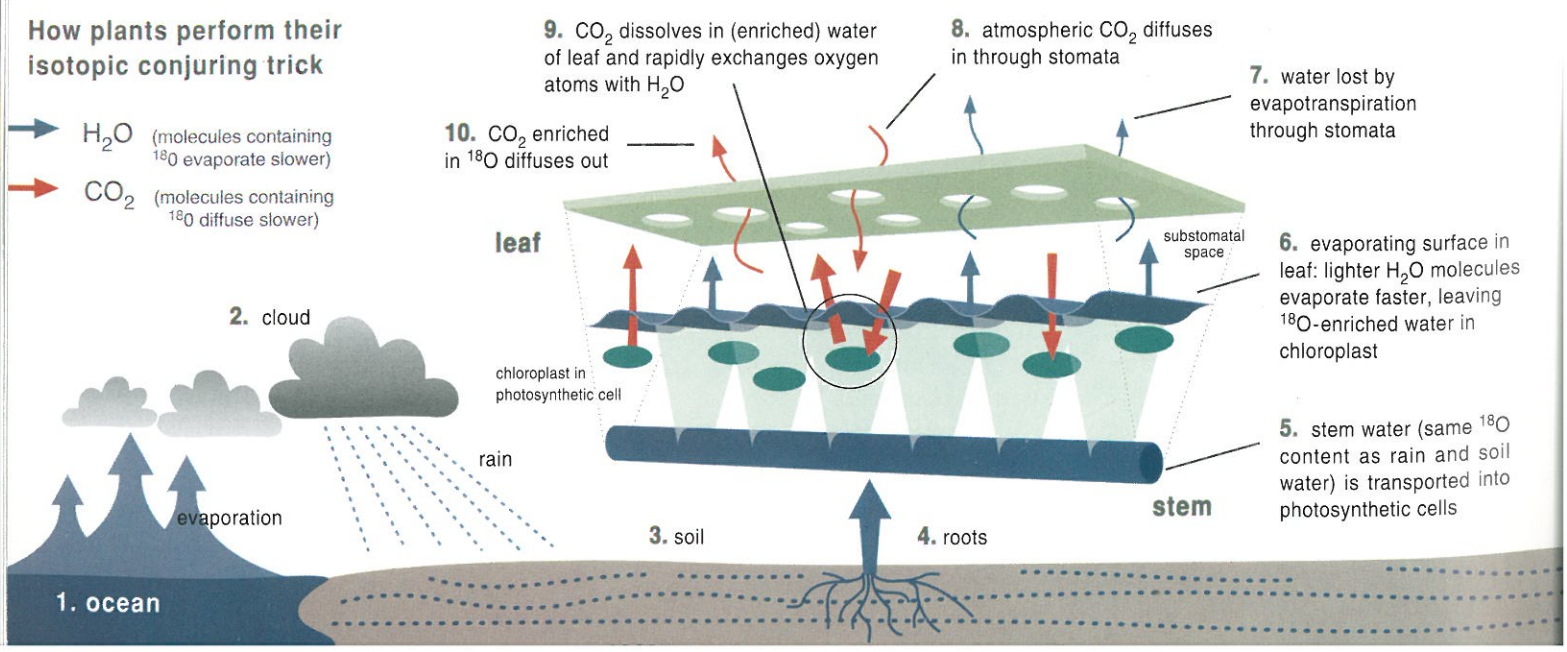
4. Balance between chloroplast and ambient air

Naturally, any concentration of 'heavy' CO_2 built up at the chloroplast will tend to dissipate due to diffusion away from it; so we need to allow for isotopic discrimination as CO_2 molecules diffuse through the intervening leaf water and out the stomata.

In essence, the required equation states that discrimination at the chloroplast is the net result of diffusion into and out of the leaf. This equation (number 2 in the box) expresses the plant's overall discrimination against ^{18}O, and includes terms for the relative diffusion rates of the two CO_2 types (about 7‰), and the degree of stomatal opening — which determines the partial pressure of CO_2 in the substomatal cavities and chloroplast. A simple stomatal model will provide the required relationship so long as we specify the vegetation type and whether it uses a C_3 or C_4 pathway.

This equation tells us that when the stomata are shut, CO_2 fluxes are zero, and the discrimination at the chloroplast has to be the same as that of the stem water. On the other hand, when stomata are wide open, discrimination can produce enrichment or depletion of oxygen-18 depending on whether inwards diffusion of CO_2 molecules exceeds, or falls short of, outwards diffusion.

In general, for most plants under most situations, the tendency is for stomatal visiting to enhance the concentration of heavy CO_2 in the atmosphere. As an illustration of this, the graph on page 7 shows measurements made at RSBS on the isotope discrimination of peach, grapefruit, and lemon trees. You can see that these plants effectively discriminated, by 20–50‰, against oxygen-18 containing carbon dioxide, the spread in values largely depending on the photosynthetic activity (degree of stomatal opening) of the plants — high in sun, low in shade.



This graph is also of interest in showing the results expected if CO_2 were in full equilibrium with stem water (lower curve) or with water at an evaporating surface (top). Clearly, the measured values came closer to the latter, indicating that chloroplasts do in fact operate very close to the evaporation sites in a leaf.

To enlarge the picture to the entire planet, we can put some global-scale numbers into the equations. For instance, we know the world-wide figures for soil-water enrichment (equation A and the map at the top of page 4), so we can employ equation 1 to give us corresponding values for chloroplast water — and hence chloroplast CO_2.

Then to get closer to the heart of the matter — to gauge the region-by-region power of stomatal visiting in altering the isotopic ratio of the air's CO_2 — we plug the last-derived numbers into equation 2. Thanks to Francey and Tans, the atmosphere's isotopic characteristics are already known, at least averaged across latitude and year, and they are pretty uniform. All that's needed now is data on vegetation types — as a proxy for photosynthetic activity — and this is answered by delving into the literature.

What emerges is the startling picture reproduced at the bottom of page 4. It vividly shows that, when considering stomatal visiting, the world's vegetation types vary markedly in their isotope-swapping tendencies. The blue–magenta regions — chiefly arctic tundra — cause a severe depletion in oxygen-18 (up to 12‰ annually averaged); in contrast, the red areas — the dry steppes of Kazakhstan and Ukraine — have the opposite effect, enhancing the relative abundance of oxygen-18 by 32‰.

Of course, counting up areas, it is obvious that depletion of oxygen-18 dominates in the Northern Hemisphere, but not so strongly in the Southern. By comparing the maps on page 4, it is readily apparent that the isotope balance of the originating soil water is a major factor.

Although we expect stomatal visiting to dominate the exchange process, to wrap up the story there is one last factor — plant respiration — that we have to consider.

