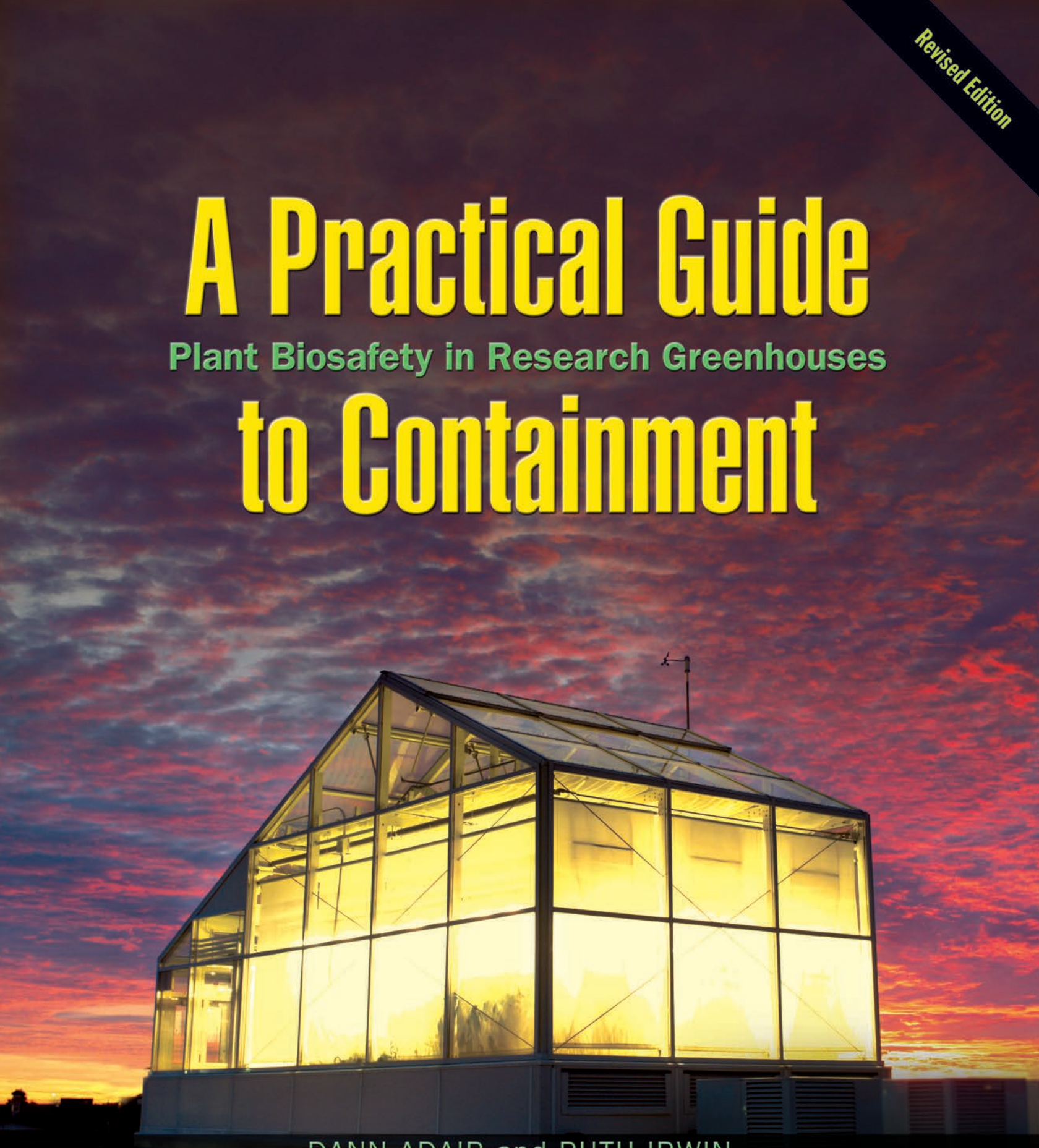
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A Practical Guide to Containment

Plant Biosafety in Research Greenhouses



DANN ADAIR and RUTH IRWIN

Information Systems for Biotechnology



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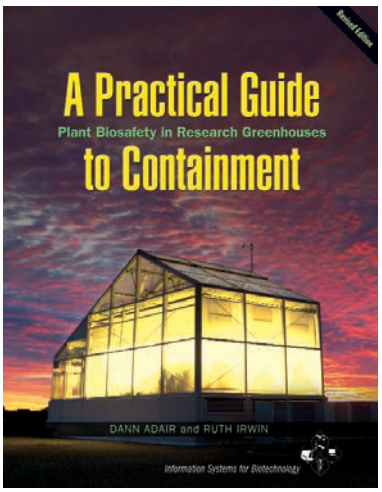
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The information contained in this manual is accurate to the best knowledge of the authors. Any errors or omissions are their sole responsibility. The views presented here do not represent those of Virginia Tech, USDA, or other state or federal regulatory agencies. Readers are advised to consult directly with relevant national or state agencies or authorities for official guidance.

