A Practical Guide to Containment

Greenhouse Research with Transgenic Plants and Microbes

Patricia L. Traynor | Dann Adair | Ruth Irwin

Information Systems for Biotechnology

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Patricia L. Traynor

Dann Adair

Ruth Irwin

Information Systems for Biotechnology

Virginia Tech

Information Systems for Biotechnology
207 Engel Hall, Blacksburg VA 24061
tel: 540-231-3747 / fax: 231-4434 / email: isb@vt.edu
http://www.isb.vt.edu

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Table of Contents

Section I. Introduction

THE USE OF BIOTECHNOLOGY TO MODIFY PLANTS has become a common practice in agricultural and horticultural research. Unlike ordinary research materials used in laboratory, greenhouse, and field studies, transgenic (genetically engineered, genetically modified)[1] organisms are subject to special rules intended to ensure that they are used in a way that does not pose an unacceptable risk to human health or the environment.

Methods for the safe handling of transgenic materials in laboratory settings are described in the National Institutes of Health's *Guidelines for Research Involving Recombinant DNA Molecules* (NIH Guidelines). Regulations and guidance for the safe release of genetically modified organisms (GMOs) into the environment are implemented by the Animal and Plant Health Inspection Service of the US Department of Agriculture (USDA/APHIS) and the Environmental Protection Agency (EPA). Genetic modifications include, but are not limited to, those made by recombinant DNA (rDNA)[2] methodologies.

Information about handling transgenic plants in greenhouses, however, is relatively sparse. Appendix P of the NIH Guidelines[3] specifies facilities and practices for meeting containment standards appropriate for each of four biosafety levels. Presently, though, there is no single source of practical guidance on managing greenhouses containing GMOs, nor on the requirements for building or renovating plant growth facilities to make them suitable for containing transgenic plants and associated organisms.

This Guide is intended as a simple and convenient reference on appropriate biosafety and containment levels for GMO research conducted in greenhouses. There may be a broad range of guesses and opinions among scientists and greenhouse managers regarding what is needed. Some may harbor a misunderstanding that all GMOs must be grown in a highly contained 'clean-room,' while others may be completely unaware that certain cases require specific containment measures in order to protect the surrounding environment. The Guide will help clarify what level of containment is needed and what measures are sufficient to achieve the various biosafety levels.

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1 In this Guide, the terms "transgenic," "genetically engineered," and "genetically modified" are used interchangeably.

2 Recombinant DNA molecules are defined as: "(i) molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or (ii) molecules that result from the replication of those described in (i) above."

3 http://www4.od.nih.gov/oba/appendix_p.htm

Scope

This Guide applies to greenhouses—controlled environment structures having a transparent or translucent covering and used for growing plants—that contain genetically modified plants or plant-associated organisms. The wide range of microorganisms that are plant-associated include viruses, bacteria, fungi, protozoa, mycoplasma-like organisms, nematodes, insects, mites, and others.

Screenhouses—structures that are screened for insect or plant containment (or exclusion) but which offer little environmental control—are suitable for temperate climates or warm seasons in zones subject to colder temperatures. Screenhouse construction details and upgrades are briefly described in this Guide.

Other contained plant growth facilities, such as growth chambers, biosafety cabinets, incubators, and tissue culture tables or rooms, often are an integral part of the process leading to the preparation of GMO materials for greenhouse studies or field tests. These facilities are mentioned in passing; a detailed description is not within the scope of this Guide.

This Guide includes:

- Relevant information on four levels of biosafety containment;
- Physical and biological strategies that provide containment;
- Suggested facility modifications to achieve prescribed containment levels;
- Suggestions for day-to-day greenhouse management;
- Methods for proper handling of GMOs;
- Discussions of selected design issues for new or renovated facilities;
- Descriptions of equipment and supplies;
- A sample floor plan and;
- Sources for additional information.

The Guide is organized in seven sections plus two Appendices. Section I contains introductory information and a brief discussion of the contents. Section II covers the regulation and oversight of GMOs by government regulatory and research agencies, and outlines the roles and responsibilities of institutional personnel. Section III presents descriptions of four biosafety levels affording increasing levels of containment, together with examples of studies that may be conducted at each level. Physical, biological and combination containment strategies are given in Section IV, followed by suggested management practices for greenhouses containing GMOs in Section V. Section VI discusses options for retrofitting existing facilities to meet containment standards, and Section VII addresses the design of new facilities. Two Appendices provide facility inspection checklists and a list of supplemental information resources.

This Guide was written so that anyone who works in a greenhouse that houses transgenic materials will be better informed about the purpose of containment, the variety of methods used to achieve it, and the facilities and practices that satisfy the requirements of established guidelines and regulations. It is intended as guidance and should not be a considered an authoritative source. Readers are encouraged to seek additional guidance from institutional authorities and USDA/APHIS officials whenever questions arise.

Audience

Greenhouse managers, facility staff, and research scientists are the primary audience of this Guide. Managers, being responsible for the overall operations of a greenhouse facility, will benefit from a clear description of when, where, and why additional containment measures should be instituted, as well as practical guidance for managing the facility and persons working in it. Greenhouse staff who are involved in the day-to-day care of transgenic organisms will gain a better understanding of what tasks, if any, should be modified when the experimental materials have been genetically engineered. Researchers who work with

GMOs, together with members of Institutional Biosafety Committees and students, will likely find it a simple and convenient reference on the various levels of containment and the types of experiments appropriate to each level.

In addition, designers working on retrofits to existing greenhouses or on new construction will find specialized information that pertains to meeting non-standard structural requirements for containment facilities. Others who work in and around such facilities, including tradespeople, maintenance personnel, and adjacent residents, will benefit from a basic understanding of the purpose of containment. Such understanding will help ensure that GMOs are handled in an environmentally responsible manner.

Section II. Regulation and Oversight of GMOs

TRANSGENIC PLANTS ARE SUBJECT TO FEDERAL GUIDELINES, regulations, and rules pertaining to their containment, movement, and release into the environment. In addition, a few states, notably Florida and California, have applicable regulations as well. Institutions where biotechnology research is conducted are expected to have an institutional biosafety committee (IBC) serving as the local authority. Ultimately, responsibility for the safe handling of transgenic materials lies with the principal investigator and other individuals who manage any part of the research.

THE NIH GUIDELINES AND APPENDIX P

Guidelines first published by the NIH in 1976 address the safe conduct of laboratory research involving the construction and handling of rDNA molecules and organisms containing rDNA. They are advisory in nature, rather than legally binding. However, all federal agencies that support or conduct rDNA research agreed to abide by the NIH Guidelines and require institutional compliance as a condition of funding. Thus, failure to comply may result in the suspension, limitation, or termination of financial support for rDNA research at the institution. The current version of the NIH Guidelines can be accessed on the Internet[4].

The NIH Guidelines discuss risk assessment and recommend containment measures for various biological experiments. They set forth facility specifications and practices for conducting experiments classified according to four levels of biosafety containment; a fifth class encompasses experiments that are exempt. Although originally focused on rDNA microorganisms, the NIH Guidelines have undergone numerous revisions and now cover plant, animal, and human gene therapy research to accommodate the wide range of federally funded research projects.

The Guidelines were expanded in 1994 by the addition of Appendix P, Physical and Biological Containment for Recombinant DNA Research Involving Plants. The term “plants” includes, but is not limited to, mosses, liverworts, macroscopic algae, and vascular plants including terrestrial crop, forest, weed, and ornamental species. Recommended

4 http://www4.od.nih.gov/oba/guidelines.html

containment conditions for experiments involving plants together with plant-associated microorganisms or small animals in which any organism may be genetically modified are also found in Appendix P.

Plant-associated microorganisms include those known to cause plant disease, such as viroids, virusoids, viruses, bacteria, and fungi, as well as protozoa, and microorganisms that have a benign or beneficial association with plants, such as certain *Rhizobium* species. Microorganisms that are modified with the objective of fostering an association with plants are similarly subject to the terms of Appendix P. Plant-associated small animals include those arthropods that: (1) are in obligate association with plants; (2) are plant pests; (3) are plant pollinators; or (4) transmit plant disease agents, as well as other small animals such as nematodes for which tests of biological properties necessitate the use of plants. Microorganisms associated with such small animals (e.g., pathogens or symbionts) are included.

Appendix P describes practices for conducting experiments to construct, use experimentally, and propagate genetically engineered plants. It specifies physical and biological containment measures and management protocols applicable to each of four biosafety levels designated BL1-P, the lowest level of containment, through BL4-P, the highest level.

FEDERAL REGULATORY AGENCIES

Under the Coordinated Framework for Regulation of Biotechnology, three US governmental agencies regulate GMOs: the Department of Agriculture, the Environmental Protection Agency, and the Food and Drug Administration (FDA). Greenhouse research is not generally subject to federal regulation; however, the following brief summary provides the broad context for regulatory review of transgenic plants associated with testing in the environment and commercialization. More complete information about these agencies and their roles with respect to products derived from biotechnology, with links to the laws, rules, and regulations that they administer, can be accessed at the US Regulatory Oversight in Biotechnology site on the Web[5].

USDA/APHIS

The USDA's Animal and Plant Health Inspection Service (APHIS) has authority under the Federal Plant Pest Act to protect US agriculture from pests and diseases. Under the Coordinated Framework, this authority was extended to cover rDNA-containing plants and other potential plant pests. USDA also regulates veterinary biologics such as recombinant vaccines. The Plant Protection and Quarantine division is the lead regulatory office for GMOs. APHIS also adheres to international standards created by the International Plant Protection Convention. Any *introduction* of a GMO, defined as importation, interstate movement, or release to the environment, requires either notification to APHIS or application for a release permit, depending on the nature of the plant and the genetic modification made to it. APHIS has an extensive biotechnology Web site describing their regulations[6].

EPA

The EPA regulates the use of two categories of GMOs. The first encompasses novel microorganisms (formed by deliberate combinations of genetic material from different taxonomic genera) that contain or express new combinations of traits and are intended for commercial use as biofertilizers, biosensors, waste treatment or pollutant degradation, or for commodity or specialty chemical production. The second category consists of plants and microbes producing pesticidal substances, such as plants expressing insect control proteins derived from *Bacillus thuringiensis* (Bt). More information on these topics is available through the EPA's Toxic Substances Control Act Biotechnology Program[7] and their Biopesticides Program[8].

5 http://www.aphis.usda.gov/biotech/OECD/usregs.htm

6 http://www.aphis.usda.gov/bbep/bp

7 http://www.epa.gov/opptintr/biotech/index.html

8 http://www.epa.gov/oppbppd1/biopesticides/index.html

FDA

Commercial products modified by genetic engineering for human and animal consumption, food additives, human and veterinary drugs are subject to regulation by the FDA. Their oversight does not apply to the R&D phases of product improvement. Nevertheless, developers are expected to consult with the FDA during the development phase for guidance on what types of data will be needed at the time of product safety review. An overview of the FDA's policies on food and feed from GM plants can be found on the Internet[9].

Table 1 shows a concise overview of USDA's, EPA's and FDA's overlapping regulatory authorities.

TABLE 1. Multiple Regulatory authorities oversee certain GMOs

NEW TRAIT/ORGANISM	REGULATORY REVIEW CONDUCTED BY:	REVIEWED FOR:
Viral Resistance in food crop	USDA EPA FDA	Safe to grow Safe for the environment Safe to eat
Herbicide Tolerance in food crop	USDA EPA FDA	Safe to grow New use of companion herbicide Safe to eat
Herbicide Tolerance in ornamental crop	USDA EPA	Safe to grow New use of companion herbicide
Modified Oil Content in food crop	USDA FDA	Safe to grow Safe to eat
Modified Flower Color in ornamental crop	USDA	Safe to grow
Modified Pollutant Degrading soil bacteria	EPA	Safe for the environment

[9] http://vm.cfsan.fda.gov/~lrd/biotechm.html

INSTITUTIONAL BIOSAFETY COMMITTEE

Any institution where research involving transgenic organisms is conducted and which receives federal funding for research is required to appoint an Institutional Biosafety Committee (IBC). The committee is to consist of at least five persons, two of whom are "citizen members" not affiliated with the institution. Preferably they are familiar with biosafety issues and have a demonstrated commitment to the surrounding community, especially as it pertains to human and environmental protection. Local government officials, state environmental agency staff, or persons in the medical, occupational health or environmental areas are among those individuals suitable for IBC membership. The committee should also include at least one member having expertise in plant, plant pathogen, or plant pest containment principles.

The IBC reviews recombinant DNA research programs or proposals and confirms the research leader's assignment of the appropriate containment level for the proposed work. Commonly the IBC first considers the proper containment level for the unmodified organism, and then considers whether or not the proposed change to the organism could increase, decrease, or leave unchanged the organism's necessary containment level. The Committee ensures compliance with the NIH Guidelines by evaluating facilities, procedures, and the expertise of personnel involved in the research. In addition, the IBC is responsible for adopting emergency plans for responding to an accidental release from containment. To facilitate timely disposal of residual transgenic experimental materials, the IBC may adopt a closeout policy that provides the project leader with written notice of project termination dates. The Committee is responsible for maintaining and/or verifying documentation of rDNA research at the institution, and acts as a point of contact for NIH and other agencies.

BIOLOGICAL SAFETY OFFICER

If research is conducted on organisms that require special containment conditions designated as BL3-P or BL4-P (described later), or if large-scale microbial research is conducted, a Biological Safety Officer (BSO) must be appointed. This person, who also serves on the IBC, acts as a technical liaison between researchers and the IBC, develops emergency plans, and periodically inspects facilities and protocols. Because higher containment levels require more scrutiny, the BSO serves as an additional contact beyond the IBC.