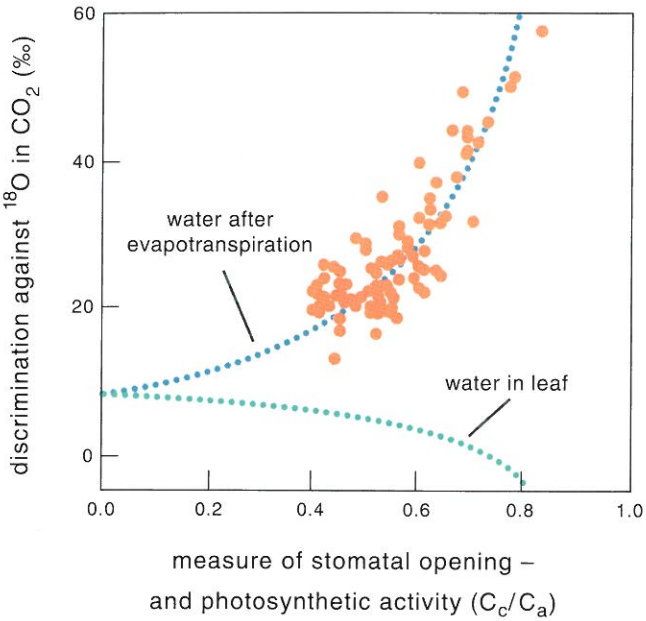
5. Effect of plant respiration

Two fates await a CO_2 molecule within striking distance of a chloroplast: it can either be captured by

(continued on page 18)

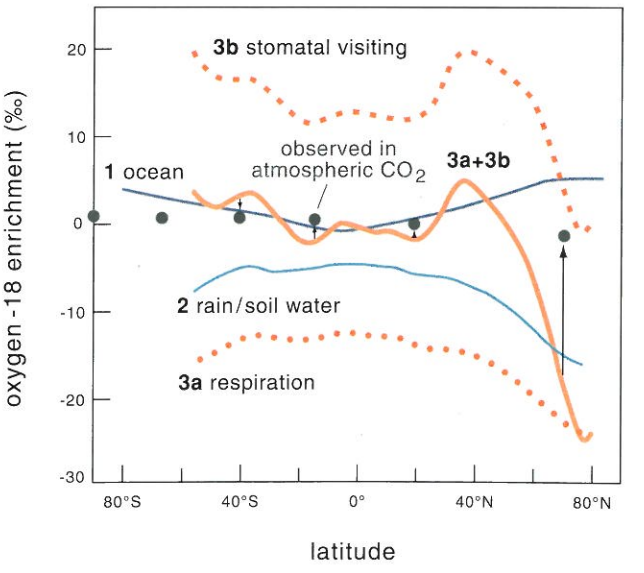
< When atmospheric CO_2 diffuses into a leaf though the stomata, it dissolves in leaf water and oxygen atoms are exchanged. Promptly re-released, the gas is now typically enriched in oxygen-18. Outlined here are the 10 major steps. Key factors in enrichment are (1) slower evaporation of H_2O containing the heavier ^{18}O isotope, and (2) slower diffusion through the stomata of CO_2 containing ^{18}O. The crucial exchange between H_2O and CO_2 occurs at step 9, an exchange accelerated a million-fold by the powerful plant enzyme carbonic anhydrase, which resides mostly in the chloroplasts.

Isotope exchange depends on stomatal opening



Close monitoring of growing trees showed that isotope enrichment of CO_2 due to stomatal visiting depends on how widely the plant opens its stomata (data points for three tree species). The data lie close to the values expected (dashed curve) if CO_2 molecules had exchanged oxygen atoms with water enriched in ^{18}O by evapotranspiration. This confirms that the chloroplasts, where exchange occurs, must lie close to the evaporating surface in the leaf.

Pulling it all together: from ocean, via plants, to CO_2



To see how isotope discrimination progresses, step by step, we combine latitudinal averages of the previous maps with broad estimates of plant respiration. Starting from the oceans (1), evaporation gives rain depleted in ^{18}O (2).

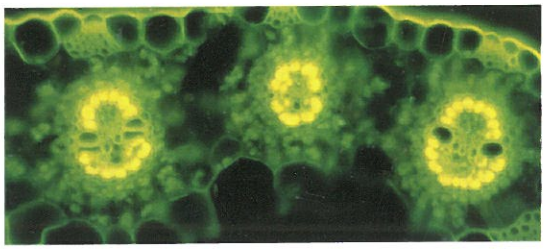
Plants take up this water from the soil and exchange its oxygen atoms with CO_2 in two ways. Respiration depletes CO_2 in ^{18}O (3a), but stomatal visiting enriches it (3b). The two effects largely cancel (bold line), except for large depletions at high Northern latitudes. Cross-latitudinal mixing (arrows) takes us from the activity of the plant to the observed atmospheric levels (circles).

And the winner, *in the fight for supremacy between* C_3 *and* C_4 *plants,* **is...**

Because carbon dioxide is so vital for plant growth, all the world's plants are constantly waging a life-and-death contest with each other for a bigger share of that precious resource, which presently makes up only 0·03% of the atmosphere.

Those plants that can grab a larger portion are rewarded by evolutionary success, and so it was that the so-called C_4 plants emerged on the scene — probably on several separate occasions — some 20–70 million years ago when atmospheric concentrations of CO_2 were very low. Unlike the majority of plants that use the usual C_3 photosynthetic cycle, C_4 plants have developed a CO_2-concentrating mechanism that permits, under the right conditions, more efficient photosynthesis.

Essentially, C_4 plants have invented a CO_2 pump. (It's worth noting that this feaure, first described 25 years ago by Australian scientists — in laboratories at CSR, CSIRO, and RSBS — put this country at the forefront of plant biochemistry.) Carbon dioxide diffusing into the leaf via the stomata is initially captured in outer mesophyll cells as four-carbon acids (hence the name C_4). These acids diffuse to adjacent bundle-sheath cells, where CO_2 is released again and fixation by Rubisco, the key photosynthetic catalyst, takes place (photo above). In this way, the bundle-sheath cells enjoy a 10–100 times higher concentration of CO_2, allowing Rubisco to operate at full tilt and virtually eliminating the wasteful photorespiration that this catalyst is prone to (see the article in the July 1993 issue of *Biologic*).



Paul Hattersley

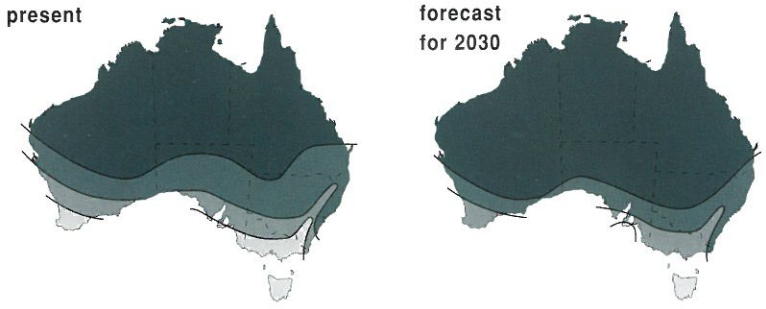
Lighting up yellow, the key photosynthetic catalyst (Rubisco) glows brightly in this cross-section of a C_4 grass treated with fluorescent antibodies. The fluorescence highlights the bundle-sheath cells, where C_4 plants concentrate CO_2.

Compared with their C_3 counterparts, C_4 plants can assimilate CO_2 faster and more efficiently, and they also transpire less water. These advantages hold only above about 20°C, however, and so we find that C_4 plants luxuriate in the tropics, whereas C_3 plants always hold their own elsewhere. Among the grasses, the contest is pretty evenly matched, and of the world's 10 000 species, half belong to the C_4 camp and half to the C_3.

But given that humans are now releasing CO_2 so liberally into the atmosphere, which way will the balance tip? Will the hard-won competitive advantage of the C_4 plants soon come to nought?

The C_4 plants cannot benefit from higher CO_2 levels because the leaf interior already contains saturation levels of the gas; in comparison, C_3 plants increasingly thrive as ambient CO_2 levels rise. Ultimately, then, with CO_2 levels set to double next century, will the improved relative fitness of the C_3 plants be such that we see the demise of many out-classed C_4 species?

The southerly march of the C_4 grasses



Map at far left shows the present relative distribution of C_4 and C_3 grass species. Applying temperature forecasts for 2030, based on expected greenhouse scenarios, gives the other map. It assumes, of course, that species could migrate — at short notice! — and re-establish.

