

Proceedings of the UK Controlled Environment Users' Group

2009 SCIENTIFIC MEETING

“CONTROLLED ENVIRONMENTS FOR CONTAINMENT”

Volume 20

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UK CONTROLLED ENVIRONMENT USERS' GROUP**2009 SCIENTIFIC MEETING****CONTROLLED ENVIRONMENTS FOR CONTAINMENT**

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

SUMMARIES OF PAPERS

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Construction and establishment of a containment level 3 plant growth facility at Rothamsted Research

A high end controlled environment and containment facility at Rothamsted Research was designed and built from 2005 to 2008 to support diverse research projects with agricultural plant pathogens and fulfil DEFRA/HSE requirements for category level 3 containment. The facility is part of the Jenkinson Building at the Rothamsted Research campus in Harpenden, Herts and was fully funded by the Biotechnology and Biological Sciences Research Council (BBSRC) at a cost of £4.4 m. The building has been designed to be adaptable for the future and features a robust design with brick walls and diverse energy saving aspects to reduce maintenance costs. The main contractor was SCD Ltd with Sanyo-Gallenkamp as the sub-contractor for the plant growth rooms. The containment facility will be the first public sector containment level 3 facility for agricultural pathogens within the UK. The licensed work involves the development and use of virus-induced RNA gene silencing (VIGS) for the analysis of plant gene function in cereal species. VIGS is a newly emergent technique in agricultural plant research which is taking advantage of the increasing volumes of gene sequence and genomic information. In addition, the insertion of genes coding for easily detectable reporter proteins will allow the monitoring of plant virus movement using, for example, the green fluorescent protein (GFP) from jelly fish (Fig. 1). Also, within this containment facility, we will explore plant infection by transgenic and non-native fungal pathogens of UK crop plants in combination with the VIGS technology.

The containment category level 3 facility has a total area of 374 m² and contains four large 'walk in' growth rooms of 21 m² each fitted with a lobby and two associated laboratory areas for microscopy/seed storage (dry lab) and microbiological cultures (wet lab) (Fig. 2). All of these rooms including the main hall containing the plant growth rooms are sealed and held at negative pressure with respect to the external atmosphere. The final pressure in the plant growth space is held at -45 Pa below outside ambient air pressure and has been achieved by three sequential step downs. All air leaving the building is filtered through HEPA filters. All air coming into the building is both filtered to remove particles >5 µm, humidity controlled and conditioned to 21°C. Within the containment facility there are two growth rooms with tiered shelving suitable for growing seedlings of cereal and *Brassica* crop species or *Arabidopsis*. There are another two growth rooms where the single shelf is created with trolleys or where the plants can be grown in pots on the floor. These rooms are suitable for

flowering wheat, barley, rice and maize. Access to the containment area is via a lobby fitted with interlocked doors, security locks, air showers and sticky mats.

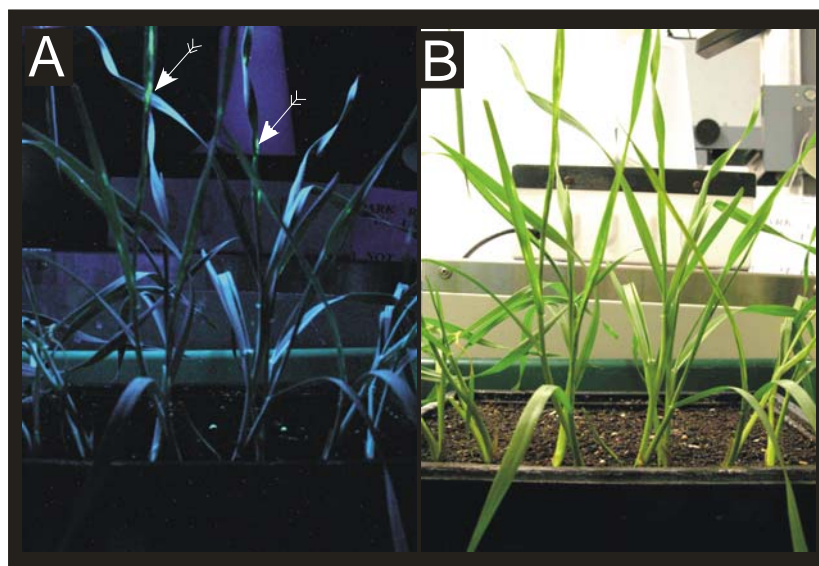


Figure 1. Expression of the green fluorescent protein in young wheat plants using the Barley stripe mosaic virus (K. Kanuka, unpubl.). White arrows in panel A indicate green fluorescent leaf sections. The same plants are viewed under UV light (panel A) and in white light (panel B).