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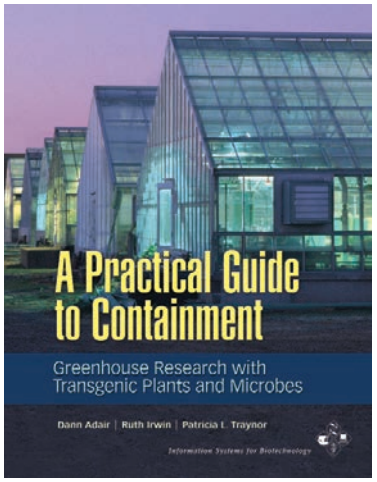
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Preface

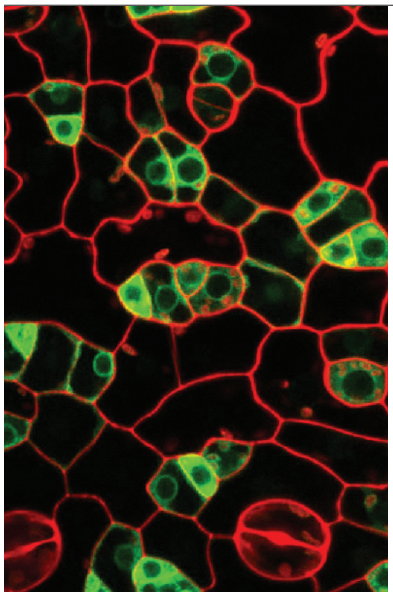
Research conducted in greenhouses involves many biological systems that benefit from containment. Guidance literature on containment strategies for the greenhouse is limited, though it is rapidly expanding as new facilities and practices emerge and experiences are shared. The original version of this Guide is testament to the dearth of printed material covering the principles of containment in research greenhouses, based on the multiple printings required to fill demand around the world.



This Guide was originally published in 2001 as *A Practical Guide to Containment: Greenhouse Research with Transgenic Plants and Microbes* and primarily addressed containment of plants and plant-associated organisms containing recombinant DNA (rDNA). Researchers, facility managers, and regulators have subsequently encouraged the authors to expand this Guide beyond containment of solely transgenic organisms. Therefore, the reader will find new information on containment strategies for research with exotics (non-native invasive species), pathogens, insects, and genetically engineered (GE)[1] plant-manufactured pharmaceuticals and industrial compounds, and on high containment for quarantined organisms, including those on the Select Agent list. Material was obtained from many individuals, primarily those acknowledged on page v, as well as from regulatory agencies, the literature, personal experience from planning and constructing facilities, and shared 'lessons learned' from the research community.

We emphasize working closely with regulatory authorities when using this Guide to develop containment strategies for research greenhouses. Although we refer by default to agencies within the United States, we also welcome people residing outside the United States to use the Guide freely. It is our sincere desire that this updated Guide will be of even greater service to the research community.

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



Section I. Introduction

RESEARCH IS VITAL TO AGRICULTURE. HOWEVER, INHERENT in research are certain risks to the natural environment and agricultural crops and markets. Research involving plant diseases and pests, often microscopic and motile, require containment within research facilities. As an example, Asian soybean rust infection can reduce the yield of soybeans by over 80%. The fungal pathogen was first identified in Japan in 1902 and has subsequently spread throughout Asia and Africa, and is now found in the southeastern United States. One can quickly appreciate how effectively a microscopic windblown spore may become a pandemic disease, spreading around the globe and threatening a vital agricultural commodity. Consequently, greenhouses and similar plant growth facilities are required for studying the biological attributes of plant diseases and pests. The challenge is to contain these organisms within a secure facility.

In addition to plant pathogens, other organisms—genetically engineered and exotic organisms, and plant associated insects and mites, for instance—require containment. However, unlike other research materials, transgenic organisms are subject to special rules intended to ensure that they do not pose an unacceptable risk to agriculture or the environment. Genetic modifications of transgenic organisms include, but are not limited to, gene insertions made by recombinant DNA (rDNA)[2] methodologies.

Methods for safely handling 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 importation, interstate movement, and release into the environment of genetically engineered organisms are implemented by the Biotechnology Regulatory Services within the Animal and Plant Health Inspection Service of the US Department of Agriculture (USDA-APHIS). USDA-APHIS also regulates and guides the movement certain non-GE plants, pathogens, and related insects and microbes. Products of biotechnology are also regulated by the US Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA) under the Coordinated Framework for the Regulation of Biotechnology.

[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."

Information about handling organisms that require containment in greenhouses, however, is relatively sparse. Appendix P of the *NIH Guidelines*[3] specifies facility features and practices for meeting containment standards[4] appropriate for each of four biosafety levels. APHIS has published containment facility guidelines that suggest ways to meet containment standards. Presently, though, there is no single source of practical guidance on managing containment within research greenhouses, 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 a simple and convenient reference on appropriate biosafety and containment guidelines for research conducted in greenhouses. There may be a broad range of opinions among scientists and greenhouse managers regarding what is needed. Some may harbor a misunderstanding that plants under containment protocols 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. This Guide will help clarify the level of containment and measures needed for each biosafety level.

Scope

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

Screenhouses—structures that are screened for insect or plant containment (or exclusion) but that 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.

Growth chambers and growth rooms—controlled environments created specifically for plant research—are commonly used for containment. Information is included on these types of equipment as well. Biosafety cabinets, incubators, and tissue culture tables or rooms are mentioned in passing, however, a detailed description is not within the scope of this Guide.

