

Proceedings of the UK Controlled Environment Users' Group**1999 SCIENTIFIC MEETING****“CONTROLLED ENVIRONMENTS AT YORK AND IN GERMANY”****Volume 10**

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UK CONTROLLED ENVIRONMENTAL USERS' GROUP**1999 SCIENTIFIC MEETING****“CONTROLLED ENVIRONMENTS AT YORK AND IN GERMANY”**

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

SUMMARIES OF PAPERS

Maggie Smallwood, Dawn Worrall, Louise Byass, Lot Doucet and Luisa Elias (The Plant Laboratory, Department of Biology, University of York, York YO1 5YW, UK) **Plant antifreeze proteins.**

1. Biology

Many organisms adapted to live at sub-zero temperatures express antifreeze proteins (AFPs) as part of their survival strategy. Antifreeze proteins exhibit two related properties, thermal hysteresis and inhibition of ice recrystallisation (RI). Thermal hysteresis is the non-colligative depression of the freezing temperature of aqueous solutions below their melting temperature in the presence of ice. Ice recrystallisation is the growth of large ice crystals at the expense of smaller ones, a process, which is inhibited by antifreeze proteins. Both properties are thought to result from direct interaction of antifreeze proteins with ice crystal surfaces.

Five structurally unrelated antifreeze proteins from cold-water fish have been described and a further two from insects. Thermal hysteresis has been identified in a number of plants in Duman's laboratory (Duman *et al.*, 1993, Duman & Olsen, 1993) and activity in winter rye further characterised in Marilyn Griffiths' laboratory where pathogenesis related proteins (PRPs) have been identified as potential antifreeze proteins (Griffith *et al.*, 1997). In general the thermal hysteresis values of plant extracts are rather low in the context of the temperatures to which plants are exposed. This has led to speculation that it is the RI activity of antifreeze proteins which is important in plants, where modulation of the growth and deposition of ice within the apoplast may be important especially within dense tissues such as roots.

With this background, we have used a modified version of Charles Knight's splat assay to screen a wide range of cold-acclimated plant species for RI activity (Smallwood *et al.*, 1999). Biochemical characterisation of the 45 active proteins identified during the screen indicates that, as in fish, a diverse range of proteins have been co-opted into antifreeze function in plants. Further, an antifreeze protein expressed in the apoplast of cold-acclimated carrot roots has been purified, the cognate gene cloned and expressed in an heterologous plant system and extracts from the transgenic plant shown to possess RI and thermal hysteresis activity. The sequence of the carrot antifreeze protein is similar to that of polygalacturonase inhibitor proteins (PGIPs) but it does not inhibit polygalacturonase from *Erwinia*, tomato or *Aspergillus niger*. The native protein is N-glycosylated but its RI activity is not affected by removal of the glycan moiety. The carrot antifreeze protein is a cold-induced, single-copy gene, which contains no introns.

Acknowledgements

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Garry Taylor (Sanyo Gallemkamp) and **Colin Abbott** (Department of Biology, University of York, York YO1 5YW, UK) **Plant antifreeze proteins.**

2. Technology

To carry out such research it is necessary to be able to hold plants at temperatures below 0°C continuously but to have normal light/dark cycles and to enable plants to continue to transpire. In order to maintain truly sub-zero growth conditions, even during defrost, the refrigeration system required two evaporator coils. One would be cooling whilst the other was de-icing. A complicated air changeover system was employed to redirect the circulating air over the appropriate cooling coil and to allow de-icing to take place. Special drainage systems were designed to allow water to drain away from the chamber at sub-zero temperatures without icing. The light box was separated by a triple-glazed system to prevent condensation/ice forming in the light box. Philips High Frequency lamps were used in order to allow low temperature ignition.

The specifications of the cabinet built by Sanyo Gallenkamp to achieve these conditions were as below (modifications in italics).

CABINET SPECIFICATIONS

General

Controlled variables	Temperature, relative humidity, light
Temperature: range	– 5°C to +40°C (lights on) – 10°C to +40°C (lights off)
Humidity: range	30% to 95% rh at +40°C 56% to 95% rh at +20°C 67% to 95% rh at +10°C

Typical values at 25°C ambient temperature; actual ranges depend upon actual ambient conditions.

Dimensions: internal	1200 x 600 x 900 mm (w x d x h)
Growing height	850 mm

Dimensions: external	1870 x 820 x 1860 mm (w x d x h)
Construction	
Interior	Stainless steel with a reflective coating in the growing chamber
Shelves	Two separate half-depth shelves
Access	Full-width insulated door Viewing window with light shield 66 mm cable entry port in the left hand side
Airflow	Vertical, 0.2 m/s, turbulent
Attitude	Protected against electrical overload
Safety	Thermostatic temperature protection
Glazing	<i>Triple-glazed panel</i>
Heating and cooling	
Heater type	1 inconel sheathed element, operating at black heat
Cooling	Single-stage mechanical refrigerator. R134A refrigerant <i>Dual-coil defrost facility to prevent the progressive build up of ice</i>
Humidification/dehumidification	
Humidification method	Vapour phase generation
Dehumidification method	Condensation on to a cold surface (dewpoint)
Lighting	
Method	12, 32 W <i>TLD 83 fluorescent tubes</i> , plus 4 incandescent lamps, in air-cooled light box, variable intensity from 10% to 100% of maximum
Maximum	300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at 300 mm from base of the chamber, with a supply voltage of 230 V and at 25°C ambient
Electrical	
Supply	220/240 V, 50 Hz, 1 phase
Demand	As stated on the cabinet rating plate
Additional connection	Earth

R. Matyssek & H. Blaschke (Lehrstuhl fuer Forstbotanik, Ludwig-Maximilians-Universitaet Muenchen, Am Hochanger 13, D-85354 Freising, Germany) **Testing growth chambers: the distribution of light, temperature and air humidity in walk-in cabinets.**

Testing controlled-environment rooms, in particular phytotrons, for the distribution of relevant microclimatic factors like light, air temperature and air humidity as well as wind velocity is a pre-requisite for becoming acquainted with the operational range and the extent of uniformity in their growth conditions. The latter issue is crucial for the reliability of plant physiological research, even though the microclimatic conditions may be significantly biased by the number and structure of plants being exposed in the phytotrons. In this case, two of four temperature- and humidity-controlled walk-in cabinets (YORK, Germany), each 2.2 m in width, 2.8 m in depth and 2.0 m in height, needed to be rebuilt to install a lighting system suitable for plant

growth. The installation of the lamps required the heat exchange capacity (and hence, the demand for electrical power and cooling water) to be enhanced substantially.