PRINCIPAL INVESTIGATOR

The Principal Investigator (PI) ultimately is responsible for the research project and for ensuring compliance with biosafety standards. The PI functions as a project manager as well as a researcher, bearing responsibility for training and supervising personnel, communicating with the IBC, BSO, greenhouse manager and staff, and correcting any operations that may result in a loss of containment. Based on the nature of the transgenic organism, the PI determines the proper containment level for the project and, in accordance with the NIH Guidelines, develops the necessary experimental protocols; he submits this information to the IBC for review.

For all experiments to be conducted with plants, the Principal Investigator must file a notification document with the IBC. Notification is made either at the time the work is initiated or prior to the start of the experiment, depending on the level of containment required. In some cases, the investigator may need to obtain further approvals before initiation, in addition to that of the IBC. Details of approval requirements are given in Section III of the NIH Guidelines. The IBC can assist the PI in obtaining requisite approvals.

GREENHOUSE STAFF

Greenhouse staff may range in experience from part time student workers who water plants to skilled tradesmen who maintain the facility's structure and mechanical systems. Regardless of individual duties, all staff should become familiar with any differences between caring for GMOs and conventional plants that may affect their own work. In most cases, a brief orientation session is sufficient to explain the nature of the plants (or other transgenic organisms) and any special practices to be employed when handling or working around them. For example, where transgenic microbes are being tested for their ability to associate with roots, the PI may require that runoff from watering is collected and treated prior to disposal. Both the greenhouse manager and the PI should work with the staff to ensure compliance with safety procedures and standards.

Section III. Biosafety Levels

THE PURPOSE OF CONTAINMENT IS TO PREVENT the transfer of recombinant DNA from transgenic organisms inside the greenhouse to populations outside the greenhouse. Section III of the NIH Guidelines describes four physical containment levels for experiments involving recombinant DNA molecules. It further categorizes experiments according to specific risk criteria and assigns them to one of the four biosafety levels, BL1-P through BL4-P.

Appendix P of the Guidelines specifies the physical and biological containment conditions and practices required for greenhouse experiments for each biosafety level. A brief description of the four biosafety levels and the criteria used by the NIH Guidelines for assigning experiments to each category are provided here. It is the responsibility of the IBC and PI to determine the appropriate biosafety level. When making a biosafety level assignment, consider the following criteria:

- Source and nature of the introduced DNA: whether from an exotic infectious agent or pathogenic organism; and whether a fragment of DNA or complete genome;
- Recipient organism: mode and ease of dissemination; invasiveness; whether a noxious weed or capable of interbreeding with noxious weeds; potential for outcrossing between recipient organisms and nearby related species; and potential for detrimental impact on natural or managed ecosystems;
- Nature of expressed protein: whether a vertebrate toxin or potential or known allergen; and whether toxic to other organisms in local environment;
- Local environment: nature and importance of nearby crops; presence of sexually compatible wild or weedy species; and
- Experimental procedures: transfer to or from greenhouse; and necessary containment measures.

Sound scientific principles and a thorough knowledge of the recipient organism and its mode of dissemination are the basis for designating a suitable level of containment. A brief comparison of criteria used in the Guidelines to assign an appropriate biosafety level is shown in Table 2.

The table shows that as the potential risk to the environment increases, increasingly stringent requirements for containment are indicated. When applicable, physical containment requirements may be eased by the addition of measures for biological containment, indicated by the "+" sign. (Biological containment is described in Section IV, Elements of Containment.)

TABLE 2. Suggested criteria for assigning biosafety levels

CRITERIA	TRANSGENIC PLANTS	TRANGENIC MICROBES		TRANSGENIC INSECTS/ANIMALS/ ASSOC. MICROBES
		Exotic	Non-Exotic	
Not a noxious weed or cannot outcross with one	BL1-P			
Not easily disseminated			BL1-P	
No detriment to environment		BL2-P or BL1-P +	BL1-P	BL2-P or BL1-P +
Noxious weed or can interbreed with weeds	BL2-P or BL1-P +			
Contains complete genome of non-EIA*	BL2-P or BL1-P +			
Contains genome of EIA	BL3-P or BL2-P +			
Treated with an EIA	BL3-P or BL2-P +			
Detriment to environment		BL3-P-4**	BL2-P or BL1-P +	BL3-P or BL2-P +
Involves EIA with detriment to environment	BL3-P or BL2-P +			
May reconstitute genome of infectious agent *in planta*	BL3-P or BL2-P +			
Contains Vertebrate Toxin	BL3-P	BL3-P	BL3-P	

*EIA – Exotic Infectious Agent

**BL4-P containment is recommended only for experiments with readily transmissible exotic infectious agents whether transgenic or not, such as air-borne fungi or viruses *in the presence of their arthropod vectors* that have the potential of being serious pathogens of major US crops.

Experiments that Are Exempt

Experiments that do not present a risk to health or the environment are exempt from the NIH Guidelines and do not require the approval of the local IBC. For example, research using synthetic DNA molecules that are not part of any organism or virus, or research using only DNA segments from a single nonchromosomal or viral source, are exempt. Also exempt are experiments in which the DNA from a particular host organism is propagated only in that same organism, as would be the case for research designed to splice DNA segments taken from wheat into the genome of the same or another wheat variety. This exemption applies to DNA segments regardless of whether they were obtained from the host chromosomes, chloroplasts, mitochondria or plasmids, as long as the fragment is propagated only in that same host, and that no other DNA is used, including promoters and enhancers. Finally, the Guidelines exempt research involving the transfer of DNA between two different species if they are known to exchange DNA by well-established physiological means. Appendix A of the NIH Guidelines contains a periodically revised list of these natural exchangers[10]. Currently, most organisms on this list are bacteria and yeast species, but some genera of plant pathogenic bacteria are included.

Biosafety Level 1 for Plants (BL1-P)

The BL1-P designation provides for a low level of containment for experiments involving transgenic plants in which there is no evidence that the modified organism would be able to survive and spread in the environment and, if accidentally released, would not pose an environmental risk. For example, an experiment designed to study transgenic potato plants containing cloned genes for insect resistance obtained from primitive potato cultivars would be classified as BL1-P.

BL1-P also applies to DNA-modified common microorganisms that cannot spread rapidly and are not known to have any negative effects on either natural or managed ecosystems, such as *Rhizobium* and *Agrobacterium*. A BL1-P designation would be assigned, for example, to an experiment that uses a transgenic strain of *Rhizobium* containing A*grobacterium* genes known to affect root colonization, or plants using *Agrobacterium* DNA segments as part of the transformation process.

Biosafety Level 2 for Plants (BL2-P)

BL2-P is assigned to experiments with transgenic plants and associated organisms, which, if released outside the greenhouse, could be viable in the surrounding environment but would have a negligible impact or could be readily managed. BL2-P is required for transgenic plants that may exhibit a new weedy characteristic or that may be capable of interbreeding with weeds or related species growing in the vicinity. For example, greenhouse tests of transgenic sunflower containing wheat genes intended to confer resistance to the fungus *Sclerotinia* would be classified BL2-P because sunflower is capable both of hybridizing with wild relatives, and becoming established as a volunteer weed.

BL2-P containment is assigned to transgenic experiments that use the entire genome of an indigenous infectious agent or pathogen. This level of containment is also appropriate for transgenic plant-associated microorganisms that are either indigenous to the area and potentially harmful to the environment but manageable, or are exotic but have no potential for causing serious harm to managed or natural ecosystems. The BL2-P classification likewise applies to experiments using plant-associated transgenic insects or small animals as long as they pose no threat to managed or natural ecosystems.

Biosafety Level 3 for Plants (BL3-P)

BL3-P facilities are designed to prevent the accidental release of transgenic plants, plant pathogens, or other organisms that have a recognized potential for significant detrimental

[10] http://www4.od.nih.gov/oba/appendix_a.htm

impact on the environment. This category also applies to non-GMO plant research that involves exotic infectious agents capable of causing serious environmental harm. In these cases, it is the pest or pathogen that requires containment; the transgenic plant itself may pose no threat. BL3-P is also recommended for transgenic plants containing genes from an exotic infectious agent in which a complete functional genome of the infectious agent could possibly be reconstituted. Experiments using transgenic plants or organisms that contain genes coding for vertebrate toxins are likewise conducted at BL3-P. Lastly, BL3-P is recommended for experiments using transgenic microbial pathogens of insects or small animals that associate with plants, if the pathogen has the potential to cause harm to the local environment.

Examples of research requiring BL3-P facilities:

- Testing citrus plants engineered to be resistant to Asiatic Bacterial Canker by infecting them with the disease pathogen, which, if released in Florida, could devastate the commercial citrus crop;
- Inoculating transgenic peanut plants containing fungal resistance genes with A*spergillus flavus*, the organism responsible for producing the potent vertebrate mycotoxin, aflatoxin.

Biosafety Level 4 for Plants (BL4-P)

BL4-P is recommended for experiments on certain exotic, readily transmissible infectious agents that are potentially serious pathogens of major US crops, such as soybean rust fungus, maize streak, or other viruses, *and* that are performed in the presence of their arthropod vector. For example, an experiment to test the efficacy of the maize streak virus coat protein to protect corn plants against infection by that virus would necessarily use its leafhopper vector, *Cicadulina* spp., in challenge inoculations. This devastating virus is not found in the United States, however leafhopper species capable of transmitting it are present. Thus the experiment using both virus and vector poses a significant risk should either escape the containment facility; in this case, the transgenic maize plant does not itself pose a risk.

Section IV. Elements of Containment

APPENDIX P OF THE NIH GUIDELINES addresses the containment conditions and practices required for recombinant DNA research involving plants. Achieving containment for genetically modified organisms is an exercise in risk management.

The Guidelines state that the principle purpose of GMO containment is to:

1. Avoid unintentional transmission of rDNA-containing plant genomes or release of rDNA-derived organisms associated with plants;
2. Minimize the possibility of unanticipated deleterious effects on organisms and ecosystems outside of the experimental facility;
3. Avoid the inadvertent spread of a serious pathogen from a greenhouse to a local agricultural crop; and
4. Avoid the unintentional introduction and establishment of an organism in a new ecosystem.

Environmental protection is the predominant goal; the key to achieving it lies in understanding the biological systems involved and accepted scientific research practices. Containment is accomplished through a combination of management practices, physical barriers, and biological methods intended to prevent GMO transfer or survival. In general, containment requirements are more stringent if plant-*associated* materials, such as insects and microorganisms, are included in the experiment. If insect quarantine measures are required, regardless of the presence of rDNA material, managers should contact APHIS for guidance.

Research involving transgenic plants at the BL1-P or BL2-P containment levels requires little more than the basic facilities, equipment, and protocols common to most research greenhouses. However, greenhouses that offer high-level BL3-P and BL4-P containment are expensive to build and operate. The cost of greenhouse containment at these levels may be prohibitive for many institutions. Other means of attaining a high level of containment, such as use of a growth chamber or growth room, may provide a suitable alternative at a fraction of the cost. The book, *Containment Facilities and Safeguards For Exotic Plant Pathogens and Pests*[11], offers descriptions of high security containment and quarantine facilities operating around the world.

11 Kahn, R. P. and S. B. Mathur. 1999. *Containment Facilities and Safeguards: For Exotic Plant Pathogens and Pests.* St. Paul, MN.: APS Press.

Growth chambers, tissue culture rooms, incubators, and biological safety cabinets are commonly used in developing GMOs. Biosafety regulations for these facilities are included in Appendix G of the NIH Guidelines,

TABLE 3. Comparison of standard practices for containment of plants in greenhouses

BIOSAFETY LEVEL 1-P	BIOSAFETY LEVEL 2-P
discretionary access	access limited to individuals directly involved with experiments
personnel must read and follow instructions	personnel must read and follow instructions
procedures followed are appropriate for organisms	greenhouse manual to advise of consequences; give contingency plans
record kept of experiments in facility	record kept of experiments and movement in/out of greenhouse
	containment required for movement in/out of greenhouse
biologically inactivate experimental organisms at end of experiment	biologically inactivate experimental organisms at end of experiment; decontaminate gravel periodically
pest control program	pest control program
appropriate caging and precautions for escape of motile organisms	appropriate caging and precautions for escape of motile organisms
	sign for restricted experiment in progress with plant names, person responsible, special requirements

which specifies physical containment standards for the laboratory. Standard practices for plants in greenhouses are summarized in Table 3.

BIOSAFETY LEVEL 3-P	BIOSAFETY LEVEL 4-P
access restricted to required persons only	access restricted; secure locked doors; record kept of all entry/exit; clothing change/shower room through air-lock is only means of entry/exit
personnel must read and follow instructions	all who enter advised of hazards and safeguards
greenhouse manual to advise of consequences; give contingency plans	greenhouse manual prepared and adopted; personnel required to follow contingency plans
record kept of experiments and movement in/out of facility	record kept of experimental material moving in/out of greenhouse
containment required for movement in/out; external decontamination	special packaging containment for in/out; airlock or decontamination for removal
	entry of supplies/materials through special chamber
biologically inactivate experimental organisms at end of experiment (including water runoff); decontaminate equipment & supplies	decontaminate experimental materials prior to removal from area by autoclave/other means all runoff water collected and decontaminated
pest control program	chemical control program for pests and pathogens
appropriate caging and precautions for escape of motile organisms	appropriate caging and precautions for escape of motile organisms
sign for restricted experiment in progress; person responsible, special requirements; biohazard symbol if a risk to humans	sign for restricted experiment in progress; special requirements, person responsible; biohazard symbol if a risk to humans
minimize aerosol creation to reduce contamination	standard microbiological procedures to decontaminate equipment and containers
protective clothing worn to minimize dissemination; hands washed before leaving facility	street clothing removed; complete change to lab clothing which is autoclaved before laundering
	report/record accidents

FIG 1. Caulking around service intrusions

FIG 2. Sill caulking

PHYSICAL CONTAINMENT

Physical containment is achieved through facility design and equipment. Choices in the type of glazing, sealing, screening, air flow system, and other features all affect the degree to which a greenhouse is capable of isolating transgenic plants, plant parts, and associated organisms from the surrounding environment. These systems are also effective in keeping unwanted pests out of the greenhouse.

