Proceedings of the UK Controlled Environment Users' Group

2000 SCIENTIFIC MEETING

"CONTROLLED ENVIRONMENTS FOR SPECIALIST APPLICATIONS"

Volume 11

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L.D. Incoll (editor)

UK CONTROLLED ENVIRONMENT USERS' GROUP

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The scientific part of the annual meeting consisted of five invited contributions. Summaries of these, supplied by the speakers, follow.

SUMMARIES OF PAPERS

J. Franklin (IACR Rothamsted, Harpenden AL5 2JQ, UK) **Controlled environment glasshouses for GMO research.**

Introduction

In recent years, the development of research involving the genetic modification of plants has required the use of specialised glasshouse facilities to grow them. A main requirement of these facilities has been to meet the conditions imposed on growing genetically modified plants by the Genetically Modified Organisms (Contained Use) Regulations 2000 (Anon, 2000a). These regulations supersede those issued in 1992 and the subsequent amendments in 1996 and 1998. Any glasshouse facility growing genetically modified plants has to meet the above regulations. Advice can be found in the Compendium of Guidance from the Health and Safety Commission's Advisory Committee on Genetic Modification (ACGM) (Anon, 2000b).

Genetically Modified Organisms (GMOs)

Genetic engineering is the production of new combinations of genetic material by transferring genes, that one organism does not normally possess from another. Methods used to modify organisms may include cell bombardment with DNA, electroporation, Agrobacterium infection or viral transfer. Within the context of contained use within a glasshouse, genetically modified plants are the main organisms to be considered. However, research may include genetically modified insects, fungi, bacteria or viruses, interacting with plants. Where these organisms are plant pests or pathogens then a Plant Health Licence will also be required.

Requirements of a contained use glasshouse for GMOs

The primary requirement of the glasshouse will be to grow good plant material reliably. It must be remembered that before genetically modified plants are grown in a glasshouse there has been a considerable investment in manpower and general research facilities to produce these plants. Loss of plant material could require an experiment to be repeated at a cost of over £10,000. Current research in this area is competitive and delays due to loss of plant material could cost the research teams involved research contracts and scientific kudos. The costs of growing the plant material are important, as research teams have to remain competitive.

A secondary requirement, but viewed by some as important, is preventing the escape of GMOs. This can result in the environmental contamination of the surrounding area. The effects of such an escape is dependant on the nature of the organism that has escaped and its potential to impact on the environment. If GM engineered Banana pollen was to escape, then the risk in the UK is minimal, however if a virulent mildew strain able to infect a wide range of cereals escaped, then this would be very serious. As important in the current political climate is the public perception of

any escapes and the measures taken to prevent them. In a commercial sense as well GMOs have a potential value and measures to prevent theft are important. The financial implications of a requirement to pay for damages resulting from an escape can be considerable. As well there are the effects of bad publicity and the loss of intellectual property.

In addition to the above is the requirement to fit within a budget when building a contained use facility. In most cases, there is a balance between the requirements of the research, the space that can be built and the funding available.

Prevention of Environmental release of GMOs

Generally, genetically modified plants are relatively easy to contain. Whole plants cannot move and disposal via an autoclave prevents an escape. It may be possible to just compost the plant material if this is done in a controlled way with restricted access until composting is complete. Seeds, tubers etc can generally be treated as whole plants. However, risks of small seeds being transferred by being washed down the drains, by vermin or on shoes, must be considered. To prevent these, sticky mats on floors, floor drain traps and good structural integrity have to be allowed for in any facility. The risk of plants pollinating others outside the glasshouse must also be considered, in terms of providing a filtered air supply or just bagging flowering plants and preventing the movement of insect vectors.

The other GMOs that might be grown or reared in a glasshouse each have risks in escaping from the facility. In preventing any escape, each organism has to be examined as to the particular risks associated with that organism. Generally, the facility should prevent dispersal of the GMO by good structural integrity, traps and good housekeeping.

Experience within IACR has shown that most pest and disease contamination within a glasshouse environment has been via people transferring a pest or disease inadvertently on their clothes or by moving contaminated plants. People management, especially controlled access, is therefore very important.

Containment Levels.

The Genetically Modified Organisms (Contained Use) Regulations 2000 (Anon, 2000a) outline various levels of containment. The containment level however is only a guideline to what can be grown at that level. Each GMO must be individually assessed for the level of risk and assigned to a facility with the appropriate level of containment. There are two levels of containment that apply to just plants (A and B) and four levels that apply to GMOs in general (1 to 4).

Containment A	Low risk, limited risk of genetic transfer to UK species. Good structural integrity. Good housekeeping. Screening to prevent ingress of vermin. Labelled plants. Filters to drain or use of soakaway/sump. Controlled access. Monitor pests. Sticky floor traps if appropriate. GMOs destroyed or removed in sealed container before removal.
Containment B	High risk, with ability to transfer genetic material to UK species. As per containment A plus: Named access via keypad/card. Good structural integrity to withstand extreme weather and vandalism. Sterilisation of glasshouse equipment as appropriate. Air filtration as appropriate with airlocks. Monitor host plants in vicinity.
Level 1	Low risk, little risk to the environment. Does not have to be a permanent

	structure, polytunnel will suffice as long as it is structurally sound. Surfaces should be cleanable. No seed or pollen should be produced. Good protocols in place to handle GMOs. GMOs destroyed or removed in sealed container before removal.
Level 2	Risk to the environment. As per level 1 plus: Permanent secure structure. Immediate area monitored. Restricted access. Contained drainage. Filtration as appropriate. Autoclave clothing.
Level 3	Considerable risk to the environment. Will include GMOs transmitted by invertebrates. As per levels 1 and 2 plus: Highly engineered structure. Electronic interlocking of doors. Vector barriers in place. Negative pressure. Facility must be capable of fumigation.
Level 4	No level 4 in UK as yet. Unlikely that glasshouse structure would be suitable.

