

# Proceedings of the UK Controlled Environment Users' Group

## 1994 SCIENTIFIC MEETING

### “CONTROLLED ENVIRONMENTS FOR TRANSGENIC PLANTS”

#### Volume 5

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## CONTROLLED ENVIRONMENT USERS GROUP

### 1994 SCIENTIFIC MEETING

#### CONTROLLED ENVIRONMENTS FOR TRANSGENIC PLANTS

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

#### SUMMARIES OF PAPERS

**D A Bosworth** (Principle Specialist Inspector, Health and Safety Executive, Bootle, Merseyside) **i. Legislation relating to the Containment of Transgenic Plants and ii. How to design and manage Plant Containment Facilities to achieve compliance with the Legislation.**

The legislation that regulates the contained use of genetically modified organisms is part of the hierarchy of UK legislation set out below:

- Health and Safety at Work etc. Act 1974;
- Control of Substances Hazardous to Health Regulations 1988;
- The Management of Health and Safety at Work Regulations 1992; and
- The Genetic Modified Organisms (Contained Use) Regulations 1992 and 1993.

The duties of a person who is carrying out genetic modification work are clearly outlined in the HSE guidance leaflet which is included with the minutes of the meeting, and I will not duplicate that information here. To achieve compliance with the regulations, contained facilities (appropriate to the level of risk to humans and the environment posed by the work) must be provided, managed and used correctly. The degree of containment required for adequate control of the work to be undertaken is determined by the risk assessment, carried out as required by the regulations. The risks identified in the assessment can be controlled by a combination of measures which may involve the following:

- 1) Physical containment with or without the addition of biological containment measures;
- 2) Safe system of work; and
- 3) Administration/management controls.

The facilities required for the contained growth of transgenic plants are outlined in ACGM/HSE/Guidance Note 10, which deals with work on plants and plant pests and can be obtained free from the HSE. However before providing containment facilities the first step is to determine what work is intended to be carried out in the facility and what risks e.g. pollen escape, soil borne pathogen escape etc., have to be controlled. The key person who should be consulted would be the Biological Safety Officer, who should have knowledge of the proposed work, legislation and appropriate control measures. However all containment is underwritten by good glasshouse practice, which can be defined as;

- adequate training of personnel;
- high standards of hygiene and cleanliness;
- the wearing of adequate and suitable protective clothing; and
- suitable pest and disease control.

The guidance distinguishes between two levels of containment facility, Containment Level A and Containment Level B, the level required depends upon the perceived risk from the work.

#### Containment A

Suitable for experiments involving:

- plants but no plant pests;
- plants with limited ability to transfer genetic material to UK plant species;
- plants incapable of surviving outdoors in the UK;
- plant cells transformed using a plant pest e.g. *Agrobacterium tumefaciens* which was subsequently removed during the plant regeneration procedure and where the risk is assessed to be low to humans, other plants and the environment.

#### Containment B

Experiments involving:

- plants with genetically modified plant pests;
- species that are potential pests under glass or in similar artificial environments;
- plants with ability to transfer novel genetic material to UK plant species.

The differences between a level A containment facility and a basic level B facility is essentially concentrated on the management of the facility e.g. treatment of materials and waste, provision of adequate disinfectants etc. What can make a level B distinct is the addition of additional measures to control identified risks.

Where airborne dispersal is of concern, the facility may have to have a filtered ventilation system, which for the majority of applications would be under negative pressure to contain the hazard. However negative pressure may not be the only option, adequate control may be achievable by;

- bagging plants or their flowers;
- debudding plants;
- using male sterile plants; or
- altering the flowering time of the plants such that no viable pollen receptors exist within range of the dispersed pollen.

#### Experimental use of arthropod vectors

- control may be able to be achieved by the use of:
  - restricted mobility forms e.g. wingless aphids;
  - water moat or adhesive boundary to experimental area;
  - glasshouse/environment temperature differential by heating a glasshouse in winter;
  - internal insect proof cages.

#### Water borne vectors

- control may be able to be achieved by the use of:
  - absence of drains;
  - provision of a kill tank in the drainage system with appropriate disinfection; or
  - effective sealing of the drainage system and disinfection of water in the glasshouse.

In summary, compliance with the regulations begins with the completion of a risk assessment, reviewed by the local genetic modification safety committee, which determines the containment required by the work and the type of notification to be made to the HSE.

Containment is achieved by the application of appropriate physical measures which may be supplemented with appropriate biological/chemical measures to achieve adequate control. The maintenance of effective control depends upon a safe system of work and effective management of the facility and staff who operate within it.