Glazing

The term glazing refers to any transparent material (as glass) used for windows. Properly installed and regularly maintained greenhouse glazing of any typical material can provide a suitable barrier for transgenic research materials. The type of glazing most commonly used consists of single panes of tempered glass installed by lapping each pane over the one below. The care taken in installing and maintaining the glazing determines its overall effectiveness. Improperly installed or loose-fitting glazing material can leave gaps through which transgenic materials could be released unintentionally.

Caulking and Sealing

Caulking materials are commonly used to seal glass panes, sills, and small openings in and around greenhouse structures. Caulking and sealing restricts the passage of insects and assists with temperature control within the greenhouse; however, it should not be considered a substitute for well-fitting structural components. Additional caulking and sealing can help to upgrade a conventional facility to meet the standards of an approved containment facility. Typical situations where the addition of caulk provides an extra measure of containment are illustrated in Figures 1 and 2.

Screening

When properly sized, installed, and maintained, screen can keep pests and pollinators out of a greenhouse or, conversely, keep experimental organisms in. The integrity of a screening system is determined by several factors including the nature of the material, the size and morphology of the insects being excluded, the hole shape and size, and the air pressure applied on either side of the screen. The maximum hole size generally capable of restricting certain insect species is shown in Table 4. Anti-Virus™ screening[12] is a commercial product advertised to be 100% effective in excluding leafminers, melon aphids, and whiteflies.

TABLE 4. Mesh sizes* for insect containment[13]

ADULT INSECT	SCREEN HOLE SIZE		
	mesh	microns²	inches²
Leafminers	40	640	0.025
Silverleaf Whiteflies	52	460	0.018
Melon Aphids	78	340	0.013
Flower Thrips	132	190	0.0075

*The number of threads per linear inch defines the mesh size of the screen; e.g., a 30-mesh screen has 30 threads per inch.

12 Gintec Shade Technologies Inc.: http://www.gintec-shade.com/greenhouse-screens.html

13 Adapted from "Greenhouse Screening for Insect Control." Rutgers Cooperative Extension. http://www.wvu.edu/~agexten/hortcult/greenhou/fs640.htm

FIG 3. Negative pressure bench-top containment unit

Negative Air Pressure

Containment of airborne pollen, spores, and insects is a significant challenge. One strategy to help achieve it is to create negative air pressure within a facility. Negative pressure exists when the amount of air exiting a space exceeds the air intake. Negative pressure bench-top chambers can increase containment of pathogens and insects within greenhouses, screenhouses, and laboratories. A chambered wood and clear plastic box fitted with a blower and filtration system can produce negative pressure on a small scale and at a relatively low cost (Fig. 3).

Cages

Insect cages, when properly used, can increase the containment level of a particular experiment as long as the factors listed above pertaining to screen characteristics and sizing are respected. Though researchers may fashion their own cages out of metal, wood, glass, or screen, commercial models are also available. The Bugdorm™ insect cage (Fig. 4) is a type of cage available from biological and greenhouse supply companies.

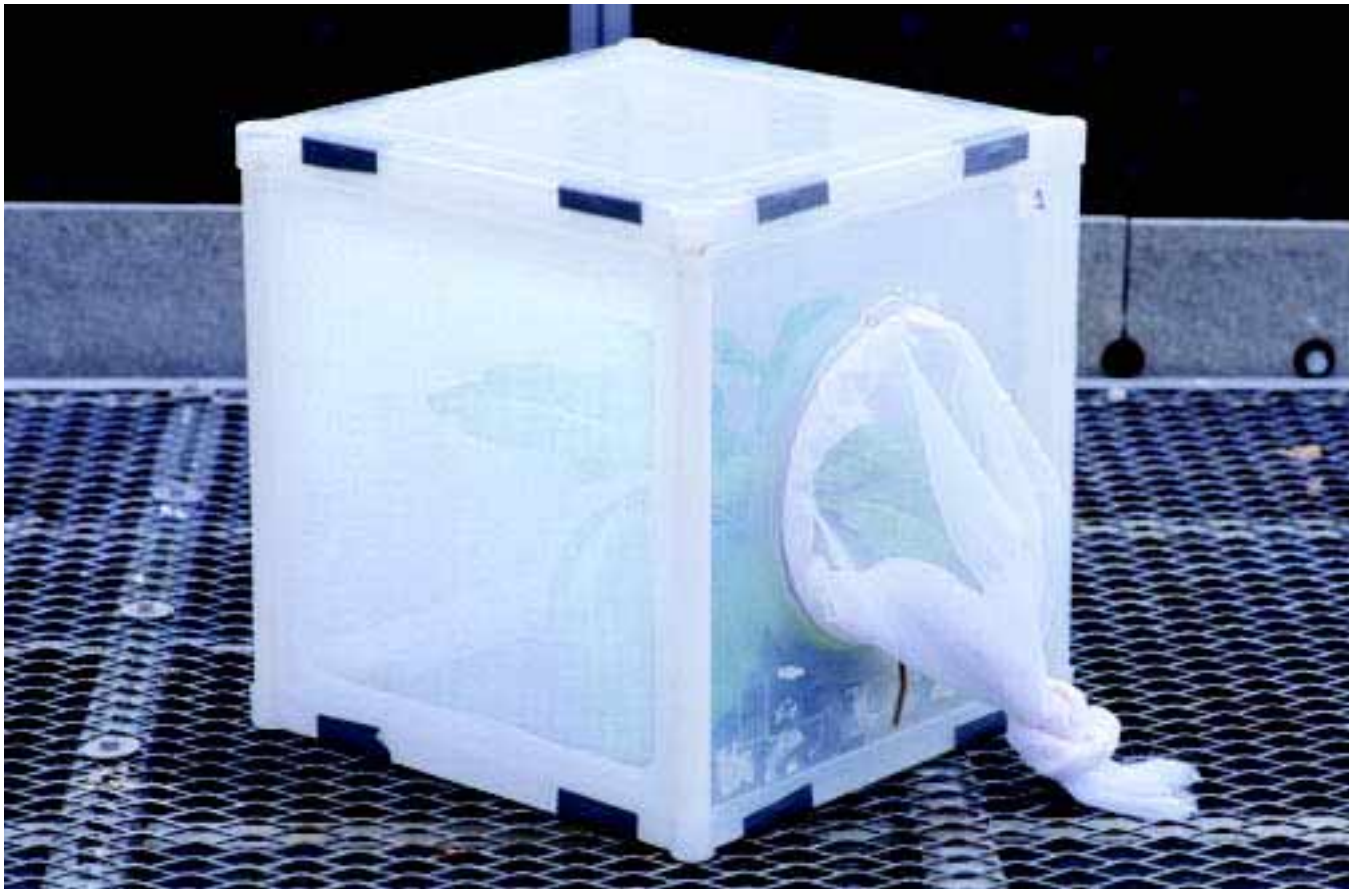


FIG 4. **Bugdorm™ insect cage**

Location

The geographical location of a greenhouse provides an element of physical containment. Research involving a crop pest or noxious weed, for instance, presents a greater risk if the facility is located in an area adjacent to large cropping areas susceptible to the pest. When planning new facilities, it is important to determine what type of agricultural activities will be occurring in adjacent areas before siting. Most work with GMOs, however, does not require remote or otherwise special siting since other safeguards are usually adequate.

BIOLOGICAL CONTAINMENT

Biological processes can provide a highly effective means of preventing unintended transmission of genetic material. Biological containment methods include reproductive, spatial, and temporal isolation. Appendix P of the NIH Guidelines provides a partial list of the biological containment practices appropriate for plants, microbes, and insects. Scientists and technicians conducting transgenic research generally best understand the biological systems involved. They are at liberty to devise other means of biological containment in their experimental protocols, subject to review by the IBC.

Fig 5.1—5.2 Bagging flowers for biological containment

Plants

One or more of the following procedures can prevent dissemination of genetic material by pollen or seed:

- Cover or remove flower and seed heads to prevent pollen and seed dispersal;
- Harvest plant material prior to sexual maturity or use male sterile lines;
- Control the time of flowering so that pollen shed does not coincide with the receptive period of sexually compatible plants nearby;
- Ensure that cross-fertile plants are not within the pollen dispersal range of the experimental plant; or
- Use genetic modification techniques that localize transgenes in non-propagative plant parts.

Bagging flowers is a standard practice used by breeders to prevent the contamination of selected plants with pollen from adjacent plants. Female flowers can be covered to prevent insect pollinators or windblown pollen from landing on the receptive surface. Male flowering structures can be bagged to prevent pollen from being disseminated by insect vectors, wind, or mechanical transfer (Fig. 5.1-5.2).

TABLE 5. Isolation distances (in feet) from contaminating sources for selected groups

CROP	FOUNDATION	REGISTERED	CERTIFIED
Alfalfa	600[1,2]	300[1,2,3]	165[1,4]
Corn (inbred lines)	660[5,6]	—	—
Corn (hybrid)			660[6,7]
Cotton (hybrid)	0[8]	0[8]	0[8]
Grasses (cross pollinated)	900[9, 10, 11]	300[9,10,11]	165[9,10,11,12]
Mung Beans	0[13]	0[13]	0[13]
Onion	5280	2640	1320
Peanuts	0[13]	0[13]	0[13]
Pepper	200[14]	100[14]	30[14]
Rape (self pollinated)	660[15]	—	330[15]
Rape (cross pollinated)	1320[15]	—	330[15]
Rice	10[16]	10[16]	10[16]
Soybeans	0[13]	0[13]	0[13]
Sunflower	2640[17,18]	2640[17,18]	2640[17,18]
Sunflower (hybrid)	2640[17,18]	—	2640[17,18]
Tomato	200[14]	100[14]	30[14]
Watermelon	2640[19]	2640[19]	1320[19]

Source: Modified from "Genetic and Crop Standards" of the AOSCA: http://aosca.org/g&ccont.htm

Paper and glassine bags are most commonly used to cover flower heads. Flower heads can be removed prior to pollen or seed production in cases where the research protocol does not require seed collection.

Genetic and Crop Standards of the AOSCA[14], published annually by the Official Seed Certifying Agencies, describes the isolation distances required to avoid genetic contamination by pollen dispersal. Table 5 shows isolation distances for selected crops. In order to be considered an environmental risk, transgenic pollen must be able to fertilize plants of a sexually compatible species growing in the vicinity.

Crop breeders have identified numerous crops with sexually compatible wild or weedy relatives. Examples of crops that outcross with wild relatives are given in Table 6.

Depending on the location of the containment facility, the choice of season in which to conduct an experiment may constitute an appropriate biological containment method for plants. For instance, growing transgenic sunflowers only during the winter in northern climates insures that any escaped pollen would be of no consequence to local plants or weeds.

14 Association of Official Seed Certifying Agencies: http://aosca.org/g&ccont.htm

1. Distance between fields of Certified classes of the same variety may be reduced to 10 feet regardless of class or size of field.
2. This distance applies for fields over five acres. For alfalfa fields of five acres or less that produce the Foundation and Registered seed classes, the minimum distance from a different variety or a field of the same variety that does not meet the varietal purity requirements for certification shall be 900 and 450 feet, respectively.
3. Isolation distance for Certified seed production of varieties adapted to the northern and central regions shall be 500 feet from varieties adapted to the southern region.
4. There must be at least 10 feet or a distance adequate to prevent mechanical mixture between a field of another variety (or non-certified area within the same field) and the area being certified. The 165 feet isolation requirement is waived if the area of the "isolation zone" is less than 10 percent of the field eligible for the Certified class. The "isolation zone" is that area calculated by multiplying the length of the common border(s) with other varieties of alfalfa by the average width of the field (being certified) falling within the 165 feet isolation. Areas within the isolation zone nearest the contamination source shall not be certified.
5. No isolation is required for the production of hand-pollinated seed.
6. When the contaminant is of the same color and texture, the isolation distance may be modified by (1) adequate natural barriers, or (2) differential maturity dates provided there are no receptive silks in the seed parent at the time the contaminant is shedding pollen. In addition, dent sterile popcorn requires no isolation from dent corn.
7. Where the contaminating source is corn of the same color and texture as that of the field inspected or white endosperm corn that is optically sorted, the isolation distance is 410 feet and may be modified by the planting of pollen parent border row.
8. Minimum isolation shall be 100 feet if the cotton plants in the contaminating source differ by easily observed morphological characteristics for the field to be inspected. Isolation distance between upland and Egyptian types is 1320, 1320, and 660 for Foundation, Registered, and Certified, respectively.
9. Isolation between classes of the same variety may be reduced to 25% of the distance otherwise required.
10. Isolation between diploids and tetraploids shall at least be 15 feet.
11. Border removal applies only to fields of five acres or more. These distances apply when there is no border removal. Removal of a 9-foot border (after flowering) decreases the required distance to 600, 225, and 100 feet for cross-pollinated species, and to 30, 15, and 15 feet for apomictic and self-pollinated species. Removal of a 15-foot border allows a further decrease to 450, 150 and 75 feet for cross-pollinated species.
12. Application to establish pedigree must be made within one year of seeding. The crop will remain under supervision of the certifying agency as long as the field is eligible for certification.
13. Distance adequate to prevent mechanical mixture is necessary.
14. The minimum distance may be reduced by 50 percent if different classes of the same variety are involved.
15. Required isolation between classes of the same variety is 10 feet.
16. Isolation between varieties or non-certified field of the same variety shall be 10 feet if ground drilled, 50 feet if ground broadcast, and 100 feet if aerial seeded.
17. Does not apply to *Helianthus similes*, *H. ludens* or *H. agrestis*.
18. An isolation distance of 5,280 feet is required between oil and non-oil sunflower types and between either type and other volunteer or wild types.
19. The minimum distance may be reduced by 50 percent if the field is adequately protected by natural or artificial barriers.