The installed lighting system consists of a total of 24 high-pressure halogen lamps (MTD 400DL/BH, spectrum 300-700 nm) which can be switched by six blocks of four lamps each. Combinations of these blocks yield photosynthetic photon flux densities (PPFD) between about 170 (four lamps) and $900 \mu\text{mol m}^{-2} \text{s}^{-1}$ (24 lamps) at 1 m from the light sources.

The operational range of the temperature control is 5 to 35°C ($\pm 1^{\circ}\text{C}$) at maximum PPFD, however, -5°C can be reached and maintained for several hours with the lamps turned off. Maximum ranges in air humidity of 30 to 80% ($\pm 7\%$) can be achieved at high PPFD, and up to 95% is achievable in darkness. Accordingly, the range in dewpoint temperature is between 7 to 31°C , with the air humidifier having a capacity of 15 L h^{-1} . The wind velocity can be set to reach more than 4 m s^{-1} , while the charcoal-filtered air in the phytotrons is exchanged at a rate of $60 \text{ m}^3 \text{ h}^{-1}$.

The above listed operational ranges represent nominal values. The actual distribution of the microclimatic conditions was examined by subdividing the chamber floors into a co-ordinate grid with 20 measuring positions. Above each of these positions, two heights were defined for further measurements (at 1.0 m and 1.5 m from the light sources in the chamber ceiling, the latter distance representing the usual foliage height of woody plants when grown in containers on the chamber floor). A bar was prepared which carried five PPFD sensors and five electrical thermometers so that five positions of the co-ordinate grid could be assessed synchronously within one out of four transects. Portable humidity sensors and anemometers were exposed at selected grid positions. All tests were conducted in empty cabinets. Although only a selection of tests can be demonstrated in the following, the findings are representative for both phytotrons.

Light distribution is of paramount importance for plant physiological studies. Although the nominal PPFD of about $900 \mu\text{mol m}^{-2} \text{s}^{-1}$ was reached at 1 m from the lamps (Fig. 1), this maximum only occurred with the central transects and close to the left chamber walls. PPFD was reduced by about $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ along the front and rear transects, and most importantly, there was a marked depression in light by more than $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ in the centre of the chambers, along a line normal to the four transects. This bias in light distribution was also indicated at 1.5 m from the ceiling and at low light levels and related to a very regular alignment of the lamps along the chamber walls (Fig. 2, open circles). The manufacturer's sub-contractor responsible for the lighting system individually repositioned the lamps as indicated by the asterisks in Fig. 2. Measurements conducted after this re-arrangement (Fig. 3) yielded an acceptably uniform light distribution in the chambers, which is remarkable, given the minor changes in lamp position and the absence of lamps in the central part of the ceiling.

The wiring of the power supply has turned out, however, to result in interactions between temperature control and PPFD. Changes in air temperature by 15°C influence maximum PPFD by about $180 \mu\text{mol m}^{-2} \text{s}^{-1}$. Such changes are of relevance when simulating diurnal courses of air temperature in the phytotrons. The light spectrum provided by the chosen lamp type (see above) covers the wavelength range of the photosynthetically active radiation (PAR) well when examined with a light spectrometer. However, marked peaks in PPFD are detectable at about 470, 530 and 560 nm, whereas a rather low minimum is found at 630-660 nm. Above 660 nm, PPFD distinctly increases to rather high levels. Irradiance approaches zero at 360 nm so that a major influence of UV radiation can be excluded.

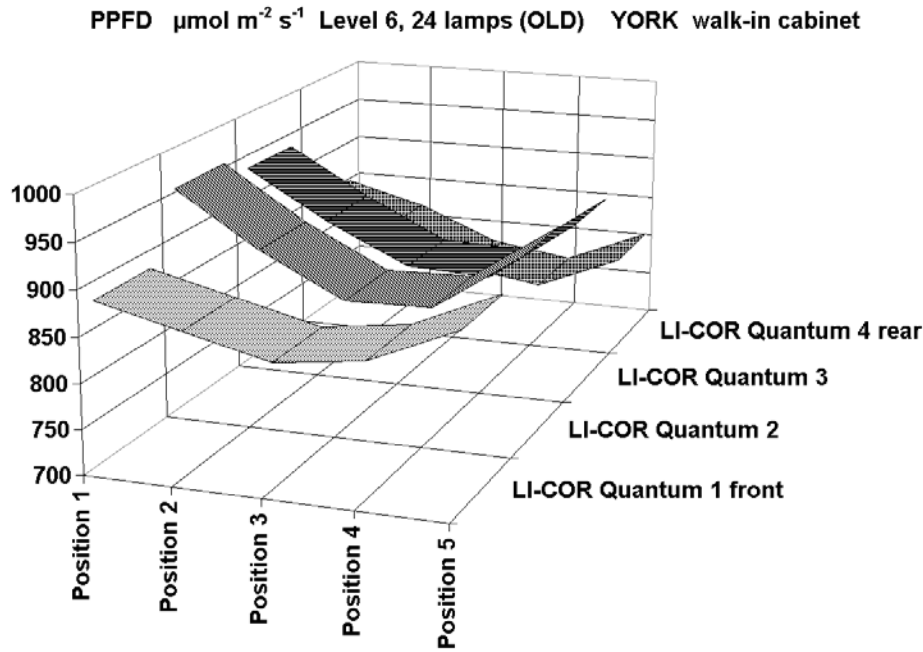


Fig. 1. Distribution of photosynthetic photon flux density (PPFD) in the phytotron before repositioning of the lamps in the chamber ceiling (all 24 lamps switched on, measured at 1 m from the lamps).