This dramatic question is posed by Dr Sally Henderson and colleagues — Dr Paul Hattersley, Dr Susanne von Caemmerer and Professor Barry Osmond — in a recent paper. (The authors were all at RSBS when the paper was written, although Dr Henderson has moved to the University of Queensland and Dr Hattersley is now with the Queensland Department of Primary Industries.) Their paper doesn't rule out that possibility, but they do suggest that the new balance that will be struck between the two sorts of plants will probably depend more on powerful climatic changes brought about by inexorable increases in CO_2 levels than by the direct effect of higher concentrations of the gas.

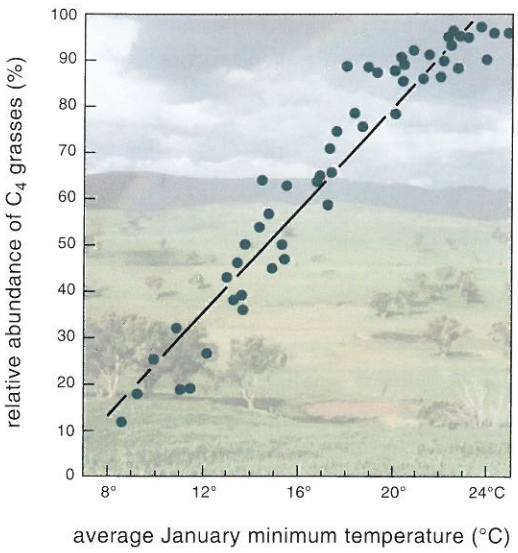
In this context, the best predictions we have for climatic effects of enhanced CO_2 levels point to generally higher temperatures. This being so, the authors see the likelihood that the C_4 plants, in particular the grasses, are going to have, well, 'a field day'. They suggest that more and more of the continent's grasslands could become dominated by C_4 species.

After evaluating the cellular machinery of the two competing pathways, and the influence of various climatic factors on them — such as CO_2 level, rainfall, and temperature — the researchers come to the conclusion that the over-riding factor, so far as they can see, is temperature. As a climatic zone's mean annual temperature rises, so does the abundance of C_4 plants. By no means are all tropical plants C_4, but whenever a plant family comes under intense competition, the winners at higher temperatures are the C_4 members.

This outcome is most apparent for the grasses. The accompanying graph shows the remarkably clear-cut effect of minimum summer temperature on the percentage of C_4 species in a region's grass flora. The data refers to various Australian regions, and although we must be a bit circumspect in attributing the effect to any one underlying cause, it remains that the relative fitness of C_3 and C_4 plants, or at least the grasses, depends critically on temperature.

And, of course, one virtually incontrovertible effect of raised CO_2 levels is global warming — the greenhouse effect. According to CSIRO predictions for 2030, Australia's mean annual temperature is likely to increase by 1–2°C in northern coastal areas, 1–3°C in southern coastal regions, and 2–4°C inland.

How temperature controls relative abundance of C_3 and C_4 grasses



When data on the relative abundance of C_4 grass species in 51 Australian regions are plotted against minimum January temperature, a surprisingly good correlation emerges (Hattersley, 1983). Warming due to the greenhouse effect is therefore expected to favour C_4 grasses over their C_3 competitors.

By applying these figures to the previous graph, Dr Henderson and colleagues predict that an expansion of C_4 grasslands is extremely likely. As the map indicates, the line marking 90% or more of C_4 grasses is (if all other factors stay the same) expected to move southwards by some 200–400 km.

The authors suggest that monitoring programs should be set up in key parts of the country to track, over the ensuing decades, the expected changes in relative abundance of the two sorts of grasses. However, contrary to what most other people think, their money is on the supremacy of C_4 types, and not C_3.

If you want to know more

- Are C_4 pathway plants threatened by global climatic change? S. Henderson, P. Hattersley, S. von Caemmerer, and B. Osmond. In: Ecological Studies, vol. 100 (Ecophysiology of Photosynthesis), ed. E.-D. Schulze and M. Caldwell (Springer: Berlin, 1993).
- The distribution of C_3 and C_4 grasses in Australia in relation to climate. P.W. Hattersley. *Oecologia* **57** (1983) 113–128.

Dr Sally Henderson.

Moths

and their remarkable carbon dioxide sensors

In the same way as hungry female mosquitoes zero in on us by detecting our exhaled carbon dioxide, so it seems many nocturnal moths locate their plant food by sensing the carbon dioxide respired by plants at night.

Studies by Dr Gert Stange of the Research School of Biological Sciences have shown that the moths' carbon dioxide receptor is exquisitely sensitive — for example, that of the cotton boll-worm (*Helicoverpa* [previously *Heliothis*] *armigera*) can detect changes in concentration of 1 part per million or less. With such a sensitive on-board sensor — equal to anything modern-day technology can provide — the moth should have no trouble locating the most actively growing vegetation on which to feed and lay its eggs.

Indeed, experiments by Dr Stange, following a suggestion by Professor Barry Osmond, indicate that the sensitive CO_2 detector of the *Cactoblastis* moth was behind its ability to wipe out the notorious prickly pear cactus plague earlier this century (see the box on page 13).

And yet, in a recent letter to *Nature* (21 October), Dr Stange and colleague Dr Chin Wong point out that the CO_2 detector of the *Helicoverpa* moth has a major drawback, a failing that also presumably applies to all other insects that employ CO_2 detectors, such as butterflies, bees, flies, ticks, centipedes and termites.

The weak point of all CO_2 detectors, biological and physical, is that they invariably require compensation for the effects of temperature. That is, all CO_2 detectors respond to temperature as well as to CO_2 concentration, so unless some adjustment for temperature is made, an inaccurate CO_2 reading results.

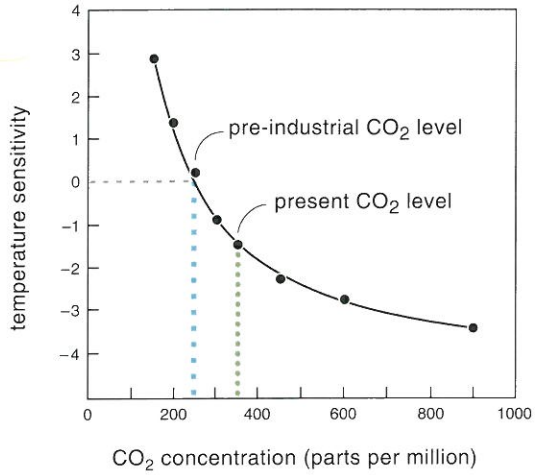
he finds that the moth's detector possesses a major deficiency.

Dr Stange has found that although *Helicoverpa*'s detector employs a temperature-compensating system, it possesses a major deficiency: the system assumes that the background level of carbon dioxide in the atmosphere is 270 parts per million — a level that prevailed before the Industrial Revolution. Presumably, the moth's genetic mechanism hasn't had time to adjust to the rapid increase in atmospheric CO_2 levels created by our burning of fossil fuels. The present background level is 350 p.p.m., and rising fast.

Colin Beaton, CSIRO Division of Entomology

Head of a *Helicoverpa* moth, showing the two palps (above the coiled proboscis) used for sensing CO_2 concentrations. The open-ended palps give access to the hollow interior.

How temperature now confounds moths' CO_2 detectors



At a fixed CO_2 concentration, temperature fluctuations generally cause the firing rate of a moth's CO_2 detector to increase or decrease. Only at 270 p.p.m. — equal to pre-industrial levels in the atmosphere — was firing rate insensitive to temperature. These laboratory findings, when translated to the outside world, imply that as atmospheric background levels continue to rise from their present 350 p.p.m., moths will have increasing difficulty telling apart changes in CO_2 concentration from temperature fluctuations.

