"The Power to Control"

A Report on the International Controlled Environment Conference, Brisbane, March 2004

by

Martyn Stenning
School of Life Sciences
John Maynard Smith Building
University of Sussex
Falmer
Brighton
BN1 9QG

Introduction:

Plant scientists worldwide have sought to control environments for growing plants since Greek and Roman times (Enoch & Enoch 1999). However, early attempts at testing the idea that growing sensitive plants in heated courtyards could improve growth and survival can only be classed as scientific in a loose sense. Things have moved on over 2,000 years and we now have international conferences on how we grow plants in controlled environment (CE) facilities, and perhaps more importantly, why. We (Earth dwellers) are even exploring systems to grow plants on the Moon and Mars. In September 2001 the UK Controlled Environment Users' Group (UK CEUG) and the North American Committee on Controlled Environment Technology and Use (NCR-101), organised an international meeting in Norwich, UK, on controlled environments, at which they resolved to meet again in Brisbane in 2004. The International Controlled Environment Meeting in Brisbane (Australia) in March 2004, "The Power to Control", organised by the Australasian Controlled Environment Working Group (ACEWG), included about 100 delegates from around the world who gave some 40 presentations on their areas of interest and responsibility. Talks varied from Mark Roehrs of Queensland, who gave the opening address, describing how to build a plant research unit where adaptability was the key, with principles such as sustainability, affordability, efficiency and availability being major factors. We heard from Neil Yorio from Florida how light emitting diodes may be a key to growing lettuces (*Lactuca sativa*) in space and from Kevin Sawford of England how controlled vernalisation in sugar beet (*Beta vulgaris*) is the key to quick plant-to-seed turn around. The variety of talks was startling, but common ground was the need to control plant growth to discover more about the phytosphere and how best it can serve both mankind and environment. This report covers some key issues raised at the meeting

Showcasing technology

There has been a new wave of investment in CE facilities, especially in the Pacific Rim countries, and at the Kennedy Space Centre in Florida. The Commonwealth Science & Industrial Research Organisation (CSIRO) has recently extended its already impressive facilities at Brisbane, which was good for us delegates as we were able to use them for the conference. The facility investigates appropriate crop development for Australia including the native *Macadamia* nut. To date, this humble native Australian fruit has been the preserve of the wealthy and aboriginal people, but is now the subject of intense investigation because of its value as a commercial crop. This is a progression from past preoccupations with sugar cane (*Saccharum officinarum*).

The GMO revolution is just one driving factor that attracts investment, and besides Brisbane, the newly refurbished Canberra CSIRO phytotron is pushing forward GM research into the model plant thale cress (*Arabidopsis thaliana*). In addition, the recently extended Adelaide facility of SARDI has the responsibility of supporting the lion's share of Australia's crop production, particularly grapes (*Vitis vinifera*) for wine. Extensively upgraded Tasmanian facilities too have had an Au$1million facelift to investigate ecophysiology and photomorphogenesis. However, perhaps the most exciting work there was the development of a narrow-band monochromatic light emitting diode (LED) system to ensure avoidance of light contamination in experimental modules.

In Palmerston North Research Centre, New Zealand, there is an impressive array of facilities for research on GMOs and topically, on the impacts of climate-related environmental factors on plants, materials, humans and other animals. Of particular interest is the increasing capability to conduct low temperature (-25°C) work such as black frosting events and snow. Also, there is increased use of aeroponics so that the temperature of different parts of the plant above or below ground can be controlled. Interestingly, 75% of use is by external clients, and use is fully recharged to them.

Perhaps the most futuristic (and best funded) research is being conducted at the Kennedy Space Centre where they are proposing to grow plants on the Moon and Mars for life support systems. A unique capability there is the totally sealed Orbiter Environmental Simulator. Here too, LEDs seem to be the answer to low heat/voltage and high accuracy illumination. A major problem with closed systems, where gases (such as CO_2) are fed into the chambers, is contamination with such gases as ethene. Potassium permanganate filter/scrubbers may be the answer.

What Controlled Environments can/can't do technically

Light is arguably the most important environmental variable to plants. Appropriate light sourcing continues to be controversial. Some experiments, especially those on *Arabidopsis* use higher light levels than is absolutely necessary. There is little difference in photosynthetic rate whatever type of lamp is used (Bugbee *et al*. 1994). Running costs can be reduced by two thirds. However, responses observed in crude CEs are interacting with the genetics being studied, which could create false conclusions. There is a need for flexibility and the use of appropriate technology, especially where crop plants are concerned according to Rob Kerslake (CSIRO, Brisbane, Australia). Radiation mainly alters growth due to changes in branching or internode elongation, which affects radiation absorption.