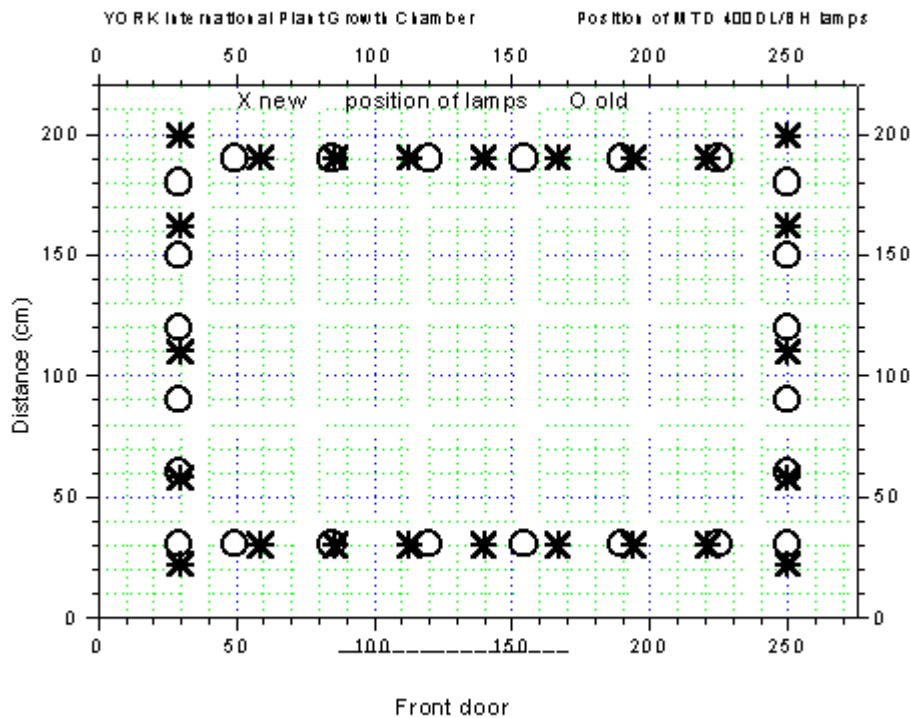


Fig. 2. Arrangement of the lamps along the edges of the chamber ceiling, as aligned to the front, rear and lateral walls (open circles = original lamp positions, asterisks = new lamp positions).

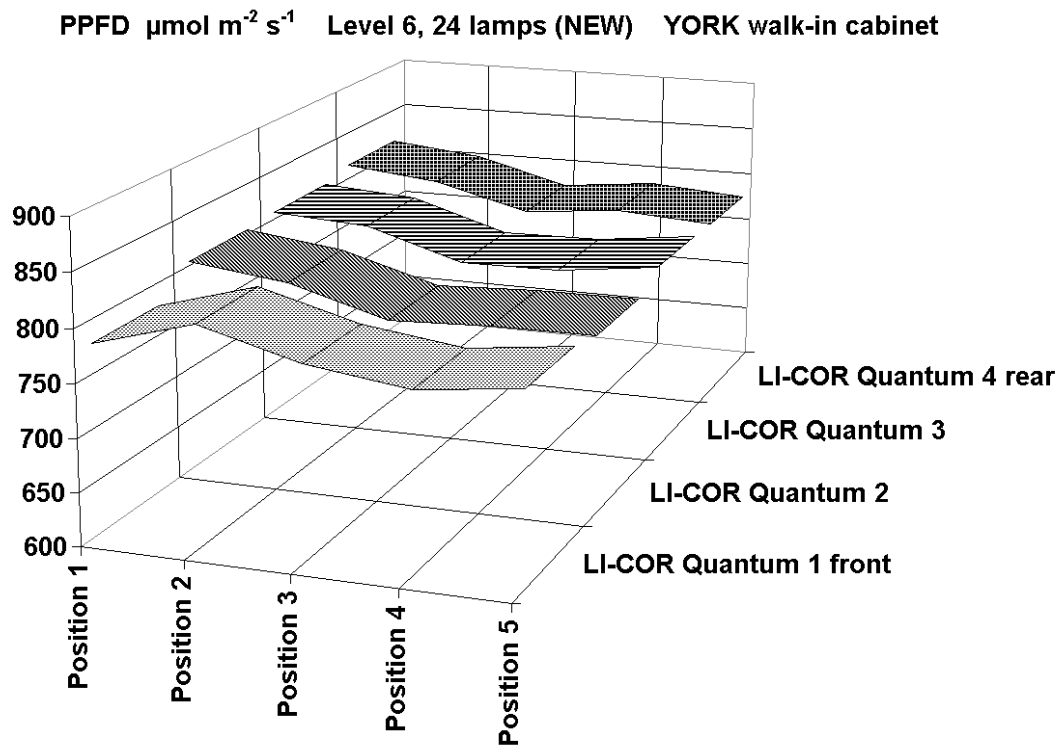


Fig. 3. Distribution of photosynthetic photon flux density (PPFD) in the phytotron after repositioning of the lamps in the chamber ceiling (all 24 lamps switched on, measured at 1 m from the lamps).

The uniformity in air temperature increased after repositioning of the lamps (see above), but strongly depended on the air circulation in the phytotrons. Fresh air is released into the chambers at the bottom, and sucked out at the top of the rear wall. At about 20 cm below the lamps, a transparent plastic shield oriented in parallel to the ceiling, forces the air circulation towards the front wall. This shield, however, consists of a number of individual slat-like plates, which can be changed in position relative to each other to allow for the setting up of variable gap sizes. The amount, width and distribution of these gaps strongly influence the temperature gradient between rear and front wall. The meticulous adjustment of the slats still causes the temperature transect along the front wall to be consistently lower by about 1°C when compared with the temperature levels of the other three transects (Fig. 4). This drop in temperature occurs even with the air circulation running at maximum rate, and the front door tightly sealing the chamber. In the best case, the temperature variation of the other three transects is uniform within 1°C (Fig. 4). Minor changes in gap width between the slats may cause the temperature of the front transect to drop by more than 2°C .

Relative humidity was closely determined by the distribution of the air temperature. When cooling the temperature to -5°C (see above), the humidity control needed to be turned off, which in fact represents a minor shortcoming only because the absolute humidity is inherently low at low temperatures. A set frost temperature could be maintained throughout the night periods of several consecutive days (up to one week) without ice forming on the heat exchanger. Temperatures below 0°C could be achieved also at low PPFD (running one block of four lamps).