Response to requirements to grow GMOs at IACR.
The brief at IACR was "to construct a facility for growing genetically modified plants and genetically modified organisms that is capable of securing them in an environment that enables good plant growth but which prevents escapes to the environment and is physically reliable in terms of structures and environmental controls." In addition, "the facility would be cost effective both in terms of construction, maintenance and running costs."

Initially steps were taken to identify the crops required, the types of GMO research that would be conducted and the scale of requirement. A budget of £1.5 million was available with a requirement for over 150 m^2 of growing space.

A requirement was identified for a facility capable of growing to containment A but with containment B capability with minimal upgrading. Consideration was given to growing in a controlled environment facility rather than a glasshouse. Capital costs were almost doubled for a controlled environment facility at £3 million + and expected running costs would probably be doubled from £4.50 per week per m^2 to £9 per week per m^2. Additional CE costs would have been offset against better secondary containment within a CE environment and better environmental control. Another factor was the increasing costs of highly skilled maintenance support required within a CE facility. Overall given the budgets available, running costs and the research requirements, a glasshouse facility was ordered.

Given the decision to build containment glasshouses, consideration was given to the preferred temperature control system. To maintain good temperature control at UK late spring temperatures (20-15°C), all year round, it was decided to use air-conditioned glasshouses with heat pumps. The use of heat pumps reduced the number of entryways for pipes etc as well as giving potentially good temperature control throughout the year. Because air exchange in the air-conditioned space is kept to a minimum (up to 4 air changes an hour) the cost of pressurising the glasshouses is minimised, as is the cost of air filtration. The use of air-conditioned glasshouses offered a cost-effective solution for providing a relatively large amount of contained glasshouse space.

The facilities at IACR Rothamsted consist of 20 air-conditioned compartments varying in size, each with 5 to 12 m^2 of growing space. Heating and cooling is provided by a heat pump, giving a temperature range of 15 to 25°C assuming outside ambient temperatures are in the range −5 to 35°C. Variation within in the room is ± 2° C. The compartments are computer controlled.

Average running costs per m^2 of bench per week are about £4.50 including lighting. Air make up is controlled from 0 to 4 air changes an hour and filtered through a HEPA filter, if required, on both entry and exhaust. The compartments can be pressurised to give a slight negative or positive pressure if required. There are automatic blinds to reduce solar radiation gain. The supplementary lighting consists of 400W SON-T lamps to provide a minimum 300 µmol m^{-2} s^{-1} light. The structure is of steel and aluminium with 16 mm double polycarbonate "glazing". The polycarbonate has a U value half that of glass (4 v. 7) and is installed in large sheets, reducing gaps. Double polycarbonate is extremely resistant to damage from bricks or stones, hence reducing damage from vandalism. Drains are blockable and they go to a 12-m-deep soakaway. The floor is impervious concrete. The access corridor is sealed and fitted with filtered fans for temperature control. Digital locks are fitted to individual compartments and electronic locks to the access corridor and service areas. There are 120 m^2 of support area including an autoclave facility, potting area, laboratory, vernalisation room and office. Intruder sensors and alarms are fitted and CCTV monitors the area. Areas around the glasshouses are gravelled.

Current concerns with the approach adopted are the costs of maintenance. The heat pumps have a life of 5-10 years, about half that of conventional glasshouse temperature control equipment. Energy costs as a proportion of overall costs to users of this facility are about 50%, the remainder being labour and maintenance costs.

The capital cost per unit area was high, largely because of the relatively large number of compartments. Although this allows better containment between experiments and reduces the risk of disease or pest problems it is a high cost. Halving the number of compartments could have reduced costs by 15%. Capital costs (1999) were split approximately as follows: Glasshouse Structure £300K, Support area structure £200K, Refrigeration £150K, Mechanical and electrical services £550K, Fees and VAT £300K.

The above facility provides a Containment A facility with the ability given some management and engineering changes to be a Containment B facility. As yet, IACR is only working with plants, but given the requirement that these facilities are capable of operating at Category 2. The changes required to operate at a higher level relate to the need to put in HEPA filters of the required level in the air inlet and outlet filters, as well as blocking the drains and putting in procedures for cleaning the surfaces of the containment glasshouse compartments. However, each GMO must have its own assessment of risk and appropriate measures put in place.

The IACR facility represents, I believe, an attempt at providing a Containment A/B facility at reasonable cost which will grow plant material reliably all year round.