Reg Quiring (Conviron, Winnipeg, Canada) reported that relative humidity (RH) or more appropriately, vapour pressure deficit (VPD) control is gaining increasing importance in plant stress work. Most plants require RH control of between 40% and 80%. However, some experiments require higher or lower RH. Lighting, plant load, irrigation systems and air changes all affect RH. VPD and dew point is illustrated by use of a psychometric chart, showing how changes in one parameter affect others. Good control is expensive, but mechanisms such as steam injection and atomising liquid water can be used to raise RH and chilled heat exchanger coils or desiccant dehumidifiers are available to reduce it.

New components of CE cabinets include CO_2 sensors and controllers. Only 5-15% of chambers and 20-25% of greenhouses include such kit according to Mark Romer (McGill University, Canada). Small changes in CO_2 concentration can dramatically change plant growth rate, emphasising the importance of strict control in plant growth experiments. Multiplex systems exist that draw samples from different CE compartments to a single analyser. An integrated controller can then control additive CO_2 levels to the range of 350 to 3000 $\mu\text{mol mol}^{-1}$ to a degree of accuracy of 5-20% from bottled carbon dioxide supplies. Reduced CO_2 levels in CEs can be achieved using scrubbing agents such as soda lime.

Paul Austin (HortResearch, Palmerston North, New Zealand) showed that complex temperature patterns are required to reveal the effects of response dynamics or metabolic control under fluctuating environments. Recent work at NZCEL has used a complex system of pseudo-random ternary temperature sequences to study room thermodynamics and plant responses under fluctuating environments. Achieving rapid rates of temperature changes (typically between 15°C, 22.5°C and 30°C) requires very accurate control systems with fast response times to avoid conflicts between heating and cooling systems. Encouraging results have led to the CE rooms being upgraded to allow faster response times to frequent temperature changes. Condensation has been a problem due to slow response time of the humidification system. Improved insulation of the CE is also required to reduce under- and over-shoot of temperature. This will provide better conditions for further trials studying effects of fluctuating environmental conditions on plant response dynamics (e.g. bud break, stress responses, etc).

Precise control of air movement within a crop canopy is difficult according to Yoshiaki Kitaya (Osaka University, Japan), especially with large numbers of plants. This causes low air movement, light level and CO_2 concentrations, and high air temperature and water vapour pressure, relative to the conditions above the plant canopy, and also reduces transpiration and net photosynthetic rate. This reduction is closely related to increased boundary layer resistance, which is proportional to plant layer thickness. Increased airflow (above 0.2 m s^{-1}) within the plant canopy (by increasing airflow above it) reduces boundary layer thickness and hence reduces variations within the canopy. In tissue culture vessels, increasing airflow over containers also increases air movement within the vessel, but the presence of plant material reduces airflow relative to an empty container.

Enhancing Controlled Environments Capabilities to Assist Plant Science.

Paul Austin described an investigation into growing *Arabidopsis* in tropical latitudes, at the National Institute of Education in Singapore. A new aeroponic unit was available, where chilled nutrient solution is used to cool the root-zone to ensure normal plant development. Plant development and photosynthesis were monitored. Level of uniformity of expression of green fluorescent protein was measured using fluorescence microscopy. Once established, cool root-zone conditions ensured vigorous root growth allowing shoots and rosettes to grow normally, and recover turgor despite repeated wilting of leaves and bolts in the middle of the day. Similarly, Dennis Greer (Charles Sturt University, Australia) maintained that root-zone temperature reduction from 25°C to 7°C delayed bud break in apple (*Pyrus malus*) by at least six days. Temperature control of ±1°C was achieved using water circulating around heating and cooling elements. Other effects of low root-zone temperature included shorter & fatter shoots, delayed flowering, decreased soil respiration rate and reduced photosynthesis. Also, leaf gas exchange rates varied with root-zone temperatures, which may relate to source-sink demands between root and shoot. Daily crop growth and transpiration rates can be measured non-destructively using whole canopy gas exchange according to AJ Both (Rutgers University, USA). This method removes variation between leaves, captures diurnal changes and includes effects of plant respiration.