Wind velocity was the least uniform property in the phytotrons. Relative to the maximum measured in the centre of the chambers, the velocity could be more than 70% less in the corners.

To prevent such effects, additional fans can be positioned in the corners of the chambers to increase the uniformity of wind movement across the entire chamber volume. An improved mixing of the air is important for envisaged experiments exposing plants to elevated levels of ozone or CO₂. On the other hand, exposure to high wind has been observed to be detrimental during the establishment of tree seedlings. A windshield needed to be employed in front of the air inlet at the bottom of the rear wall to ensure undisturbed seedling growth.

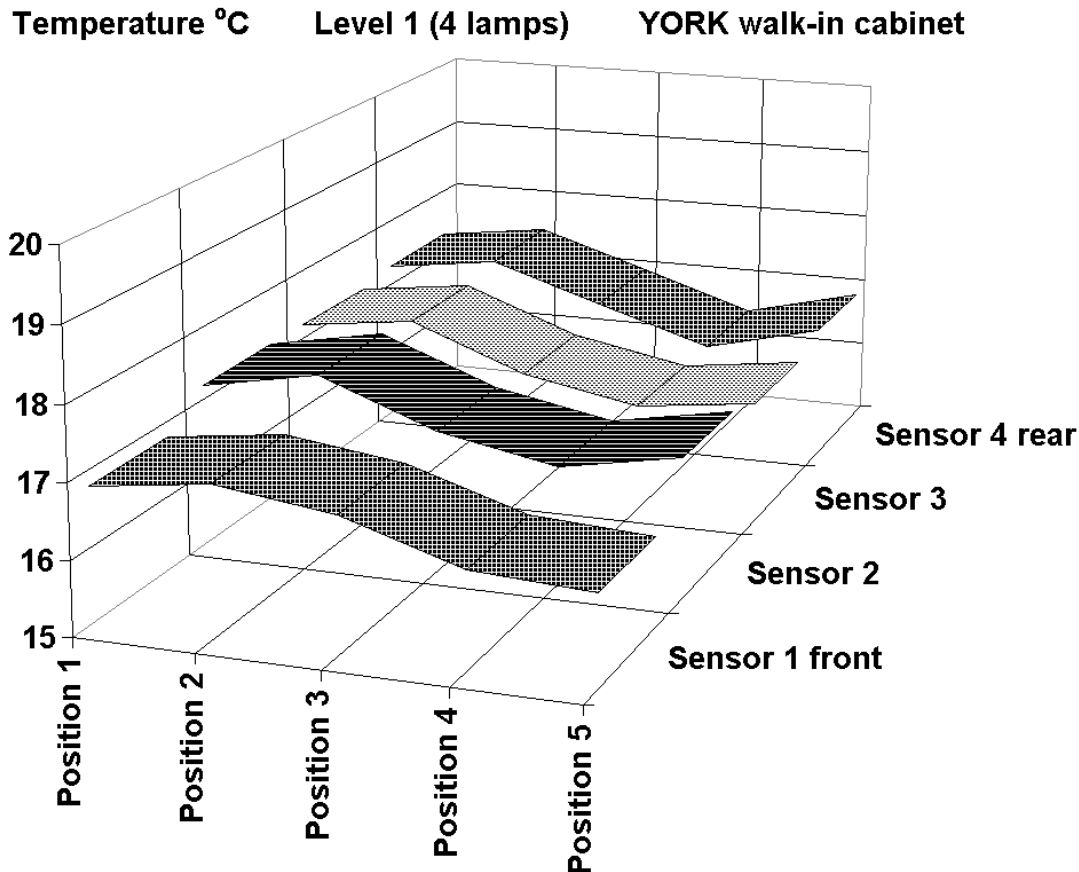


Fig. 4. Distribution of air temperature in the phytotron after repositioning of the lamps in the chamber ceiling (all 24 lamps switched on, measured at 1 m from the lamps with shaded sensors; wind velocity in the central part of the chamber about 3.5 m s^{-1}).

A brief overview was given on the concepts of currently running experiments in the phytotrons. In co-operation with Prof. Dr. Oßwald the effects of root infestation by pathogenic fungi on the biochemistry and ecophysiology of trees is being studied in the one experiment. In a joint research project with Prof. Dr. Fromm mechanisms of rapid signalling of physiological changes from the root to the leaves are under investigation in herbaceous and woody plants.

After overcoming problems in the technical operation as detected by the test procedures, the phytotrons have subsequently allowed continuous experimentation without major failure over several months.

Acknowledgment. The language editing of the English text by Mrs. Schuck is highly appreciated.

C. Bell (Central Science Laboratory MAFF, Sand Hutton York YO4 1LZ) **Fumigation research in controlled environments.****Introduction**

Fumigation is long-standing control procedure using a gas to penetrate infested commodities or buildings, or, in the case of pre-plant treatments in agriculture, soil. It is an effective last-ditch action where other measures have failed or are known to have limited prospects for successful control. The technique is almost exclusively conducted against insect and mite pests in commodities and structures. It is important to differentiate between true fumigation and several other operations, which have often been referred to erroneously as fumigation. The use of smoke generators has, for example, often been loosely termed as fumigation, but is a quite different operation both in terms of the physical state of the toxicant and in terms of efficacy. A smoke is the dispersal of a solid (particulate) phase in a gas. It lacks all the penetrative properties of gases and as a result is not suitable for use in bulk or packaged commodities or to permeate through void spaces in buildings. Similarly mists and sprays (liquids dispersed in gas) cannot operate as true fumigants. There are in fact only a small number of compounds that are used as true fumigants and we will consider work on these here, as well as work on modified atmospheres, which utilise elevated levels of carbon dioxide and nitrogen gases to decrease the oxygen available to the insects. Both types of work have illustrated the need for closely controlled conditions in some current and recently completed research topics.