References

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Anon (2000b) ACGM Compendium of Guidance. Guidance from the Health and Safety Commission's Advisory Committee on Genetic Modification. ISBN 1-7176-1763-7. HSE Books, PO Box 1999, Sudbury, Suffolk CO10 6FS (£12.50). Available on the web at: http://www.hse.gov.uk/hthdir/noframes/acgmcomp/acgmcomp.htm

J. Boccon-Gibod (Institute National d'Horticulture, 2 Rue Le Notre, 49045 Angers CEDEX 01, France) **Tissue culture rooms for commercial micropropagation and research: diverse technical solutions.**

History of tissue culture
From 1939 to the beginning of the seventies, recommendations for tissue culture environment control were rather simple and approximate. Professor Gautheret wrote in his book "La culture des tissus végétaux "(1959) that a temperature of 25°C is optimum, that 500 lux of light is enough and that jars should have a sufficiently high air relative humidity for achieving good tissue culture. Professor Street added some technical specifications in his book "Plant Tissue and Cell Culture" (1973): set temperature +/– 1°C, good internal air circulation (low speed, large-bladed fan), and a second thermostat for high temperature security (2-3°C above set point).

Biology of *in vitro* grown plants
The biology of a plant grown *in vitro* (Fig. 1) is characterised by growth and morphogenesis. Growth is determined by carbon assimilation (carbon is provided mainly by the sucrose included in the medium), nutrient supply (mineral salts, organic compounds) and the physical environment. The room temperature (temperature inside the jar is usually up to 2 to 6°C above room temperature) and the relative humidity inside the jar will influence the transpiration of the plants and consequently their growth. Morphogenesis is determined by the presence of hormones and sugars in the medium and physical environment: light and temperature are essential factors.

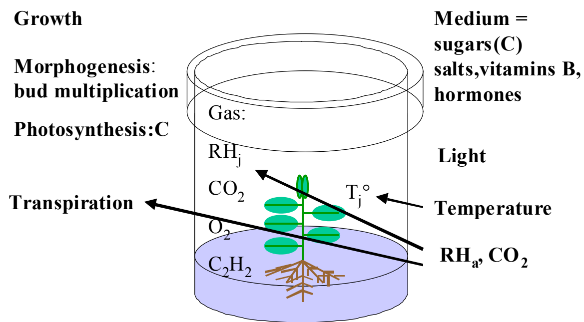
Technical requirements of tissue culture
The control of air relative humidity (RH) is not usually required, except if RH inside the culture room is too low (below 40%), and if tropical species are cultivated. Arid zone species cultivated *in vitro* can normally stand low RH inside the culture jar.

Gas exchange is also an important problem in tissue culture rooms: ethylene is often produced and accumulates, oxygen is required and is exchanged by diffusion, and carbon dioxide accumulates. Gas exchange depends on a good air circulation and this feature has to be carefully designed.

Tissue culture room designs for commercial purposes or research purposes
Four designs of tissue culture rooms can be distinguished as far as air circulation is concerned: standard (Fig. 2), horizontal air flow (Fig. 3), bottom-cooling (Fig. 4), and rotating shelves (Fig. 5). Two different air cooling systems are currently used: Freon gas, and water-glycol. Altogether this gives eight different technical designs of tissue culture rooms that have different physical characteristics for maximum temperature deviation (TDM), temperature heterogeneity, and the control of the rate of temperature change between the two set points.

Commercial laboratories are commonly equipped with the standard air circulation (Fig. 2) and with Freon gas cooling units. With these facilities you can only expect a TDM of 2 to 4°C, a high temperature gradient and serious condensation problems on jars, culture tubes, and petri dishes.

Some commercial laboratories are now equipped with a horizontal air flow system (air pushed horizontally through a perforated vertical panel at 20/30 cm before the wall, Fig. 3). This system allows one to reduce drastically the temperature gradient and also to lessen condensation



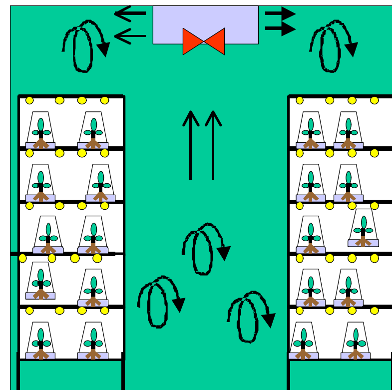
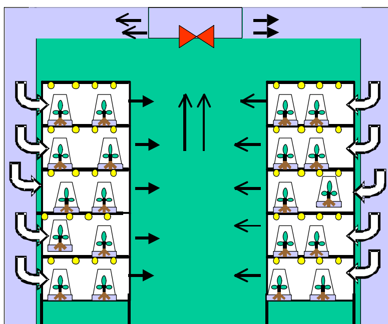


Fig. 1. *In vitro* plant physiology.

Fig.2. Air circulation: standard cooling system = Freon.