This Guide includes:

- Relevant information on biosafety containment levels
- Physical and biological strategies that provide containment
- Suggested facility modifications to achieve prescribed containment levels
- Suggestions for day-to-day greenhouse management
- Management tools to ensure proper handling of biological materials
- Guidance for developing or renovating facilities
- Descriptions of equipment and supplies
- Sample floor plans
- Sources for additional information

The Guide is organized in six sections plus five Appendices. Section I contains introductory information and a brief discussion of content. Section II covers regulation and oversight by government regulatory and research agencies, and outlines the roles and responsibilities of institutional personnel. Section III presents descriptions of biosafety levels together with examples of studies that may be conducted at each level. Physical, biological, and layered containment strategies are given in Section IV, followed by suggested management practices in Section V. Section VI discusses designing and building for containment, including retrofitting existing facilities to meet containment standards. The Appendices provide a facility inspection checklist from USDA-APHIS-BRS, a sample biosafety review, an outline for creating Standard Operating Procedures (SOP) from USDA-APHIS-PPQ, an SOP for a specific activity, and

[3] http://www4.od.nih.gov/oba/rac/guidelines_02/Appendix_P.htm

[4] http://www.aphis.usda.gov/plant_health/permits/organism/containment_facility_inspections.shtml

selected organizational resources. A reference list and glossary are also included.

This Guide is written so that anyone who works in a greenhouse with transgenic or other containment-eligible 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 considered the only authoritative source. Readers are encouraged to seek additional guidance from institutional authorities and APHIS officials whenever questions arise.

Audience

The primary audience of this Guide consists of greenhouse managers, facility staff, regulators, and research scientists. Managers, who are 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 its personnel. 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 experimental materials have been genetically engineered. Researchers and students who work with GEOs (genetically engineered organisms) and other containment-eligible systems, together with members of Institutional Biosafety Committees, will likely find this Guide is a simple and convenient reference on the various levels of containment and the types of experiments appropriate to each level. Regulators may find the Guide is a useful training tool for staff and clients.

In addition, designers working on retrofits to existing greenhouses or on new construction will find specialized information that pertains to meeting specialized 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 research material is handled in an environmentally and legally responsible manner.



Section II.
Regulation and Oversight

TRANSGENIC PLANTS AND PLANT PESTS ARE SUBJECT TO federal guidelines, regulations, and rules pertaining to their containment, movement, and release into the environment. States may have applicable regulations as well. Federally funded 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 these 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 molecules and organisms containing recombinant DNA. These Guidelines are advisory in nature, rather than legally binding. However, all federal agencies that support or conduct rDNA research agree 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 updated version of the *NIH Guidelines* can be accessed on the Internet[5].

The *NIH Guidelines* discuss risk assessment and recommend containment measures for various biological experiments. They delineate 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 address 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

[5] http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html

terrestrial crop, forest, weed, and ornamental species. Also found in Appendix P are recommended containment conditions for experiments involving plants, together with their plant-associated microorganisms and small animals, any one of which may be genetically modified.

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 to foster 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 also 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. Appendix P also very briefly describes how growth chambers may be used to meet containment standards. However, when plants are grown in the laboratory (as opposed to the greenhouse), whether in growth chambers, tissue culture rooms, or on open benches, they are regulated according to the guidelines contained in Appendix G, Physical Containment.

FEDERAL REGULATORY AGENCIES

Under the Coordinated Framework for Regulation of Biotechnology, three US governmental agencies regulate GEOs: the Department of Agriculture; the Environmental Protection Agency (EPA); and the Food and Drug Administration (FDA). The Department of Health and Human Service's Center for Disease Control (CDC) is also involved in regulation as it relates to biosecurity, specifically of plant pathogens (in conjunction with USDA-APHIS) that may be used as biological weapons. **TABLE 1** (*see page 8*) displays an overview of the overlapping regulatory authorities.

Greenhouse research is not generally subject to federal regulation. The following outline provides a broad context for the regulatory oversight of the field testing and commercialization of transgenic plants, and of plants provoking biosecurity and biocontrol concerns. Though this Guide illustrates the general purview of the federal regulatory agencies, guidance is always determined on a case-by-case basis; hence it is imperative that anyone contemplating work with regulated material should consult with the appropriate agencies very early in the planning stages. Detailed information about these agencies and their oversight of products derived from biotechnology, with links to the laws, rules, and regulations that they administer, can be accessed at the US Regulatory Agencies Unified Biotechnology website[6].

USDA-APHIS

The USDA Animal and Plant Health Inspection Service (APHIS) has authority under the Federal Plant Protection Act (a subsection of the Agricultural Risk Protection Act) to protect US agriculture from pests and disease. Under the Coordinated Framework, this authority was extended to cover recombinant DNA-containing plants and other potential plant pests. APHIS also adheres to international standards created by the International Plant Protection Convention.

Within APHIS, two operational programs are primarily devoted to plants and plant-related organisms—Plant Protection and Quarantine (PPQ) and Biotechnology Regulatory Services (BRS). PPQ focuses on the "the risks associated with the entry, establishment, or spread of animal and plant pests and noxious weeds to ensure an abundant, high-quality, and varied food supply"[7]. BRS is the lead

[6] http://usbiotechreg.nbii.gov/

[7] http://www.aphis.usda.gov/plant_health/

program on plant biotechnology and regulates the introduction of genetically engineered organisms that may pose a risk to plant health[8]. The USDA also regulates veterinary biologics such as recombinant vaccines; when produced in plants, these plant-made pharmaceuticals (PMPs) fall within USDA jurisdiction.