Fumigant dosages and work on the thresholds for effective action

A fumigant dose can be increased in two different ways. Firstly the concentration level, in grams per cubic metre or other suitable units, is an obvious component of the dosage, but secondly of equal importance is the time that the concentration is held in the treatment enclosure. It is of paramount importance that a good seal is achieved of the area to be treated before fumigant is applied. The longer the planned exposure period, the better the seal required. The effectiveness of a fumigation is usually assessed as the concentration-time product (time (T) in hours multiplied by concentration (C) in mg/L or g m^{-3}) achieved by the end of the fumigation. To assess this value it is necessary to monitor concentration levels at each strategic point within the enclosure throughout the fumigation and calculate concentration time products (CTPs) for each.

Although a useful starting point for assessing fumigation efficacy, there are several factors to be borne in mind with the CTP. Firstly there needs to be a threshold level for the toxic action to be complete. Insects can to some extent metabolise or eliminate low concentration levels and survive more or less indefinitely in their presence. Hence the basic formula $C \times T = k$, a constant representing a particular mortality level (say 95%), needs some modification. A factor C_0 needs to be introduced to represent the level the concentration needs to reach before the expected response of the target pest is evident. Similarly, a minimum period of exposure is often required as insects may be able to exclude gases or prevent uptake for certain periods, and also because there are often short periods in the insect's life cycle when there is high tolerance to the fumigant, i.e. times when the "active site" is protected or even absent. This minimum time can be referred to as T_0 . Hence the relationship for $C \times T = k$ becomes instead $(C - C_0) \times (T - T_0) = k$, the constant for mortality. Where C_0 is large in relation to T_0 , then metabolism or elimination is the limiting factor for fumigant toxicity, whereas if the reverse is true, the problem is one of active exclusion or retarded accumulation within the insect. When either T_0 or C_0 is very small in relation to the other, the CT relationship may be expressed as $C^n T = k$, where n is referred to

as the toxicity index. As with a dominant T_0 , when n is <1 , uptake is the limiting factor for toxic action while if n is >1 , it is a question of metabolism or excretion.

It is of obvious importance to conduct studies to gain insight into fumigant mode of action and the parameters for effective fumigant action. Both the compounds most commonly used as fumigants, methyl bromide and phosphine, have been investigated in this way at CSL (Bell, 1988; 1992). Currently similar studies are under way with sulphuryl fluoride. To explore the full ranges for effective fumigant action, very long exposures are required at constant conditions, and here high quality controls are needed. The factors controlling insect and mite survival are closely linked with active metabolism of one kind or another and as organisms without temperature control, they rely on ambient conditions to govern metabolic rate. Experimental programmes are conducted under finely controlled conditions of temperature and humidity. Constant environment rooms containing chambers are provided with up to 4 air changes per hour to remove any gas from the room, which leaves the chamber during transfer of insect samples. All other gas removal from the chambers is conducted via a separated evacuation system exhausting to the atmosphere via 9-m high roof stacks.

Fumigant repellency

In these studies the objective is to deduce whether insects can respond to fumigant concentration gradients and there is a reliance on stabilisation of pressure as well as temperature and humidity in order to control the rate of gas diffusion. A special apparatus (Bell, 1987) is used for these studies featuring a long (2 m) tube fitted with a terminal capillary tube and sampling points at regular intervals. The tube is attached to a fumigation chamber port fitted with a tap to allow gas to enter the tube from the chamber. Pressure in the chamber monitored by an attached manometer requires balancing frequently and the problem is one of maintaining even pressures with a minimum of 2 air-changes an hour in the constant environment room. Again a high degree of control is needed.

Resistance testing

A third area of research is the conducting of resistance tests and the preparation of strains homozygous for resistance to a particular fumigant (e.g. phosphine). Here test reproducibility is of paramount importance and there is a need for exact temperature, humidity and concentration level control in selection studies and in tests at the discriminatory dose level. Recently tests have switched to assessment tests based on insect activity (Bell *et al.*, 1994; Savvidou *et al.*, 1994; Wontner Smith *et al.*, 1999) and again exact temperature control is relied on during test exposures. These tests are conducted in glass desiccators sealed with grease and, where observations are needed for extended periods of time, a slight positive pressure can be created in the test room to aid in the retention of the gas. In addition, because insect activity is influenced by light (Bell, 1991), there is a need for standardisation of lighting conditions for the test regimes.

Modified atmospheres

All the specifications in the controlled environment rooms which assist in fumigant testing are also relevant when using other gases, such as carbon dioxide and nitrogen. There are supplies of both these gases and air piped to all the CE rooms in the fumigation suite of rooms.

Safety aspects

In the event of a fan failure, the air exchange mechanism in the fumigant chamber rooms is alarmed to alert people working in the rooms and anyone about to enter a room. In addition, there is a separate emergency button which will cause immediate, rapid changes of air in the

room until turned off manually and also a personal assistance button for use should anyone require urgent assistance.

Conclusion

The fumigant toxicity programme is heavily dependent on closely controlled environmental conditions in almost every aspect of the work. There is a demand for control of temperature, humidity, light and pressure, both in large-scale chamber experiments and for the smaller scale tests on insect behaviour.

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H. Rennenberg and J. Kreuzwieser (Institut für Forstbotanik und Baumphysiologie, Albert-Ludwigs-Universität Freiburg, Am Flughafen 17, D-79085 Freiburg, Germany) **Analysis of reactive trace gas exchange between plants and the atmosphere under controlled environmental conditions with acetaldehyde emission by trees as an example.**

The exchange of trace gases between vegetation and soils on the one side and the atmosphere on the other has received considerable attention, because the compounds involved in this exchange include photo-oxidants, such as NO, NO₂, O₃, or volatile organic compounds, that largely determine the oxidation capacity of the troposphere, and greenhouse gases, such as CO₂, CH₄ and N₂O, that control the radiation budget of the atmosphere. Vegetation and soils can be both sources and sinks for such compounds with the rate and direction of flux depending on intrinsic

and environmental factors that can be modulated by anthropogenic activities. Current research on the fluxes of atmospheric trace constituents under controlled environmental conditions focuses on identification of the effects of specific environmental conditions on flux rates, parameterisation, and measurement of trace gas fluxes.

These investigations can only be performed under controlled environmental conditions, if the following requirements are met:

- (1) Low boundary layer resistance,
- (2) low trace gas conversion inside the chamber,
- (3) no memory effects, and
- (4) no effects of chamber architecture.