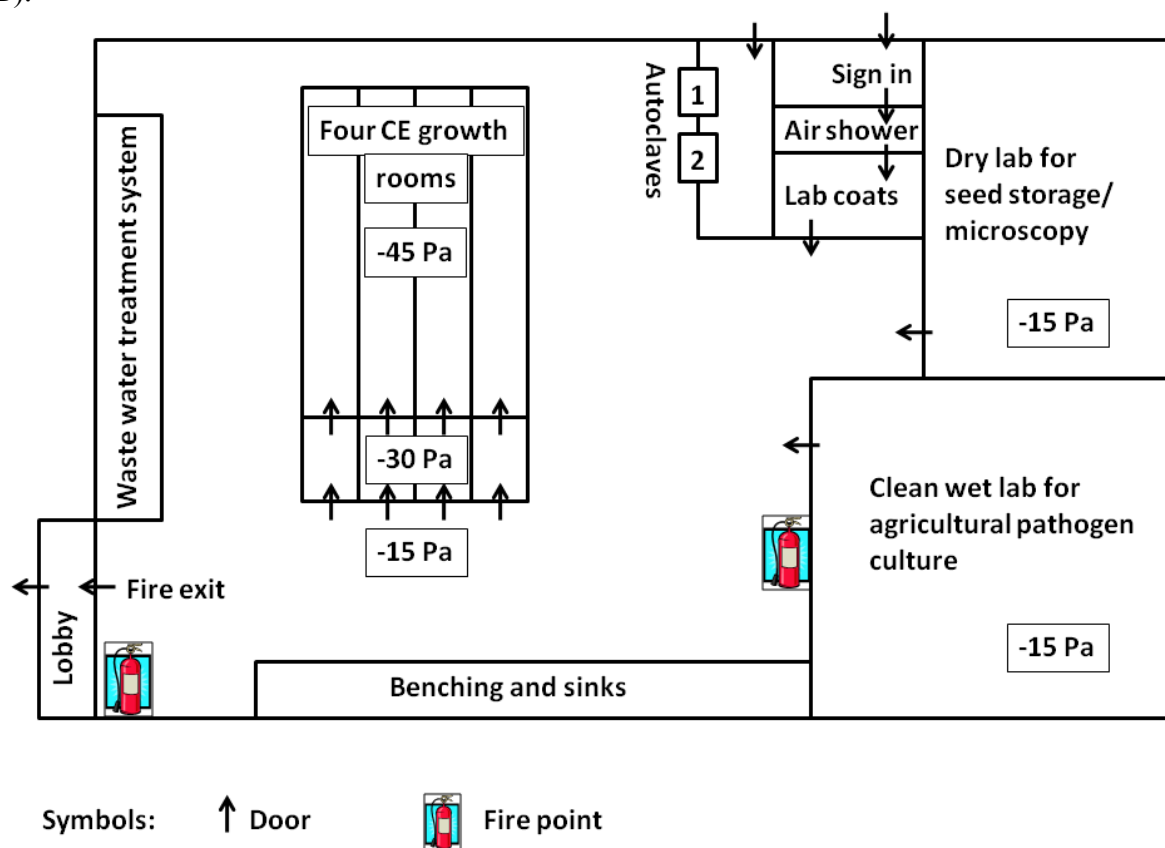


Figure 2. Floor plan of containment facility (not to scale). The three different air pressures through the building are indicated in boxes.

Waste disposal is via two 'through-the-wall' autoclaves (supplied by Priorclave) into a room only accessible from outside of the facility by a separate door. The main containment hall is fitted internally with a perimeter drain which runs to a sump, and is pumped out via a consecutive UV treatment system followed by a chemical dosatron-treatment system (Fig. 3). All water feeds into the facility are linked to float switches in the sump to prevent water flow if the sump is full. General waste from the growth rooms and all used plants and pots are destroyed by steam sterilisation in the autoclave area located within the facility. The outside of the facility is laid out with stones and concrete, so that in the unlikely event of a "seed escape" from the facility this could easily be spotted. A series of standard operating procedures (SOPs) have been developed describing all experimental work procedures in the facility and are counter-signed by staff to ensure that microbial inocula and GM plant waste are kept to a minimum. A second series of SOPs is currently being developed to cover routine and non-routine maintenance of the facility and the training of existing and new staff.

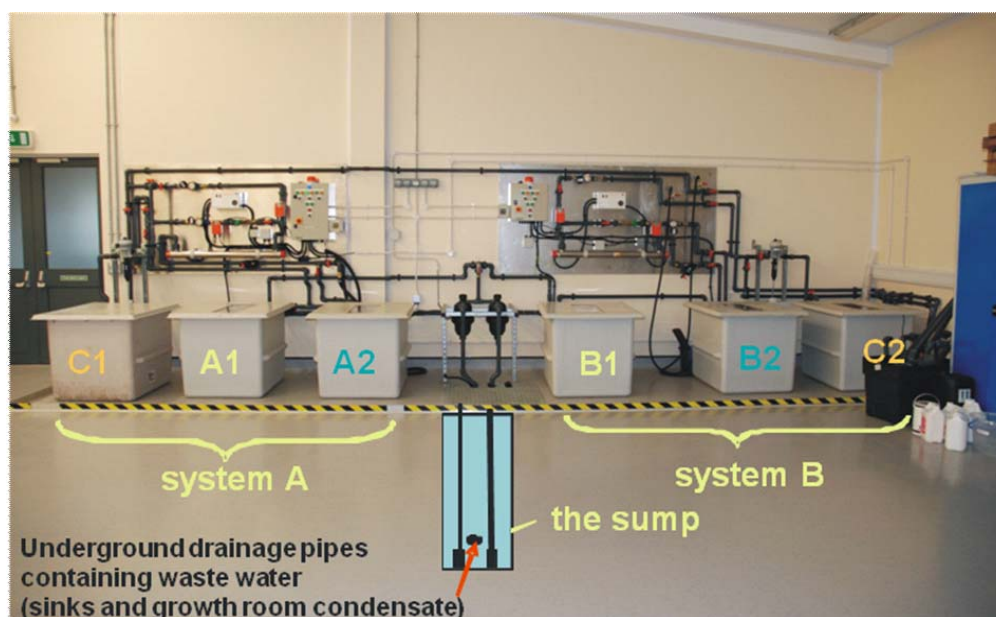


Figure 3. The two independent UV and chemical treatment systems for decontamination of waste water inside the containment facility.

A1, B1 – water collection tanks; A2, B2 – treatment tanks for batch process; C1, C2 – Dosatron chemical inactivation tanks

After the 2007 Foot and Mouth virus outbreak at the Institute of Animal Health at Pirbright, where an animal virus was unintentionally released, containment facilities for work with agricultural pathogens are under increased scrutiny and are required by DEFRA/HSE, to demonstrate that effective methods are in place to ensure containment and for waste water treatment. The waste water treatment system established at Rothamsted Research with the help of UNIGRO, combines sedimentation, filtration, UV irradiation and chemical inactivation to prevent escape of licensed agricultural pathogens and plant materials. Waste water first passes through soil traps in the sink areas of the facility, where large particles are deposited. Water is then collected in the sump. Automatic duplicated pumps then pass the waste water through 50 µm filters where fine deposit is extracted. In this way, turbidity of the waste water is kept below 30% and microbial aggregates are broken up. The water is collected in duplicate tanks with a maximum capacity of 400 litres (tank A1, B1 in Fig. 3). Every 24 hours the 400 litre tanks are then pumped over to treatment tanks (A2, B2) to start a

batch inactivation of the waste water. The treatment tank water is pumped continuously through a UV irradiation chamber and back into the treatment tank. The 24-hour long UV irradiation causes theoretically a greater than 7-fold \log_{10} reduction in microbial populations. The irradiated water is then pumped through a dosatron and mixed with a 1:50 dilution of Microsol 3 decontaminant (Anachem) to start a chemical inactivation process during a 12-hour inactivation period. Both UV and chemical treatment regime effectively ensures a two-kill step procedure. This is a requirement of the Defra/HSE licence. Based on current use, the volume of liquid coming from the four growth rooms is negligible because of the use of conditioned incoming air.

While chemical treatment is a well established technique to inactivate microbial populations, less is known about the effectiveness of the UV treatment system. Firstly, we tried to establish UV inactivation parameters for several fungal plant pathogens. In bench scale UV inactivation experiments we determined logarithmic reduction rates for the agricultural plant pathogenic fungi *Fusarium graminearum*, *Fusarium culmorum*, *Mycosphaerella graminicola*, *Magnaporthe oryzae*, *Leptosphaeria maculans* and *L. biglobosa* and the cereal viral pathogen Barley stripe mosaic virus (BSMV), the vector for the VIGS technology (Fig. 4, and data not shown).