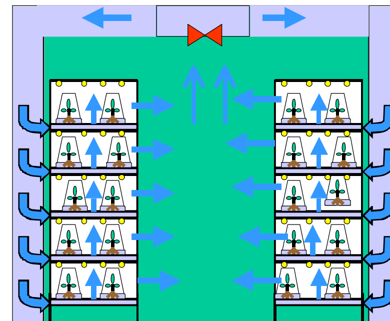


Fig. 3. Air circulation: horizontal air flow over each shelf

Fig.4. Bottom-cooling: air flow through each shelf

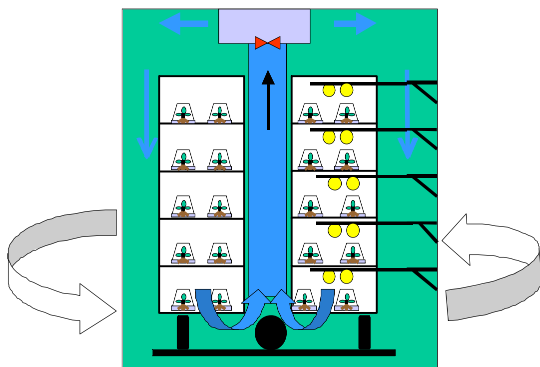


Fig.5. Rotating shelves, intermittent light over right hand side.

problems, provided that the cooling power is exactly matched to heat to be extracted. Air cooling with water-glycol gives better results than Freon gas cooling.

A third design is based on bottom-cooling equipment (Fig. 4): air is pushed through the shelf itself, or the shelf can be cooled with water-glycol. This system will completely eliminate condensation risks, and is only used for research purposes.

A new design, completely different from the others, uses rotating shelves (Fig. 5): a group of five to six circular shelves rotate at a rate of 1 to 2 rev min^{-1} and light is provided by only two fluorescent lamps on one side of the culture room. The light regime is rather peculiar (with alternating light and shade) but light is distributed homogeneously. This system, now used in several laboratories in France, seems to be well suited to tissue culture.

Light is a major factor for tissue culture, a factor to which usually little attention is paid by scientists. Different kinds and makes of fluorescent lamps used in laboratories can have drastic effects on morphogenesis and growth. Some laboratories are equipped with a mixture of 4500°K and 6500°K lamps, other with a mixture of 6500°K and Gro-Lux lamps. It is also possible to add incandescent lamps in the tissue culture room that will provide far red light (often programmed at the end of the light period). Far red light is essential for phytochrome-dependant physiological processes, especially for photomorphogenesis. It is now possible to modulate light intensity with electronic ballasts and high frequency lamps. This is very useful for research. Light homogeneity is a very difficult problem: usually we have a 20% intensity difference between the centre of the shelf and borders. Some equipment uses reflectors that can improve light distribution, but the simplest system consists of using mobile fluorescent lamps.

Conclusion

For commercial tissue culture rooms, if Freon gas is used as the air cooling technique, it is most important to calculate the cooling power exactly corresponding to the amount of heat created by fluorescent lamps and ballasts (if they are inside the room). Water-glycol can be also used. A temperature deviation of +/– 1°C is OK, and rate of temperature change has to be effectively controlled (very simple now with microprocessor technology). Horizontal airflow patterns greatly improve temperature characteristics and will thus facilitate the programming of cultures. The control of air humidity is usually not necessary, except if the relative humidity of the tissue culture room falls below 40%.

For research purposes, the cooling technique advised is obviously water-glycol, which will allow sophisticated temperature control. Shelves equipped with a bottom-cooling system will result in the complete elimination of condensation problems. Light also is an important variable to take into account (see above). Electronic ballasts and the use of high frequency fluorescent lamps will give the greatest possibilities for lighting. The technology of rotating shelves seems to be interesting, and more research is needed to evaluate the impact of this new technology on *in vitro* culture.

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M.J.W. Copland and M.J Varley (Imperial College at Wye, Ashford Kent TN25 5AH)
Environments for biocontrol research.

Our work has focussed on predatory and parasitic insects that attack plant pests in protected cultivation. In temperate countries many glasshouse pests have been introduced from warmer climates. Away from natural enemies they multiply freely and devastate crop plants. Most pests can survive and reproduce over a wide temperature range, but the main biological control agents are predatory and parasitic insects, which generally require warm sunny conditions for effective use. Biocontrol works during the summer but fails in winter and is often unpredictable during the spring and autumn.

To try to improve biocontrol in the glasshouse, we study insect biology in controlled laboratory environments. It is well known that growth in all living organisms has a strong relationship with temperature. The life cycle has a thermal constant, which requires so many day degrees above a threshold temperature, for instance, we may know that a particular species might have a threshold of say 10°C. If we could log the temperatures in the glasshouse we could accumulate the number of hours above 10°C and predict when the insect would become an adult. This in turn helps us to understand when biocontrol would be successful as many parasites and predators attack specific stages of the pests' life cycle.

We have therefore developed three types of controlled environments for our work in insect biocontrol. These include glasshouses, rearing rooms and experimental incubators.

Predictive glasshouse control system

Our first controlled environment project started in 1980 and was a new multi-span glasshouse structure comprising 32 cubicles. The system was installed with conventional motorised valves and vents controlled by individual thermostats and time switches. Our first challenge was to design a computerised environmental control system which could run the glasshouse and log environmental parameters in each cubicle every hour, and, in the event of computer failure, automatically hand back control to the original system.

Our laboratory work showed that temperature affected not only development of insects, but length of adult life and the development of eggs within the body. Similarly sunlight had an effect on dispersal and searching for pests. During the early 1980s, we began to make simulation models of the interactions between a plant and pests like mealybugs, scale insects, greenfly, whitefly, leafminer, plant hoppers and spider mites and their specific predators and parasitoid wasps. We could validate these models by setting up caged populations in the glasshouse and using logged environmental data to see if actual emergence and offspring number corresponded with our predictions.