The technical consequences for controlled environment chambers used in such studies include high rates of gas exchange (requirement 1), the use of specific materials for a particular trace gas (requirements 2 and 3), uniform conditions in terms of light, temperature, humidity, gas exchange and composition inside the chamber (requirement 4) and high precision of trace gas analysis (requirements 1, 2, and 4). These requirements are usually not met by commercial chambers, especially if reactive atmospheric trace gas species such as photo-oxidants are to be studied. To overcome these problems, chambers that meet these requirements for individual atmospheric trace gases are designed and put into commercial chambers that provide a uniform light and temperature environment. Figure 1 shows such a chamber system that, for example, is used for the analysis of acetaldehyde emission by plants. The commercial growth chamber



Fig. 1. Chamber system used for the analysis of the reactive atmospheric trace gas acetaldehyde.

contains two identical, home-made sub-chambers that are designed in a way that the gas inside these chambers only makes contact with PTFE (Teflon) (requirements 2 and 3). The air inside the chamber is thoroughly mixed by a fan (requirement 1 and 4). Gas is drawn through these

chambers at a rate such that the chamber volume is exchanged 1 to 3 times per minute (requirements 2 and 4). The gas outlet ports are connected to an infrared CO₂/H₂O analyser for the analysis of photosynthetic CO₂ exchange and transpiration and to devices for either continuous (Kreuzwieser *et al.*, 1999a) or semi-continuous determination (Kreuzwieser *et al.*, 1999b) of acetaldehyde. One of the two sub-chambers contains the shoot of the plant to be studied, the other is empty. Flux rates of CO₂, H₂O, and the trace gas (here acetaldehyde) are calculated from the difference in gas concentration at the gas outlets of the two sub-chambers. In this way chamber effects are eliminated from the calculation of gas fluxes (Rennenberg *et al.*, 1996, Kreuzwieser *et al.*, 1999b).

The chamber system shown in Fig. 1 was used to test the hypothesis that acetaldehyde emitted by the leaves of plants originates from alcoholic fermentation in the roots (Kreuzwieser *et al.* 1999a,b). To obtain the anoxic conditions required for fermentation, the roots of young poplar (*Populus tremula x Populus alba*) and pedunculate oak (*Quercus robur*) plants were flooded. As a consequence of flooding, acetaldehyde emission by the leaves of poplar strongly increased within 5 h, whereas significant acetaldehyde emission by the leaves of oak was only observed after 24 h. After this period of flooding more than 10-fold higher rates of acetaldehyde emission were determined for poplar as compared to oak. Feeding of ¹⁴C-labeled ethanol to the xylem of the petiole of a poplar leaf resulted in a release of ¹⁴C-labeled acetaldehyde of similar specific radioactivity to the ethanol fed. This result indicates that the acetaldehyde emitted by the leaves originates directly from ethanol transported in the xylem to the leaves. It is consistent with the observation of a constitutive alcohol dehydrogenase in the leaves that apparently operates in the reverse direction to fermentation; it is supported by the observation of ¹⁴C-labeled ethanol in the xylem sap of flooded poplar fed ¹⁴C-glucose to the roots.

Thus, acetaldehyde emission by the leaves can be considered an indicator of altered carbon cycling at the whole plant level, when the roots of trees experience anoxic conditions. As in herbaceous plants, glucose will be converted to ethanol with low efficiency of energy production and hence, high glucose turnover. In contrast to herbs, ethanol or other fermentation products are released into the soil to a minor extent only. Most of the ethanol is loaded into the xylem and transported to the leaves with the transpiration stream. In the leaves, ethanol supplied by transpiration seems to be converted to acetate via acetaldehyde and may be incorporated into the carbohydrate metabolism of the leaves. In this way, most of the energy of glucose is conserved, despite fermentation in the roots, and depletion of carbohydrate in the roots is prevented despite high glucose turnover, provided enhanced sucrose transport takes place in the phloem. The loss of the volatile intermediates, ethanol and acetaldehyde, from the leaves is minute when compared to its turnover, but still highly significant for the oxidation capacity of the troposphere. Whether the mechanism described above mediates flooding tolerance in woody plants is under investigation.

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Justine Head (Central Science Laboratory MAFF, Sand Hutton, York YO4 1LZ) **Quarantine insects in controlled environments.****Introduction**

The international trade in plant material and produce brings with it the risk of introducing foreign plant pests into the UK. Subsequently where outbreaks of such quarantine pests occur, containment or eradication programs are implemented by MAFF. These control programs are underpinned by scientific research which itself utilises controlled environment facilities. CSL uses a purpose-built insect quarantine unit for such research.

Plant Health

EC legislation is in place to prevent the introduction of specific 'quarantine' pests, for example the Colorado beetle, *Leptinotarsa decemlineata*. The Plant Health and Seeds Inspectorate of the Ministry of Agriculture, Fisheries and Food (MAFF) routinely inspects plant material and produce entering the UK. In instances where quarantine pests are detected on a growing crop, eradication and/or containment programmes are implemented to prevent the establishment of any harmful alien pests.

Plant Pest Research

Research is conducted at CSL on a range of non-indigenous plant pests including the South American leafminer (*Liriomyza huidobrensis*), the tobacco whitefly (*Bemisia tabaci*) and the Palm thrips (*Thrips palmi*). To enable the secure maintenance of these often small (<2 mm long) quarantine pests, CSL has a purpose-built and licensed insect quarantine unit. The unit consists of 5 plant growth rooms, a cold corridor and a general laboratory space containing several controlled environment (CE) cabinets, bench space and appropriate equipment for experimental purposes. Four of the CE rooms open onto the cold corridor which is maintained at 4°C during the day and at -15°C during the night and these rooms house the higher quarantine risk insects such as *T. palmi*. If any insects were to escape into the cold corridor they would be immobilised and subsequently frozen.

The insect quarantine unit is entered through an air lock and the air supply to the unit itself passes through a series of fine filters. All doors within are alarmed to prevent more than one door being open simultaneously thus not allowing a direct path for any rogue insects. Furthermore, a series of operating procedures are implemented to eliminate the risk of insects escaping. For example, all waste materials must be frozen for a minimum of 48 hours prior to removal. Specially designed culturing cages are used which enable insects and/or plants to be added/removed from the cultures without actually being opened in the rooms.