Any 'introduction', defined as importation, interstate movement, or release to the environment, of a plant, plant pest, or GEO requires either an APHIS Notification or an application for a Release permit, depending on the nature of the plant and the genetic modification (see 2008 USDA-APHIS Biotechnology Regulatory Services User's Guide on Notifications[9]). Permit applications can be completed online or by mail[10]. APHIS-BRS requires a permit to introduce a 'regulated article', which they define as "an organism that has been genetically engineered (via recombinant DNA techniques) from a donor organism, recipient organism, vector, or vector agent that is a plant pest or contains plant pest components"[11]. APHIS maintains and updates a searchable list of plant pests on their website[12].

APHIS does not regulate the use of transgenic organisms within contained facilities and does not evaluate the adequacy of research and storage facilities to prevent release into the environment. However, unauthorized release (exhibited by the presence of survivable material outside containment) of regulated material from such facilities is a violation of APHIS regulations. APHIS strongly encourages applicants to ensure that destination facilities follow containment guidelines established by the National Institutes of Health or other similar protocols. The USDA-APHIS does have, however, the authority to inspect any facility receiving regulated material that is shipped interstate or imported. APHIS will occasionally inspect facilities receiving materials shipped under Notification; however, they regularly inspect facilities receiving material shipped under a movement permit (M), release permit (R), or movement and release permit (R/M). These inspections occur prior to receipt of the first shipment and then again every 2–3 years. If the facility does not pass the inspection, APHIS will not issue the permit until changes are made by the applicant to ensure proper containment.

In some cases researchers may need more than one permit from APHIS if they are importing regulated material. Individuals often mistakenly think that just because they have a permit from BRS they don't need one from PPQ (such as a 588 or 526) and vice versa. Applicants are cautioned to be aware that they may need to obtain multiple permits from APHIS, depending on the circumstances. Applicants should also be aware that BRS regulations will be changing in the near future (as of the time of initial printing of this Guide, June 2008). Under the new regulations, there will be no Notification process and everything will be regulated under permit using a tiered permit structure. Information about BRS regulation changes can be found on the BRS website. Researchers are strongly advised to contact BRS or PPQ if they have any questions about the processes and the applicable permits required for their research.

EPA

The EPA's Biopesticides and Pollution Prevention Division (BPPD) of the Office of Pesticide Programs (OPP) regulates two categories of GEOs: PIPs and GE microbes The first encompasses genetically engineered microbial pesticides, that is, 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 pesticides. The second category consists of plant-incorporated protectants (PIP), which are pesticidal substances produced within the plant. An example is a plant expressing insect control proteins derived from *Bacillus thuringiensis* (Bt). More information on these topics is available through the EPA's Biopesticides and Pollution Prevention Division (BPPD) of the Office of Pesticide Programs (OPP)[13].

FDA

Commercial products modified by genetic engineering for human and animal consumption, food additives, and human and veterinary drugs are subject to regulation by the FDA. Their oversight

8 http://www.aphis.usda.gov/biotechnology/brs_main.shtml

9 http://www.aphis.usda.gov/brs/pdf/Notification_Guidance.pdf

10 http://www.aphis.usda.gov/permits/index.shtml

11 http://www.aphis.usda.gov/biotechnology/permitqa.shtml

12 http://www.aphis.usda.gov/plant_health/plant_pest_info/

13 http://www.epa.gov/pesticides/biopesticides/

TABLE 1. Regulatory Oversight by Multiple US Government Authorities

TRAIT CATEGORY	EXAMPLE	REGULATORY PURVIEW*
Viral Resistance in food crop	PRSV-resistant transgenic papaya	USDA, EPA, FDA
Insect resistant food crop	Bt maize	USDA, EPA, FDA
Herbicide Tolerance in an ornamental crop	Glyphosate-tolerant marigold	USDA, EPA, FDA
Herbicide tolerance in a food crop	Glyphosate-tolerant maize	USDA, EPA, FDA
Agronomic traits	High laurate oil canola	USDA, FDA
Phytoremediation	Transgenic poplar	USDA, EPA
Plant-made pharmaceuticals	Antibody producing Lemna sp.	USDA, FDA
Transgenic insects	GFP-expressing pink bollworm	USDA, EPA (in some cases)
Select Agent plant pathogens	Causal agents of Huanglongbing disease of citrus	USDA or CDC

* USDA provides for safety for agriculture and the environment; FDA provides for safety of food and feed use; and EPA provides for safety for the environment, food and feed safety of PIPs, and safe use of companion herbicides.

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 the product safety review. An overview of the FDA's policies on food and feed from GE plants can be found on the Internet[14].

CDC

Antiterrorism legislation, begun in 1996 after the bombing in Oklahoma City, was extended to include the recognition of plant pathogens as potential terrorist tools. The CDC created the National Select Agent Registry program for permitting and tracking agents and toxins that may be a threat to the health of the public, animals, or plants, or to animal or plant products. APHIS, which is the lead agency for regulating agricultural pests and products, became involved when legislation was updated in 2002. The program is now jointly administered by the CDC and APHIS. Guidance documentation for complying with security requirements can be found on the Internet[15]. Currently, there are eight listed plant pathogen Select Agents[16].

INTERNATIONAL REGULATIONS AND GUIDELINES

The international community cooperates in many ways to prevent the introduction of organisms that may cause disruption to the local environment or economy. For example, the International Plant Protection Convention (IPPC), which currently has 167 government consignees, is a treaty concerned with preventing the introduction and spread of pests to plants and plant products[17]. The IPPC has developed phytosanitary guidelines and serves as a reporting center as well as an information source. Seven regional phytosanitary organizations have been established under the umbrella of IPPC. The North American Plant Protection Organization[18] (NAPPO), for example, consists of the US, Canada, and Mexico, who participate through APHIS, the Canadian Food Inspection Agency[19] (CFIA), and the Plant Health Directorate, respectively[20]. The European and Mediterranean Plant Protection Organization (EPPO) is an intergovernmental organization, also under the IPPC, which is responsible for cooperation in plant protection among 50 countries in the European and Mediterranean region[21].