These simulation models showed that for biocontrol to succeed, we needed a critical amount of heat and light each day. The problem was that this might use far more energy than we were using at present. We began to think about a different kind of environmental control program. Up to now, computer systems acted like a rather expensive set of thermostats and time switches. Our idea was that if we were given an energy budget, perhaps we could use it creatively to increase the success of the biocontrol.

For our biocontrol insects to work it is only worth raising the temperature if the light conditions are bright - but you don't have to have a good day every day. The system might 'spend' energy by

day but energy could be reclaimed by using a lower night-time setpoint. Alternatively energy could be 'carried forward' to the next day and if necessary 'spent' on artificial light to supplement dull conditions.

Our first task was to calculate an acceptable energy budget. We needed to log outside air temperatures, wind speed and direction, and surface area of glass and then work out how much energy our conventional system would have used to achieve fixed set points. This seemed to work reasonably well.

Next we included the biological simulation programs and databases in our control program. The computer now had several things to do:

1) Accumulate the conventional energy budget;
2) Initially run the system to conventional set points;
3) Every hour run simulations of performance of the pests and our biocontrol agents;
4) If biocontrol performance was below the optimum, we could start to make decisions about whether to use more energy to increase it or begin to save our energy budget for a later 'spend';
5) Revise the settings to switch valves, vents and lights.

In general, the more energy or (beneficials) you could throw at the system, the more successful the biocontrol. So we thought "suppose here's an energy budget for this cubicle - now use it to promote both plant growth and the efficiency of the biological pest control". A new idea was born!

With this system it was possible that, with the given energy budget, biological control could never be effective. Our system gave nightly reports. These would give three options:

1) To increase the energy budget;
2) To consider releasing more biocontrol agents;
3) To reach for the spray can.

Our system ran until the late 1990s. Then our A/D interface died, our Commodore computers were no longer made and we haven't yet found the time to update to a PC platform.

Insect rearing rooms

Our second set of environmental controls are installed in a set of 12 shipping containers, in which we rear all our insect cultures and conduct many of our experiments. We needed a controlled temperature environment, modest lighting, and a defined photoperiod. Most of all we needed to have a system that would be cheap to run and easy to maintain.

The 12 containers are of the insulated type and occupy a space 30 m long, 6 m wide and 2.4 m high. They are painted white on the outside to reflect heat and each has a door cut in the back for easy access. The system is computer-controlled using our own design of daisy-chained serial ports to operate 96 channels of input and output. The insulated containers have a white interior and each has four 100 W computer-controlled fluorescent lamps above insect cages. The lamps give off more than enough heat to maintain 25°C in depths of winter but the rooms need a small supplement of heating during the dark period.

Cooling is accomplished by drawing outside air through each container from end to end with a powerful fan, while at the same time air is circulated in the opposite direction to each

experimental cage by small blower fans. We monitor outside air temperature, then control the cooling fans and the heater on a pulsed time-proportional basis so as to ensure the most effective use of energy. While the control cycle period is about one minute, the fans and heaters might switch on for only a few seconds depending on outside air temperature and distance from set point. We run the photoperiod largely at night on cheap rate electricity, and students only get illuminated access in the morning. This also means that the lights are off when the summer sun is at its hottest. Provided the outside air conditions are not above the control temperatures, we achieve an accuracy within 0.5 K of our target temperatures.

Our main concerns are failsafe systems. If the computer stops, the system falls back to analogue control circuitry in aspirated screens. If the screens fail and the temperature rises above a set limit, a conventional bimetal room thermostat takes over control, disconnects all solid state relays, shuts off lighting and operates heating and cooling circuits. All problems result in an automatic call-out via a telephone.

The system had lots of interesting problems to solve. Our control program used Microsoft BASIC which seems to have undocumented bugs when using system timers for long periods, which caused the serial port to hang up every few days. We overcame this by making the computer reset itself every night at midnight.

Our solid-state relays were often damaged by power cuts or by the operation of manual over-ride thermostat switches leaving them in the on condition. Metal Oxide Varistor protection eventually cured all these problems but it took a long time to sort out.

Computer-controlled incubators

Our latest controlled environments are a suite of computer-controlled incubators. Most cooled illuminated incubators work on the principle of running the compressor continuously and using heaters to achieve the desired temperatures. This means they are wasteful of energy and ice up over prolonged experiments. In addition the lighting has limited on/off control and time switches usually give only two temperature settings over the 24-hour period. When problems occur the failsafe controls are unsuitable when dual temperatures are in operation.

Our incubators are based on a domestic larder fridge cabinet which is fitted with six 8 W fluorescent lamps with ballasts and starters outside of the cabinet. A recirculating fan is positioned inside at the top of the cabinet to blow air down over the cooling plate. A standard PC is used for control, equipped with interface boards (National Instrument). The control program is written using the Lab View Programming language. The computer monitors temperatures via PRT100 sensors and addresses solid-state switches in each incubator to control lighting, heating and cooling.

To make our system more energy efficient, we needed the ability to switch the cooling on and off whenever we wanted. However, a domestic compressor will not restart under pressure so we have incorporated a bypass valve which, depressurises the system as soon as the compressor switches off. This rapidly stops cooling and enables an instant restart, as well as automatically defrosting the cooling plate.