The research conducted on the non-indigenous pests covers a wide range of aspects of their biology with the aim of developing commercially viable control strategies. The range of work includes:

- determining the insects' responses to environmental conditions;
- identifying the efficacy of pesticides against alien pests, often for immediate use in the field;
- determining the presence of insecticide resistance expressed by pest species. Often insect populations are exposed to pesticides before entry to the UK and pesticide resistance may have developed;
- developing non-chemical alternative control strategies where few or no effective chemical controls exist. For example, the use of entomopathogenic nematodes as foliar treatments for leafminer larvae.

In summary, CSL has quarantine facilities unrivalled in the UK which enable research to be conducted to develop rational pest management strategies with minimal use of harmful chemical insecticides. These can be utilised to prevent entry of quarantine pests and also to eradicate outbreaks of quarantine pests in the UK when they occur.

R. Matyssek¹, R.T.W. Siegwolf², M. Saurer², M.S. Günthard-Goerg³, S. Maurer², P. Schmutz³ & J.B. Bucher³ (¹) Forest Botany, University of Munich, Am Hochanger 13, D - 85354 Freising, Germany; (²) Paul-Scherrer-Institute (PSI), CH - 5232 Villigen, Switzerland; (³) Swiss Federal Institute for Forest, Snow & Landscape Research (FSL) Zürcherstr. 111, CH - 8903 Birmensdorf, Switzerland) **Nitrogen dioxide - fertilizer or pollutant in trees? A case study in a controlled chamber environment.**

During the last few decades, nitrogen dioxide (NO₂) has been recognised as one of today's major gaseous air pollutants. NO₂ released from traffic exhaust and industrial combustion processes is involved in the formation of tropospheric ozone - another toxic air pollutant - it contributes to the regionally high nitrogen (N) deposition, which may accelerate soil acidification and induce nutritional imbalances in forest ecosystems. Findings in literature, however, conflict with respect to the toxicity of NO₂, which often has been reported to act as a fertiliser in plants. The case study presented here was conducted in Switzerland where NO₂ levels may reach more than 130 nL L⁻¹ during morning and evening hours, and may stay as high as 80 nL L⁻¹ over midday. Therefore, the toxic potential of NO₂ regimes of Swiss urban areas was of great interest. We examined the hypothesis, therefore, that such high NO₂ regimes could cause injury in trees if soil-N availability is also high (e.g. as a consequence of persistently high N deposition) - and that trees could profit from high NO₂ exposure if the N supply from the soil is low. The study was conducted on hybrid poplar (*Populus x euramericana*), a tree species known for high productivity and, therefore, potentially highly susceptible to pollutant impact.

Two groups of a clone were cultivated, one under high, the other under low fertilisation conditions. The total N supply differed between the two groups by a factor of 6 (and in ammonium by a factor of 11), whereas other relevant macro- and micro-nutrients were provided in equal amounts for both treatments. The plants were well watered throughout the experiment. Each soil fertilisation treatment was split into two NO₂ exposure regimes, one mimicking the high diurnal NO₂ regimes as occurring in Swiss cities (see above) with a daily mean NO₂ concentration of about 100 nL L⁻¹, the other representing NO₂-free air as a control. Plants of both soil-N treatments were distributed between two walk-in phytotrons, one in which the urban NO₂ regime was applied, and in the other NO₂-free air. For 15 weeks, the trees were exposed to the four soil-N/NO₂ regimes, 10 plants to each one, and were harvested at the end of the experiment, by which time they had grown to a height of 2 m. Because only two phytotrons were available, the investigation was conducted a second time with a completely new set of plants.

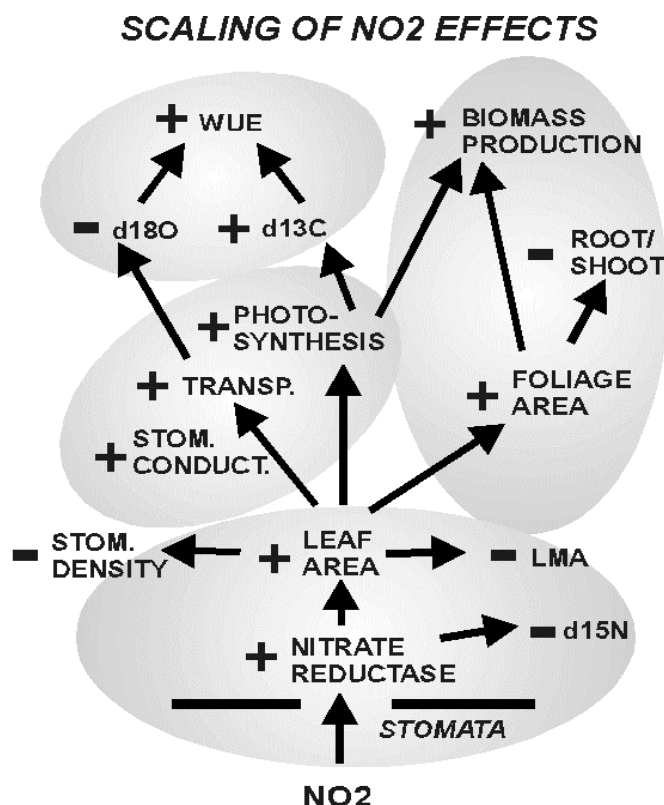
The study was performed at the Swiss Federal Institute for Forest Snow & Landscape Research in Birmensdorf, Zurich, where the two phytotrons (BBC-York, Germany) were located. Each cabinet had a ground area of 9.7 m² and a height of 2.0 m. The photosynthetic photon flux density (PPFD) was provided by 25 1000 W lamps in each chamber (Power Star HQI-E, Osram, Germany). Diurnal courses in PPFD were simulated, reaching a maximum of 800 μmol m⁻² s⁻¹ at foliage height. Air temperature and relative humidity smoothly followed the diurnal course in PPFD (night/day: 80/70%, 12/20°C). Each chamber was supplied with filtered air (charcoal, Purafil) at a turnover rate of 100 m³ h⁻¹ (wind speed about 0.5 m s⁻¹), and NO₂ levels were monitored by a NO₂ analyser (Model 8840, Monitor Labs Inc., USA). NO₂ was mixed into the

air of the one phytotron, and the release from a pressurised gas tank (containing 1% NO₂ in 99% N₂) was feedback-controlled by a computer system and operated through a mass flow controller (Brooks 2560, The Netherlands).