LOCAL OVERSIGHT

Institutional Biosafety Committee

Any institution where research is conducted with transgenic organisms and that receives federal funding for research is required to appoint an Institutional Biosafety Committee (IBC). 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. The institution may ask its IBC to review other research that requires biosafety considerations. The committee must consist of at least five persons, two of whom are "citizen members" not affiliated with the institution. Preferably members are familiar with biosafety issues and have a demonstrated commitment to the surrounding community, especially pertaining 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 suitable for IBC membership. The committee should also include at least one member with expertise in plant, plant pathogen, or plant pest containment principles.

The IBC reviews recombinant DNA research programs or proposals and evaluates the research leader's containment level designation for the proposed work (see **Appendix I – Sample Biosafety Review**). Commonly the IBC first considers the proper containment level for the unmodified organism, and then considers whether the proposed manipulation could increase, decrease, or leave unchanged the organism's required level of

14 http://vm.cfsan.fda.gov/~lrd/biotechm.html

15 http://www.selectagents.gov/securitydoc.htm

16 http://www.aphis.usda.gov/programs/ag_selectagent/ag_bioterr_toxinslist.html

17 https://www.ippc.int/IPP/En/default.jsp

18 http://www.nappo.org/

19 http://www.inspection.gc.ca/

20 http://www.senasica.gob.mx/2007/190607/

21 http://www.eppo.org/index.htm

containment. The Committee ensures compliance with state, federal, and 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 breach of containment. To facilitate timely disposal of experimental materials, the IBC may adopt a closeout policy that provides the project leader with written notice of project termination dates.

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.

Containment or Quarantine Officer

A designated containment or quarantine officer is required for USDA-inspected containment facilities housing materials under permit. They are at minimum responsible for the daily operation and maintenance of the containment facility. It is not uncommon for this individual to be a researcher, principal investigator, BSO, greenhouse manager, permittee, or director of a larger unit. Responsibilities often include creating and implementing standard operating procedures, ensuring that guidelines are followed, responding to security or containment breach issues, training others, and handling packages under permit.

Principal Investigator

The Principal Investigator (PI) is ultimately responsible for the research project and for ensuring compliance with biosafety standards. The PI functions as project manager as well as researcher, bearing responsibility for training and supervising personnel, communicating with the IBC, BSO, regulators, greenhouse manager and support staff, and correcting any operations that may result in a loss of containment. Based on the nature of the research, the PI recommends a containment level designation for the project and, in accordance with the *NIH Guidelines* and/or APHIS requirements, develops the necessary containment protocols. The PI is also responsible for all APHIS-regulated materials. An IBC review can verify or modify the protocols and containment level recommended by the PI.

For all experiments using GE plant material, 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 beginning the experiment, in addition to those 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 the containment requirements of the ongoing research. In most cases, a brief orientation session is sufficient to explain the nature of the research, the plant material (or other contained organisms), and any special procedures to be followed when handling or working around them. For example, if transgenic microbes are tested for their ability to associate with roots, the PI may require that runoff water is collected and treated prior to disposal. A basic understanding of the biological systems involved considerably helps the staff comply with containment procedures. Both the greenhouse manager and the PI should work with the staff to ensure compliance with safety procedures and standards.



Section III. Biosafety Levels

BIOSAFETY LEVELS PROVIDE A DESCRIPTION OF A combination of administrative controls, work practices and procedures, equipment, and facility features required to achieve a designated level of containment. The purpose of containment is to prevent the transfer of propagules and other organisms from inside the greenhouse to receptive environments outside the greenhouse.

Confusion often arises over what constitutes a particular biosafety level, especially when planning to design or retrofit a containment facility. Section III of the *NIH Guidelines* describes the four physical containment levels for experiments involving recombinant DNA molecules. Appendix P of the *NIH Guidelines* was created to categorize experiments for recombinant DNA research involving plants according to specific risk criteria. Experiments may be assigned to one of four biosafety levels, BL1-P through BL4-P, using the criteria in Appendix section P-II. The *Guidelines* also specify the physical and biological containment conditions and practices required for greenhouse experiments for each biosafety level.

It should be noted that USDA-APHIS does not designate a biosafety level for research when issuing permits. They instead publish guidelines for a construction standard for containment, with suggested methods for achieving the standard. Containment measures for regulated articles are implemented on a case-by-case basis. Experiments that have 'nonregulated' status (see Glossary) are exempt from APHIS oversight. The USDA-APHIS will inspect containment facilities prior to issuing permits and may inspect at any time while the permit is active. The permit holder is responsible for ensuring that containment is not breached.

Laboratory biosafety, which uses the BMBL designations BSL-1 through BSL-4[22], is primarily concerned with worker and research subject protection as well as environmental protection. When working with plant materials, environmental protection is the primary concern, though worker protection can be a concern in rare situations. The USDA-ARS created the biosafety level designation BSL-3Ag for special situations in which high containment is required in an agricultural setting[23]. BSL-3Ag was created as part of an internal agency security protocol and has become widely accepted. Although used primarily for animal and zoonotic diseases, plant work has sometimes been placed under the designation.