The computer program allows us not only fixed or dual temperatures but sine-wave shaped temperature curves and manually designed changes over the 24-hour period as well as the ability to read data logged in the field or glasshouse so that we can exactly reproduce outside conditions.

Because of the delays inherent in a compressor system, we cannot use direct proportional control about a set point. To overcome this problem, we have an upper and lower switch point, currently set 0.25°C above and below the set point. On switch-on, heaters or coolers drive the temperature through the switch point, past the set point to the other switch point and the time taken is recorded. The reverse time taken back across the switch points is then measured and the computer then calculates an oscillating cycle of heating and cooling which will keep the temperature within the switch points.

If these timed cycles result in an overshoot or undershoot of the switch point they will be adjusted until the temperatures stay within range. To our surprise, the inside cabinet achieves an accuracy of around +0.25°C either side of the set point.

The advantage of the central computer is that it can make intelligent decisions about control and when (and how) to call for help if the temperatures are outside the set limits, for instance, if there is a malfunction in heating or cooling, a sensor fault, or simply if the doors have been left open. These computer-controlled incubators are cheap to run, cheap to make, and give us facilities that no one else in the market offers.

In conclusion

We continue our work to evaluate new predators and parasites for biocontrol. We can design experiments to look at the interactions with environmental parameters at constant temperatures then validate our assumptions by mimicking real life variable conditions. Linking our systems with field data loggers is really useful. Our controlled environments don't use computers to mimic switches but rather set up dynamic responsive environments, which reflect real life conditions.

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K. Manger (Royal Botanic Gardens, Kew, Wakehurst Place, West Sussex RH17 6TN) **The Millennium Seedbank - a technical perspective.**

The Millennium Seed Bank is the largest international millennium project. Over the next ten years, the project aims to conserve 10% of the world's flowering wild plant species.

The Wellcome Trust Millennium Building (pictured) is the latest seed bank based at the country estate of Wakehurst Place, which has been the home of the Royal Botanic Gardens seed bank since the early 1970s. The original seed bank at Wakehurst was no more than a small refrigerator with seed drying being achieved using silica gel. Through research and technological advances, the new facilities are equipped with in excess of 100 m^2 of mechanical drying and 135 m^2 of cold storage secure within a 900 m^2 underground vault, where the collections will be safe from all reasonable risks.

What are seed banks, and why do we need them?
In essence, our seed bank is a secure, long-term, cost effective store for the world's wild plant germplasm in the form of seeds. The main pressures on the wild plant populations are due to the effects of human population growth and climatic change. It has been estimated that a quarter of the worlds plant species will be under threat of extinction within the next 50 years. We are dependent on plants not only for oxygen, food and fuel, but also for many other benefits including medicine. In excess of one fifth of western prescribed medicine is of plant origin and a greater proportion of the developing world relies directly on plants for their pharmaceutical properties.

It has been estimated that perhaps as many as 90% of seeds from wild plant species can be dried to low moisture contents (*circa*. 5% wet mass basis) without them being killed. Once dry these seeds can be frozen to −20°C and in this state will remain viable for tens or hundreds of years. Such desiccation tolerant seeds are termed 'orthodox'. Desiccation intolerant seeds are termed 'recalcitrant' and cannot be successfully frozen using standard seed bank techniques due to the harmful formation of intracellular ice crystals.

Research has shown that between about 5% and 20% moisture content (wet mass basis), there exists a negative linear relation between orthodox seed longevity and moisture content. Drying increases seed longevity by two to three orders of magnitude. Rapid drying is also important; although seed size and structure influences the rate of drying, the majority of seeds dry to *circa.* 5% moisture content with in two to five days. Freezing dried seed to −20°C further improves seed longevity of all orthodox seeds by several orders of magnitude.

Having obtained permission to collect seed from a particular country, mature seed is collected from wild plant populations by expert botanists. It is rapidly air freighted to Wakehurst Place where it undergoes initial drying at 15°C / 15% relative humidity (RH) for up to one month.
At this stage the seed is contained within moisture permeable cotton bags.

The diverse size and shape, and the delicate nature of some seed prevents large-scale automation of the seed cleaning process. The samples are hand cleaned using graded sieves and aspirators to remove debris, chaff and empty seed. This work is carried out in a controlled access laboratory with dust extract facilities to protect staff from potentially harmful allergens or dusts.

A sub-sample from each cleaned seed batch is subjected to x-ray analysis in order to assess seed quality. Poorly developed or insect infested seed are clearly shown on the resultant x-ray. The x-ray equipment is specially designed and housed in a purpose-built dark room.

Following x-ray analysis, the collection is counted and then further dried at 18°C / 10% RH prior to sealing in uniquely labelled, suitably sized, moisture tight glass containers and banked at –20°C. It is of note, that the air within the cold rooms is at approximately 60% RH, it is therefore critical that seed is held within moisture tight containers in order to maintain its long term viability.

Post freezing, a small sample of seeds is removed for viability testing. Seeds are grown under optimal conditions on 1% agar in a variety of fan-assisted cooled incubators, capable of either constant or alternating temperature control with +/– 1°C temperature tolerances. The photoperiod can also be controlled with cool white light, saturating any light requirement of the seed. In some instances nitrate or gibberellic acid is added as a growth stimulator. In order to maintain a high quality seed collection an initial germination of at least 75% is required wherever possible. Collections are subsequently retested every ten years.