The leaves of plants exposed to NO₂ displayed a reduced stomatal density (i.e. number of stomata per unit of leaf area), as both NO₂ exposure and high soil-N supply had led to increases in leaf area. The mass per area ratio of the leaves (LMA) was decreased, but the dry-mass-related photosynthetic CO₂ assimilation rate (A) increased under high soil-N supply, whereas NO₂ had no apparent effect on these two leaf properties. When expressed, however, on a leaf area basis, photosynthetic rate and stomatal conductance (g_{H_2O} , a measure of the extent of stomatal opening in a leaf) were distinctly elevated in NO₂-exposed plants (relative to the control in NO₂-free air) when growing under a low N supply from the soil. Relative to this latter soil fertilisation treatment, high soil-N supply increased A and g_{H_2O} by a factor of about 2, each in the absence however, of any effect of NO₂ exposure. At experimentally enhanced CO₂ levels in the air (400 - 1500 $\mu\text{L L}^{-1}$), high soil fertilisation distinctly elevated the water-use efficiency of the leaf gas exchange (WUE i.e. photosynthetic CO₂ uptake per unit of transpired water) and a minor increase in WUE by NO₂ exposure was indicated in plants under low soil-N supply. The NO₂ flux into the leaves, estimated throughout the experiment from g_{H_2O} under ambient CO₂ levels of 350 $\mu\text{L L}^{-1}$, was 63 mmol m⁻² in the low soil-N regime and, similarly, 70 mmol m⁻² in the high soil-N regime. The presence of NO₂ appeared to facilitate NO₂ uptake by enhancing stomatal opening.

The actual NO₂ uptake into the leaves can be confirmed by responses in the nitrate reductase enzyme (NaR) which catalyses the assimilation of NO₂ into the plant metabolism. Another confirmation of NO₂ uptake is the fluctuation of the $\delta^{15}\text{N}$ value in the foliage biomass. This is expressed by shifts in the ¹⁴N/¹⁵N stable isotope ratio caused by N incorporation from atmospheric NO₂ and soil nitrogen. The activity of NaR was stimulated to a similar extent by both high soil-N supply and the presence of NO₂ in the air. In parallel, $\delta^{15}\text{N}$ was distinctly reduced in the plant biomass due to a rather negative $\delta^{15}\text{N}$ signature of the applied NO₂ fumigation gas. In contrast, incorporation of soil N was reflected by an increase of $\delta^{15}\text{N}$ in the biomass, as the $\delta^{15}\text{N}$ signature of the soil fertiliser was positive. Hence, NO₂ gas and soil fertiliser served as markers for assessing the different pathways of N incorporation into the plant. Although high soil-N supply was more effective than NO₂ uptake, the incorporation of N increased the foliage area and, as a consequence, the whole-plant biomass production, regardless of the N source. These increases in foliage area and plant biomass were associated with similar declines in the root/shoot biomass ratio, indicating a shift in the whole-plant carbon allocation by both N uptake from the soil and NO₂ uptake. Parallel to the changes in biomass development, decrease in $\delta^{18}\text{O}$ (a measure of the ¹⁶O/¹⁸O stable isotope ratio) indicated increases in transpiration at the foliage level under both high soil-N supply and NO₂ exposure. However, only exposure to NO₂ increased WUE under both soil fertilisation treatments, as reflected by elevated $\delta^{13}\text{C}$ (representing the ¹²C/¹³C ratio in the plant). The combined analysis of $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ was consistent with the measurements of leaf gas exchange, but achieved a higher resolution in detecting changes in WUE. Isotope analysis provided an integration of the carbon and water flux over the entire life span of the experimental plants.

The effects of NO₂ uptake can consistently be scaled from the enzymatic to the whole-plant level (Fig. 1). Uptake of NO₂, being restricted to the pathway through open stomata, stimulates NaR activity as the first step in metabolic NO₂ incorporation. The latter is documented by a decrease in $\delta^{15}\text{N}$. N incorporation from NO₂ causes enlargement in leaf size, which leads to a decrease in



stomatal density and LMA, but to an increase in whole-plant foliage area (leaf formation rate remaining unchanged). Along with a stimulation of the area-related photosynthetic rate, which occurred at low rather than high soil-N supply, whole-plant biomass production was enhanced, while carbon allocation was altered at the expense of root growth. Increased single-leaf area was also linked to increasing stomatal conductance and transpiration. The latter was reflected by a decline in $\delta^{18}\text{O}$. Nevertheless WUE was increased at the foliage level, because apparently NO_2 stimulated photosynthesis to a higher degree than transpiration, which is indicated by an increase in $\delta^{13}\text{C}$. Overall, NO_2 uptake induced interactions between structural and physiological plant properties as those typically caused by N fertilisation.

Fig. 1. Scaling of NO_2 effects from the enzymatic to the whole-plant level: arrows show cause-effect relationships leading to stimulation (+) or decrease (-) in the depicted plant parameters ($d15\text{N} = \delta^{15}\text{N}$; $d18\text{O} = \delta^{18}\text{O}$; $d13\text{C} = \delta^{13}\text{C}$).

In conclusion, it became necessary to reject the hypothesis posed at the beginning, because, regardless of the soil-N supply, NO_2 exposure stimulated rather than inhibited the physiological performance and biomass production of the experimental plants. Thus, NO_2 acted - in this case study - as a fertiliser rather than a pollutant, which would have caused limitation or injury. One needs to be aware, however, that controlled chamber experiments, which are typically restricted to young trees and short-term exposures can reveal only principles in plant response. Such principles cannot be extrapolated to forest stands without experimental validation under site conditions. There are good reasons, though, to assume that chronic (i.e. long-term) 'leaf fertilisation' by NO_2 will render trees more susceptible, i.e. predispose them, to further abiotic and, in particular, biotic stress.

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