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Air today – gone tomorrow?*

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(Dedicated to William Lewis Thomas, who knows more than most about the air we have to breathe)

SUMMARY

This paper discusses ways in which plant metabolism, development and survival influence, and are influenced by, gaseous pollutants. When amino acids are used as carbon and energy sources by plants and other organisms, reduced inorganic nitrogen is released, with wide-ranging implications for the N cycle. Plants can detect the presence and amounts of deposited N and ozone in the environment, not only by direct metabolic incorporation but probably also via distinct signalling systems. Low-nutrient communities and ecosystems, such as temperate conifer forest, are often protected against N overnutrition by adaptations that insulate plant metabolism and growth from the fertilizing effects of deposited pollutant N. Changes in ecosystem balance and biodiversity are not related in any simple way to differential injury responses of constituent species. The question of what happens when a particular component of a changing plant community fails is considered in terms of the relationship between phases of development and the turnover of tissues and organisms.

Key words: Eutrophication, deamination, cell signalling, resource allocation, turnover.

PRELIMINARIES

This contribution presents the prejudices and perspectives of a participant who approached the Symposium as a (comparatively informed) outsider. This discussion does not pretend to be comprehensive or balanced. In particular, it is largely preoccupied with plants, even though soils, the atmosphere, micro-organisms and animals are all deeply implicated.

The words 'issues' and 'disturbance' in the Symposium title imply that we are dealing with some kind of problem for mankind, or the natural world, or both. The N cycle operates on a pan-continental scale and alterations to it automatically become factors in the broad topic of Global Environmental Change. The problem, if such it be, has four aspects.

1. Human behaviour directly disturbing the cycle without further biological intervention; burning fossil fuel, for example.

2. Human perturbation of the cycle mediated by other living organisms; for instance, emissions from, and applications to, farmlands.

3. Evolutionary reactions, in which changes in the N cycle exert selection pressure on natural species and ecosystems; for example, biodiversity under eutrophication conditions.

4. Responses of natural and domesticated organisms that serve to attenuate changes in the N cycle; assimilation and immobilization of N by plants, for instance.

Biology does not figure highly amongst currently favoured technological solutions to the problems arising from the burning of fossil fuels, which is short-sighted. But physiology and ecology can provide us with understanding of the remaining aspects and show how, through altered practice as well as with the genetic tools of modern biology, we might make changes for the better.

* Professor Thomas was invited to attend the meeting and to offer some personal 'afterthoughts'.

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SKELETONS

Ammonia is the odour of affluence. The people, farm animals and crop plants of the developed world metabolize protein for energy on a scale comparable with that of the other great N-releasing process, combustion. People of the disadvantaged nations struggle to obtain calories and have to consume nutrient-poor bulk to meet even basic needs. By contrast, the appetite of the rich countries for energy is so avid and indiscriminate that organic N compounds are plundered for their C skeletons, leaving inorganic N to build up; which is why a sensitive nose can evaluate economic circumstances. To put it bluntly, poor countries smell of faeces, affluent countries of urine. The relationship between inorganic N and C skeletons is central. Everywhere you look, the making and breaking of C–N associations causes problems. Emissions from cattle, for example. Ideally, carbohydrates and lipids in the diet should meet all the energy requirements of rumen micro-organisms. But forages often contain high levels of low-molecular-weight organic N compounds as a result of fertilizer application and post-harvest proteolysis. Faced with the choice between getting C from relatively inaccessible cell walls and membranes on the one hand or from amino acids on the other, rumen microbes will preferentially ferment the latter, releasing NH_x, which is poorly utilized by the animal and emerges in the form of malodorous high-N slurries and emissions (Lockyer, Pain & Klarenbeek, 1989). In intensive production systems, there are economic pressures to supplement forage with extra protein and, as the United Kingdom has learned to its cost, when the additional protein is of animal origin, the relationship between the N cycle and human affairs takes on a new and sinister dimension. Photochemical smog is far from being the only, or even the most alarming, health risk associated with anthropogenic emissions of nitrogenous compounds.