TABLE 6. Commercially important species that hybridize with wild relatives in the USA[15]

CULTIVATED SPECIES[16]	WILD RELATIVE
Apium graveolens (celery)	Same species
Daucus carota (carrot)	Same species (wild carrot)
Chenopodium quinoa (quingua [a grain])	*C. berlandieri*
Beta vulgaris (beet)	*B. vulgaris var. maritima* (hybrid is a weed)
Chicorium intybus (chicory)	Same species
Helianthus annuus (sunflower)	Same species
Lactuca sativa (lettuce)	*L. serriola* (wild lettuce)
Brassica napus (oilseed rape; canola)	Same species, *B. campestris*, *B. juncea*
Brassica rapa (turnip)	Same species (= *B. campestris*)
Raphanus sativus (radish)	Same species, *R. raphanistrum*
Cucurbita pepo (squash)	Same species (= *C. texana*, wild squash)
Vaccinium macrocarpon (cranberry)	Same species
Vaccinium angustifolium (blueberry)	Same species
Trifolium spp. (clover)	Same species
Medicago sativa (alfalfa)	Same species
Liquidambar styraciflua (sweetgum)	Same species
Juglans regia (walnut)	*J. hindsii*
Asparagus officinalis (asparagus)	Same species
Picea glauca (spruce)	Same species
Avena sativa (oat)	*A. fatua* (wild oats)
Cynodon dactylon (bermuda grass)	Same species
Oryza sativa (rice)	Same species (red rice)
Sorghum bicolor (sorghum)	*S. halapense* (johnsongrass)
Amelanchier laevis (serviceberry)	Same species
Fragaria sp.(strawberry)	*F. virginiana, F. chiloensis,* others
Rubus spp. (raspberry, blackberry)	Same species
Populus alba x P. grandidentata (poplar)	*Populus* species. (ten species listed as weed of unknown status in U.S.)
Nicotiana tabacum (tobacco)	Same species (escapes cultivation)
Vitus vinifera (grape)	*Vitus* spp. (wild grape)

15 Adapted by permission of the authors (Allison Snow and Pedro Moran Palma, Department of Plant Biology, Ohio State University) from their publication titled "Commercialization of Transgenic Plants: Potential Ecological Risks." Bioscience (1997), Vol. 47, pp. 86-96.

16 This is not an exhaustive list, especially with regard to commercially important grasses and woody species, which often occur in unmanaged populations. Also, for many cultivars the extent of hybridization with wild relatives has not been studied.

Microorganisms

Effective physical containment of bacteria, viruses, and other microbes can be extremely difficult because they cannot be seen and, once dispersed, cannot be recovered. However, many will not survive and persist if they are dispersed. Biological measures often provide better containment options. The following methods may help prevent dissemination of genetically modified microorganisms:

- Avoid creating aerosols when inoculating plants with transgenic microbes;
- Provide adequate distance between an infected plant and another susceptible host, especially if the microorganism can be disseminated through the air or by leaf contact;
- Grow experimental plants and microbes at a time of year when nearby susceptible plants are not growing;
- Eliminate vectors for insect-borne microorganisms;
- Choose microorganisms having an obligate association with the host plant;
- Genetically disable the microorganism to minimize survival and reproduction; and
- Treat or evaporate runoff water.

Insects

Insect and mite containment is difficult in a greenhouse facility. Entomologists who raise insects on greenhouse plants work constantly to prevent their escape and to control disease and parasites. The following procedures can be used to prevent dissemination of arthropods and other small animals:

- Choose or create non-flying, flight-impaired, or sterile strains;
- Conduct experiments at a time of year when survival of escaping organisms is impossible;
- Choose organisms that have an obligate association with a plant not found in the vicinity;
- Treat or evaporate runoff water to eliminate viable eggs and larvae;
- Avoid use of small-sized insects in experimental greenhouse cages; and
- Destroy pollinating insects in experimental cages after pollen transfer to eliminate potential for dissemination of transgenic pollen into the environment.

COMBINING PHYSICAL AND BIOLOGICAL CONTAINMENT

Using biological and physical containment measures in concert offers two advantages when planning how to achieve a specified level of containment. First, combining methods reduces the physical requirements to those of the next lower biosafety level. A greenhouse experiment using transgenic sunflowers, for example, located where wild sunflowers are endemic and found within the isolation distance used by breeders, requires BL2-P containment so that outcrossing does not occur. Alternatively, physically removing all wild sunflowers within the isolation distance allows BL1-P physical standards to be used. If the experiment does not include seed collection for subsequent trials, adding biological containment by removing the flower heads before pollen shed similarly allows use of the less stringent BL1-P physical standards.

Second, the ability to do BL2-P transgenic research in an existing BL1-P facility may be achieved simply by incorporating biological containment practices. Consider an experiment designed to evaluate tomato plants genetically engineered for resistance to tomato spotted wilt virus (TSWV). The protocol involves three organisms: tomatoes, the virus, and thrips, the insect vector that transmits TSWV. Physical containment would be provided by a greenhouse fitted with AntiVirus™ screening or by conducting the experiment in insect-proof cages within the greenhouse. Biological containment could be added by removing alternate host plants for the virus both in and outside of the greenhouse and by applying stringent insect control measures in the surrounding area.

Section V. Management Practices

CONTAINMENT STRATEGIES ARE EFFECTIVE ONLY when greenhouse personnel understand and adhere to established procedures for handling transgenic material. Before entering the greenhouse, all staff working around transgenic organisms should be fully informed about the containment measures applicable to a given research project. Prescribed procedures and practices should be appropriate for the assigned biosafety level; those that appear excessive for the needed level of containment may discourage compliance.

Access

Access to greenhouses housing transgenic research materials is restricted, regardless of the biosafety level. Such restrictions are intended to minimize the spread of transgenic pollen, seed, or other propagative material that could be carried by people moving between rooms or facilities. At BL1-P, access is limited or restricted at the discretion of the greenhouse manager or PI when experiments are in progress. At BL2-P, the manager is required to limit greenhouse access to individuals directly involved with the experiments, and at BL3-P, the manager, in consultation with the PI, should determine access authorization on an individual basis. Discretionary access is generally reserved for maintenance personnel and accompanied visitors who have a special interest in the research.

If the greenhouse consists of one large room as opposed to individual compartments, access to the entire facility may need to be restricted; all authorized personnel should have access to a key to enter. Signs must be posted at the entries to the greenhouse indicating that access is restricted for the experiment in progress. These signs may also contain access instructions. An entry and exit logbook is required for BL4-P greenhouses only. However, when exotic infectious agents are present in the research facility, APHIS recommends keeping a record of the personnel who regularly work there, visitor, and service personnel visits. The log should include the names, dates, and times of everyone entering and exiting the facility.

Apparel and Hygiene

Personnel entering BL1-P and BL2-P facilities may wear their usual street or lab clothing. For entry into BL3-P greenhouses, disposable lab gowns or the equivalent may be required at the discretion of the greenhouse manager. If special clothing is required, it must be removed before leaving the facility and decontaminated (usually by autoclaving) before washing or disposal. Users are also required to wash their hands before leaving BL3-P restricted areas.

BL4-P facilities maintain strict apparel and hygiene protocols. All users are required to enter only through the dressing/shower rooms and must shower when leaving the facility. Users are also required to remove all street clothing and don protective clothing before entering. Likewise, personnel leaving the facility must remove protective clothing before showering and exiting. The clothing must be stored in the inner change room and autoclaved before laundering. Showering before entering is required only when there is concern that organisms will be brought into the containment area from the outside.

FIG 6. GMOs marked with colored stakes

Signage

No special signs are required for BL1-P containment greenhouses. Entryways into BL2-P and higher facilities should be posted with signs indicating that access is limited to authorized personnel only. If the experiment uses organisms that pose a risk to the local ecosystem or

agriculture, a sign so stating must be placed on the access doors to the greenhouse. A description of the potential risk may be posted on the restricted access sign as long as this is not confidential information. The sign should state the name and telephone number of the responsible individual, the plants in use, and any special requirements for using the area. It may include contact information for the greenhouse manager and others to be called in case of emergency.

Transgenic material in a greenhouse room must be marked to distinguish it from non-transgenic organisms such as plants serving as experimental controls or those not involved with the experiment. Individual pots, bench sections, or entire benches can be marked with stakes or signs that identify the plant and the primary genetic modification, for example, "Soybeans with viral coat protein gene" (Fig. 6). All organisms in the room must be treated in accordance with the highest level of containment standards required by any experimental material present.

Seed Storage

Transgenic seed should be stored in a locked cabinet located preferably in the greenhouse room so as to minimize handling in unconfined spaces. When stored or handled outside the area of confinement, such as in a cabinet or on a potting bench in a headhouse corridor, the seed should be in a spill-proof container. The transgenic seed should be clearly identified and labeled to distinguish it from other stored seeds or materials in the cabinet. Greenhouse personnel should take ordinary precautions to prevent seed germination in unwanted locations.

Transfer of Materials

The NIH Guidelines specify requirements for transporting experimental materials to and from a greenhouse for levels BL2-4P. For BL2-P and higher facilities, transgenic material in the form of seeds or propagules, potted plants, trays of seedlings, etc. are to be transferred in a closed non-breakable container. For BL3-P and BL4-P containment, the guidelines require an additional sealed secondary container for movement of experimental materials. The exterior surface of the secondary chamber should be decontaminated either chemically or in a fumigation chamber if the same plant, host, or vector is present within the effective dissemination distance of the propagules of the experimental organism.

Termination and Disposal

To prevent the unintended survival of GMOs outside the greenhouse environment, all experimental materials must be rendered biologically inactive (devitalized) before disposal. Termination procedures for the safe disposal of soil and plant material should be part of the experimental plan for a research project. The IBC may institute a policy that outlines acceptable disposal procedures for GM research materials, taking into consideration the biosafety level of the experiment and the volume of material to be handled. Devitalization of plant material and soil should be completed before it leaves a greenhouse or laboratory and goes to a landfill.

Plants and associated organisms can be inactivated though steam or chemical sterilization procedures. Steam forced into special carts or boxes has traditionally been used in greenhouses for treating growing beds, pasteurizing or sterilizing media, and disinfecting containers, thus it is likely to be available. Materials from smaller experiments can be inactivated by autoclaving all plants, plant parts, containers, and potting media. For larger volumes, composting is an acceptable treatment for experimental plant and soil materials that pose no recognized harm to the environment. Plants can be devitalized through desiccation simply by withholding water or they can be chopped or minced to pieces unable to grow independently under natural conditions. Incineration may also be used to destroy easily combustible, dry plant material; however, incineration must be used with caution since not all seeds are easily burned, e.g., cottonseed.

At higher containment levels, it is recommended that all materials leaving the greenhouse be sterilized in an autoclave.

Disposing of very small transgenic seeds requires special care. Fine mesh bags can be secured around flower heads prior to disposal; a sheet of dampened white paper such as BenchKote™ placed on the work surface facilitates recovery of easily scattered seeds. The gravel under benches in BL2-P facilities should be decontaminated by, for example, treatment with a sodium hypochlorite (household bleach) solution. Catching liquid in a large open pan and allowing it to evaporate is a simple alternative.

Abandoned or forgotten experimental materials are not an infrequent problem for greenhouse managers. An IBC policy stipulating that GMO material is the responsibility of the PI would clarify authority in disposing of neglected or abandoned materials. This policy would preclude a source of gene escape that may occur when a PI leaves transgenic material in the greenhouse due to death, resignation, or simple oversight.

Pest Control

The NIH Guidelines call for a pest control program for all biosafety levels when working with transgenic organisms in a greenhouse setting. Rodents and birds can transport transgenic seed outside the facility; insects and other organisms can transfer pollen to receptive plants located within or outside the containment area. A stringent pest control program, using physical, chemical, or biological control measures, alone or in combination, should be implemented and monitored for effectiveness. Screens are recommended for BL1-P and required for BL2-P to exclude pollinating insects and birds; BL2-P facilities must have louvers fitted on exhaust fans that are open only when fans are running. The perimeters of greenhouses of every containment level should be sealed to prevent rodents and other large pests from entering. Fumigation can be used to control certain insect pests such as whiteflies. Biological control measures may involve the introduction of predators, parasites, and parasitoids to control pests.

Greenhouse research commonly uses insect pests as part of the experimental protocol, such as in testing plants for disease or insect resistance. In these cases, selective control measures are needed to eliminate the unwanted pest without killing the required pest organism. When insect vectors are used to transmit genetically modified viruses, particular care should be taken to eliminate the vector once the transmission has been accomplished.

Training and Reference Manuals

Personnel instruction is an important component of good management practices. A reference manual should be prepared containing directives covering all safety considerations pertaining to the transgenic research being conducted. Staff are required to read, understand, and follow the instructions provided in the manual before entering the greenhouse. Personnel training is best accomplished through interactive sessions that include the PI, greenhouse manager, or other safety-management staff.

For BL2-P and higher facilities, emergency and contingency plans, as well as documents pertaining to routine operations, are required to be included in the reference manual. It is not necessary to include experimental protocols in the manual, however researchers and greenhouse staff may find that a copy of the experimental protocol aids compliance with containment procedures. Conversely, relevant portions of the manual may be included in the project documents submitted for IBC approval.

Monitoring Containment Effectiveness

Escaped organisms may be detected by placing susceptible host plants, insect traps, or spore/pollen-catching devices both inside and outside the containment area. Traps and bioindicator plants can be used to detect unintended virus transmission, insect migration, and pollen or spore spread. For example, if an experiment involves a caged insect-

vectored plant disease system, uninfected plants placed in the same greenhouse but not in the caged area can be monitored for evidence of disease transmission. Light traps placed in corridors and operated at night are useful to indicate the presence of insects that have escaped the greenhouse rooms.