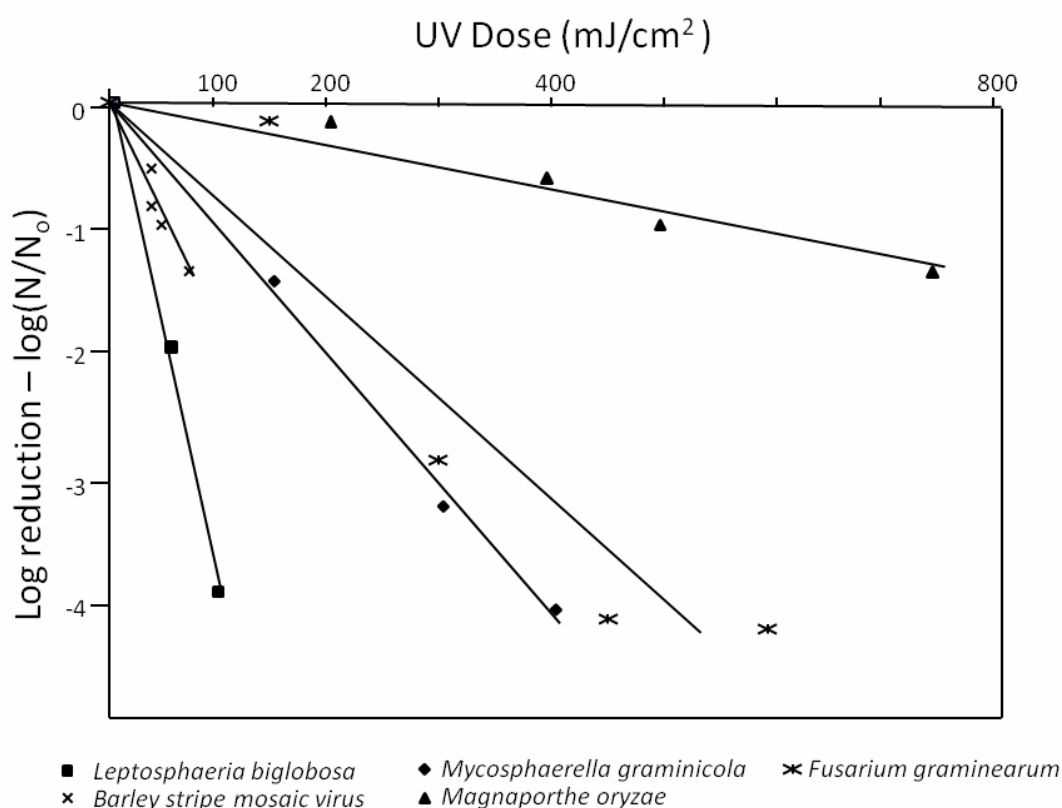


Figure 4. Ultraviolet light inactivation curves of agricultural pathogens in bench scale experiments

All tested organisms were susceptible to UV irradiation. Secondly, we tested the 400-litre batch UV irradiation system by deliberate inoculation with unlicensed microbial tester strains into the collection tanks (A1, B1 in Fig. 3). Water samples were then taken after 24 hours from treated waste water pumped to drain and analysed for the presence of surviving

microbes. Interestingly, for *F. graminearum*, *M. graminicola*, *M. oryzae* and for BSMV, UV treatment decreased microbial populations to undetectable levels as expected, while for *L. maculans* / *L. biglobosa* and *F. culmorum*, water samples retained viable microbes. This unexpected result conflicts with the data obtained in the bench scale UV sensitivity tests, where *M. oryzae* was the least sensitive to UV irradiation, *F. culmorum* showed intermediate, and *L. maculans* / *L. biglobosa* even showed the highest sensitivity to UV. It is possible that for some microbial species like *F. culmorum* and *L. maculans* / *L. biglobosa*, their spores may either float or stick to mechanical parts in the treatment tank and are not pumped repeatedly through the irradiation chamber. In contrast *M. oryzae* has heavy spores and is unlikely to float. A further outcome of an additional experiment is that the viral plant pathogen, Barley stripe mosaic virus, which has a tripartite RNA genome, can effectively be inactivated by 512-fold dilution in water alone. All three viral genomes are required for establishing an infection. It would appear that the three viral genomes of BSMV disassociate upon dilution and the diluted inoculum becomes uninfected. Therefore dilution in the bulk waste water represents a further level of containment for the VIGS vector BSMV.

We conclude from our experimental data, that UV irradiation can effectively inactivate several microbial species, but that some microbial species cannot be killed using a batch UV treatment process alone. A more fail-proof approach to complete microbial deactivation of waste water can only be ensured by a combination of filtration, UV irradiation and chemical treatment as established in the category level 3 containment facility at Rothamsted Research.

Acknowledgements

We thank the following for all their help, assistance and advice whilst establishing this new facility: L. Benjamin, I. Crute, A. Cuzick, K. Kanyuka, H-C. Jing, B. Kerry, K. Law, A. van de Meene, J. Motteram, I. Pearman, C. Peters, G. Smith and J. Townsend. The staffs at SCD Ltd, Sanyo-Gallenkamp, UNIGRO, Priorclave and the dialysis unit at the Queen Elizabeth Hospital, Welwyn Garden City, and M. Penrose of HSE are also thanked for their considerable help and advice.

K. Gorman (Plant & Invertebrate Ecology Department, Rothamsted Research, West Common, Harpenden AL5 2JQ, UK; E-mail: kevin.gorman@bbsrc.ac.uk) **Containment for insects**

Introduction and insect diversity

Entomological research is an integral component of a wide range of scientific disciplines including amongst others; ecology, agriculture, public health, veterinary medicine and forensic science. For each of them, the primary goal of the insect rearing facility is the production and containment of consistently high quality insects.

There are numerous contrasting insect examples to draw upon, one that demonstrates a well-known insect life cycle, complete metamorphosis, is the common housefly (Figure 1). Sexual reproduction between a male and a female leads to egg fertilisation, the female lays the eggs from which larvae hatch and develop through several immature instars into sessile pupae. Finally, after a complete transformation of form and function within the pupae, adults emerge. However, sexual reproduction is by no means a prerequisite for all insects. Females of some aphid species are able to reproduce asexually (parthenogenesis), resulting in genetically identical, clonal progeny. The female does not lay eggs but instead gives birth to

live young, akin in form and function to the parent and without the need of pupal transformation prior to adulthood (incomplete metamorphosis).



Figure 1. Examples of diversity: houseflies (left), aphids (left centre), parasitic wasps and aphid hosts (right centre), spider mites (right).