Plants in space

Gary Stutte & Ray Wheeler (NASA, USA) described research into effects of micro-gravity on plant growth. Initially, experimentation in space was difficult due to time limitation in the space shuttle, biosatellite programmes and inadequate environmental control. Recent development of manned space stations has enabled longer duration experiments under better environmental conditions. As constraints of micro-gravity (e.g. different gas/fluid dynamics, lack of convective currents, etc) have become better understood, methods were developed to improve the growing environment. These have enabled plants to be grown in space that are comparable to ground control plants when similar environmental conditions are applied. As manned flights go deeper into space, it will be necessary to grow food to sustain human life over long periods. Problems such as watering and providing adequate lighting are still being investigated. Much has yet to be done, but experiments with potato (*Solanum tuberosum*) wheat (*Triticum aestivum*) and soybean (*Glycine max*) are already encouraging. Many cultivars and some GMOs are being developed to produce dwarf plants producing high yields.

Ted Tibbitts (University of Wisconsin, USA) described research to establish the potential of potatoes as food and life support in long-term space programmes. Using disease free micro-propagated potato cultivars, he showed that tuber formation is temperature, variety and photoperiod dependent. Evidence was given of differences in growth shown by different cultivars grown in different temperature and photoperiodic conditions. Early varieties and varieties from northern latitudes tolerate 24 hours of lighting and constant 18°C; others do require a dark period and lower night temperatures.

Back to Earth, how can we adapt plants to marginal habitats?

There are productivity problems associated with growing sugar cane at the extremes of its range according to Graham Bonnett (CSIRO, St Lucia, Australia). Sugar cane is often exposed to temperature extremes at some time during development at the margins of its range. Using tall greenhouses at Brisbane, experiments studied genotypic variation for resistance to frost and photosynthetic rate during cooling. Wild types perform better than cultivars at low temperatures but not high temperatures with or without acclimatization of 20/12°C compared with control (30/25°C). No differences in frost tolerance were found.

Long-day plants are profoundly affected by light quality. Erik Runkle (Michigan University, USA) also stressed the importance of red/far-red light ratio in promoting plant growth. He explained how red and far-red were absorbed by phytochrome photoreceptors, which in many plants regulate growth and development. Using plastic sheets that selectively reduce transmission of far-red light (700 to 800 nm) and neutral sheeting, a number of greenhouse experiments determined that as red to far red ratio increased (i.e. less far-red) stem extension decreased and flowering was delayed. Exposure of plants to far-red light has a facultative effect on flowering.

When transporting propagules, there is a need for quality transplants both from in-vitro culture and from module raisers (plant propagators). Cheri Kubota (University of Arizona, USA) presented data emphasising that both types of transplants must have abundant starch/sugar to grow well in glasshouse or field. Light is important during rooting and transport of modules long distances. Low light (approx 9 to 18 μmol m^{-2} s^{-1}) was sufficient to improve plant take. Where light cannot be supplied during transport, then chilling to 10-12°C reduces respiration losses, maintains starch levels and improves plant take on transplanting.

Plant Stress research at the University of Sussex began by searching for salt tolerant rice (*Oryza sativa*) and tomato (*Solanum lycopersicum*) strains. Objectives include developing strains that grow in high soil salinity, and improved crop yield. Additionally, (*Agave tequilana*) research, using greenhouses at Sussex that replicate the semi-arid neo-tropical Mexican habitat they come from, is yielding possible treatments for diabetes. The research demonstrated how a variety of CEs could be used to investigate socio-economic problems in marginal habitats.

Bruce Bugbee (Utah State University, USA) reported how spectral images of plant canopies, using a multi-element array spectroradiometer, are highly correlated with radiation capture and growth rate. They could also be used to provide spectral signatures for major plant nutrients, enabling early identification of deficiencies (e.g. nitrogen deficiency shows as increased green reflectance). New low-cost line quantum sensors and digital cameras allow real-time measurements of radiation capture and ground cover. These measurements are easier to make than canopy photosynthesis and because biomass yield directly correlates to intercepted radiation (in the absence of stress) are a very good guide to growth rate prior to canopy closure.

Since the beginning of the industrial revolution ambient carbon dioxide levels have risen by 31% to 367 $\mu L\ L^{-1}$ according to Sukumar Chakraborty (CISRO Queensland, Australia). Doubling the availability of carbon dioxide increases plant biomass by 30%, but the resultant enlarged canopy offers a microclimate that encourages disease. Using anthracnose, a disease of tropical legumes and a rust disease of woody weed rubber vine (*Cryptostegia grandiflora*), it was shown that the fecundity of rust disease increases, and rust pustules increase spore production by up to 112%, at twice ambient carbon dioxide levels. However, the doubling of carbon dioxide levels can also increase a plant's ability to resist disease infection. The severity of infection increases from increased disease pressure after several initial cycles, as the pathogen overcomes host resistance. This is a dynamic system in need of field trials, but initially using CEs, hypotheses can be formed before expensive field trials are started.