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[22] U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes of Health. 2007. *Biosafety in Microbiological and Biomedical Laboratories*, 5th edition.

[23] http://www.ocio.usda.gov/directives/doc/DM9610-001.pdf

There are other biosafety level designations found in the US as well as around the world for large animals, plant pests, and arthropods. In all cases, containment measures increase as the numerical designator increases. The NIH biosafety levels are generally universally accepted as the most relevant for plant work and thus are used in this Guide.

A brief description of the four biosafety levels and the criteria used for assigning experiments to each category are provided here. When making a biosafety level assignment, the IBC members consider the following criteria:

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

The determination of the appropriate level of containment is based on sound scientific principles and a thorough knowledge of the recipient organism and its mode of dissemination. A brief comparison of criteria used to assign an appropriate biosafety level is shown in **TABLE 2** (*see right*). 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 with the addition of biological containment measures, indicated by the "+" sign. (Biological containment is described in Section IV, Strategies of Containment.)

According to the *NIH Guidelines*, 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 are potentially 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 oversight under the *NIH Guidelines* and do not require the approval of the local IBC. However, the USDA, EPA, or local regulators may make their own determinations of experiments that are exempt from oversight. According to the *Guidelines*, 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 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

TABLE 2. Suggested Criteria for Assigning Biosafety Levels

CRITERIA	TRANSGENIC PLANTS	TRANSGENIC MICROBES		TRANSGENIC ARTHROPODS AND THEIR 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			BL2-P or BL1-P+	BL3-P or BL2-P +
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	
PMP & PMI	BL3-P			
Select Agent plant pathogens	BL3-P	BL3-P	BL3-P	BL3-P+ or BL4-P

*EIA – Exotic Infectious Agent

to exchange DNA by well-established physiological means. Appendix A of the *NIH Guidelines* contains a periodically revised list of these natural exchangers[24]. 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 is used to provide 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. This designation also includes sterile plants or those rendered non-propagative.

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 *Agrobacterium* genes known to affect root colonization, or plants using *Agrobacterium* DNA segments as part of the transformation process.

The *NIH Guidelines* note in Section III that physical containment requirements may be reduced to the next lower level by applying appropriate biological containment practices. For example, using a genetically attenuated strain of a viral pathogen would reduce a BL2-P level experiment to a 'BL1-P + biological containment' (BL1-P+) designation.

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 also assigned to transgenic research that uses the entire genome of an indigenous infectious agent or pathogen. This level of containment is likewise 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. In addition, the BL2-P classification applies to experiments using plant-associated transgenic insects or small animals if they pose no threat to managed or natural ecosystems. Again, the addition of a biological containment measure can often reduce the biosafety level to the next lower designation.

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 impact on the environment. This category also applies to non-GE 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

[24] http://www4.od.nih.gov/oba/rac/guidelines_02/APPENDIX_A.htm

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 *Aspergillus flavus*, the organism responsible for producing the potent vertebrate mycotoxin aflatoxin

Biosafety Level 4 for Plants (BL4-P)

BL4-P is recommended for experiments involving certain exotic, readily transmissible infectious agents that are potentially serious pathogens of major US crops. Additionally, human pathogens or vaccines made in plants could, in some cases, cause serious human illness and would certainly be designated BL4-P. To date, few greenhouses have been built that satisfy BL3-P and BSL-3Ag criteria and even fewer that meet BL4-P criteria, as they are difficult and expensive to design and build. In addition, only a very small percentage of greenhouse experiments require such a high level of containment. An example of research at the BL4-P level could be an experiment designed to test the ability of the maize streak virus coat protein to protect corn plants against infection by that virus, using its leafhopper vector, *Cicadulina* spp., in challenge inoculations. This devastating virus is not found in the United States; however leafhoppers capable of transmitting the virus are present. Thus an experiment using both a serious pathogenic virus with its vector poses a significant risk should they escape the containment facility. Note that the transgenic maize plant does not pose a risk itself. In this case, the greenhouse is the primary containment barrier to insect vector escape.

Biosafety Level 3 Agriculture (BSL-3Ag)

BSL-3Ag is a unique containment designation developed by the USDA Agricultural Research Service (ARS) for work that involves certain biological agents in large animal species. The need for high level containment prompted ARS to apply some of the same design principles used with animal containment to plant facilities. As in the NIH-P biosafety levels listed above, the emphasis is primarily on environmental protection. In fact, BSL-3Ag shares many of the requirements found in BL3-P and BL4-P, with facilities themselves acting as the primary containment barrier (see **TABLE 6** *Important Features of a Containment Greenhouse Facility* in section VI). The complete requirements for attaining BSL-3Ag are found in the *ARS Facility Design Standards, 2002*[25]. Appendix D of the CDC/NIH biosafety manual, *Biosafety in Microbiological and Biomedical Laboratories* (BMBL)[26] also describes BSL-3Ag requirements.

[25] http://www4.od.nih.gov/oba/rac/SSSept04/pdf/USDA%20BSL-3(Ag).pdf

[26] U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes of Health. 2007. *Biosafety in Microbiological and Biomedical Laboratories*. 5th edition.



Section IV. Strategies of Containment

THE BROAD ARRAY OF BIOLOGICAL RESEARCH PROGRAMS encountered in greenhouse settings necessitates the implementation of an equally wide variety of containment strategies. There are many examples of both organisms and applications requiring minimal to maximal containment. In addition, organisms and applications are often combined, generating additional containment issues. One can quickly appreciate that containment strategies and measures may vary widely to accommodate everything from whole plants with large, flying insects to microbial pathogens and pollen. A few of the organisms and applications requiring containment are listed here.