Throughout the history of plant biochemistry, synthetic and assimilatory processes have usually been more intensely studied than catabolism and emission, and this was certainly true of N metabolism. By the mid-1970s the primary route of inorganic N into organic combination via the GS-GOGAT pathway was well established (Miflin & Lea, 1976). The release of inorganic N when amino acids are respired was understood long ago by plant scientists such as A. Chibnall and W. O. James (Chibnall, 1939), though it must be said that even such illustrious practitioners were not always correct in their interpretation of respiratory quotient (Gut & Matile, 1988). But it was only after the existence of N assimilation was established that it became possible to propose a metabolic pathway that would separate C skeletons and NH_x (Thomas, 1978; Feller & Fischer, 1994). This scheme provides functions for GDH and GS1, as well as for aminotransferases and deaminases. Many of the genes encoding the enzyme system that removes inorganic N from amino acids have now been cloned and some have been shown to be turned on when the pathway becomes active (Kamachi *et al.*, 1991; Szamosi, Shaner & Singh, 1993; Watanabe *et al.*, 1994). Modification of the process by plant breeding or genetic manipulation is already being explored, though reducing the contribution of plant NH_x to the totality of N emissions is not yet a primary objective in most of these projects. Once again, N assimilation shows the way; the barley mutation programme described by Joy, Blackwell & Lea (1992) is a model of how basic understanding of critical biochemical processes and means of their modification can be gained using the genetic approach.

SIGNALS

As well as trans- and de-amination, N and C metabolism interact in more subtle ways. Nitrogen assimilation is responsive to photosynthesis and it has long been known that nitrate reductase is subject to pronounced daily fluctuations. An elegant control system is emerging in which reversible phosphorylation of regulatory polypeptides is fine-tuned through alterations in gene expression, post-transcriptional modifications and redox sensitivities (MacKintosh, 1998). Such signalling pathways remind us that the obvious properties of an environmental stimulus are not necessarily those to which plants are responding. For example, although light drives photosynthesis, photoperiodism is neither perceived nor transduced through interactions between light and the photosynthetic apparatus. Furthermore, there seems to be no distinction in plants between substances that function as raw materials and those that are morphogenetic. Sucrose, for instance, is not just the mobile and short-term storage form of C, it is an initiator of new gene expression, and manipulating its supply can modify differentiation of cells and tissues *in vitro* (Koch, 1996). Nitrate is similarly influential on growth and development (Crawford, 1995).

Ozone is a potent source of active oxygen species and free radicals, but is it this characteristic of ozone that plant cells perceive and respond to? Similarly, NH_x and oxides of N can enter metabolism through the N-assimilation sequence, but is this how their presence is detected and their influence exerted?

Perception and metabolism are clearly connected, but the relationship might be a complex one. It is an established rule of plant hormone action that the tissues most responsive to a given regulator are those that most actively metabolize it. Either metabolism modulates signalling by regulating the stoichiometry of stimulus and receptors, or else entry into metabolic transactions is itself a signalling act. The position is further complicated when metabolism becomes autocatalytic, as it does in the case of ethylene, for example (Zarembinski & Theologis, 1994). The astonishing variety of processes modulated by NO in animal cells and the importance of NO synthase (Nussler & Billiar, 1993) are reminiscent of the ethylene story in plants. It can only be a matter of time before NO synthase becomes an important new element in our understanding of how N oxides might be detected by plants and turned into adaptive physiological responses beyond direct biochemical adjustments of N metabolism (Ninnemann & Maier, 1996).

All of which goes to show that plants get every bit of information they possibly can from environmental stimuli, that this often involves physiological reactions other than through obvious adjustments of primary metabolism, and that this is probably essential to make a success of an immobile lifestyle.