Manipulating Genes in Controlled Environments

Julian Franklin (Rothamsted Research, UK) discussed the value of CEs in Genomics, Proteomics and Metabalomics. The need of this type of research for CEs with uniformity, repeatability, as well as specificity and extremity in temperature, humidity and light was highlighted. Factors such as air movement, watering and CO_2 were important especially when uniformity and repeatability were considered. The need to grow large numbers of plants, under standard conditions underpinned much of the work on genomics and metabalomics. When considering the acquisition of CEs for such work many factors, including optimum size, uniformity of conditions, sophistication of control, range of control and, importantly, reliability should be considered. Quality control was seen as of being of increased importance, and increasing dependence of funding on good quality control processes was stressed. The new NCR-101/UK CEUG/ACEWG reporting guidelines were cited as an aid to the quality control process. These guidelines were launched at this conference.

Rod King (CISRO Canberra, Australia) explained that seasonal control of flowering in perennial plants involves sensing low temperature and daylength while in annuals it may be daylength alone which is critical. Using darnel (*Lolium temulentum*) grass in a long-day environment, he showed that certain gibberellic acids increased in leaf and later in shoot tissue to induce flowering, and this was more pronounced when low intensity incandescent lamps were used. Vernalisation responsive genes, unlike those involved with day length responses, are localized in the shoot apex. Cold treatment promotes early flowering by suppressing flowering locus C gene in the shoot. Whereas without cold treatment (vernalisation), the flowering locus C gene is present in the shoot, and late flowering occurs.

Although there is usually plenty of phosphate (P) in soil, in many locations it may not be available for plant growth as it is strongly absorbed onto iron and aluminium, leaving soil solution concentrations of less than 1 micromolar, according to Frank Smith (CSIRO Queensland, Australia). A Dilute Flow Culture was used to measure uptake of ^{32}P labelled phosphate in hydroponically grown *Arabidopsis* and cereals. Phosphate co-transporter proteins form pores in cell membranes enabling the proton pump to drive P transport against the concentration gradient. This is regulated by environmental, chemical and developmental signals stimulating production of transcription factors. Reporter genes link the promoter gene to a coding region producing a product, which can be visualised e.g. by green fluorescence protein. In high P there is little expression but in low P there is high expression and an increase in numbers of root hairs, which aid uptake by releasing organic anions to complex with iron and aluminium thus releasing P for plant uptake.

To produce an appropriate response, changes in extra-cellular environment must be signalled from outside the cell to the inside according to Kamal Kazan (CSIRO Queensland, Australia). There is evidence that signal transduction of genes is important to changes in gene expression patterns that underlie plant responses to external cues. The aim is to identify molecular events involved in the regulatory network connecting plant responses to biotic stimuli such as pathogen infection in model plant *Arabidopsis* genes. It is evident from microarray analyses of *Arabidopsis* genes that special emphasis should be given to the reproducibility of microarray experiments. This can be achieved by reducing variation due to differences unrelated to the actual treatment. Good CE facilities can help to reduce environmental variation in gene expression observed between the repeated experiments by strictly adhering to identical plant growing conditions.

Andrew Allan (HFRI Auckland, New Zealand) reported genes of apple, kiwi fruit (*Actinidia* spp.) and blueberry (*Vaccinium angustifolium*) have been inserted into *Arabidopsis* using agrobacteria to study chosen fruit genes e.g. dwarfing, cold tolerance and branching. 90% of genes do not express in the *Arabidopsis* phenotype. However, because *Arabidopsis* is so easy to grow, poorly controlled or variable environments are often used in research. This masks or falsely enhances true expression of the transgene. Experiments at the New Zealand facility that characterised small changes in the *Arabidopsis* genotypes were also described. Expression of branching, fruiting and stress in the phenotype demonstrated that precisely controlled environments are required in order to avoid ambiguous or meaningless results.

Although the photon-based quantum system of light measurement for plants has been around for over three decades its dissemination and use outside circles of plant scientists and agricultural/biosystems engineers remains very limited. Joel Cuello (University of Arizona, USA) reported problems from the quantum system being not quite intuitive and by the difficulty of linking it to the universally adopted photometric system developed for human vision applications. A comparison was made between the response curves for human eye and that of a photosynthesising plant curve. A mathematical formula was proposed that unified the different response curves to allow one measurement system called Phytometric. The phytometric system allows for conversion of units to quantum system, radiometric system, and photometric system. Quite simply, he proposed, the phytometric system is more versatile, comprehensive and compatible with existing standard measuring of light than the quantum system - a contentious point of view.