As CO_2 levels continue to rise, the moth will increasingly confuse fluctuations in temperature with changes in CO_2 concentration. Next century, when CO_2 levels are predicted to reach 700 p.p.m., we might expect the moth to erroneously interpret a temperature fluctuation of 1 degree (often found within the microclimate of a plant canopy) as a CO_2 concentration change of 7 p.p.m. The moth's ability to track down plants may therefore be hampered.

Studies on three other moth species, of different genera (*Cactoblastis, Phalaenoides* and *Precis*), showed similar confusion of temperature and CO_2 concentration, with compensation points lying between 190 and 320 p.p.m.

What effect this will have on the moths' behaviour is anybody's guess, but it has the potential to disrupt their plant-finding abilities. So far as the cotton bollworm is concerned, this is probably a good thing. Indeed, Dr Stange is hoping that his research may lead to a way of turning the tables against this troublesome pest. But on the other hand, the effect on honey bees, for example, could be disastrous.

Experiments are now being done to assess the CO_2-sensing function of other insects, and tests are underway to see if they too may be put off the scent by temperature.

Finding out how moths respond to CO_2

Moths, butterflies, flies, bees, mosquitoes, ticks, centipedes and termites all have the special ability to monitor the CO_2 levels of their environment. The CO_2 sensors of moths and butterflies are particularly well developed, and Dr Stange's experiments suggest that the insects can detect changes in CO_2 levels of less than 1 part per million.

Leaves, the most nutritious and metabolically active parts of plants, absorb and give off the most CO_2. In bright light, a typical plant leaf can process about 1 milligram of carbon dioxide per square meter per second. Hence, a device for detecting differences in concentrations of this gas is a good way for an insect to find nutritious food to eat, or the best place to lay eggs on.

A moth uses special structures called labial palps, located on either side of its proboscis, to detect the gas. In *Helicoverpa*, the palp is a small hollow cylinder about 1 mm long. At its tip is a pore, normally covered with scales, which leads into a cavity lined with hundreds of tiny hair-like protrusions called sensilla. It is these sensilla, seen in the accompanying electron micrograph, which respond to carbon dioxide.

To study how these receptors respond to various stimuli, Dr Stange immobilises moths in paraffin wax and inserts microelectrodes into the palp to record action potentials. In searching for active units, human breath (about 50 000 p.p.m. CO_2) was the most potent stimulus, giving spike rates of up to 120 impulses per second. Ambient air, with background levels of 350 p.p.m., gave spike rates of about 10 per second. Activity ceased altogether at CO_2 levels of 100 p.p.m. and below.

To examine the effects of CO_2 concentration and temperature, separately and together, gas

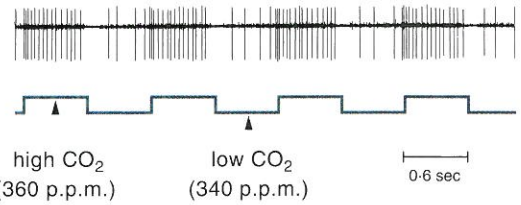
from CO_2 cylinders was fed through sensitive flow regulators and passed over finely adjusted electric heating elements. Gas concentrations were measured with an infrared gas analyser, which is able to detect differences in CO_2 concentration of 1 p.p.m. — that is, this fine example of human ingenuity has about the same sensitivity as does the moth's detector.

The adjacent figure shows the pattern of action potentials recorded from *Helicoverpa* when the concentration of CO_2 around the moth was alternated from 340 to 360 p.p.m. every 0·6 second. Clearly, the moth has no problem discerning the difference, especially when it is remembered that the creature has about 500 similar receptors. Presumably, all the receptors convey the same message, and the outputs are simply pooled. In this way, Dr Stange calculates that the creature should be able to distinguish changes in CO_2 concentration as small as 0·5 p.p.m. The only fly in the ointment associated with this scheme is that temperature also affects the firing rate, as the main story explains.

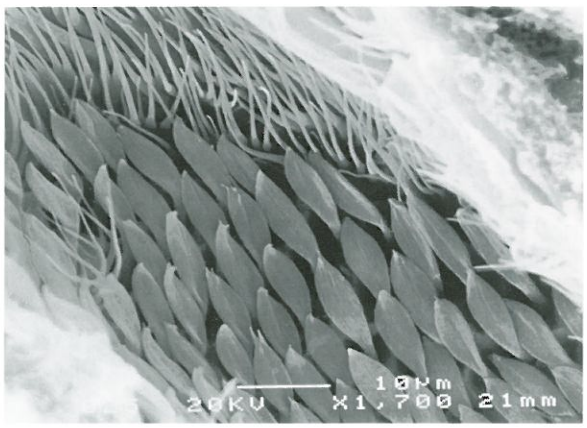
Another interesting aspect of this work concerns how general anesthetics work. Despite their widespread use, there is no convincing theory of how these powerful agents act. Clearly, volatile general anesthetics must act somewhere on the cell membrane of a neuron, but where? The insect's olfactory receptor neurons are ideal for studying the question, because their dendrites are, of necessity, exposed to volatile substances.

In conjunction with Prof. Karl-Ernst Kaissling of the Max Planck Institute for Behavioural Physiology, Dr Stange has examined the effects of a wide range of volatile anesthetics on the carbon dioxide receptors of moths. The effects were complex, but it was clear that a simple mechanism involving dissolution of the anesthetic in the membrane lipids was inadequate. Indeed, the work tends to support a mounting body of evidence that anesthetics act by influencing membrane proteins.

A moth's CO_2 detector in action



The spike train, recorded from electrodes in a moth's labial palp, shows how CO_2-sensitive neurons respond to small rapid changes in concentration of the gas.



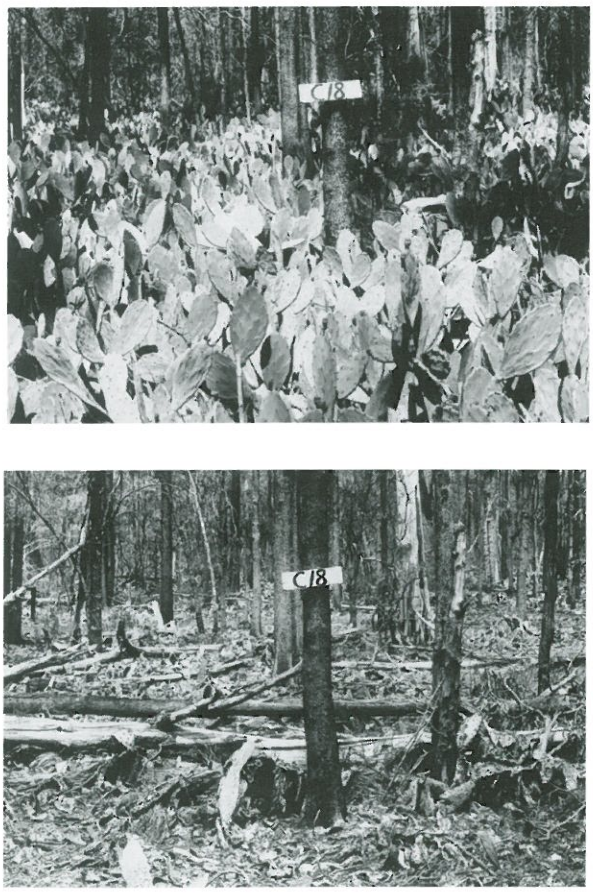
Sally Stowe, ANU E.M. Unit

Sensilla, the leaf-like protrusions found inside the hollow palp. Each sensilla is a miniature CO_2 sensor.