Procedures for Loss of Containment

The integrity of containment measures is susceptible to equipment malfunctions, acts of nature such as fire, flood, and storm damage, and human error. A loss of BL1-P containment due to any of these factors would likely have only minor environmental consequences, if any, and would not require any response. At BL2-P or higher, such events may present larger concerns.

Facilities operated above BL1-P should be equipped with an alarm system designed to alert someone when mechanical or weather-related events causing a loss of containment occur. Greenhouse systems that monitor automated environmental controls should have built-in local and remote alarms. Instances of human error, such as a door left open or ordinary disposal of unlabeled transgenic materials, is actually a more common cause of containment loss than facility malfunctions or structural damage. Designated people who are promptly alerted to problems can make timely decisions in regards to contacting or dispatching appropriate response personnel.

The NIH Guidelines require BL2-P and higher facilities to have contingency plans for handling emergency situations that also apply in cases of theft or vandalism. These plans, drawn up by the BSO and/or IBC in consultation with the PI, must include measures to contain the breach, a personnel notification sequence, and decontamination procedures. In addition, the plans should include names and contact information for repair personnel, researchers, relevant authorities, and greenhouse staff.

Should an inadvertent release of transgenic material at BL2-P or higher occur, the Principal Investigator must immediately report the incident in writing to the Biological Safety Officer (if assigned), the greenhouse manager, the Institutional Biosafety Committee, NIH Office of Biotechnology Activities, and/or other designated authorities. Greenhouse managers should be advised that any plant material governed by APHIS permit that escapes or is stolen must be reported to Dianne Hatmaker, Biotech Permits, APHIS, PPQ[17] (telephone 301-734-5787) within 24 hours of the incident.

Records

The extent of record keeping required for research using transgenic organisms is commensurate with the level of biosafety. Records of experiments in progress must be kept for all biosafety levels. At BL2-P and higher, additional records must be kept of all plants and plant-associated organisms entering or leaving the greenhouse. A record of the dates and times of personnel visits must be kept for BL4-P facilities.

Although the NIH Guidelines do not specify who should keep records, the PI is the logical choice as he/she is responsible for tracking experimental material. It is also appropriate that someone stationed in the facility (e.g., the greenhouse manager or equivalent) has responsibility for entry and exit logs when required.

Inspections

Greenhouses should be inspected periodically to ensure that containment measures appropriate for the transgenic plants and other organisms held inside are being rigorously followed. Inspections should be conducted on a regular schedule and whenever new types of experimental materials are brought into the facility. Inspectors may include the greenhouse manager, BSO, IBC representative, or state agriculture officials. Officials from USDA/APHIS may, upon request, visit a facility to observe containment features. However, USDA does not certify or otherwise designate a greenhouse's suitability for research materials requiring a specific

[17] http://www.aphis.usda.gov/ppq/

biosafety level(s) unless there is present a plant pest requiring a permit from APHIS-PPQ.

Inspection checklists help ensure that a greenhouse complies with all necessary physical, biological, and managerial requirements for a given Biosafety Level. Inspection checklists facilitate IBC approval, provide an outline for internal monitoring, and serve as documentation of compliance. A sample of an APHIS "Facility Inspection Checklist for Containment of Genetically Engineered Organisms" is included as an Appendix. Public and private sector research organizations usually develop their own in-house checklists. Checklists may be customized by combining items from the APHIS checklist, other lists, and the list below. Where several levels of containment are provided by different rooms within a single facility, checklists tailored to each level simplify the inspections.

For each room or research project, an inspection checklist may ask:

- Who is the responsible party? Is their contact information posted on the door?
- What is the nature of the GMO and how is it identified?
- What is the prescribed level of containment? Do the physical facilities meet this level?
- What specific physical and biological measures are being used to achieve that level of containment?
- Are prescribed practices being followed?
- Is there any evidence of deficiencies with regard to containment?
- How is the area secured? What security is required?
- Is there a written plan for responding to loss of containment? What is the most likely containment breach?

If GMOs under APHIS permit are in a greenhouse with the same species of non-GMOs, APHIS recommends that the two groups (or more) be well separated to avoid inadvertent cross pollination. Also, it is recommended that the GMOs have some designated boundary on the bench such as color-coded markers. Additionally, Plant Protection and Quarantine Officers of APHIS may conduct unannounced re-visits to facilities housing GMOs under federal permit. The unannounced inspections occur during normal business hours and are a Standard Permit Condition.

Periodic reinspections of the greenhouse should be conducted. The presence of light, heat, and water within a facility promotes gradual deterioration of equipment and structural features over time. Additionally, an inspection serves as an opportunity to review any special practices that may be required, as staff adherence to non-standard procedures may tend to relax over time.

A Note about Vandalism

Vandalism is an increasing concern for greenhouse managers. Some individuals and organizations opposed to recombinant DNA research have targeted greenhouse and field trial research projects, causing substantial damage. Determined individuals gain entry either by force, by defeating security hardware, or they may be admitted inadvertently by authorized personnel—self-closing doors may be propped open, rooms and entries left unlocked, and strangers not always confronted. Facility users should be advised that they share responsibility for maintaining security.

When the threat of vandalism is politically motivated, a situation termed "domestic terrorism" by the US Federal Bureau of Investigation, an institution may wish to create a response team. This group typically is composed of a high level administrator, a public information officer, the facility manager, legal counsel, and relevant others whose job it is to review physical deterrents and develop public relations strategies. Because political actions generally are designed to garner sympathy for a cause via the news media, it is important that an institution have an opportunity to respond quickly and clearly to threats or acts of vandalism.

Section VI. Retrofitting for Containment

GREENHOUSES

Retrofitting a conventional greenhouse to meet BL1-P and BL2-P containment standards is far cheaper than building a new facility. Requirements for meeting BL3-P standards are more extensive and may involve basic structural changes; therefore, retrofitting may not be feasible or cost-effective. Similarly, if a greenhouse is structurally unsound or suspect, rebuilding may be the best option. BL4-P standards require a dedicated, highly engineered, and isolated facility, which excludes the possibility of retrofitting existing greenhouses. Accordingly, this section primarily concerns modifications that would bring a conventional greenhouse up to the containment standards appropriate for the lower biosafety levels.

Existing greenhouse facilities should be carefully inspected to determine if they are suitable for retrofitting. Structurally sound buildings in good condition are often adequate, or nearly so, in terms of containment. Necessary modifications, if any, are usually simple, straightforward, and involve readily available materials. Before deciding to retrofit an existing greenhouse, the cost should first be compared to that of building a new structure. If retrofitting costs fall within 20% of the price of new construction, renovation generally is not recommended. It is advisable to contact a greenhouse builder, engineer, architect, or experienced consultant before proceeding with any major renovation.

Upgrades needed to meet specified containment standards are shown in Table 7.

TABLE 7. Enhanced features of containment greenhouses

	CONVENTIONAL	BIOSAFETY LEVEL 1-P	BIOSAFETY LEVEL 2-P
STRUCTURE	Framing may be aluminum, steel, wood, or pipe		
ENTRY	Hinged or sliding entry doors		Locks on entry doors
GLAZING	Standard greenhouse glass or plastic material		
SCREENING	If used, standard 30 mesh fly screen	Recommended	30-mesh or higher required
VENTILATION	Roof or side vents, fans, cooling pads, fog system, or a combination of these		
BENCHING	Any material; solid or porous bottoms		
FLOORS	Gravel (most common), soil, or concrete throughout	Impervious walkways recommended	Impervious material; collection of runoff water may be required
DRAINS	Discharge into groundwater or sanitary/storm sewer		
OTHER	Automatic control and utility systems meet basic operating requirements		Autoclave available

BIOSAFETY LEVEL 3-P	BIOSAFETY LEVEL 4-P
Rigid, wind resistant frame preferred; internal walls, ceilings, and floors resistant to liquids and chemicals	Reinforced, rigid frame required; walls, floors, and ceilings form sealed internal shell, resistant to liquids and chemicals; see Appendix P for others
Double set of self-closing, locking doors	Double set of self-closing, locking doors with air-lock; shower and changing rooms
Laminated, strengthened, sealed	Double-paned, laminated, strengthened, sealed
Not permitted	Not permitted
Separate negative pressure system; air supply fans with back-flow damper; exhaust air HEPA filtered	Air-conditioned and HEPA filtered, closely monitored negative pressure, no roof or side vent allowed
Seamless water and chemical resistant bench tops	Seamless water and chemical resistant bench tops
Impervious material; for microbes, runoff water collection and decontamination	Sealed floors as part of internal shell; runoff collection and decontamination
Provision for collection and decontamination of runoff	Runoff collection required, sewer vents filtered
Autoclave within facility; hand washing with hands free on/off; filtered vacuum lines; disinfectant traps for liquid lines	Double-door autoclave; self-contained vacuum system; in-line filters and back-flow protection for all liquid/gas services

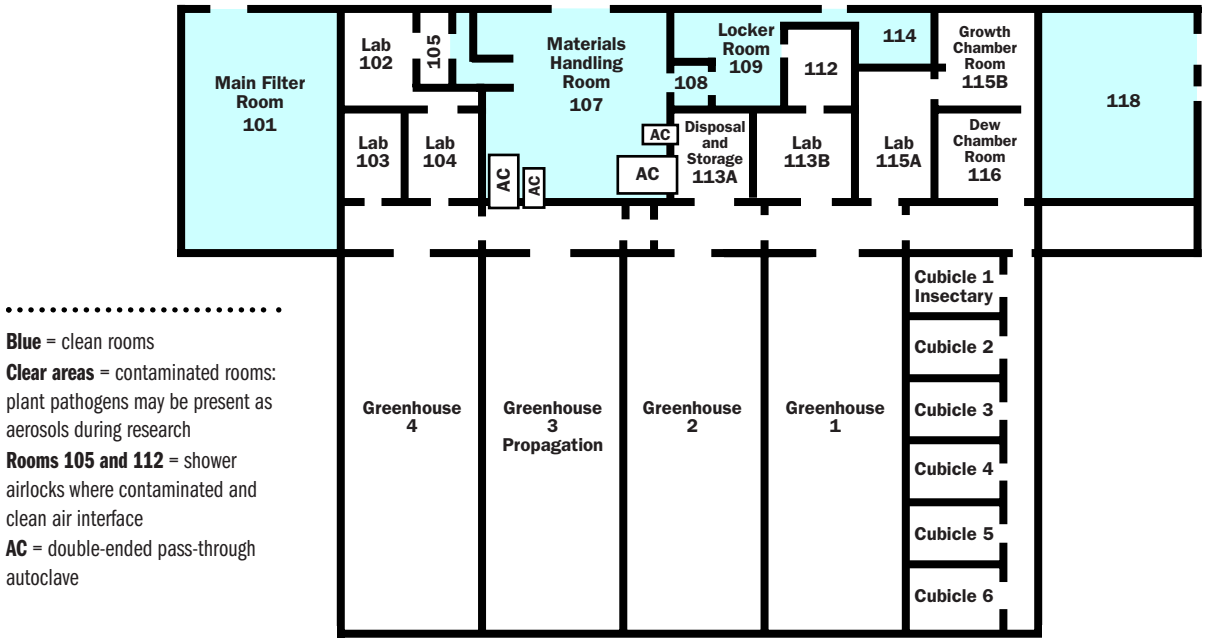
Layout

A greenhouse can be an inhospitable environment for people and equipment because of the humidity, temperature, light, chemicals, and soil. The headhouse, an enclosed area within or adjacent to the greenhouse facility, provides cleaner, more comfortable space for offices, labs, equipment, supplies, and control systems.

When upgrading a conventional greenhouse to accommodate transgenic materials, traffic patterns, process flow, and security measures should be analyzed to determine if the layout should be modified. The configuration should be optimized to provide variable levels of containment and growing conditions, control of access, and ease of movement. The NIH Guidelines stipulate that all plant material within a greenhouse room must be maintained at the highest level of containment required by any organism in the room. Thus in a large room housing BL1-P and BL2-P experiments, all plants must conform to BL2-P containment standards. A compartmentalized arrangement of small rooms allows the facility to provide a variety of containment levels as well as individualized environmental conditions.

In many standard greenhouses, interior space is divided into relatively large rooms with a common central corridor running through them. This arrangement forces personnel to pass through each room to get to the succeeding one, making it difficult, if not impossible, to restrict access to an individual room. A more efficient and manageable layout has an array of small rooms and cubicles opening off one or more common walkways (Fig. 7).

FIG 7. Floor plan of the USDA Foreign Disease and Weed Science Research Unit BLP-3 Containment Facility in Frederick, Maryland[18]



Note that major changes to the layout can necessitate further structural modifications, such as the addition of partitions and/or hallways within a previously undivided greenhouse. These changes may in turn call for revamping environmental control schemes, utilities, ventilators, and primary structural components as well.

18 Kahn, R.P. and S.B. Mathur. 1999. *Containment Facilities and Safeguards for Exotic Plant Pathogens and Pests*. The American Phytopathological Society, St. Paul, MN. Reprinted with permission.

FIG 8. Neoprene door sweep

Entry doors and locks

Standard lockable hinged doors can be used for exterior and corridor entrances. Sliding doors are acceptable at BL1-P and BL2-P but do not seal tightly enough for higher containment levels. Both styles of doors can be fitted with locks to limit access. For security reasons, the distribution of greenhouse keys should be carefully controlled and monitored. Greenhouse rooms dedicated to transgenic research could be re-keyed to assure access is limited to authorized personnel only. It is also advisable to restrict the total number of keys issued to a practical minimum and to strictly limit the number of master or sub-master keys made.