Organisms

Insects and mites
Microbes
Nematodes
Plant propagules, especially seeds and pollen
Whole plants

Applications

Biocontrol
Research with exotics
Plant-made pharmaceuticals and industrial compounds
Quarantine
Traditional and transgenic research

Almost any organism, from microbes to whole plants, can be easily transported into and out of a containment facility in a multitude of ways. When planning an experiment or constructing a containment facility, one must carefully consider all the many ways in which an organism can breach containment. The predominant route of inadvertent dissemination is via opportunistic organisms that hitchhike on personnel and their clothing, shoes, and personal items, poor adherence to prescribed protocols by staff, and air currents created when passing

through doorways. Other routes of escape include small animal intruders (birds, rodents, insects, etc.), irrigation and waste water, ventilation air currents, material handling equipment, refuse removal, and maintenance products and equipment. Another type of containment breach occurs when mobile research organisms confined to secondary cages or units within the facility escape and cross contaminate other experiments.

In general, containment is more difficult and requirements are more stringent if plant-*associated* materials, such as insects and microorganisms, are included in the experiment. If insect quarantine measures are required, managers should contact APHIS for additional guidance and, if an APHIS permit is required, be prepared to describe precisely the planned containment strategies.

Environmental protection is the primary goal of containment. The key to achieving this goal lies in acquiring a working knowledge of the factors that impinge on containment, including organism characteristics and behavior, biological interactions, experimental protocol, greenhouse qualities and limitations, routes of escape, and human factors. Although it is beyond the scope of this book to familiarize the reader with the biology and interactions of all possible organisms and research applications, the primary risks posed by representative classes of organisms requiring greenhouse containment are presented. The physical measures and equipment used to preclude the most frequent means of escape of these organisms are then considered.

ORGANISMS AND APPLICATIONS

GEOs

Recombinant DNA technology can be employed in almost every type of research organism and application system listed above. Containment of genetically engineered organisms is an exercise in risk management. The purpose of GEO containment, according to the *NIH Guidelines* 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 the experimental facility;
3. Avoid the inadvertent spread of a serious plant pathogen from a greenhouse to a local agricultural crop; and
4. Avoid the unintentional introduction and establishment of an organism in a new ecosystem.

These principles summarize the intent of all containment situations described in this guidebook.

Plants Engineered to Produce Pharmaceuticals and Industrial Compounds

Plants and associated organisms genetically engineered to produce plant-made pharmaceuticals (PMP) or plant-made industrial compounds (PMIC) are afforded special containment and regulatory scrutiny, even though greenhouse protocols are not extraordinary. The APHIS-BRS document *Draft Guidance for APHIS Permits for Field Testing or Movement of Organisms with Pharmaceutical or Industrial Intent*[27] outlines unique considerations for working with this emerging technology.

A draft Guidance for Industry document entitled *Drugs, Biologics, and Medical Devices Derived from Bioengineered Plants for Use in Humans and Animals*[28] was created collaboratively by the FDA and APHIS. This document is primarily concerned with field-grown material but mentions research conducted in greenhouses. Plants or other articles producing PMP and PMIC are considered 'in containment' when in a greenhouse or growth chamber, and as such are subject to the *NIH Guidelines*. Likewise, personnel must adhere to Good Laboratory Practice (GLP) or Current Good Manufacturing Practice (cGMP) standards. Although a full description is beyond the scope of this guidebook, briefly stated, GLP and cGMP codes stipulate that facilities must be easily disinfected, all

[27] http://www.aphis.usda.gov/brs/pdf/Pharma_Guidance.pdf

[28] http://www.fda.gov/cber/gdlns/bioplant.htm

activities are well documented, and the plant growing environment is precisely controlled and uniformly maintained throughout the facility.

Exotic Organisms

The containment, control, or eradication of non-native plants and related organisms that are or may become plant pests has been the focus of APHIS-PPQ since its inception. The most famous example of destruction caused by an exotic plant pathogen would likely be the potato blight epidemic of the 1840s in Ireland that resulted in famine and migration for 1.5 million Irish. A good source of information on exotic and invasive species can be found at *Invasive.org*, which is a joint project of The University of Georgia's Bugwood Network, USDA Forest Service, and USDA-APHIS-PPQ.

The high level of risk to agriculture that may be posed by exotic organisms necessitates the careful inspection and regulation of items intentionally brought across borders. Further, if material is permitted for a containment facility, APHIS will require inspection of the laboratories, tissue culture rooms, growth chambers, screenhouses, or greenhouses for containment integrity. Inspections are often conducted before the research is initiated, but are also likely to occur at any time during the permit period. Although fines and legal action can be levied for permit violations, APHIS would much rather assist researchers in achieving containment of exotic organisms. Permit applicants are encouraged to seek advice and schedule inspections to ensure sufficient containment measures are in place.

Insects and Mites

The regulation of plant-associated insects and mites and the strategies used to restrict their movement is a broad subject. A large number of these species carry not only plant diseases but human and zoonotic diseases. Because of this, containment guidelines were developed by members of the American Society of Tropical Medicine and Hygiene (ASTMH)[29], which are accessible on their website. Four Arthropod Containment Levels (ACL1-4) outline the suggested standard and special practices, equipment (primary barriers), and facilities (secondary barriers) needed for containing insects and mites. APHIS also offers permitting and containment guidance for non-indigenous arthropods[30] and for transgenic arthropods[31].