Controlled Environment Technologies to Assist Plant Science.

This session explored new ideas for improving the life and facilities of plant researchers, and started with a limitation often experienced, namely for sub-zero temperature work. Mick Fuller (University of Plymouth, UK) reported that radiation freezing is a very damaging event in temperate climates in spring and in sub-tropical climates in mid-winter. Radiation freezing cabinets are unavailable on the open market so must be custom built. Radiation freezing also requires ice nucleation before damage occurs and this is temperamental. In controlled freezing experiments the ice nucleating bacteria, *Pseudomonas syringae* Cit 7, is used and ice nucleation and ice spread monitored using Infrared Thermography. These techniques and the radiation chamber enable a better understanding of the process of radiation freezing damage to non-hardy plant tissues. Intervention strategies to avoid radiation freezing damage using a hydrophobic particle film were showing promise in potatoes and grapevine. The technique reduces the risk of freezing by encouraging the shedding of dewdrops from the leaf surfaces which are implicated in ice nucleation in the field.

There were further presentations that discussed air conditioning for research glasshouses and ways of getting light to plants tightly packed in chambers such as those proposed for use in space. There was also discussion of appropriate watering strategies in CEs and the need to monitor water stress to optimize any automatic watering system.

The conference concluded with a masterly summary of the state of CE technology in the world by Cary Mitchell (Wisconsin, USA), and how this specialist but 'provincial' area of science could move forward. He covered a wide range of CE issues raised during the conference. CE skills were not typical. They cover a wide range of techniques from plant processes to physical processes with knowledge of engineering along with horticulture and computing. The global CE community is limited, and awaits the discovery by biotechnologists of a need for facilities to study gene interaction with the environment. This is an opportunity for CE staff to stress the value of its skills, offering solutions and overcoming preconceived or ill-conceived perceptions. There is a poor perception among our institutions on the value of CE as well as under investment in some areas. There is a need for leadership as well as involvement of CE professionals in writing funding proposals. Mitchell discussed the need for successful models for managing CE facilities as part of clusters or centralised facilities, the way they were funded and recharged. Light, temperature, water, nutrients and atmosphere were cardinal factors affecting plant growth, but physical forces of wind, vibration, touch and gravity have to be considered also. The ‘added value’ of CE in terms of increased yield per unit area, both in terms of crop yield as well as crop cycles is recognised. The value of producing consistent reliable crops, with high value components reliably to high quality standards was also being exploited commercially. The 'pharma' plant defined in part by genetic modification plus modification of the environment to produce nutraceuticals and pharmaceuticals was an exciting opportunity for CE researchers. Space applications, especially regenerative support will enable man to reach into the solar system. These were the ultimate CEs and opportunities for ground-breaking developments were likely to occur. New technologies are evolving more applications for CE in terms of the scope for research as well as in improvements to CE facilities themselves. Aquaponics, bio-derived sources of energy, gene environment optimisation, and reclamation technologies are examples of areas where CE will be involved.

Acknowledgements:
Grateful thanks to New Phytologist Trust and the UK Controlled Environment Users' Group for sponsorship of me and 8 other UK delegates, who all contributed to this paper, these are:
Barry Robertson (John Innes Centre, Norwich),
Graham Pitkin (Scottish Crop Research Institute, Dundee),
Ian Pearman (Rothamsted Research, Harpenden),
Joe Aldous (John Innes Centre, Norwich),
Julian Franklin (Rothamsted Research, Harpenden),
Kevin Sawford (Broom's Barn Research Station, Bury St Edmunds),
Malcolm Pratt (University of Leicester).
Mick Fuller (University of Plymouth),

Thanks also to Lynton Incoll, chairman of the UK CEUG Bursary Committee for encouragement and for useful comments on the manuscript. References to work in the text only mentions a key selection of reporting speakers, however, much research reported includes co-workers too numerous to be individually named in this paper, their contribution is gratefully acknowledged.

References:

Enoch, H.Z. & Enoch, Y. (1999). The History and Geography of the Greenhouse. Chapter 1 in: Ecosystems Of The World. Stanhill & Enoch (Eds.). Elsevier Science

Bugbee, B. 1995. Effects of Radiation quality, Intensity, and Duration on Photosynthesis and Growth. pp. 39-50. In: Proceedings of the International Lighting in Controlled Environments Workshop. T.W. Tibbitts (ed). NASA Ames Res. Center, Moffett Field, CA.