Doors should fit tightly against the jamb and have a sweep at the threshold. The most commonly used standard door sweep consists of a neoprene or rubber strip or a short plastic brush attached to an aluminum holder that can be fastened to any relatively flat surface (Fig. 8). Although sweeps cannot restrict all small insects that are intent on entering or exiting a space, they can easily exclude rodents, birds, and larger flying insects.

The NIH Guidelines stipulate a double set of self-closing and locking doors for BL3-P and BL4-P containment. Building codes prescribe the presence and placement of emergency exits regardless of contain–ment needs. Therefore local officials must be consulted before amending or creating entrances and exits.

Glazing

The condition of the glazing and bedding putty should be carefully evaluated before conducting transgenic research. Properly installed glazing provides low infiltration and generally affords a high degree of containment. Bedding putty for standard lapped glass greenhouses, however, wears out long before the glass, a condition that may precipitate glass breakage and cracking. If a glass greenhouse needs new bedding putty, which is a very labor-intensive job, it may be economically advantageous to consider reglazing at the same time with sheet materials, new styles of glass, or inflated films. Simultaneously replacing bedding putty and glazing would provide tighter containment, better environmental control, and energy cost savings.

Standard greenhouse glazing material will satisfy the requirements for BL1-P and BL2-P. Glass glazing is the most enduring material and provides the greatest amount of natural light. Laminated and heat-strengthened glass is preferred or, depending on building codes, may be required. Standard tempered glass is more prone to spontaneous breakage and shattering which can both breach containment and create a hazard. Glass can be manufactured in lengths that extend from the eaves to the ridge, though lengths over eight feet become impractical. Sheets of rigid plastics such as Lexan ™ polycarbonate or Exolite ™ acrylic also are commonly used for glazing. Polycarbonate costs less and is more fire resistant than acrylic; acrylic glazing, however, lasts longer and permits better light transmission. Double-walled sheets of rigid plastic glazing shift significantly within their framing with temperature fluctuations; therefore, inspections should be made seasonally for openings in these materials.

Various types of film plastic glazing are commonly available, e.g., p1olyester, polyethylene, polyvinyl chloride, and so on. Double-layer plastics rely on a fan to inflate the space between the sheets, and require regular inspections to detect loose hold-down clamps and tears. Film plastics also have a relatively short life (less than four years on average), become brittle with age, and are easily penetrated, accidentally or intentionally. Therefore they are not the preferred choice for a containment greenhouse.

Reglazing with the new generation of film plastics may be a viable option; project managers are advised to consult contractors and institutional officials. Films such as Hostaflon™ can be installed in three layers and still transmit light as efficiently as glass. Longevity with some of the new films has increased from four to 20 years, and they can resist hail damage better than most rigid materials.

Standards for BL3-P and BL4-P require windows to be closed, sealed, and resistant to breakage. This requirement can be met by using double-paned sealed glass or rigid, double-walled plastic panels. Examples are Sedo™, a brand of double-paned glass that contains an inert gas between the panes, and two readily available sheet materials, Lexan™ and Exolite™, as noted above. Reglazing with double-paned sealed glass is likely to require extensive structural renovations to bear the additional weight.

The National Greenhouse Manufacturers Association published glazing standards that allow manufacturers to run standard tests on their products[19]. Test results allow consumers to make comparisons between various glazing products on the market.

Screening

Screening is an especially important consideration when retrofitting an existing structure to attain a higher containment level. Screening should be carefully installed on all ventilation intake vents. Figure 9 demonstrates a method of screening around moving vent arms. For containment purposes, screening side vents is recommended for BL1-P and required for BL2-P. If evaporative cooling pads made of aspen fiber or corrugated cellulose are used on the intake side vents, screening is still useful since insects can find their way through these materials.

Screen mesh size should be gauged relative to the size and shape of the organisms to be contained or excluded. A comparison of commercial screening materials[20] indicates that in some instances screens with a larger hole size may have exclusion

19 *Book of Standards*. National Greenhouse Manufacturers Association (NGMA).

20 Bell, M.L., and J.R. Baker. 1997. Choose a greenhouse screen based on its pest exclusion efficiency. *North Carolina Flower Growers' Bulletin* 42(2):7-13.

FIG 9. Screen panels over ridge*

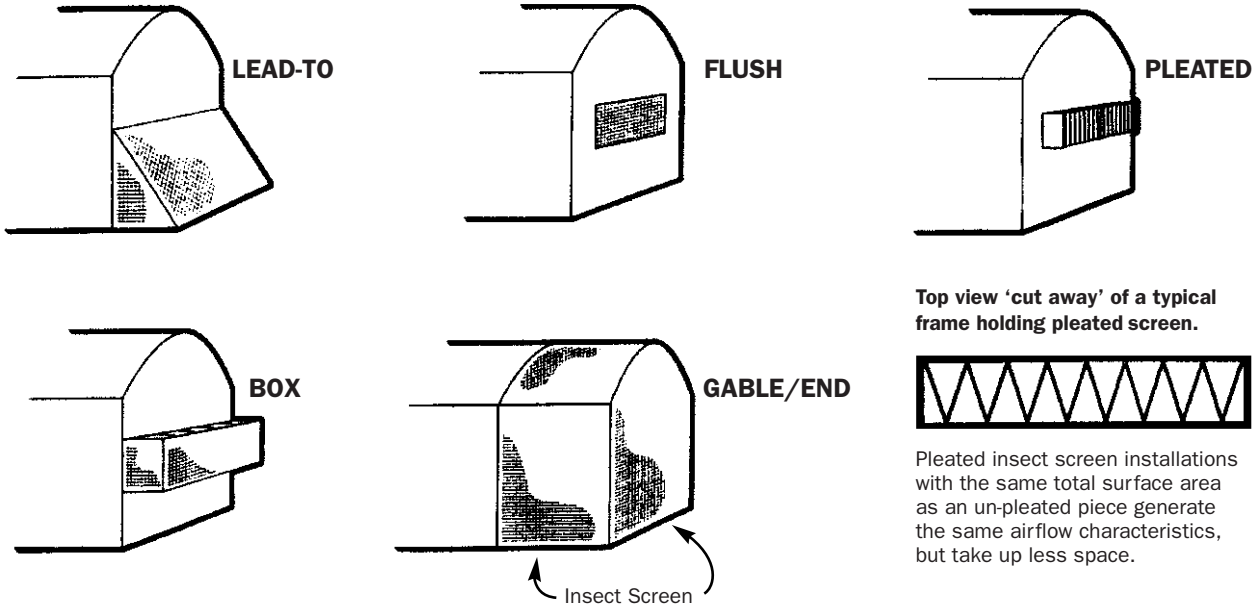
efficiencies similar to those with smaller holes. This is because holes are not always perfectly square in commercially-made screens, a factor that may or may not favor insect exclusion, depending on hole shape. Further, thread diameter and mesh material also influence exclusion properties. Relatively rigid stainless steel mesh may offer better exclusion than softer mesh with a similar hole size. Fine mesh screen requires high maintenance; therefore consideration should be given to ease of replacement and cleaning.

Screen size can greatly affect airflow, cooling efficiency, CO_2 retention, humidity level, and light transmission. Proper sizing of screen to the ventilation system is critical, regardless of the type of cooling systems installed—passive, fan only, fan and pad, or mechanical (air-conditioned). A piece of 64-mesh screen with a thread thickness of 0.008" has a total of only 23.8% open space. Dust accumulation on screens can also affect their efficiency—as the screen opening size decreases, the need to keep the screens clean by washing or vacuuming increases.

Regardless of where screening is placed, airflow considerations are paramount because of temperature changes associated with reduced air movement. Airflow, cooling, and fan performance are significantly affected by the installation of any screen, especially when using the finer mesh sizes. One solution to the airflow restriction problem is to build a "screen box" outside the cooling pad frame (Fig. 10) to provide adequate surface area for airflow though the cooling pads.

* Reprinted with permission of Agritechnove, Inc., St. Anselme, QUE., CA.

FIG 10. Typical insect screen installation shown on intake vent end of greenhouse*



Ventilation, Cooling and Heating

Motorized and/or manual hinged vents, located at the roof ridge and/or sidewall, are a common feature of most greenhouse cooling and ventilating systems. The passive ventilation afforded by vents can be activated with the addition of exhaust fans and evaporative cooling systems. Air intake screening (but never air outlet) and motorized or gravity-driven exhaust fan louvers are recommended for BL1-P and required for BL2-P. Motorized louvers should be interlocked so they open and close with fan startup and shutdown. Gravity-operated louvers are also adequate. The vent operator arms or racks that pass through screen are generally fitted with brushes or flexible barriers to prevent rodents and other large pests from entering the greenhouse. Fog cooling systems, if suitable for the structure and climate, may offer a better and more convenient alternative to evaporative cooling pad systems. Fog cooling should always be used in conjunction with a good control system to insure precise relative humidity measurement and proper fog delivery. Screens should be made to fit all vent openings, fans sized accordingly, and the system installed inside the greenhouse as specified by the manufacturer. Recirculating fans and curtain systems are also used to help control temperature.

The use of mechanical cooling, i.e., air conditioning, is the only option for higher levels of containment. Construction and operation costs are very high due to the enormous heat load of a greenhouse.

Typical greenhouse heating systems include hot water radiation, steam radiation, infrared electric, solar, and forced air. All are adequate for every containment level.

Benching

Standard greenhouse benches are adequate for most GMO research projects though wood is not recommended. Benching made of expanded

**Book of Standards*. National Greenhouse Manufacturers Association. Reprinted with permission.

FIG 11. Ebb and flow bench

galvanized steel or aluminum is preferred since these materials are resistant to water and most chemicals. In addition, such benches are readily available, meet higher containment standards, and allow for thorough cleaning, which contributes positively to a pest control program regardless of the research protocol. In some cases, benches with solid tops have adequate framing to allow replacement with expanded metal.

In cases where the IBC, as recommended by the NIH Guidelines, stipulates collection of runoff water, a solid bench may be installed that drains runoff into a holding tank for treatment with chemicals or heat before being released to the sewer or ground. A bench that collects water for recirculation, also called an Ebb and Flow bench (Fig. 11), could also be modified to collect runoff for subsequent treatment, or simply desiccation, if that renders the propagules in question inactive.

At BL3-P, other provisions may be needed to collect and treat runoff water. These may involve collection from the bench and consequent treatment but would more likely involve whole room collection using a sewer. Higher levels of containment also require seamless bench tops and other work surfaces that are impervious to water and chemicals and can withstand mild heat. These requirements may make retrofitting for high levels of containment cost prohibitive.

Floors and Drains

Requirements for greenhouse floors vary according to the biosafety level indicated (Table 7). Floors and drains may need to be renovated to meet containment standards for transgenic greenhouses. Gravel and soil beds can be used under benches in BL1-P greenhouses only if experimental material cannot travel through these beds and leave the greenhouse; concrete walkways are preferred. A BL2-P greenhouse must have an impervious floor surface. Retrofitting a greenhouse with concrete floors and walkways can substantially improve containment and sanitation practices. Coatings can be applied to concrete surfaces to make them easier to clean and disinfect.

If a new floor is to be installed, it may be advantageous to install floor drains designed to collect all runoff. This is particularly true if research projects that use genetically engineered microbes are underway or expected. Retrofitting with a biowaste collection and treatment system can be prohibitively expensive if the existing concrete slab and underground piping must first be removed and reinstalled. Such renovations could easily push the cost of retrofitting an existing facility to exceed that of new construction.

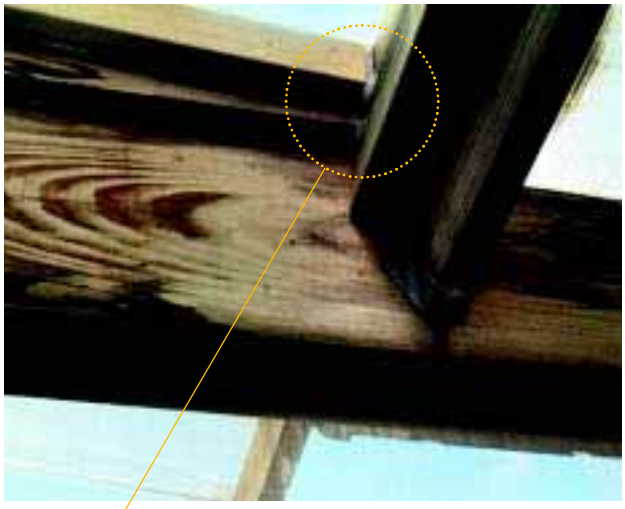


FIG 12. Sealed framing joints in a containment screenhouse

Control Systems

Standard digital, analog, pneumatic, or mechanical greenhouse control systems are suitable for GMO research at BL1-P and BL2-P. Computer or other control systems that incorporate alarms and interact with headhouse systems are recommended for BL3-P and BL4-P. Sensors, usually under computer control, are also required for high containment facilities to monitor differential air pressures. Sensor technology has become prevalent and could be employed in any modern research greenhouse. Control systems can be easily upgraded in most situations.

Greenhouse control systems technology has become highly advanced, reliable, and cost effective. It is strongly recommended that any control system used in the greenhouse itself be designed and manufactured exclusively for greenhouses, in contrast to building control systems, which cannot meet the exacting specifications for a research greenhouse.

Piping systems

Heating, watering, and fertilizing systems are typically piped into and throughout the greenhouse. Automatic watering and fertilizing systems are advantageous because they reduce the amount of traffic into the greenhouse, thus decreasing the opportunity to spread transgenic pollen, seed, and other propagative materials. The relative ease and affordable cost of installing these systems makes them an attractive option. However, for containment reasons, new piping should be installed with a minimal number of intrusions. All new and existing intrusions should be sealed with a durable material to help ensure containment (see Fig. 1).