RESOURCES

To scientists brought up on ideas about how plants work that are based on crop species, it sometimes comes as a surprise to encounter resource *rejection* as a shaping force in physiology and ecology. Crop species are generally freaks, selected to be oversexed and hypertrophied. To use their physiological characteristics as a guide to how plants in general conduct themselves is at best questionable. In particular, the useful concept of *resource capture* (Monteith, 1994), when applied to crops, all too often implies maximization rather than optimization, reflecting perhaps the imperatives of farming rather than ecology and evolution. As someone once remarked, in a rather different context, the meek do not necessarily want to inherit the earth.

Regarded with a perverse eye, angiosperm physiology seems to be directed towards unloading raw material as much as towards acquiring resources. Amongst the processes and structures that can be reinterpreted this way are futile metabolic cycles, turnover of gene products, accumulation of reserves, secondary compounds, production of wood, the alternative respiratory pathway, even roots (buried heterotrophic biomass) – Thomas (1994*a*). As discussed in this volume by Rennenberg *et al*. (1998), communities such as forests are adapted to N limitation and can protect themselves against overnutrition. Exposure of roots to N during N circulation reduces NO_3^- uptake. There is also lower investment of C below ground, with consequences for root growth and mycorrhizal associations. Another excellent example of adaptive resource rejection by natural communities was provided at this meeting by Lee & Caporn (1998). The nitrate reductase activity of mosses from pristine sites generally tracks deposition events, whereas the enzyme of mosses from polluted locations is inhibited, thereby limiting N uptake.

A fundamental principle of plant physiology is that when one limiting factor becomes non-limiting, another takes its place. If N deposition becomes excessive, the reaction of a particular plant might be to display a stress response typical of some other nutrient or environmental input. So N fertilization might tend to extend leaf lifespans, but in a phosphorus-limited environment, say, the need to mobilize and recycle P would be overriding, and leaves might senesce without wholesale protein breakdown. *Arabidopsis*, a non-mycorrhizal nitrophobe, shows just this kind of foliar senescence pattern under some circumstances. Plenty of species shed leaves when they still contain considerable amounts of potentially recoverable N; for example, alder (a N-fixer) and some genotypes of ash (Bortlik, Gut & Matile, 1987; Neave, Dawson & DeLucia, 1989). This might be another example of dumping raw materials and regulating overnutrition. Agronomic plants displaying this kind of behaviour, which can be used to improve excessively eutrophic soils, are sometimes called 'green sponges'. The NO_x-surviving barley mutants described in this volume by Wellburn (1998) have notably green-sponge-like characteristics.

INJURIES

Are we clear what worries us about human interference with the environment? Ecosystems have always been changing and any impression we get of stability is a product of the parochiality of the human view of timescales. If it is possible to generalize, we might say that the concern is with an acceleration and/or redirection of the intrinsic processes of species, community and flora turnover. Earlier, I made the point

that we know far more about synthetic than degradative aspects of metabolism. This imbalance in understanding seems to extend to competition and survival at higher levels of biological organization. It is fairly easy to see how an organism or a community thrives; it grows, reproduces, occupies space and so on. But what is happening to plants that are *failing*?

The example of forest death illustrates the not unreasonable belief that decline is linked to damage. But it turns out to be difficult to integrate all the individual instances of injury at the organ, tissue and cell levels into a clear picture of organism-level and community-level behaviour that explains the observations. In this meeting, Davison & Barnes (1998) described instances of ozone-induced injury to parts that seem not to have compromised significantly the survival of whole plants. Conversely, other plants decline but show no visible injury symptoms. At this point, we should recall that most plants experience non-optimal conditions for most of their lives, and many of them actively require deviations from the optimal to read the environment and adapt to it. Concepts such as relative growth rate (RGR), that allow the quantification of successful performance are well established and understood. But are plant decline and diminishing RGR the same thing, and are cause and effect at work here? There are one or two examples of researchers employing 'relative death rate' (RDR) as an index (for example, Runeckles (1982)). At the whole organism level, are RGR and RDR simply reciprocally related? This point is laboured because it raises a physiological issue very familiar to those of us who work on senescence and death at the subcellular and genetic level. These processes are known to be mostly programmed, which means that they do not happen just because growth and differentiation are curtailed: positive (gene expression) events have also to be initiated. Otherwise the mode of death is that of the herbarium specimen, the frozen pea or boiled spinach (Thomas & Smart, 1993).