Parasitic insects, such as the numerous species of aphelenid wasps, are able to exploit other insects (e.g. aphids) as a food source for their own immature offspring. A mated female wasp pierces the adult aphid's body with her ovipositor, laying an egg inside. The egg hatches and develops through to adulthood as an immature larva feeding on the host's internal fluids and organs, ingeniously inducing host mortality just at the final stages of its own development. A fourth distinct example comes from a familiar insectary resident that is actually not an insect. Spider mites are arachnids, having eight legs rather than six, but do possess other insect-like characteristics such as a structural exoskeleton formed of chitin. Also in common with several insect species, some employ a haploid-diploid breeding system termed arrhenotoky. Unmated females give rise to male offspring (asexual reproduction) and mated females give rise to both male and female offspring (sexual reproduction). Eggs are laid, hatch and after several nymphal stages develop into adults following incomplete metamorphosis.

With more species than all other animals combined and the broadest of all habitat ranges, diversity within insects is readily evident. It is their innate ability to adapt rapidly that has made them so evolutionary successful.

Key considerations

Despite the tremendous diversity within the insect class, there are generic, interdependent biological characteristics and environmental requirements that determine some of the key considerations for a rearing facility

Size: has implications for effective containment and likely population densities

Reproductive system: has implications for the stability of genes and denotes population size needed to maintain genetic equilibrium

Life-stages: complexity of cycle affects food, environmental and labour requirements

Generation time: impacts productivity and lead times

Mobility: has implications for effective containment and environmental design

Diet: live, perishable, artificial - choice will affect labour requirements.

Temperature: affects health, vitality, metabolic rates and generation time

Humidity: affects health, vitality, food sources and pathogen build-up

Day length: affects health, vitality, insect-insect and insect-plant interactions

In addition to the above, there are a range of other factors that may or may not be relevant to the facility in question, but are often critical when rearing multiple insect species or types in a commercial environment.

Contaminants: viruses, bacteria, fungal pathogens, other insects

Compatibility: antagonism or synergism between species is common

Quarantine: affects containment measures, only applies to the culture of exotic species licensed by Plant Health Inspectorate

Monitoring: manual or automated, to diagnose and react to facility variables

Costs: long-term or short-term strategies, energy efficiency, etc.

With all of the above considerations imposing themselves upon insectary production, good design features will strive to maximise versatility, consistency, and efficiency.

Levels of containment

In order to appreciate the environmental relationships between different insect holding areas within an insectary, it is sometimes useful to consider the levels of containment present (Figure 2). Each level plays a pivotal role with regard to the environmental stability, effective containment, and overall usability. Level 1 is the primary insect cage or chamber, designs vary yet ventilation, access and maintenance are usually key considerations. Level 2 is the immediate room space, frequently with a regulated or controlled environment (CE). Manipulating level 2 air pressure can provide additional containment benefits as the escape of attractant volatiles can be minimised. Level 3 is the suite in which the room is located.

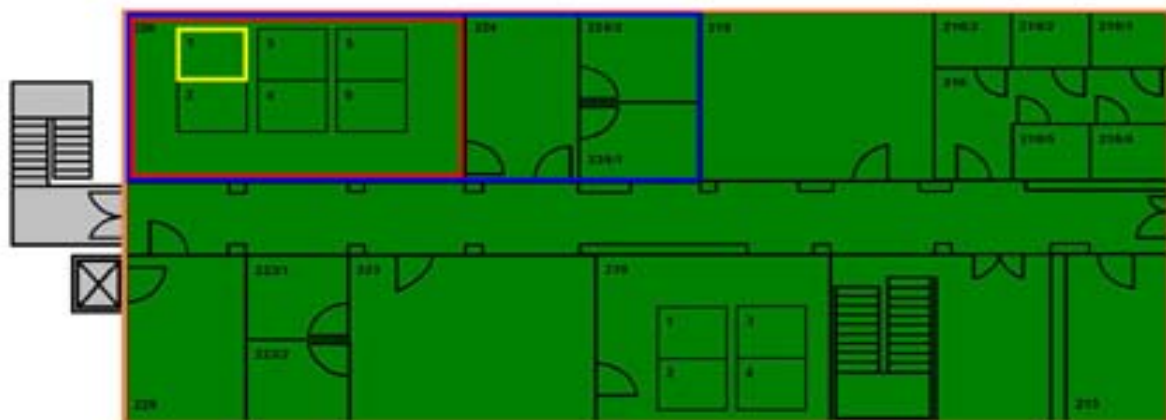


Figure 2. Levels of containment exemplified by a section of the Rothamsted Research insectary, Harpenden, UK. Level 1 = yellow, level 2 = red, level 3 = blue, level 4 = orange.

Although this is often a luxury some building designs do not afford, it can help isolate areas and mitigate the risks resulting from frequent user access. Level 4 is the building itself. As a standalone entity within an external environment, windows, doors, and ducting present a significant threat to effective containment.

Less is more

Reliable data is only attainable if experimental parameters are consistent. The only consistent level of insect quality is 100% healthy and this should always be a primary objective. Maximising this, both in terms of integrity and health, is much more realistic when populations are small to medium in size, uncrowded in respect of their cage, and uncrowded in respect of their CE environment. As exemplified by contemporary commercial insectary facilities, replicates of small age-structured populations, housed in small CE rooms, coupled with small laboratories, frequently offer the most versatile, cost efficient and productive option (Figure 3).

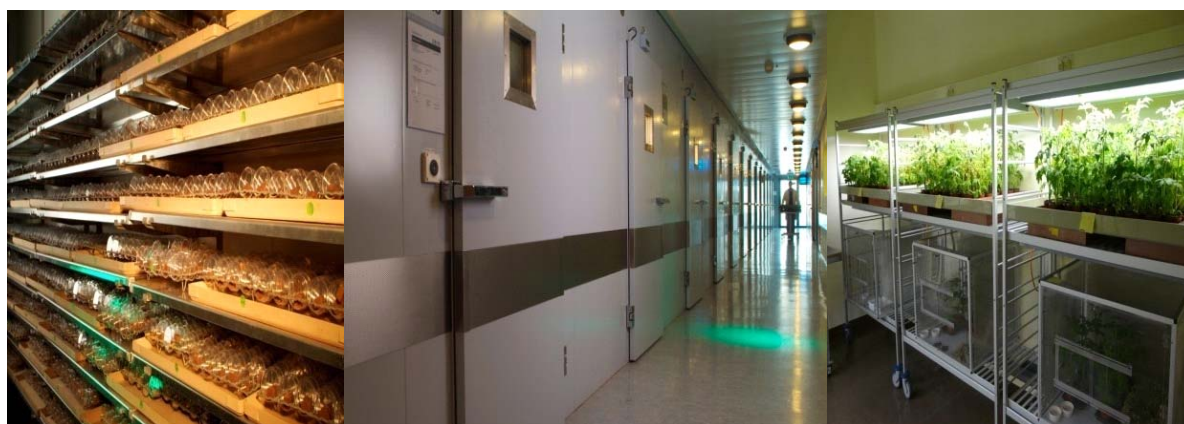


Figure 3. Insectary facilities at Syngenta, Stein, Switzerland and Bayer CropScience, Monheim, Germany.