SCREENHOUSES

Screenhouses are acceptable for GMO research only when they meet the requirements for BL1-P or BL2-P level greenhouses, including floors, and contain organisms that would have minimal impact on the environment, if released. Though they have limited utility for research, screenhouses may offer a low cost alternative to greenhouses when sited in an appropriate climate. Retrofitting screenhouses involves many of the same measures listed for greenhouses. Upgrades could include the addition of concrete floors, well-fitting, lockable doors, individual compartments, sealed joints (Fig. 12) and utility intrusions, and specialized screening. BL3-P experiments would likely not be approved for screenhouse containment.

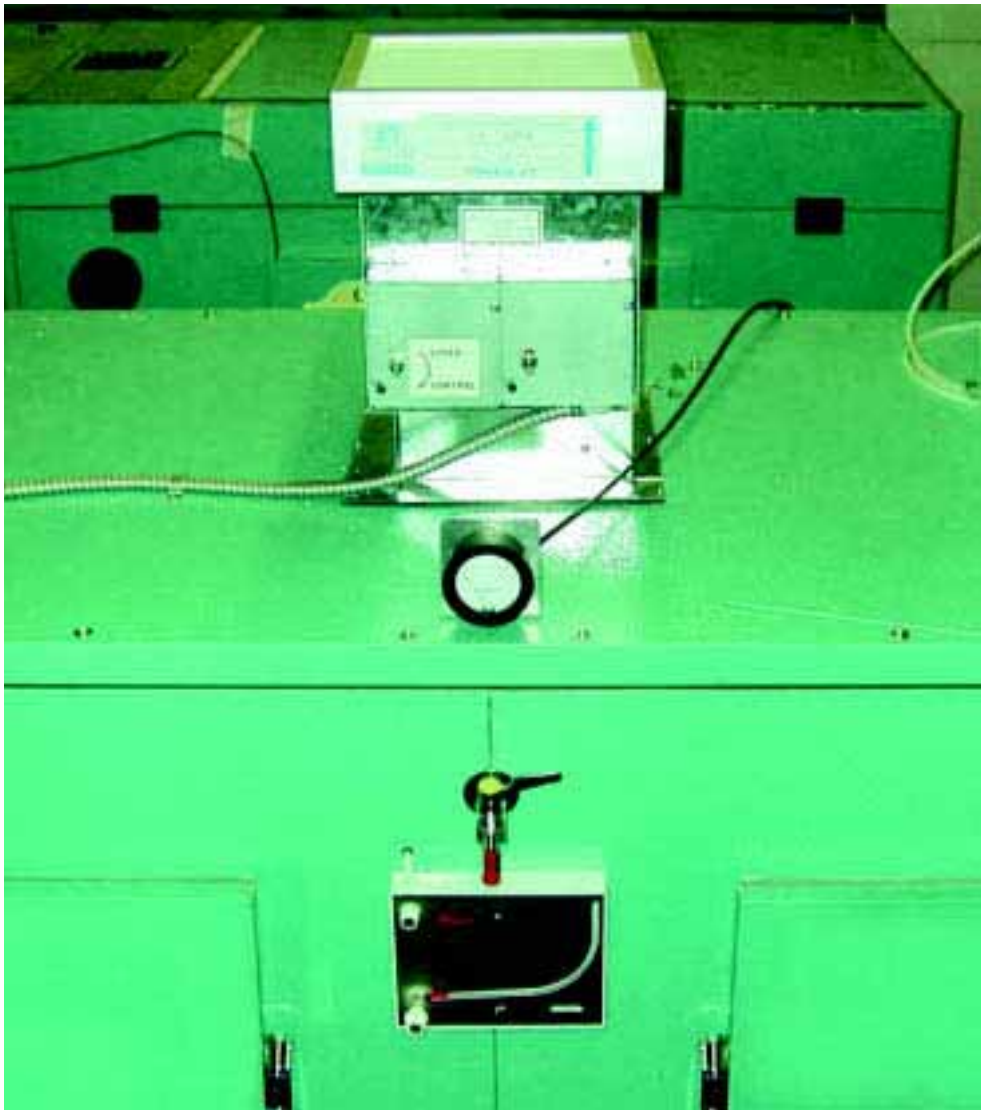


FIG 13. Growth chamber with HEPA filtration*

GROWTH CHAMBERS

If the quantity of plant material is not large, use of a growth chamber or growth room may be the best option for containment at the higher levels. A growth chamber modified to meet BL3-P requirements is shown in Fig. 13. The two main retrofits to the chamber are a HEPA filter and a system for collecting runoff water. If large quantities of plant material are produced, then renovation of existing facilities may be as cost effective as retrofitting the growth chamber.

*Reprinted with permission of Conviron, Inc., Winnepeg, Man., CA.

Section VII. Design of New Containment Facilities

A new greenhouse intended for research with transgenic organisms should be designed and built to maintain containment for the life of the facility. A greenhouse built to BL1-P or BL2-P containment standards costs little more than a standard research greenhouse; relatively small differences in design details may add slightly to the total cost. For the most part, structural features for newly constructed lower level containment greenhouses are covered in Section V, Retrofitting for Containment, and are not repeated here.

The main focus of this section is on the design of higher-level containment greenhouses. Because of more stringent design requirements, greenhouses built to BL3-P or BL4-P specifications will cost significantly more than conventional facilities. For the same reason, a qualified and experienced team of designers must render the detailed plans for such facilities.

BUILDING A DESIGN TEAM

The creation of a specialized greenhouse facility requires a team of experts. Experienced architects and/or engineers are pivotal members of the team and generally are hired independently of the construction firm. The design team creates the documentation that allows construction firms to bid on the project. Construction firms specializing in greenhouses may have an engineering staff; however, the construction of laboratories and other specialized rooms may require the skills of an architect as well.

Researchers and staff who will be using, operating, and maintaining the facility should be included in the planning process. IBC members and regulators from USDA/APHIS and state Agriculture Departments should be notified and updated regularly and may also be invited to join the design team. A commissioning agent with experience in testing greenhouse systems would also be a useful team member, though such services would be most valuable at the conclusion of the project. Consultation with users and managers at other research greenhouses is valuable when designing new facilities. The Association of Education and

Research Greenhouse Curators[21] offers an electronic mail forum, web pages, and annual meetings from which detailed information can be gathered.

CONSTRUCTION OVERVIEW

Framing Materials

Typical construction styles for research include even-span with a standard peak, Venlo, and ridge and furrow with gutter connects. Roof styles include the standard peaked, as well as arched, mansard, and Quonset-style. Figure 14 shows examples of greenhouse exterior structures. A headhouse and hallways that are immediately contiguous to the greenhouse are considered part of the containment area.

Modern greenhouse structures are framed with aluminum (Fig. 15) or galvanized steel; however, many older facilities are framed in wood or metal pipe. Wood and pipe framing are still being used in new construction of some plastic film greenhouses. A reinforced, rigid frame is preferred for BL3-P and required for BL4-P. The latter requires additional strength and rigidity to accommodate the weight of double-paned, break-resistant, sealed glass.

Use of aluminum or galvanized steel truss framing allows a prefabricated frame to be quickly erected. The rigid frame, coupled with purlins, glazing bars, and other framing members, creates a quality, long-lasting structure that can be covered with various glazing materials. Environmental control and containment is enhanced through proper installation and fitting of all materials. Information on structural materials, as well as other relevant topics, can be found in the *Book of Standards* authored by The National Greenhouse Manufacturers Association[22] or in the *American Society of Agricultural Engineers Standards 2000*[23].

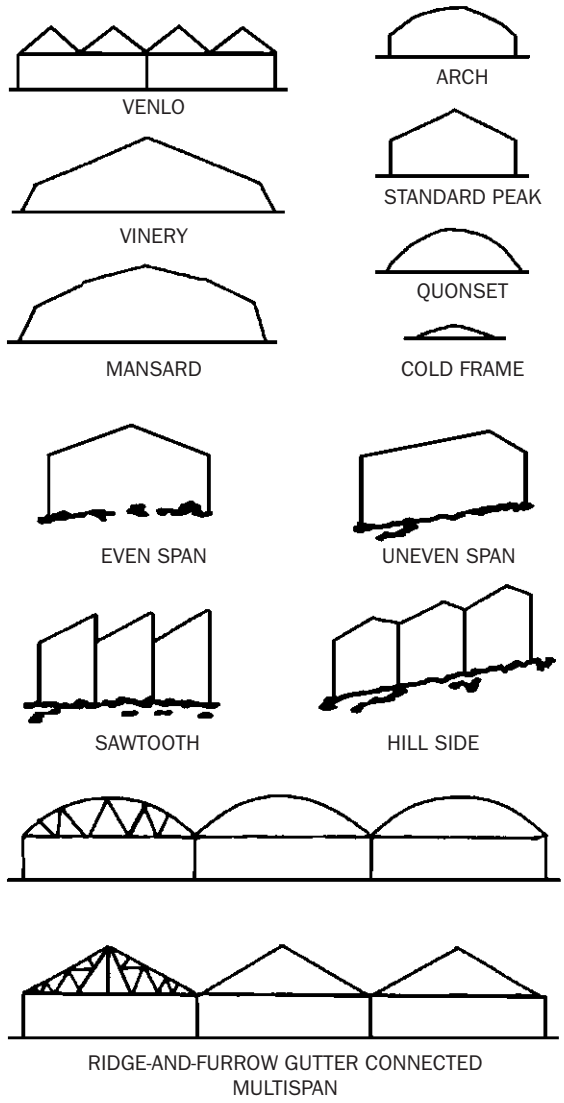


FIG 14. Greenhouse roof styles*

Entry doors and locks

The choice of greenhouse doors should receive careful consideration since containment and security breaches occur most often at points of entry.

Specifications for BL3-P and BL4-P facilities stipulate a *double* set of self-closing and locking doors. High containment facilities also require one-way emergency exit doors for personnel safety.

Traditional cylinder locks offer good security as long as good key control is implemented. Newer electronic systems such as a card swipe or Marlock™ keying provide highly restricted access and a log of

21 http://www.life.uiuc.edu/aergc

22 *Book of Standards*. 1995. National Greenhouse Manufacturers Association (NGMA). (Revised 1999) www.ngma.com

23 *American Society of Agricultural Engineers Standards 2000*. American Society of Agricultural Engineers (ASAE). asae.org

* Reprinted with permission of Hanan, Joe J., 1998. *GREENHOUSES: Advanced Technology for Protected Horticulture*, CRC Press LLC: Boca Raton, FL.

FIG 15. **Aluminum-framing under construction**

all entries and exits. Special keys or cards are programmed to allow individuals access to selected areas with one tool. Using this system, fewer keys are issued, key loss is minimized, and codes can be changed quickly and easily.

A double-door entry system, with a dark vestibule sandwiched between the doors, aids in effective insect containment. UV lights may be installed in the vestibule. Air curtains that fan individuals exiting a contained area can help blow organisms and propagules back into containment.

Benching

Many different types of benching can be found in research facilities, but when building a new high level containment greenhouse, the design and materials should be chosen so as to comply with BL-3P and BL-4P requirements. Benches must be

thoroughly cleaned and disinfected in conjunction with transgenic research at higher biosafety levels. Those made of aluminum or galvanized steel provide the longest wear, are easiest to clean, and amenable to installing systems for runoff water collection and treatment. An ebb and flow (also called ebb and flood) bench is one that can collect water and recycle it to the bench (see Fig. 11). This system can also be adapted to collect and hold water prior to subsequent decontamination by chemicals or heat.

Ventilation, Heating, and Cooling

Few conventional research greenhouses are built with sealed glazing, mechanically conditioned air, differentially controlled air pressure, and exhaust air filtered through high efficiency particulate air (HEPA filters). Thus new construction is usually needed to meet the standards for BL3-P and BL4-P facilities. Air conditioning is not strictly mandatory for higher-level containment greenhouses; however the loss of cooling efficiency due to required air-handling measures makes it a necessity in most climates.

The exhaust air produced from negative pressure systems must be filtered to prevent contained organisms from exiting. Intake air is also filtered to prevent introduction of organisms from the environment into the enclosed space. Filter systems can be designed to trap pollen, spores, and other very small particles. High efficiency particulate air (HEPA) filters can remove 0.3 micron and larger particles but still allow gases to transfer across the filter media.

It is relatively difficult and expensive to equip an entire greenhouse to restrict small particle movement. Air-conditioned greenhouses, growth chambers, growth rooms, or biological safety cabinets are alternatives to standard research greenhouses with air filtration systems. Specialists should be consulted when designing or retrofitting facilities that require a highly effective air filtration system. The engineering specifications required for air balancing, ventilating, and cooling BL3-P and BL4-P greenhouses are beyond the scope of this Guide. If this type of facility is required, it is highly recommended to involve an experienced design firm for the project.

Floors and drains

Solid concrete flooring and drains are preferred for new research greenhouses. Commercial greenhouses often use porous concrete floors to allow passage of water. However, BL3-P and BL4-P facilities must have non-porous floors that can be disinfected as well as a system to collect all runoff. The floor of a BL4-P facility must be part of an "internal shell" system that includes the walls and ceiling. Runoff is drained to a decontamination tank or treatment facility before entering a standard sewer or other disposal system. Additionally, sewer vents on BL4-P greenhouses must be HEPA filtered.

Control Systems

Normal building controls cannot readily be adapted to meet the rigorous needs of a high-level containment greenhouse; therefore dedicated controls available from greenhouse control vendors are recommended. New facilities should have control systems that incorporate the latest digital technology, and allow precise environmental control, logging, sensing, alarm, and related functions. Moreover, the security and redundancy functions that are required at higher containment levels prescribe newer digital controls and should interface with the institutional building security system.

Greenhouse managers and others involved in retrofitting existing greenhouses or building new facilities can draw on the experience of USDA officials, the NIH Office of Biotechnology Activities, architects, vendors, and professional colleagues. A partial list of these is included in Appendix II.

Appendix I.