Prickly pear v. *Cactoblastis*: *a continuing contest*

Contrary to popular opinion, *Cactoblastis* did not completely eradicate prickly pear. Like any system of biological control, both the control agent and its host have achieved a dynamic equilibrium. Both prickly pear and *Cactoblastis* are still out there, although the plant (and the moth) exist in very much reduced numbers compared to the plague proportions that prevailed in the late 1920s.

Now and again, the spectre of a cactus-ridden landscape resurfaces when rootstocks of prickly pear burst into life and grow rapidly for a few seasons. However, before long, the cactus is quelled by the attentions of roving *Cactoblastis*. But how does the moth, whose present numbers are low, find these isolated fresh outbreaks of its host?



Flashback to the 1920s: prickly pear near Chinchilla, Qld, before and after *Cactoblastis* set to work.

Prof. Barry Osmond has a long-held interest in this biological system, and believes it offers us many lessons today. Recent experiments conducted by Dr Stange provide evidence that the nocturnally active moth tracks down the most actively growing cactus by detecting carbon dioxide deficits due to the plant's absorption of the gas at night.

This distinctive feature of prickly pear — all other plants in the landscape are giving off CO_2 at this time — makes it a sitting duck for *Cactoblastis'* exquisitely sensitive sensors.

If you take the trouble to taste the tissue of prickly pear or any of its cousins like *Aloe*, you will discover a peculiar trait: they taste acid at dawn and bland at dusk. This peculiarity can be traced to their distinctive photosynthetic pathway, called Crassulacean acid metabolism (CAM) by researchers in the field. It is named after a large plant family (*Crassula*) in which the pathway is common, and it allows these plants to grow with exceptionally low water loss because they too capture CO_2 in the cool of the night.

At this time, CAM plants open their stomatal pores and take in the gas, fixing it into a four-carbon malic acid (so named because of its prevalence in apples, *Pyrus malus*) by drawing on three other carbons from its sugar and starch reserves — which it builds up again during the following day. In a fine example of Nature's economy, the night-time reactions are in fact identical to those used in the day by the ubiquitous C_4 plants.

"Because its stomata are closed during the heat of the day, prickly pear doesn't need much water. It will do quite well on a hollow fence post" says Prof. Osmond. "The ability of cacti to remain green and functional during drought, while all around wither and die, gives them a running start when water becomes available."

"Practically the whole plant is green and photosynthetically active. Prickly pear was biochemically, physiologically, morphologically and ecologically well suited to occupy millions of hectares of Queensland and New South Wales, especially the brigalow country. It occupied a niche that was

"Where is a memorial to prickly pear?" asks Professor Osmond.

virtually empty. In the evolution of land plants, nothing quite like the cacti arose in Australia."

Curiously, this continent has relatively few native CAM plants. They include epiphytic orchids and ferns from tropical Queensland and some tiny salt-loving succulents from Western Australia.

A time bomb was therefore set when a Dr Carlisle brought a potted specimen to Scone, N.S.W., in 1839. By 1925, the plant had taken over 25 million ha of northern New South Wales and southern Queensland and was advancing at 100 ha per hour, despite futile attempts to poison it with hundreds of tonnes of arsenic pentoxide, traces of which can still be detected today.

The exploits of the Queensland Prickly Pear Commission make for an intriguing story, but enough for now to relate that *Cactoblastis cactorum* finally, on the third attempt, was successfully introduced into Australia in the mid 1920s. After distribution of 2·1 billion *Cactoblastis* eggs, an observer noted that "you could actually stand in the paddocks and hear the larvae feeding — you could hear them chewing." The cactus population crashed, and by 1933 our brigalow country was saved.

"To the Australian farmer, *Cactoblastis* was a success and *Opuntia* (prickly pear) was defeated," Prof. Osmond says. "But to the biologist, both organisms were, in their own way, successful."

The persistence of *Opuntia* is dependent on the nocturnal metabolism of this succulent plant. But there is also another key and novel element. Dr John Monro found that the female moth is fussy as to where she will lay her eggs. Healthy green tissue is preferred to yellowish types, and more eggs are laid in denser stands. In this way, although the most active prickly pear is devoured, there are always a few surviving plants to sustain the moth population.

Prof. Osmond and Dr Stange have recently returned to study this remarkably stable biological system once again. In the latest set of experiments, they have shown how sensitively the moth responds to the activity of the cactus. These experiments involved placing electrodes in the palps of the moth, and recording the changes in its nerve firings in response to currents of air. When the air originated from the vicinity of a prickly pear — at its nocturnal peak of activity — pronounced changes in firing rates occurred. During the daytime, however, when the cactus' stomata are closed, the firing rate was more or less constant.

And so the researchers now have powerful evidence that *Cactoblastis* senses the 'breathing' of the cactus. After more than 60 years, *Cactoblastis* is still firmly in control, but now we have a clearer idea of how it performs this remarkable feat.

If you want to know more

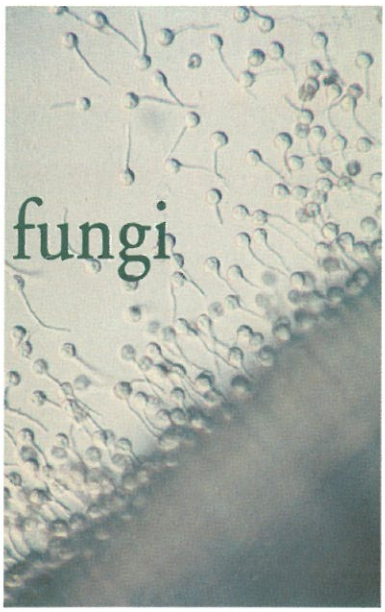
- Moth response to climate change. G. Stange and C. Wong. *Nature* **365** (1993), 699.
- High resolution measurement of atmospheric carbon dioxide concentration changes by the labial palp organ of the moth *Heliothis armigera* (Lepidoptera: Noctuidae). G. Stange. *Journal of Comparative Physiology A* **171** (1992), 317–324.

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

Dr Gert Stange.

An intricate dance:

Plants and their pathogenic fungi



The dieback fungus, Phytophthora cinnamomi, *is one of the most successful pathogenic fungi on earth, attacking world-wide more than 1000 species of plants. And yet science is still far from explaining how this troublesome fungus can so effectively cripple such a diversity of hosts.*

However, Dr Adrienne Hardham of the Plant Cell Biology group at RSBS believes that a detailed study of the fungus' cellular microstructure offers good prospects of uncovering chinks in its armour. Using modern scientific tools and techniques — such as immunofluoresence microscopy, enzyme-linked immunosorbent assay, and laser confocal scanning microscopy — Dr Hardham and her colleagues have enlarged our understanding of how the fungus goes about its life cycle. One major fruit deriving from their years of study is a diagnostic kit for detection of *P. cinnamomi* in soil, an item that is about to be offered commercially (see the box on page 17).

One clear message that comes from the research is that the cell biology of the dieback fungus is of the utmost intricacy.

"Plant–pathogen interaction produces a cascade of events," says Dr Hardham, "and in unravelling this sequence there are exciting discoveries to be made."

This article takes a look at the remarkable cellular machinery that makes the fungus tick, and provides a floor-plan of the delicate to-and-fro dance that plant and fungus execute.

At the outset, the fungus is, undoubtedly, the partner that leads, the plant following along behind. If you think fungus means mushrooms and toadstools, then you will be surprised to learn that *P. cinnamomi,* animal-like, actively seeks out its host with the help of two flagella: a smooth whip-like sort at the rear — aptly called a whiplash flagellum — and another more specialised one at the front adorned with two rows of tubular hairs — called, picturesquely, a tinsel flagellum.