Acknowledgements

Photographs for Figure 3 were kindly provided by R Slater, Syngenta AG; R Nauen and S Eilmus, Bayer CropScience.

D. Adair (Controlled Environments Inc., 11465 Loftman Trail, North Branch, Minnesota, MN 55056, USA; E-mail: dadair@conviron.com) **Containment for plants: a view from 'Across the Pond'**

Introduction

Plant research requiring greenhouse and laboratory containment is common throughout the world yet regulations and guidelines vary widely. This paper describes regulations and guidelines from the United States to illustrate differences found in the UK. In 2001, I co-authored *A Practical Guide to Containment: Greenhouse Research with Transgenic Plants and Microbes* and presented it to the first international conference on controlled environments. This meeting in 2009 was an opportunity to introduce the 2008 update entitled *A Practical Guide to Containment: Plant Biosafety in Research Greenhouses*. The new guidebook adds information on high containment applications garnered from professionals and new facilities. The publication is available from Information Systems for Biotechnology at Virginia Tech University, <http://www.isb.vt.edu>. It is hoped that practical application can be gained regardless of governing documents.

The research greenhouse and the need for containment

Plant researchers are working hard to address pressing global problems of food production, nutrient supply limitations, energy needs, climate change, and water conservation. This often

results in the need for programs that require containment. These include the use of transgenic or genetically engineered plants and related organisms (GE), the need to move material across political boundaries (exotics), plant and insect pathogens, plant made pharmaceuticals (PMP) and industrial compounds (PMIC), quarantined material, and 'Select Agents'.

Examples of exotic plant pathogens are Asian soybean rust (ASR) and the wheat stem rust, UG-99, which can travel around the globe producing devastating crop losses. GE PMP crops are being developed to save development and delivery time of 'orphan drugs' and PMIC may offer economical and fast growing fibre supplies.

The US has developed a roster of especially important plant pathogens that theoretically could become bioweapons. This list of 'Select Agents' changes with their prominence or if the agent is discovered in the outdoor environment within US borders. For these research programs, security is paramount.

2009 Select Agents List

- *Peronosclerospora philippinensis* (*Peronosclerospora sacchari*) – Sugarcane downy mildew
- *Phoma glycinicola* (formerly *Pyrenochaeta glycines*) – Red leaf blotch of soybean
- *Ralstonia solanacearum*, race 3, biovar 2 – Southern wilt/brown rot of potato
- *Rathayibacter toxicus* – Gumming disease of grasses, nematode-vectored
- *Sclerophthora rayssiae* var. *zeae* – Brown stripe downy mildew of corn
- *Synchytrium endobioticum* – Potato wart
- *Xanthomonas oryzae* pv *oryzae* – Bacterial leaf blight of rice
- *Xylella fastidiosa* (citrus variegated chlorosis strain) – Citrus variegated chlorosis

Guidelines and agency oversight

The US Department of Agriculture's Animal and Plant Health Inspection Service (APHIS) regulates the movement of plant material, pathogens, pests, and related organisms. The Plant Protection and Quarantine (PPQ) division, <http://www.aphis.usda.gov/ppq/index.html>, regulates exotics and the Biotechnology Regulatory Services (BRS), <http://www.aphis.usda.gov/brs/index.html>, regulates the introduction of GE material into the natural environment. There is overlap for inspection for both APHIS divisions. PPQ offers facility guidelines that can aid facility design and operation. BRS, as well as all other agencies, defer to the National Institutes of Health's Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines) which can be found at http://oba.od.nih.gov/oba/rac/guidelines_02/NIH_Guidelines_Apr_02.htm. Non-compliance of regulations may result in monetary penalties or incarceration. Guidelines, on the other hand, are voluntary yet non-compliance can result in the loss of government funding for an entire institution.

The Environmental Protection Agency <http://www.epa.gov/> regulates plant material that has pesticidal activity. The Food and Drug Administration <http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM055424.pdf> offers guidance for industry when producing PMP. In addition, individual states may have regulations or guidelines for handling materials requiring containment or release into the environment. State agencies sometimes act as agents for the above mentioned federal agencies.

Biosafety level designators are commonly used when discussing worker biosafety. The US Centers for Disease Control as well as the World Health Organization use designators BSL1-4. This concept has been borrowed to apply to recombinant DNA research. For example the NIH Guidelines offer BL1 to 4 when working in laboratories and offer BL1-P to 4-P when working with plants. APHIS, the primary agency for issuing plant permits, does not prescribe biosafety levels but instead offers program specific suggestions for meeting containment.

Containment objectives and elements

Regardless of the research program, regulation, or guideline, the primary reason for plant containment is to protect the environment. Worker protection is generally not at issue. Plant pathogens, insects, or rDNA material that escapes a facility may have mild to serious consequences on the natural ecosystem or on agriculture. The concept of the 'disease triangle'- the presence of the pathogen, a susceptible host, and a proper environment - is highly applicable when designing for containment. People moving pests and spores out of a contained space is likely the most common route of containment loss followed by pests themselves, water, and air. Operational standards, physical barriers, and biological interruptions, singly or in combination, are used to achieve containment.

Management considerations

Operational standards involve controlling access to the space, keeping logs when appropriate, record keeping, creating contingency plans, and developing training and reference manuals and/or 'standard operating procedures'. Apparel and hygiene and signage vary with biosafety levels or APHIS regulations. Special attention is given to the termination and disposal of material as well as the transfer of seeds and plants. Very basic or quite sophisticated security is applied as appropriate. For example, Select Agents are accessed by trained individuals who must pass through three locked rooms or containers.

Hardware considerations for physical containment

A laboratory or controlled environment chamber or room takes advantage of the 'room within a room' containment concept whereas a greenhouse itself is the primary containment barrier. The choice of greenhouse glazing and how the growing space is conditioned are additional careful choices to be made. Attention to a proper layout and site choice is critical to good design. Standard research equipment is sometimes modified to meet containment standards. For example, benches, floors, and walls that are built from materials that are easily disinfected are recommended. Pressurization of the contained space, generally negative though occasionally positive, can be useful if not required. Screen sizes for ventilation or caging are often recommended to meet specific program needs. APHIS often recommends the use of vestibules especially when working with insects.

Biological containment

Biological containment is defined as the use of biological means to block plant sexual and vegetative reproduction and to prevent the spread and persistence of genetic material in the environment. Some methods for attaining this include chloroplast engineering, Genetic Use Restriction Technology (GURT), and Virus Induced Gene Silencing (VIGS). Bagging of pollen and seed producing plant parts is also an effective method for preventing dissemination.

In conclusion, the NIH Guidelines continue to offer primary guidance for GE plant and plant-related organisms. USDA-APHIS is the primary regulatory agency for the movement of plant material, pests, and organisms that may become pests, and field release of GE material.