### Sources of Information

1. ACGM/HSE/Guidance note 10: Guidelines on work involving the genetic modification of plants and plant pests.
2. A guide to the Genetically Modified Organisms (Contained Use) Regulations 1992. ISBN 0-71-760473X.
- 3.

### **Christine H Foyer** (Department of Environmental Biology, IGER, Aberystwyth) **Experiments with Transgenic Plants: a User's Viewpoint**

In studies on primary plant metabolism associated with the partitioning of the energy and carbon skeletons produced by photosynthesis it became apparent that the answers to many unresolved questions concerning the nature and regulation of the component processes could not be obtained easily by either a purely physiological or purely biochemical approach. The ability to engineer plants with precise modifications in the amounts or regulated activities of component enzymes provided the possibility to manipulate metabolism *in vivo*. Transgenic plants therefore offered a means by which a more complete understanding of the roles of individual enzymes and their regulation could be attained. In addition it provided an opportunity to eliminate intrinsic metabolic barriers and placed within our grasp the possibility of engineering greater biomass production through more effective use of photosynthetic products or by increasing the endogenous capacity for stress tolerance. The need for innovative agricultural technology is real and, in fact, has never been more urgent since new technology is vital if food supply is to be increased. The application of genetic engineering has the potential to boost agricultural production.

Photosynthesis provides the primary source of energy and carbon compounds for both carbohydrate and amino acid biosynthesis. In higher plants these two assimilatory sequences are co-ordinated by a complex regulatory system that involves many steps of reciprocal control. Atmospheric CO<sub>2</sub> enrichment is a classical means by which plant growth may be increased in the greenhouse. The observed initial marked increases in photosynthesis and plant growth are not, however, sustained and extended periods of atmospheric CO<sub>2</sub> enrichment do not result in greatly increased yields. Many observations suggest that plants do not have an appropriate genetic response to high CO<sub>2</sub> and cannot realise the potential that improved photosynthetic capacity could offer for increased biomass accumulation. One way that the natural regulatory barriers, to improved performance at elevated CO<sub>2</sub> levels, may be overcome, is to increase the capacity for either sucrose synthesis or nitrogen assimilation. Transformed tomato plants expressing the maize sucrose phosphate synthase (SPS) gene and transformed *Nicotiana plumbaginifolia* constitutively expressing nitrate reductase (NR) were grown in air and then transferred to a 1,000  $\mu\text{L L}^{-1}$  CO<sub>2</sub> atmosphere in controlled environment chambers. SPS over-expression resulted in increased photosynthesis in air compared to untransformed controls with a much more pronounced stimulation in rate in plants exposed to high CO<sub>2</sub>. The C to N ratio was slightly lower in the SPS over-expressers than in untransformed controls in air and at high CO<sub>2</sub>. These features were independent of the promoter used to express the maize enzyme but related to the amount of extractable foliar

SPS activity. They were therefore truly associated with the increased capacity for sucrose synthesis. Long term CO<sub>2</sub> enrichment led to marked decreases in NR activity in the *N. plumbaginifolia* plants constitutively expressing NR.

Foliar glutamine and asparagine levels were also considerably decreased compared to control plants kept at air levels of CO<sub>2</sub> suggesting down-regulation of nitrogen assimilation during long-term exposure to high CO<sub>2</sub> levels. Similar observations were made on untransformed controls following CO<sub>2</sub> enrichment but the extent of the loss of NR was less marked. Selective degradation of the NR protein may occur at high CO<sub>2</sub>. A similar observation was made when the constitutive NR expressers were deprived of N.

In comparison to untransformed controls, biomass production was not significantly increased in tomato plants which had been modified such that the introduced maize SPS activity was expressed largely in the photo synthetic tissues alone. Constitutive expression, however, resulted in increases in total biomass accumulation of up to 100% in plants grown in air both in controlled environment chambers and in greenhouse conditions. NR over-expression was found to modify nitrogen metabolism, favouring decreased foliar nitrate levels and increased glutamine contents but no changes in total protein or biomass accumulation were found. Our preliminary results suggest that of the principal factors limiting biomass production, manipulation of carbon metabolism is most likely to result in increases in biomass while manipulation of NR may lead to decreased nitrate levels in food crops. In these studies we are, always mindful that an enormous variety of artifacts can arise from gene translocation; gene silencing, co-suppression, position effects etc. Therefore, large numbers of plants must be studied per transformed line and several lines bearing the same transformed gene must be assessed and preferably not primary transformants. There is a real danger that new characteristics related to somaclonal variation consecutive to tissue culture may arise. It is, therefore, important to finally ensure that new traits co-segregate with the inserted gene for example, in crosses with the wild-type. Finally, if we are to serve the needs of agriculture the products of our new technology must be designed with the requirements of the users in view rather than those of ourselves the producers. The benefits of transformation procedures studied in all model systems must ultimately prove effective in heterogenous complex and diverse farming environments where variables are intrinsic and crop vitality is site-dependent and risk prone.