The tinsel flagellum pulls the organism forward, while the whiplash one acts like a rudder to steer it. Together, these flagella propel the fungal zoospore — which is 10–15 µm across — at speeds of about 100 µm per second for many hours.

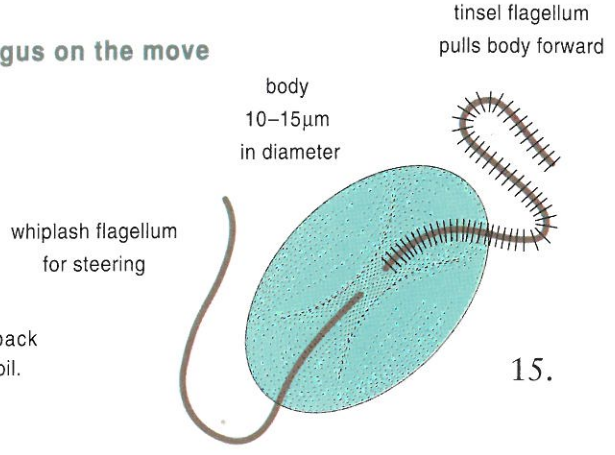
The motile zoospores help dieback spread rapidly through creeks and waterlogged soils. Motility enables the fungus to follow a chemical trail to its host and to select a suitable site for docking. For some peculiar reason, zoospores are equally attracted to roots of hosts and non-hosts, but they clearly prefer growing root ends and wound sites, where there are greater amounts of exuded chemicals.

In the laboratory, common molecules such as amino acids, sugars, and ethanol will serve as attractants, but there is something about actual root exudates that makes them even more attractive. Receptors for these attractants are probably located on the surface of the zoospore, but so far no chemoreceptor has been identified or localised.

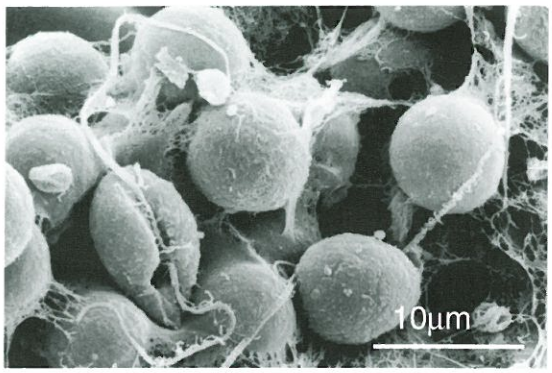
Once in the vicinity of a potential host root, plant and pathogen exchange chemical signals, and the dance has begun.

Before the life cycle has gone full circle, new genes will be expressed, new proteins synthesised, and major biochemical and structural modifications made.

A fungus on the move

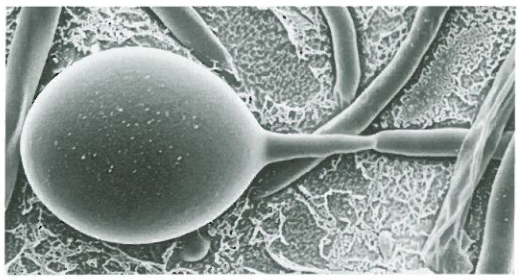


With its two tails, the zoospore of the dieback fungus can propel itself through waterlogged soil.



Kathy Pickerd, CSIRO Division of Entomology

The two tails belong to a zoospore of *P. cinnamomi* on the surface of a root. Its companions are cysts, into which the zoospore develops.



Joanna Maleszka

Like a miniature puff ball, this sporangium will open and release dozens of new dieback fungus spores.

The motile cell will discard its flagella, bond to the root surface, build up a cell wall, and within half an hour, microscopic threads called hyphae will permeate the root and tap into the plant's nutrient supply.

Moving our focus back to the time when the zoospore throws away its high performance 'legs', the identity of the fungus changes to that of a cyst. Persuasive evidence that specific receptors initiate the transformation into cysts comes from studies on the effect of monoclonal antibodies on zoospore motility. When certain such probes are allowed to bind to the flagella, cysts start to form.

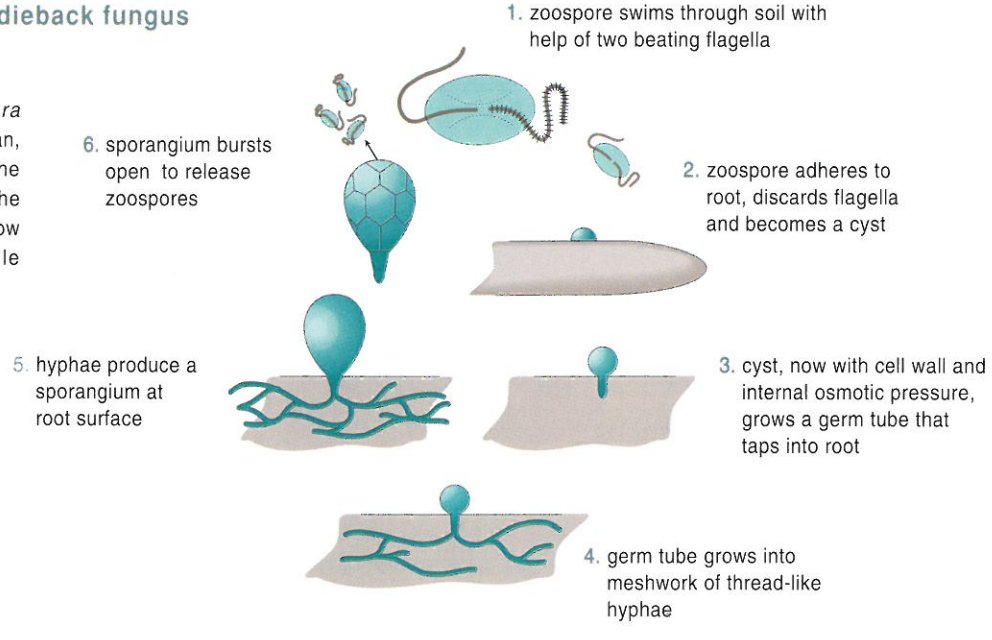
Triggering of cyst formation leads to a series of rapid and dramatic changes in the structure of the zoospore. Within 2 minutes of the zoospore losing its flagella, the zoospore releases a sticky material that glues it to the root surface. Dr Hardham and colleagues have discovered that this glue is packaged in tiny vesicles stored in the zoospore cytoplasm, close to where the flagella are attached. This configuration means that a zoospore, before it releases its glue, must carefully align itself so that the vesicle-populated region faces the root.

A few minutes later, a cell wall begins to form, osmotic pressure builds up, and the cysts become perfectly spherical, like tightly inflated basketballs. The synthesis of a cell wall and establishment of turgor pressure is vital; without a protective wall fungal zoospores would probably fail to infect plants.

As a cyst, the fungus loses many differentiated structures. Organelles are rearranged, allowing them to move freely into the hypha (or germ tube). Although formation of the germ tube — the germination stage — requires synthesis of new RNA, remarkably the fungus can do so without drawing on nutrients from the plant. In-built stores of protein, carbohydrate, and lipid are evidently enough for germination, although eventually the fungus needs to tap into the plant's nutrient supply.

The subterranean life cycle of the dieback fungus

It's hard to stop the spread of *Phytophthora cinnamomi* because zoospores of the fungus can, animal-like, swim through the soil, following the scent to a potential host. Once united with the root, the fungus forms infecting threads that grow fruiting bodies; when ripe, many more motile zoospores are released.