Facilities can be constructed with a general biosafety containment level e.g. BL2-P, yet APHIS permitted material does not prescribe levels. APHIS offers program specific suggestions for meeting containment.

Resources

- 7th Annual Biosafety and Biosecurity Training Course: 8 to 15 July 2010: <http://www.cvmb.colostate.edu/mip/crwad/BBTC.htm>
- American Biological Safety Association: <http://www.absa.org/>
- Information Systems for Biotechnology: <http://www.isb.vt.edu/>

Handbooks:

1. D. Adair, R. Irwin and P.L. Traynor (2001) *A Practical Guide to Containment: Greenhouse Research with Transgenic Plants and Microbes*. (c) 2001 by Information Systems for Biotechnology. All rights reserved. Printed in the United States of America. ISBN: 0-9703604-0-1

2. D. Adair and R. Irwin (2008) *A Practical Guide to Containment: Plant Biosafety in Research Greenhouses*. (c) 2001, 2008 by Information Systems for Biotechnology. All rights reserved. Published 2001. Second edition 2008. Printed in the United States of America. ISBN: 0-9703604-2-8

The complete text of this Guide is available on the ISB Web site (<http://www.isb.vt.edu>). Print copies may be obtained by ordering from the ISB website, or you may send your request by email to isb@vt.edu or by fax to 540-231-4434. Please be sure to include a complete mailing address.

A.N.G. Holden (Plant Health Seeds Inspectorate, Vancouver House, County Court Road, Kings Lynn. PE30 5GZ, UK; E-mail: tony.holden@fera.gsi.gov.uk) **Issues of containment, plant health licences and plant movement in England and Wales**

Abstract

This paper introduces the legal basis for plant health regulations on the movement of plants, plant pests, soil and potatoes in England and Wales, and develops the theme of licensing to permit the introduction and keeping of otherwise prohibited material.

Introduction

Around the world there are many plant pests and diseases which, if they were to become established in Great Britain, could cause serious damage to our crops and plants. To guard against the spread of harmful organisms, official controls apply to the import movement and keeping of prohibited plants, plant pests (plant pests refers to both invertebrate plant pests as well as plant pathogens) and other material such as soils. These controls are based on UK and EC legal provisions. However, the current Plant Health controls include provision, subject to appropriate precautions, for trials for scientific purposes or varietal selection work on plants, plant pests, soil and growing medium that would otherwise be prohibited.

The origins of the legislation relating to Plant Health in England can be traced back to 1877 when the first law to control Colorado Beetle was passed. Following membership of the EEC in 1973, the Plant Health Directive 77/93/EEC was formulated and in 1980 this directive was

implemented in all member states. When in 1993 Community border controls were removed and the EC became a single market, the Plant Health (Great Britain) Order 1993 came into effect. Today's legislation relating to Plant Health in England and Wales is laid out in the Plant Health (England) Order 2005 (as amended) and the Plant Health (Wales) Order 2006 (as amended) (hereafter the P H Orders). These two statutory instruments implement the European Council Directives 2000/29/EC and 2008/61/EC (these statutory instruments do not cover forestry, seeds or seed potatoes, as these areas have their own legislation. (The import, movement and keeping of seed potatoes are covered by 2000/29/EC – but the marketing of seed potatoes has separate legislation).

Essentially the P H order defines the plant pests and plants and plant products that are prohibited entry into England; the requirements and conditions required for importing certain plants and plant products; and the provisions required for movement of plants and plant products within the EU. Additionally it also defines the authority and actions for enforcement of the legislation, including obligations on Plant Pest Notification. The P H Order also allow for the authorising authority, in England and Wales, Fera (Food and Environment Research Agency), to licence the import of otherwise prohibited plant material and plant pests for scientific or trial purposes. Scotland has its own separate statutory authority and arrangements.

The P H Orders consists of two mains parts termed Articles and Schedules. The Articles contain the basic instructions, powers and requirements of the P H Orders. The Schedules contain lists of quarantine pests and specific requirements that must be satisfied regarding plants to be imported into the UK from third countries (outside the EU) and, where appropriate, for plants moving in the EU and internally within the UK.

Plant Passports

Community measures require that, in the case of certain plants, plant products and other objects originating in the Community, the material must be accompanied by a plant passport (issued under the authority of the plant health services of the Member State). For England, the plants etc. to which this requirement applies are listed in Schedules 7, Part A of the Plant Health (England) Order 2005 (as amended). In Wales, the same schedule number refers within the Welsh legislation.

In the case of certain plants, plant products and other objects to be introduced from a third country, (outside the EU) the material must be accompanied wherever possible by a phytosanitary certificate issued in the country of origin. For England, the plants etc. to which this requirement applies are listed in Schedule 5, Part A of the Plant Health (England) Order 2005 (as amended). In Wales, the same schedule number refers within the Welsh legislation.

Plant Health Licences

Plant health controls include provision, subject to appropriate precautions, for work on plants, plant pests, soil and growing medium which would otherwise be prohibited. The P H Orders provide for the granting of licences in accordance with Commission Directive 2008/61/EC, which prescribes, *inter alia*, quarantine and containment conditions to be applied as appropriate.

Under this provision, Fera can issue the following types of Plant Health licence:

1. Licences for the import, movement and keeping of prohibited plants, plant material, plant pests, soil and growing media for scientific research, varietal selection or trialling purposes.
2. Licences may also be issued for plants that are otherwise not prohibited but which cannot meet the plant health requirements of the P H Orders, such that a phytosanitary certificate cannot be issued.
3. Licences for the import, movement and keeping of soil and growing medium for physical or chemical analysis;
4. Licence for the import of prohibited potatoes: Fera only issue licences for potatoes where the material is intended solely for research purposes only. A condition for licences to import potatoes is that the licensee must inform their local Plant Health and Seeds Inspector (PHSI) on the first working day after new material has been imported, and the plant material should be kept in isolation until inspected by the PHSI. Material intended for release should be imported under a Scottish Plant Health licence and sent to SASA for testing.

Structure of the Licence

For England and Wales a Plant Health Licence comprises four parts.

1. The main body of the licence containing the licence conditions including responsible persons, containment facilities, and waste disposal procedures.
2. The Annex requirements specifying the material that can be obtained under the licence, as well as the approved activities.
3. Special precautions: This section details additional precautions that must be followed and are tailored to the risks associated with the material and work undertaken.
4. Letter of Authority, copies of which must accompany all imported consignments and be available for inspection on outside of packaging.

The Licence Application Process

Fera cannot issue a licence unless in receipt of one of the following forms, properly completed and signed, together with the appropriate fee:

1. PHI 3 – Application to import, move and keep prohibited plants, plant material, plant pests, soil or growing medium for scientific or trialling purposes and for work on varietal selections, and to import other plant material which would otherwise be prohibited.
2. PHI 3A – Application to import, move and keep prohibited soil or growing medium for physical or chemical analysis.