The two prefabricated dry rooms of the Wellcome Trust Millennium Building are well insulated with plastic clad floors and ceiling with an epoxy floor. Entry is via an air lock, which helps to maintain the internal environment. Air is thoroughly re-circulated with low level extract and high level return. The air is dried via 'ML1100 Munter' units, which use re-chargeable lithium chloride desiccant. The re-circulated dry air is cooled to the appropriate temperature prior to re-entry into the dry room.

Each of the three 45 m^2 cold rooms is accessed via the final dry room. The rooms are heavily insulated with 200-mm closed cell polystyrene. The walls and ceiling are plastic clad with a floor of stainless steel. The rooms are shelved with purpose-designed powder-coated static and mobile shelving thus maximising storage efficiency. Doors and windows are fitted with heaters to prevent icing. All door locks have an internal emergency override mechanism. The cold rooms are raised off the vault floor with an air insulation gap to prevent permafrost. Cooling is achieved using 'Prescold semi-hermetic reciprocating compressors' with 404A refrigerant. The compressors are water cooled with the recovered energy being used to heat domestic hot water.

Due to the hostile environment within the cold rooms, authorised staff wear full arctic thermal protective clothing and are only permitted to work in the environment for 20 minutes. Control of staff exposure is via a timed personnel alarm system, which also has a panic alarm facility.

The plant associated with each of the dry rooms and cold rooms is run with an alternating duty and backup system, whilst the building has its own generator back up.

The 900-m^2 underground vault which houses both the main dry room and cold rooms is constructed of 500-mm reinforced concrete. The air entering this vault is monitored for radiation. The vault currently houses three cold rooms with expansion space for another six; and has been built with a life expectancy of 500 years.

In summary, the various controlled environments within the Wellcome Trust Millennium Building support a project, which will underpin probably the largest and most ambitious *ex-situ* plant conservation project currently undertaken in the world.

D. Johnson (Post-harvest Group, Horticulture Research International, East Malling, West Malling, Kent ME19 6BJ) **Controlled environments for preserving horticultural crops.**

Biological requirements

Once harvested fruits and vegetables no longer have access to water or nutrients, the only interchanges with the environment are then loss of water and carbon dioxide and uptake of oxygen. Although anabolic processes occur during fruit ripening the balance of metabolism is catabolic and ultimately the fruit becomes senescent. The objective of storage is to delay senescence and thereby preserve the desired characteristics of fresh horticultural produce and thus provide to the market a continuous supply of product with high nutritional value that has acceptable sensory quality. In the simplest terms if the objective is to delay senescence then means should be sought to reduce the rate of metabolism of the product. This can be done by reducing product temperature or the concentration of oxygen in the environment or by increasing the concentration of carbon dioxide (Johnson, 1999). Ripening in many fruits is accompanied by a dramatic rise in respiration; a phenomenon referred to as the 'climacteric'. The more dramatic the rise in respiration rate, then the more rapid the rate of ripening. The storage of apples and pears is profoundly influenced by the date of harvest relative to the onset of the climacteric. Longest storage is generally achieved when fruit is harvested just prior to the climacteric.

Accepting that freezing of plant tissue is generally detrimental it might be expected that the lowest respiration rate and the longest storage life would be achieved at a temperature just above the freezing point of the tissue. Apples freeze at about −2°C thus the lowest temperature that can safely be used is −1 to 0°C. However many cultivars of apple grown in the UK suffer injury leading to breakdown of the tissues when exposed to low temperatures well above the freezing point. In apples suffering low temperature stress the respiration rate is higher than expected. The marked variation in susceptibility to low temperature injury that exists between cultivars complicates the recommendations that are made for the storage of UK apples. For example Gala apples can be stored in air at 0°C whereas Bramley's Seedling apples require a minimum temperature of 4°C.

In addition to temperature the composition of the atmosphere effects the rate of respiration. Decreasing the oxygen and increasing the carbon dioxide content of the atmosphere retards respiration, the effects of the two gases are additive. The use of low oxygen and/or elevated carbon dioxide levels has been exploited fully to maximise the storage quality of different cultivars of apples.

Recommendations for the storage of apples and pears that are provided to the UK fruit industry are based on factorial trials involving temperature, carbon dioxide and oxygen concentrations conducted in our experimental facilities at East Malling. Although the storage potential of a variety is genetically determined our goal is to develop storage conditions that maximise the storage life of each of the important commercial cultivars and of new selections and varieties from breeding programmes at HRI and elsewhere. Part of the variability in response of different apple cultivars to similar controlled atmosphere (CA) conditions can be explained by inherent

differences in their rate of respiration and the rate of diffusion of gases through the skin of the fruit. Fruits respond to the conditions within the tissues inside the fruit that can be very different from those in the surrounding atmosphere. Further details on the current status of CA technology for the storage of apples in the UK have been published recently (Johnson, 1999).

Storage behaviour is influenced by pre-harvest orchard and climatic factors and by the stage of maturity at the time of harvest. Consequently a programme of work to define optimum storage conditions for any given cultivar extends over a number of seasons. In addition a range of picking dates are used in order to provide reliable information to the growers. It would be foolhardy to make recommendations based on data acquired in just one year.