Facility Inspection Checklists

Courtesy of Ralph Stoaks
C/O Diane Hatmaker
USDA/APHIS
Biotechnology Programs Operations
4700 River Road, Unit 147, Rm. 5B53
Riverdale, Maryland 20737
Telephone: (301) 734-5787

FACILITY INSPECTION CHECKLIST

FOR CONTAINMENT OF GENETICALLY ENGINEERED ORGANISMS

Address of Facility

_____

_____

_____

_____

_____

_____

( )_____

Telephone Number

Applicant (Responsible Person)

Name _____

_____

_____

Address _____

_____

_____

( )_____

Telephone Number

LOCATION OF ALL FACILITIES COVERED BY THIS INSPECTION

Building Name _____

Room/Laboratory _____

Growth Chamber Identification _____

Greenhouse Number or other Identification _____

RESEARCH QUALIFICATIONS AND GENERAL BACKGROUND

1. Does this facility operate under the National Institutes of Health (NIH) Recombinant Advisory Committee (RAC) recombinant-DNA (r-DNA) guidelines? Yes____ No____

2. Is there a written policy regarding handling of rDNA at this establishment? Yes____ No____

3. Who is the chairperson of the local Institutional Biosafety Committee (IBC)?

   Name and Title_____

4. Who is the scientist who will conduct the research?

   Name and Title_____

5. Is the scientist who is conducting the research the applicant? Yes____ No____

6. What other scientists and technicians will be working on the research?

   Describe, in a general way, their experience and qualifications.

7. Do researchers and laboratory technicians practice and adhere to the NIH guidelines? Yes____ No____

PHYSICAL DESIGN AND SECURITY

8. Provide a short description of how the regulated article is physically marked and identified in the laboratory, growth chamber, and greenhouse. Provide floor plan and/or map of facilities if possible.

9. Is the general area secure from public access? Yes____ No____
   If not, please elaborate.

10. A. Is the general area secure from unauthorized personnel? Yes____ No____
    If not, please elaborate.

    B. Can individual laboratories be locked? Yes____ No____

    C. Is there at least one sign posted on the facility door stating that a regulated genetically engineered organism is present? Yes____ No____

    If not, when will a sign be installed? Date _____

11. Who is allowed in the research areas?
    Cleaning Personnel Yes____ No____ Trades Persons Yes____ No____ Other Yes____ No____

12. How distant from each other are the germination laboratories, growth chambers, and greenhouses? Be specific.

13. What kind of records, logs, or inventory are maintained regarding receipt, increase, and destruction of regulated articles?

HANDLING OF MATERIAL—GERMINATION

14. A. Is there a cabinet to store seeds, plant material, tissue cultures, etc.? Yes____ No____
    B. If yes, does it have a lock? Yes____ No____
    C. Is the storage container identified with a sign stating it contains a genetically engineered organism? Yes____ No____
    D. If not, when will a sign be installed? Date _____

15. Where will seeds, tissue cultures, plant material, etc. be grown or germinated?

16. What medium will be used for seed germination? (e.g., germination paper, perlite, sand)

17. Is there any danger of seeds, tissue cultures, plant material, etc. being lost during this germination process, or of ungerminated seed being transferred into subsequent research stages? Yes____ No____

18. Are there any cracks or irregular surfaces in the germination laboratory that could trap seeds? Yes____ No____
    If Yes, describe size and location of cracks.

19. Are there water drains in the laboratory? Yes____ No____

20. Are the drains screened? Yes____ No____
    If so, what is the size of the screen?

21. Does the drain system enter into a special waste trap? Yes____ No____

22. How will the germinated seed be moved to the growth chamber?

23. How will petri dishes, tissue cultures, spores, plant materials, etc. be moved from the laminar flow hood, to the incubator, to the growth chamber?

24. How will the regulated articles be kept separate from other organisms?

HANDLING OF MATERIAL—GROWTH CHAMBER

25. Does growth chamber have access by authorized personnel only? Yes____ No____

26. Describe the growth chamber. lab top____ walk in____ built on site____ other ____.

27. Will the material be grown with any other plant materials in the same chamber? Yes____ No____
    If yes, name the types of plants.

28. How will genetically engineered plants and/or containers be physically marked?

29. Does the growth chamber have water drains? Yes____ No____
If so, can they be screened? Yes____ No____

30. Does the drain system enter into a special waste trap? Yes____ No____

31. Where is the autoclave or incinerator in relation to the growth chamber?

32. Can the growth chamber be locked and separated from other growth chamber(s)? Yes____ No____

33. How will the material be transferred to the greenhouse?

34. How will the regulated articles be kept separate from other organisms?

HANDLING OF MATERIAL—GREENHOUSE

35. What is the name of the greenhouse manager? _____

36. Is the greenhouse accessed by authorized personnel only? Yes____ No____

37. A. Does the greenhouse have a double door entry system? Yes____ No____
B. Is the greenhouse entry through a "headhouse"? Yes____ No____

38. A. Do the greenhouse doors have locks? Yes____ No____
B. Is there a rear exit door? Yes____ No____

39. What type of greenhouse? Glass____Lexan____Plastic Poly____Screen____Other____
If screen, what size mesh? _____ If Poly, what thickness? _____

40. What are the approximate outside dimensions of the greenhouse(s)?

41. A. Do the roof vents open? Yes____ No____
B. If the roof vent opens, is it screened? Yes____ No_____ What size is the screen mesh? _____

42. What kind of floor does the greenhouse have?
Concrete____Gravel____Packed Dirt____Other (Explain)_____

43. Does the greenhouse have water drains? Yes____ No____
Do they enter into a special waste trap? Yes____ No____

44. A. Does the greenhouse have black light traps for vectors? Yes____ No____
    B. Does the greenhouse have "Sticky Board" traps for vectors? Yes____ No____
    C. Does the greenhouse have other kinds of vector traps? Describe.

45. How will the plants be grown in the greenhouse? On Benches_____ In Flats_____ In Pots_____,
    Other (describe)_____.

46. Will there be physical markers on each plant or container indicating that the plants are genetically engineered?
    Yes____ No____

47. Where is the autoclave or incinerator in relation to where the plants will be grown?

48. Are there any openings in the greenhouse through which animals and pollinating insects could enter? Yes____ No____

49. How will the regulated articles be kept separate from other organisms?

GENERAL CONSIDERATIONS

What kinds of "spill response" action plan/equipment is available for items spilled in transit between labs, chambers, and greenhouses? Items should be carried in containers so spills should not occur.

Are any similar plants growing in the area, either on the facility grounds or outside of the facility grounds?

What other factors are present which may influence the handling of seed or plants and may have an effect on containment or risk?

Inspect for other specific conditions as stipulated on the permit.

Name of State Plant Pest Regulatory Official Performing Inspection	Printed Name of PPQ Officer Performing Inspection
	Signature

Instructions to the inspector: Complete this form and return to:

Ralph Stoaks
C/O Dianne Hatmaker
USDA/APHIS
Biotechnology Program Operations
4700 River Road, Unit 147, Rm. 5B53
Riverdale, Maryland 20737
Telephone: (301) 734-5787

REINSPECTION CHECKLIST

FOR CONTAINMENT OF GENETICALLY ENGINEERED PLANT MATERIAL AND ORGANISMS

Address of Facility

_____

_____

_____

_____

_____

_____

( )_____

Telephone Number

Applicant (Responsible Person)

Name _____

_____

_____

Address _____

_____

_____

( )_____

Telephone Number

LOCATION OF ALL FACILITIES COVERED BY THIS INSPECTION

Building Name _____

Room/Laboratory _____

Growth Chamber Identification _____

Greenhouse Number or other Identification _____

RESEARCH QUALIFICATIONS

1. Who is the scientist responsible for conducting the research?

   _____

2. Who was the responsible scientist at the time of the initial facility inspection?

   _____

3. Do researchers and laboratory technicians regularly review, practice, and adhere to the permit protocol and the conditions described in the permit? Yes____ No____

4. Conditions were reviewed by applicant and/or technicians on _____(date).

5. Have any major changes occurred or new operational procedures been instituted since the initial inspection?
Yes____ No____ If YES, initiate and complete a new facility inspection checklist.

6. Are the permit articles or any other regulated organisms derived from these articles still in use? Yes____ No____
or in storage? Yes____ No____

7. Have all of the regulated articles been properly destroyed? Yes____ No____ Date_____.
If Yes, no further action is required.

GENERAL CONSIDERATIONS

Remarks and/or observations.

Other factors which may influence the handling of seed or plants and may have an effect on continued containment or risk of unwanted release.

Inspect or spot check for other specific conditions as stipulated in the permit.

Name of State Plant Pest Regulatory Official Performing Inspection	Printed Name of PPQ Officer Performing Inspection
	Signature

Instructions to the inspector: Complete this form and return to:

Ralph Stoaks
C/O Dianne Hatmaker
USDA/APHIS
Biotechnology Program Operations
4700 River Road, Unit 147, Rm. 5B53
Riverdale, Maryland 20737
Telephone: (301) 734-5787

Appendix II.

Supplemental Resources

Regulatory Contacts
National Associations
Greenhouse Construction Resources

Regulatory Contacts

Biotechnology Evaluation
USDA-APHIS-PPQ
4700 River Road, Unit 147
Riverdale, MD 20737-1236
Phone: (301) 734-8896
Web: http://www.aphis.usda.gov/bbep/bp

Office of Biotechnology Activities
National Institutes of Health
6705 Rockledge Drive, Suite 750, MSC 7985
Bethesda, MD 20892-7985
Phone: (301) 496-9838
Fax: (301) 496-9839
Web: http://www4.od.nih.gov/oba/

National Associations

USDA NCR-101
Committee on Controlled Environment Technology and Use
Mark Romer, Phytotron Manager
McGill University
1205 Dr. Penfield Ave.
Montreal, QC H3A 1B1 Canada
Phone: (514) 398-6741
Fax: (514) 398-5069
mark@bio1.lan.mcgill.ca
Web: http://www.botany.duke.edu/ncr101/

NCR-101 is a committee of the USDA's North Central Region convened to help plant scientists understand how to use controlled environment technology effectively and consistently. They discuss how to utilize growth chambers effectively to ensure consistent and comparable growth data among laboratories.

The Association of Education and Research Greenhouse Curators (AERGC)
c/o Department of Plant Biology
University of Illinois at Urbana-Champaign
265 Morril Hall
505 S. Goodwin Ave.
Urbana, IL 61801-3793 USA
Web: http://www.life.uiuc.edu/aergc/default.html

The Association consists primarily of greenhouse and plant growth facility managers, supervisors, and staff involved with the operation of college or university facilities used to grow plant materials for research, class use or plant collections. The AERGC publishes the AERGC Newsletter and sponsors an Annual Meeting at a member's institution. The AERGC also provides the AERGC Forum, an e-mail discussion group, as a service to its members.

National Greenhouse Manufacturers Association (NGMA)
20 West Dry Creek Circle, Suite 110
Littleton, CO 80120
Phone: (800) 792-6462
Web: http://www.ngma.com/

The National Greenhouse Manufacturers Association is a professional trade organization for the manufacturers and suppliers of greenhouses and greenhouse components. The Association membership brings together some of the most experienced and knowledgeable manufacturers in the industry.

Greenhouse Construction Resources[24]

DESIGN FIRMS

Agritechnove, Inc.
651 Route Begin
St-Anselme, Quebec, Canada, GOR 2NO
Phone: (418) 885-9595
Fax: (418) 885-4957
Email: agritech@total.net

Alex Turkewitsch, P. Eng. Ltd.
86 Glenview Avenue
Toronto, ON, Canada M4R 1P8
Phone: (416) 489-3816
Fax: (416) 481-3883

GREENHOUSE FABRICATORS

Ludy Greenhouse Mfg. Corp.
P.O. Box 141
New Madison, OH 45346
Phone: (937) 996-1921 or (800) 255-LUDY
Fax: (937) 996-8031
Web: http://www.ludy.com

Nexus Greenhouse Corp.
10983 Leroy Dr.
Northglenn, CO 80233
Phone: (800) 228-9639
Fax: (303) 457-2801
Web: http://www.nexuscorp.com

Rough Brothers, Inc.
5513 Vine St.
Cincinnati, OH 45217
Phone: (513) 242-0310 or (800) 543-7351
Fax: (513) 242-0816
Web: http://www.roughbros.com

SUPPLIERS

Brighton By-Products Co. Inc.
P.O. Box 23
New Brighton, PA 15066
Phone: (724) 846-1220 or (800) 245-3502
Fax: (412) 846-7240

E.C. Geiger, Inc.
Route 63, Box 285
Harleysville, PA 19438
Phone: (215) 256-6511 or (800) 443-4437
Fax: (215) 256-6110 or (800) 432-9434
Web: http://www.geigerco.com/default.html

McCalif Grower Supplies, Inc.
P.O. Box 310
Ceres, California 95307
Phone: (800) 234-4559 (hard goods)
Phone: (800) 473-7413 (plant sales)
Fax: (209) 538-2086
Web: http://www.mccalif.com/default.html

Hummert International
4500 Earth City Expressway
Earth City, MO 63045
Phone: (800) 325-3055
Fax: (314) 739-4510
Web: http://www.hummert.com

Branch-Smith Publishing
Online supplier search
P.O. Box 1868
Fort Worth, TX 76101
Phone: (817) 882-4120 or (800) 434-6776
Fax: (817) 882-4121
Web: http://www.greenbeam.com/branchsmith/default.stm

[24] Citation of greenhouse resources is merely for the reader convenience and does not imply the authors' endorsement of these firms and suppliers. Readers are encouraged to independently investigate alternative resources.

Notes

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Information Systems for Biotechnology

207 Engel Hall, Blacksburg VA 24061

tel: 540-231-3747 / fax: 540-231-4434 / email: isb@vt.edu

http://www.isb.vt.edu

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