A copy of the Standard Operating Procedures relating to the work with the material covered by the application must accompany the application form.

Usually licences will be issued only in the name of an individual, who must be a permanent member of staff. In the case of a University or research establishment this will normally be the head of the relevant department. In the case of a commercial establishment, the licence will be issued in the name of the company, with a permanent member of staff nominated as the person responsible. However, a list of all scientific and technical personnel who will be involved in the project, together with their qualifications, should be included with the Standard Operating Procedure.

Once received the application is reviewed and a scientific assessment undertaken with reference to the supporting documents / SOP / risk assessment and background papers submitted with the application.

This preliminary assessment considers if the proposed licensed organism is listed in the Order, or whether a phytosanitary certificate or plant passport is available. If the organism is not listed in the order, a further licence risk assessment may be required. Within the preliminary assessment, a licence is appropriate if the proposed organism is damaging to plants, is a non native or non-UK strain of a native plant pest, and /or has potential for survival, reproduction and establishment.

If the organism is not listed, a supplementary Risk Assessment may be required - Article 16(2) allows appropriate action to be taken against organisms which are not listed, but which pose a phytosanitary risk.

The supplementary risk assessment (if required), comprises a further assessment of containment measures, and will consider the host plant range, the global distribution of the proposed licensed organism, whether it is a vector of pathogens/disease, together with an assessment of the potential to survive in protected environments in UK/EU and of the potential to survive outdoors in the UK/EU.

Based on Fera's assessment, the Plant Health and Seeds Inspectorate inspect the proposed containment facilities. The inspection includes aspects of security, administration and recording, transport, an assessment of the containment facilities and experimental procedures, on-site hygiene and disposal and a general overview of the site and suitability for the proposed work.

In establishing contact at the start of the project, the Plant Health and Seeds Inspectorate have responsibility for the initial approval of the site and subsequently act as a point of contact with the licensee. As part of this role, the PHSI have an obligation to make annual contact with all licence holders, and, where appropriate, this involves an annual site inspection

Licences, if issued, are valid for 12 months and are subject to annual renewal if the work is ongoing.

In practice, the licence process works at the point of entry in the following ways:

Obtaining material from countries outside the EU

A copy of the Letter of Authority (LoA) must be sent to the person supplying the material and must accompany the material during import. There is NO requirement for the LoA to be endorsed by the NPPO of the country the material is being sent from. The LoA must be attached to the outside of the package/s containing the licensed material, and each individual consignment must be accompanied by its own copy of the LoA.

Bringing material into England and Wales from EU member states (including from Scotland, Northern Ireland and the Channel Islands, as well as Switzerland)

The Defra licensee should send a copy of their LoA to the intended supplier of the material. The LoA should be endorsed by the Plant Health Authorities in the Member State where the material originates and then used to accompany the material to the licensed recipient. Where more than one consignment is to be imported, each should be accompanied by a copy Letter

of Authority, separately endorsed by the relevant plant health authority in the case of material from another Member State.

Moving Licensed Material between Licensed Facilities in England and Wales

Material covered by a licence may be sent to other persons or organisations within England and Wales who hold an appropriate licence, provided written agreement has first been obtained from Fera Plant Health. To obtain written agreement, an application, using form PHI 10, must be submitted before material can be moved between licence holders in the UK.

Exporting Material

a) Sending licensed material to countries within the EU (including Scotland, Northern Ireland and the Channel Islands)

When licence holders wish to send material which is listed in 2000/29/EC, to other licensees within the EU, they should obtain a copy of the Letter of Authority from the intended recipient. This should be sent to the local PHSI to be endorsed and any necessary additional information added. The LoA should then be returned to the licensee to be attached to the consignment/s before dispatch.

b) Sending licensed material to countries outside the European Union (third countries)

Sending material to third countries falls outside of the scope of the plant health licensing system in England and Wales. It is assumed that compliance with the third country's plant health import regulations will apply, and it is the responsibility of the person sending the material to ensure compliance with such regulations.

References

The Plant Health (England) Order 2005 Statutory Instrument (2005) No. 2530 Published by TSO (The Stationery Office).

The Plant Health (Wales) Order 2006 Welsh Statutory Instrument (2006) No. 1643 (W.158).

H. Cabrera and M. Paton (Health and Safety Executive, HID Specialised Industries S14, Biological Agents Unit, Bld 1.2 Redgrave Court, Merton Road, Bootle, Merseyside L20 7HS, UK; E-mail: hector.cabrera@hse.gsi.gov.uk) **HSE and containment for Genetic Modification**

Introduction

Advances in molecular biology since the early 1970s have resulted in the growth of a wide variety of techniques, which result in genetic modification (GM). These techniques continue to develop rapidly and have been applied to a wide range of micro-organisms, plants and animals. The GM activities have resulted in increased scientific understanding of organisms, their interactions, genetics, and functions, as well as enabling production of enzymes, therapeutic agents and gene therapy vectors.

Where the activity involves the use of control measures or barriers to limit contact between genetically modified organisms (GMOs), humans and the environment, the activity is referred to as 'contained use'. In practice, this will cover any activity in which organisms are genetically modified and where GMOs are cultured, stored, transported, destroyed, disposed of or used in any other way. Contained use activities comprise the bulk of GM activities undertaken in Great Britain. The vast majority (~90%) of GM activities in Great Britain is

viewed as either low risk or as inherently safe as scientists implement strategies that severely incapacitate the organism so that it is unable to survive or proliferate outside of the laboratory without specified 'artificial' growth requirements. For some other activities involving organisms still capable of growth outside of the laboratory, a robust risk assessment must be submitted for scrutiny by the Competent Authority, before work commences. This must identify appropriate and proportionate containment measures to prevent release.

Regulation of genetic modification

The Health and Safety Executive (HSE) has lead responsibility for human health and safety throughout Great Britain and has launched a strategy in June 2009 to improve health and safety performance [1]. HSE enforces legislation as part of the Competent Authority in Great Britain that aims to control the risks to human health and the environment arising from activities involving GMOs in containment. The primary piece of legislation that applies to the use of GMOs in the workplace is the Genetically Modified Organisms (Contained Use) Regulations 2000 (as amended) [2]. The HSE and the Secretary of State for the Department for Environment, Food and Rural Affairs (Defra) form the Competent Authority in England and Wales. In practice, these functions are delegated to HSE and Defra officials. In Scotland, the Competent Authority comprises the Scottish Ministers and HSE and similarly these functions are delegated to officials of HSE and the Scottish Government. Although not part of the Competent Authority, the National Assemblies for Wales and Northern Ireland are included in all UK Competent Authority considerations.