Body-piercing and body sculpture are essential elements in plant differentiation. Complex and/or large organisms are perforated with holes and tubes. Animals generally develop holes by cell movement and cell death. Plant cells cannot move. Cell death occurs by lysogeny and schizogeny. The lytic potential of the earliest green plants, exemplified by algae such as *Chara*, is high by virtue of the large central lysosome-like vacuole (Moriyasu, 1995). The evolutionary origins of lysogeny and schizogeny might lie in the ancestral tendency to secrete highly aggressive hydrolases and oxidases from the cytoplasm into the 'inner space' of the vacuole and the 'outer space' of the extracellular matrix. And so we arrive at trees, gigantic accumulations of cellular tubes formed by a combination of autolysis and the removal of end walls. Cell separation and death are also the major means by which the external architecture of plants is reconfigured. Plants are designed along built-in obsolescence lines, an essential structural principle for sedentary organisms in a hostile, changing environment.

We can see how patterns of cell death contribute to whole organ development in the example of the leaf. Xylem cells are formed by autolysis in the very earliest stages of foliar differentiation. Mesophyll cell senescence and death occur much later. The plastids of guard cells apparently remain green indefinitely. The timing of cell death is crucial. It might be that there are different, cell-specific *die now* signals, or else the *die now* instruction is always present, different tissues acquiring competence to respond to it at different times. Instances of genetic variation related to errors in the timing and spatial distribution of leaf cell death are well known (Jones & Dangl, 1996). Scaling up from the organ to the organism, we find that the developmental sequence from initiation through maturity to death determines habit and survival. Thus, as it grows, a creeping perennial such as clover continuously moves around its environment by intruding into unexploited areas, leaving spent and discarded tissue behind it; a tree does the same thing in the vertical dimension, but here the residue of dead cells persists as wood, rather than decaying away as it does with the horizontal herbaceous perennial.

Stresses generally work by altering the progression of cells and multicellular structures through the phases of development leading from initiation to controlled terminal autolysis (Thomas, 1994*b*). It is easy to imagine that some of the injury symptoms induced by gaseous pollutants represent the premature expression of processes to which cells are predisposed as a normal part of development. Furthermore, to provoke apparently symptomless decline, such stresses do not need to intervene particularly dramatically in the turnover of cells and tissues on which whole organism development and survival depends. Difficulties in establishing the critical physiological process through which environmental perturbations like gaseous pollutants act arise because plants are developmental amplifiers and hence the dramatic whole-organism and community effects we observe originate with minuscule cellular inputs.

Resource allocation models of plant response to environmental stress are, in my view, incomplete, and would be strengthened if the developmental genetics of growth-maturity-senescence-death sequence were built in. Classical demography has always recognized the central importance of such factors in determining fitness and survival. We are gaining ever better understanding of what these processes mean in physiological and genetic terms. Some kind of synthesis seems in order.

POSTSCRIPTS

If there was one theme that recurred throughout the meeting, it was the way that dogmas were constantly under fire. Plants do not store or translocate NH_x? Think again. Ozone damages by causing oxidative mayhem in cells? Close observation reveals a much richer and more interesting story. Visible symptoms mean growth effects? Not if the responses of black cherry, blackberry and black grass are anything to go by. Above all, the meeting was a challenge to models of plant–environment interactions that are based largely on crop behaviour. If we know more about how wild plants in complex natural systems rise and fall, we will stand a better chance of solving not only ecological but, maybe, also agricultural and social problems arising from human disturbance of the environment.

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