Watch out *Phytophthora* : these diagnostic tests will find you

The cell wall of the plant presents a formidable impediment to the fungus, and to gain access to its host's resources, the fungus must breach this barrier and grow through a surface layer of root cells. To do this the hypha may penetrate between the cells, or it may use high internal hydrostatic pressure and wall-degrading enzymes to bore straight through cell walls.

Having invaded beyond the outer cell layers, the fungus grows rapidly and colonises the root tissues. The hyphae develop into an extensive network that ramifies throughout the root. Eventually, *P. cinnamomi* may kill its host, but not before an abundance of new spores have been produced and released.

Triggered perhaps by cooler temperatures, or by signals that indicate a reduction in available nutrients from the plant, the hyphae sprout hundreds of miniature 'puff balls', or sporangia, on the surface of the root. Each club-shaped sporangium, less than 80 µm across, subdivides its multinuclear cytoplasm to form dozens of uninuclear zoospores. When the sporangia all burst open, thousands of of zoospores are released back into the soil. Full-scale attack!

There are many unresolved issues at the heart of spore formation, and according to Dr Hardham a central one is the origin of the membranes that delineate the new spores. She is hopeful that understanding this particular process may open the door to a method of controlling the spread of dieback fungus.

She and her colleagues are using a combination of immunolabeling with monoclonal antibodies and freeze-substitution to probe deeper into the genesis of zoospores in the fungus. If we could determine just which components contribute to formation of the partitioning membranes, we might come closer to developing a method of disrupting this crucial step.

If you want to know more

- Cell biology of pathogenesis. A.R. Hardham. *Annual Review of Plant Physiology and Plant Molecular Biology* **43** (1992) 491–526.
- Exploitation of zoospore taxis in the development of a novel dipstick immunoassay for the specific detection of *Phytophthora cinnamomi*. D.M. Cahill and A.R. Hardham. *Phytopathology* **84** (1994), in press.

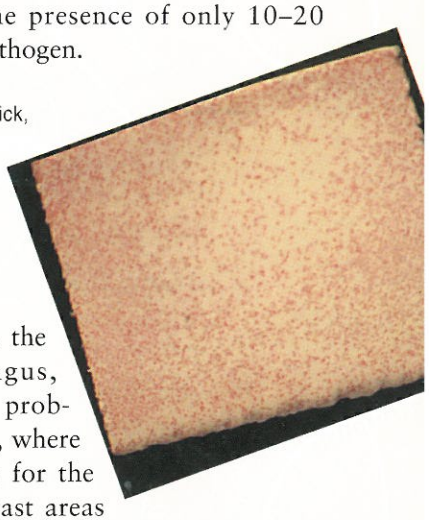
- You are welcome to contact Dr Hardham via the address and phone numbers on page 2.

Dr Adrienne Hardham.

Over the past 8 years, Dr Adrienne Hardham and her colleagues have raised more than 50 different cell lines producing monoclonal antibodies against *P. cinnamomi*. After screening hundreds of fungal isolates against the library of monoclonal antibodies, their hard work has been rewarded.

They have succeeded in designing unique diagnostic kits for identifying *P. cinnamomi* in samples of plants and soil, and negotiations are underway with companies here and overseas who are keen to offer the test kits commercially.

One of the tests comprises a simple dipstick that can be used in the field by non-technical people and, after incubating the sample in water for 24 hours to induce production of spores, gives a result in less than a hour. It is so sensitive it can detect the presence of only 10–20 spores of this pathogen.



Not measles, but a dipstick, seen magnified, after reacting positively to *P. cinnamomi*. Each spot signals one cyst of the fungus.

P. cinnamomi, the dieback fungus, poses a serious problem in Australia, where it is responsible for the destruction of vast areas of eucalypt forests in the west and south-east of the country. The fungus is also to blame for damage to pineapple, macadamia and avocado crops in Queensland, and in nursery plants such as *Rhododendron* and *Camellia*.

One of the major problems in trying to control this pathogen is the difficulty of detecting and identifying it at an early stage of infection. Up until now, the standard method involved baiting the pathogen from root or soil samples and growing them up on selective media. Once a culture had been isolated in this way, identification relied on the particular morphology of the colony and the microscopic appearance of reproductive structures. This task is a difficult one, even for an expert.

A wide range of diagnostic assays using antibodies as immunological probes is now available for the detection of a variety of plant viruses and bacteria. However, very few diagnostic kits for

the detection and identification of fungal pathogens are available. To a large extent, this is due to difficulties in producing antibodies specific to a chosen fungal species. Most of the antibodies that have been raised react with more than just the target fungus. For example, kits presently on the market for detecting *Phytophthora* are not specific for *P. cinnamomi,* but also react with other *Phytophthora* species and other fungal genera.

By raising monoclonal antibodies and screening them for specificity against a multitude of fungal isolates, Dr Hardham's group have identified probes specific to *P. cinnamomi.* Not only do the new tests make identification of the fungus rapid and accurate, but no technical expertise is required to use them.

The dipstick test exploits the propensity of the fungal spores to swim towards a source of attractant molecules, such as those that might be exuded by a plant root.

The researchers have found which chemicals are the most effective lures, and have devised ways of implanting them on an inert strip of nitrocellulose. The motile zoospores of the fungus from a soil sample are attracted to the strip and adhere. The strip is then dipped in a series of antibody solutions and, if *P. cinnamomi* is present, a tell-tale coloured stain appears. The colour can be seen with the naked eye or a simple hand lens.

The other test kit is designed for use in laboratories. It uses enzyme-linked immunosorbent assay (ELISA) to detect the fungus in plant samples as well as soil. The researchers raised antibodies against components of fungal spores; however they have since discovered that some of the species-specific molecules are present throughout all stages of the fungus' life cycle.

Screening has confirmed that the antibodies at the core of the kits have much greater specificity and reliability than any others known. Indeed, the researchers claim that two of the antibodies are 100% reliable.

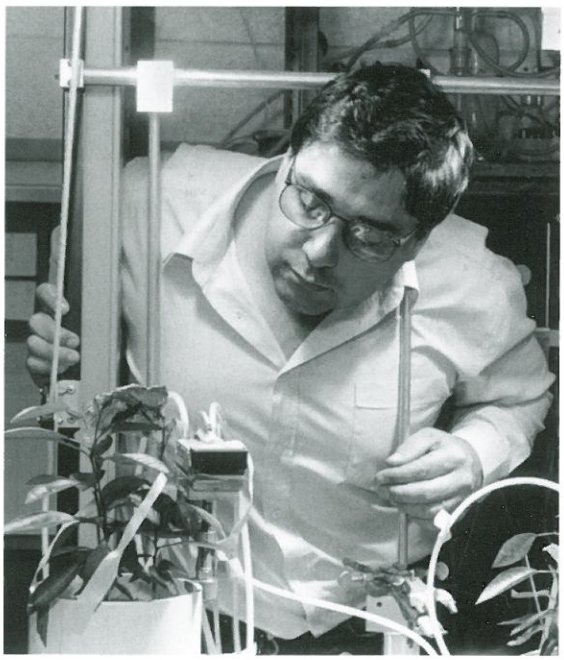
Development of the diagnostic test kits has been facilitated by funding from a syndicated R&D grant established by Bankers Trust Australia and Anutech Pty Ltd.

(continued from page 7)

photosynthetic catalysts and become assimilated into the plant or, after exchanging oxygen atoms with chloroplast water, it can diffuse out again. Studies have in fact shown that some 45% of molecules end up as part of the plant, whereas 55% are nimble enough to escape.