In addition to requirements in relation to human health and safety and environmental protection from GMOs that are microorganisms, the GMO(CU) Regulations also require protection of human health in respect of GM animals and plants in contained use. The key requirement of the GMO(CU) Regulations is to assess the risks of all activities and to make sure that any necessary controls are put in place. The GMO(CU) Regulations provide a framework for making these judgments, and place clear legal obligations on people who work with GMOs.

The GMO(CU) Regulations 2000 (as amended):

- require risk assessment of activities involving genetically modified organisms;
- introduce a classification system based on the risk of the activity. The classification is based on the four levels of containment;
- require notification of all premises to HSE before they are used for genetic modification activities for the first time;
- require notification to HSE of individual activities of Class 2 (low risk) to Class 4 (high risk). Consents are required for all Class 3 (medium risk) and Class 4 (high risk) activities. Class 1 (no or negligible risk) activities are non-notifiable, although they are open to scrutiny by HSE's specialist inspectors who enforce the Regulations;
- require the maintenance of a public register of GM premises and certain activities.

HSE administers the notification system under GMO(CU) on behalf of the Competent Authority. HSE's Biological Agents Unit (BAU) maintains the Public Register of contained use GM activities and provides technical comment on the notifications. BAU undertakes inspections of notified premises and activities to check compliance and support the GMO(CU) regulatory framework and ensure that risks in the workplace from microbiological hazards are properly controlled [3].

The protection of the environment from contained use activities involving genetically modified animals and plants is enacted through relevant sections of the Environmental Protection Act 1990 and associated regulations (the Genetically Modified Organisms (Risk Assessment) (Records and Exemptions) regulations 1996). Taken together with GMO(CU), the legislation requires that anyone carrying out any activity of this nature, must carry out a risk assessment for human health and the environment and undertake the activity in conditions of contained use, which satisfy the legislation. The Department for Environment, Food and Rural Affairs (Defra), the Scottish Government and Welsh Assembly Government (in England, Scotland and Wales respectively) have lead responsibilities for all effects from the contained use of GM animals and plants in relation to contained uses that affect the environment e.g. farmed animals (including fish and shellfish), plant varieties and seeds, veterinary medicines, fertilizers, animal feedstuffs, food and forestry, as well as the marine environment.

Defra, Scottish Government and the Welsh Assembly Government (in England, Scotland and Wales respectively) are also responsible for regulation of deliberate releases of GMOs. The deliberate release of GMOs is not considered as part of this paper. Further guidance can be obtained from the Defra website [4].

Containment

It is explicit within GMO(CU) that any contained use activity involving GMOs requires containment measures that limit their contact with, and provide a high level of protection for, humans and the environment. In this context, containment means physical, chemical or biological barriers or any combination of such barriers. Contained use facilities therefore include: laboratories; animal houses; plant growth rooms and glasshouses; industrial fermenters used for large-scale production of enzymes or therapeutics; and facilities to contain genetically modified farm animals.

The selection of containment measures needs to be based on an appropriate risk assessment that considers amongst other things, the hazardous nature of the GMO and the nature of the activity being undertaken. This risk based approach ensures that there is clear understanding of the risks that need to be controlled and which control measures need to be implemented and monitored. Experience also shows that appropriate control measures are not only physical but also systems and procedures that ensure effective management of the facility.

The 'Compendium of Guidance' represents what the regulator considers to be safe and good practice when working with GMOs in a contained use setting [5]. Whilst it is not mandatory to follow this guidance, doing so will almost certainly ensure that workers are complying with GMO(CU). The guidance was developed by the Scientific Advisory Committee for Genetic Modification (Contained Use) and is widely used by the research community. The compendium provides guidance on risk assessment for GM activities as well as selection of containment measures for a range of GM activities including: laboratory work; work with GM animals and plants; and applications in a clinical setting.

Single regulatory framework

Following the outbreak of Foot and Mouth Disease which began in Surrey in August 2007, Sir Bill Callaghan led a review of the regulatory framework for handling animal pathogens[6].

The review contained a number of recommendations, two key ones being:

- that there should be a single regulatory framework to govern work with human and animal pathogens (based on the regulatory approach for GMOs); and
- that HSE become the single regulatory body for both human and animal pathogens, with responsibility for inspection and enforcement functions.

Work is well underway to implement the review's recommendations to develop a single regulatory framework to govern work with animal and human pathogens and incorporating GMOs. The first stage of establishing the legal basis for a single framework was a complex process involving analysis of several Acts of Parliament and multiple European directives. The detailed work to shape the regulatory framework is at an advanced stage and is expected to commence public consultation in February 2010. As part of the process, HSE has sought, and continues to seek, dialogue and involvement with stakeholders throughout the development of the framework. In addition to the new regulations, the consultation package will include an associated guide to the regulations, regulatory impact assessment and guidance document on containment measures. The Regulatory Impact Assessment is intended to assess the impact, in terms of costs, benefits and risks, of any proposed regulation, on businesses, charities or voluntary bodies.

There are several predetermined procedures that have to be followed to implement new legislation and, based on past experience; it is likely that the new regulations will be in place in by October 2010 [7].

Key Messages

The key messages of this paper are:

- HSE's strategy for health and safety in Great Britain was launched in June 2009 – everyone has a role to play;
- Effective management systems are a key determinant of health & safety performance;
- The regulatory framework for genetic modification activities in Great Britain is well developed and risk based;
- Selection of containment is based on risk assessment;
- Change in legislative framework to encompass human and specified animal pathogens within the same regulatory framework as GMOs is on the horizon;
- One stop notification for GMOs, human and specified animal pathogens (October 2010)

References – web links

1. HSE strategy, 'The Health and Safety of Great Britain – Be Part of the Solution', HSE website (<http://www.hse.gov.uk/strategy/document.htm>)
2. Genetically Modified Organisms (Contained Use) Regulations 2000 (as amended). Guidance on regulations (<http://www.hse.gov.uk/biosafety/gmo/law.htm>)
3. HSE biosafety web pages (<http://www.hse.gov.uk/biosafety/index.htm>)
4. The Genetically Modified Organisms (Deliberate Release) Regulations 2002 (<http://www.opsi.gov.uk/si/si2002/20022443.htm>) and additional information genetic modification available from DEFRA website (<http://www.defra.gov.uk/environment/quality/gm/index.htm>)
5. 'SACGM Compendium of Guidance', available from HSE website (<http://www.hse.gov.uk/biosafety/gmo/acgm/acgmcomp/index.htm>)
6. Callaghan review – 'A Review of the Regulatory Framework for Handling Animal Pathogens', Executive summary available from Defra (<http://www.defra.gov.uk/foodfarm/farmanimal/diseases/atoz/fmd/2007/bill-callaghan.htm>)

7. Implementation of Callaghan recommendations – update on HSE website (<http://www.hse.gov.uk/biosafety/callaghan.htm>)
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