### **George B. Sykes FRES. Integrated Pest Management (IPM) in Glasshouses and Controlled Environments.**

#### Background

Glasshouses and other controlled environment facilities afford isolation and protection to plants grown therein. They also provide a sheltered habitat for any pests and diseases which may gain entry. Very few immigrants are needed, and in some cases only a single specimen is all that is required, to initiate the production of pest foci which, if not noticed, can quickly result in pest populations of a magnitude difficult to control by any means (i.e. physical, biological or chemical).

Growers and researchers alike, have for very many years largely relied upon chemical pesticides to control pests of protected crops and experiments. These methods, and the results

obtained have been generally acceptable. However, for a number of reasons, this may no longer be so.

### Causes for concern

The recorded number of animal species and plant pathogens that are considered 'resistant' to chemical pesticides, has been increasing year by year since the early 1950s. The list is extensive, and includes some important plant pests.

There is a growing concern amongst the public for the environment and the conservation of flora and fauna.

Consumers and the authorities show concern about potential chemical residues.

Employees in situations where chemical pesticides might be/have been used, are concerned about the effects of exposure to these substances or their residues.

Researchers may pursue lines of work requiring clean and healthy plants, but where the experimental protocol excludes the use of chemical pesticides.

### Biological control is not a new idea\

The use of biological agents to protect plants/crops from harmful organisms has been practised since ancient times, and the use of predacious insects was practised long before insect parasitism was identified. During the late 19th C and the early 20th C, there was considerable cooperation between agriculturalists and biologists, particularly in the US of A, and 'biological control' became an accepted term in the late 1920's.

More recently, the late Joe Hussey, and Mr H Gould (ADAS ret'd), did much to encourage the use of predators and parasites for pest control in the UK glasshouse industry. IPM is now widely adopted in countries where horticulture is a viable industry.

### Perceived advantages of IPM

The most serious pests of protected crops can be controlled using this technique.

'Resistant' types of pests are not a problem.

The risk of further development of resistance is minimised, as is the risk of phytotoxicity.

Many more beneficial organisms will survive.

There is a constant evaluation of potential new 'agents'.

The minimal use of chemicals results in a healthier working environment than hitherto.

### M = Management

A 'site' approach is required, and there has to be a commitment to making IPM work.

Living creatures supplied by the specialist firms need conditions suitable for their behavioural activities.

The balance of pests/beneficials, or a possible 'overkill' situation, will need careful monitoring, particularly during the initial period.

Regular introductions of some agents will be needed.

The pesticides which are used, need to be chosen with care, and guidance is available from the suppliers of the biological material.

### What is available, and from whom

Several firms produce and/or distribute biological material for use on protected edible and ornamental crops, and for some outdoor crops. Natural enemies are commercially available for use against the following major pests (in their adult or immature forms) of protected crops.

Aphids	(most spp.)
Caterpillars	(not all spp.)
Citrus mealy bug	(and some other spp.)
Glasshouse whitefly	
Leafhopper	(not all spp.)
Leafminer	(common spp.)
Sciarid fly	
Soft scale insects	
Tobacco whitefly	
Thrips	
Two spotted spider mite	(and Carmine mite)
Vine weevil	

There is little difference between the majority of the biological agents sold by the various companies, but the 'packaging' does vary, as do some of the prices charged. Most growers seem to be influenced in their choice of supplier mainly by the firms' reputation and the person that comes to see/advise them (it is not unknown for a company's invoicing procedure to be the deciding factor!)

#### Companies

AGC Microbio, Church St, Thriplow, Royston, Herts.

Applied Horticulture, Toddington Lane, Littlehampton, W. Sussex.

BCP, Acorn Nurseries, West Wittering, Sussex.

Bunting Biological Control Ltd., Westwood Park, Little Horkeley, Colchester, Essex.

English Woodlands, Graffham, Petworth, W. Sussex.

Koppert UK Ltd., 1 Wadhurst Business Park, Wadhurst, E. Sussex.

Natural Pest Control Ltd., Watertmead Yapton Road, Barnham, Bognor Regis, W. Sussex.

**Not for publication other than in these "Proceedings"**