Those that diffuse out again drive the $^{18}O/^{16}O$ ratio of atmospheric CO_2 towards the value expected from equilibrium with chloroplast water. But those that are enmeshed in the plant's metabolism are temporarily taken out of circulation, and can return to the atmosphere only during plant respiration, or after the plant dies. Carbon dioxide molecules caught up in the plant have time to reach equilibrium with stem and soil water, and hence the atmospheric $^{18}O/^{16}O$ ratio leans in this direction.

The authors develop a complex equation (not given here) that includes the effect of respiration, but are able to derive a simplification (equation 3) that says that discrimination due to respiration is basically 8‰ less than that of soil water.



Dr Jon Lloyd finding out how an individual leaf affects isotope balances.

6. Putting it all together

The net effect on the atmosphere is simply the sum of stomatal visiting and respiration. Thus, we now need region-by-region figures on the proportion of CO_2 that plants respire. The proportion relates to how much biomass plants assimilate (gross primary production) and how much CO_2 plants breathe out at night (gross minus net primary production), and works out to be anything from 0·3–0·5 for grasses, 0·3–0·6 for temperate forests, and 0·65–0·8 for tropical forests.

Published data allowed the researchers to calculate latitudinal averages of oxygen-18 discrimination for each major factor, and the resulting graph is reproduced at the bottom of page 7.

One clear message the graph conveys is that the effects of plant respiration and stomatal visiting are opposite, and tend to nearly cancel. That is, stomatal visiting inclines towards an enhancement of heavy CO_2, whereas respiration tends to deplete it.

Although the net effect is, for the most part, about neutral, the key exception is at high Northern latitudes (above 60°N) where discrimination due to stomatal visiting plummets towards zero, causing the overall figure (bold curve) to go sharply negative — in other words, we find that many molecules of heavy CO_2 go missing from the Northern atmosphere.

This result matches Francey and Tans' original observation (as marked by the dots), the puzzling anomaly that started all this work. But now we can see that the imbalance is due to the power of plants dwelling near the Arctic Circle.

Note also that if there were no atmospheric mixing, the observational points would in fact come close to the bold curve. If someone could derive a model that embodied the world's average wind regimes, and which could describe the mixing process, we would have a map of predicted isotope distributions (which we could then compare with measured values). Prof. Farquhar believes such a global model shouldn't be too far away.

And for local scales, assuming limited mixing, the calculation is very much simplified. All it requires is for an air-parcel to remain intact above a plant canopy for long enough to its oxygen-18 content to be comprehensively measured. This situation prevails wherever a sharp, low inversion layer forms. Such an enclosed boundary layer is very prominent over the Brazilian rainforest at night, when CO_2 levels build up to over 550 p.p.m.

Deep in the Brazilian jungle, mosquito-bitten researchers sit beneath this 54-m tower to measure — by day and by night — the ins and outs of CO_2 consumption.

Dr Lloyd has participated in recent experiments — in collaboration with the University of Edinburgh — designed to measure such localised effects. Using a 54-m tower built in the luxuriant (and mosquito-ridden) rainforest near Ji Parana, Brazil, he and his colleagues have made detailed measurements of the jungle's day-and-night breathing. The data, now being analysed, should help refine the model, and determine if rainforests do represent CO_2's hidden sink.

Similarly, in collaboration with the CSIRO Centre for Environmental Mechanics, RSBS scientists are trekking to the grasslands near Wagga, N.S.W., to simultaneously measure (on suitably calm days) the daily rise and fall of the inversion layer and the corresponding isotopic concentrations in the 'closed chamber' beneath it.

And so, plants quietly go about their business of intimately linking earth, water and air. With isotopic analysis, we can now appreciate what a powerful, but subtle, balance that is.

If you want to know more:

• Vegetation effects on the isotope composition of oxygen in atmospheric CO_2. G.D. Farquhar, J. Lloyd, J.A. Taylor, L.B. Flanagan, J.P. Syvertsen, K.T. Hubick, S.C. Wong, and J.R. Ehleringer. *Nature* **363** (1993), 439–443.

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

Prof. Graham Farquhar.

FUNDING AND THE ECOLOGY OF RESEARCH

How much can society afford to spend on research? This vital, but vexed, question has surfaced again with a Senate inquiry into the organisation and funding of research in the higher education sector. The inquiry was seeded, in part, by discussions initiated in this Research School.

To my mind, it helps in tackling this important national issue if we think about research funding in terms of the 'ecology' of research. What I mean is that ecological principles apply in this situation as much as they do to any other domain of life. University research can be considered part of an intellectual ecosystem supported by many educational layers underneath. Like any ecosystem, its continued fruitfulness and evolution depend critically on diversity.

Ecological principles also suggest that attempts to restrict and channel the diversity of this ecosystem is likely to destabilise the research process and lead to its degradation. In agriculture, we are all familiar with the effects of monocultures and of overcropping, and the weeds they engender. Similarly, in universities, over-emphasis on specific mission-oriented research projects is asking for trouble.

Another danger comes from the lack of diversity in funding sources. Over the last decade, 93% of tertiary-level research was funded by the federal government and only 3% by industry. The Cooperative Research Centres (involving the universities, CSIRO and industry) represent a major initiative on the part of the tax-payer to rectify this imbalance.

However, as I said in my submission to the Senate enquiry, we may now have more CRCs than the sustaining base of fundamental research will allow.

Consider the 'power of ten' rule, found in textbooks of ecology. This suggests you can only expect about 10% of energy or materials from one level to flow efficiently to the next. In the CRC context, it means that an investment of 100 units in basic research is required to sustain 10 units in applied research (which will probably lead to 1 unit of output — a single good idea or practical invention).

On this basis, there is a case for a major expansion of investment in basic research — in all sectors of the diverse tertiary research ecosystem. For example, Australian universities now need access to major new facilities to support research in global climate change. Here at RSBS, we are finding that the lack of such facilities is starting to hold back our contributions of first-rate international research on this topic, yet knowledge of impending climate change is vital to our future.

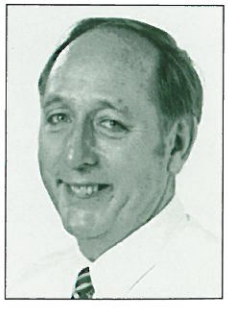
Another concern is the strongly utilitarian agendas and extraordinary bureaucratic processes that have become associated with grant disbursement. I believe the Australian Research Council has endured a succession of political and bureaucratic interventions that have left the funding process for university research floundering, belly up. If our grant-giving agencies are not radically reformed to better serve the research process — using academic principles of trust and peer review — large slabs of the Australian research ecosystem will degrade. And if political and bureaucratic agendas continue to favour CRCs in preference to basic research, then the well-spring of creativity upon which our future depends may dry up.

Acquiring new knowledge rests mainly with creative, especially gifted individuals who have time to think. Because the creativity from which their research springs is sustained by a commitment of a sort uncommon in most human pursuits, entrusting them with research funds is rarely misplaced. Trust translates to a minimum of bureaucratic procedures, especially priority setting, grant application paper-work, and reporting.

An excellent model is the 'operating grant' given by the Canadian National Science and Engineering Research Council. Reviewers of (brief) proposals for these grants, of which there are now about 7000, are reminded that

> there is no special treatment of 'priority' research areas premised on currently perceived national or industrial need; rather the emphasis is on top quality research and researchers, in a belief that it is the creative mind that is ultimately responsible for the most important advances in science and engineering and thereby technological progress. Equally, applicants are not restricted to the research outlined in their application and are free to pursue other promising lines of research as they emerge.

We could do well to follow the Canadians on this one, if only as an experiment to begin with. In the interests of national survival, we need to create and sustain the most diverse research ecosystem that we can.



Barry Osmond