Engineering requirements

Pioneering research on CA storage was carried out by Kidd and West at the Ditton Laboratory that was built in 1930 and is situated to the East of the main laboratory complex at HRI-East Malling. Ditton Laboratory was the centre of UK activity in post-harvest research until June 1991 when it was decided to construct a new facility on a different site within the East Malling complex (Jameson & Stow, 1993). The new facility was opened in 1992 and named 'The Jim Mount Building' as a tribute to Sir James Mount - a prominent Kent fruit grower and staunch supporter of horticultural research and former chairman of the East Malling Trust for Horticultural Research. The Building is steel-framed and the entire wall and roof cladding consists of 60 mm polyurethane insulation panels to provide maximum isolation from ambient conditions. Within this shell are 10 controlled temperature (CT) rooms, two service rooms, one large laboratory and the refrigeration plant room. The ground floor CT rooms, laboratory, corridors and doors are designed to facilitate easy fruit handling with fork-lift or stacker trucks. There are six CT rooms on the ground floor with dimensions of 6 m x 6 m and a clear working height of 3 m. All rooms have 125 mm polyurethane insulation to the walls, 150 mm to the ceiling and 50 mm extruded polystyrene under-floor insulation.

A centralised, tertiary refrigeration system was selected to give maximum flexibility in use for the CT rooms. A pair of standard, commercial chiller units, each incorporating an electronically controlled, variable-duty screw compressor, provides primary cooling. Each unit is adequate to cope with normal operation, so providing 100% standby capacity. The duty chiller is used to cool a 3500 litre reservoir of propylene glycol to a nominal temperature of −12°C. The CT rooms were designed to operate in the range −3 to +30°C with a spatial variation not exceeding 0.5°C. Measurements of spatial variation in temperature indicate that in the larger rooms it is unlikely that the produce temperatures will vary by more than 0.25°C from one location to another

For small-scale CA experiments two sizes of container are in regular use. The more popular size holds approximately 90 kg of fruit. Each of the CT rooms will accommodate 16 of this type of container. They are fitted with all the necessary tube connections for remote monitoring and scrubbing of CO_2 and ethylene, as well as oxygen control by ventilation with compressed air. The larger type of CA container holds approximately 400 kg of fruit. Control of the atmosphere inside these containers is the same as that described for the smaller units. Two automatic atmosphere monitoring and control systems provide information on temperature, CO_2 and O_2 status for a maximum of 80 containers. The concentrations of gases in each container are measured at approximately 3-h intervals. Deficiency of O_2 is corrected by the admission of air whilst excess CO_2 is removed by individual lime scrubbers. At present equipment is installed to allow up to 28 containers to have automatic CO_2 control. The remaining containers have continuous scrubbing and operate at CO_2 concentrations below 1%.

Twenty-four containers may be linked to variable-rate ethylene scrubbers, allowing for manual control of concentration. Concentration of ethylene inside containers is a function of both fruit production rates and scrubber capacity, but as a guide it proved possible to achieve concentrations of ethylene below 10 vpb (10 nL L^{-1}) in some instances.

Safety requirements

There are recognised hazards associated with working in the Jim Mount Building and a concomitant requirement to provide a safe working practice for all staff that enter the building. The building incorporates a comprehensive network of gas services. All CT rooms are supplied with a range of five gases to facilitate instrument calibration and manually to establish or adjust the atmosphere in storage cabinets. An extended range of eight gases is available in the instrument service room to operate CA monitoring and control systems, mixing stations for flow-through systems and gas chromatographs. To ensure the safety of those entering and working in the Jim Mount Building an improved atmosphere monitoring and alarm system has been installed recently. The new system is designed to prevent a non-breathable atmosphere developing and to fail-safe i.e. the system actively holds open valves controlling the gas supplies to the building so long as no hazard exists, but close until manually reset if a fault develops.

An industrial IBM-compatible PC monitors continuously the status of 16 alarm units located in all CT rooms, laboratories and other areas through which gas services run. If any alarms are activated the corresponding box on the PC screen will change to reflect the new status. Whenever the status of any of the alarm units changes from normal the condition is recorded with the date, time, unit identification and what the condition changed to. The maximum number of entries in the memory is 10,000. Earlier events can be viewed by pressing the history button again to page back through the memory.

Gas analysers comprising an electrochemical O_2 detector (0-25%) and an infra-red absorption CO_2 detector (0-2%) are located in each area being monitored. The analysers are fitted within a single enclosure that is heated to minimise condensation. Store atmosphere is sampled continuously through a dust filter using a small fan. Alarm level 1 activates an audible alarm at the relevant location if the O_2 concentration falls below 19.5% or CO_2 rises above 0.5%. The PC display changes from 'STATUS - NORMAL' to 'STATUS - ALARM'. Alarm level 2 activates when the O_2 concentration falls below 18% or CO_2 rises above 1.5%. This will invoke the same response as a level 1 alarm but additionally audible and visual alarms are activated at the entrance to the building, gas supplies to the building are shut-off and an alarm signal is sent to a centrally monitored building management system. A fault in the system will invoke the same response as a level 2 alarm with the exception that 'STATUS - FAULT' will be displayed in large red letters on the PC screen.

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