Organisms under Quarantine

Quarantines are established to reduce the risk of spreading insect pests, transmissible diseases, or invasive weedy species. Nursery and greenhouse plants are often subject to quarantine regulations, and containment greenhouses and screenhouses frequently serve as observation and treatment facilities of restricted materials before they are moved to non-infested locations. The materials are almost always under APHIS permit, but states or other entities may have additional regulations.

In general, inspections [of facilities housing Select Agent plant pathogens] have not resulted in major findings. Biocontainment of plant pathogens is adequately preventing accidental release of agents into the environment.

▲▲▲ **MIKE FIRKO AND CHARLES DIVAN,** USDA-APHIS

(2007 ABSA ANNUAL MEETING)



Select Agents

Certain plant pathogens that have the potential to be employed as bioterrorist weapons are placed on the HHS and USDA Select Agent and Toxin List[32], which is periodically reviewed and updated on their websites. If improperly used or released, these highly virulent pathogens could cause epidemics that would seriously harm the food supply and/or commodity markets. Regulations state that it is illegal to possess,

29 http://www.astmh.org/SIC/acme.cfm

30 http://www.aphis.usda.gov/plant_health/permits/downloads/arthropod_biocontrol_containment_guidelines.pdf

31 http://www.aphis.usda.gov/biotechnology/arthropods.shtml

32 http://www.aphis.usda.gov/programs/ag_selectagent/ag_bioterr_toxinslist.html

use, or transfer any of the listed agents without first registering with the CDC or USDA-APHIS. Researchers using agents on the List are responsible for managing both the biological and biosecurity risks associated with these pathogens. Information on compliance found on the CDC website[33] is primarily concerned with ensuring biosecurity. The 5th edition of *Biosafety in Microbiological and Biomedical Laboratories* states, "The objective of biosecurity is to prevent loss, theft or misuse of microorganisms, biological materials, and research-related information." Greenhouse containment for Select Agents would likely not be permitted below biosafety levels BL3-P or BSL-3Ag.

PHYSICAL CONTAINMENT

Primary physical containment is provided by both the facility and the containment equipment within the facility. Containment is maintained through the good laboratory practices of staff who adhere to the facility SOPs (standard operating procedures) to maintain the physical conditions required for containment and who are trained to notice when conditions are not normal and take swift action.

In order to plan and price a research greenhouse facility, the appropriate physical elements of containment must first be determined in logical order. For new construction, the initial consideration is the choice of a greenhouse site. With knowledge of the degree of containment required for the anticipated research, one must then assess the need for spatial separation from related activities, other buildings, and nearby crops, and the amount of human or vehicular traffic in the vicinity. Once the site is chosen, the next consideration is the type of plant growth facility (whether growth chamber/room, screenhouse, or greenhouse, either commercial or research, etc.), because the amount of air infiltration and hence potential routes of escape can vary widely among different design types. Floor plans, including decisions about the inclusion of vestibules and ancillary spaces, are determined next.

After the location, basic design, and floor plan of the greenhouse are determined, the selection of construction materials and features must be thoughtfully made. The type of glazing, sealing, screening, air flow system, and other features all affect the degree to which a greenhouse is capable of isolating plants, plant parts, and associated organisms from the surrounding environment. These systems also are important for keeping unwanted pests out of the greenhouse. Proper door hardware is critical at all levels of containment. It is also vital to consider utility routing plans—including plumbing, electrical, and communications—to allow maintenance accessibility without compromising natural light or containment. When air borne material is a safety issue, close attention must be paid to the specifications for air supply, exhaust, filtration, and pressurization. One must also remember that utilities and building materials can be adversely affected over time by corrosive disinfection products and systems, and choose appropriate materials wisely. Specialty hardware such as insect traps and foot baths, and the level of required security, are also important factors to consider early in the planning process.

Ancillary facilities and systems are integral to achieving high levels of physical containment in research greenhouses. These include the headhouse, equipment rooms, shower and change rooms, and laboratories attached to the greenhouse. Growth chambers, tissue culture rooms, incubators, and biological safety cabinets are commonly used for containment purposes. Biosafety regulations for these systems are included in Appendix G of the *NIH Guidelines*, which specifies physical containment standards for the laboratory.

Research carried out under BL1-P and BL2-P containment levels requires little more than the basic facilities, equipment, and protocols common to most research greenhouses. However, greenhouses that provide higher level (BL3-P, BL4-P, and BSL-3Ag) containment require advanced features that are expensive to build and operate. Retrofitting existing facilities to meet high containment standards is at best expensive and may be impossible from a design standpoint; consequently, the cost of greenhouse containment at these levels may be prohibitive for

[33] http://www.selectagents.gov/securitydoc.htm

many institutions. The book *Containment Facilities and Safeguards for Exotic Plant Pathogens and Pests*[34], which unfortunately is no longer in print, contains descriptions of some high security containment and quarantine facilities operating around the world. (The reader should know that several high containment facilities have been constructed subsequent to the printing of the book but, for a variety of reasons, will not be catalogued.)

Glazing

'Glazing' refers to any transparent material (such as glass) used for windows. Properly installed and regularly maintained greenhouse glazing of any typical material can provide a suitable barrier for a variety of research materials. The type of glazing most commonly used is single panes of tempered glass installed by lapping each pane over the one below. The degree of care taken in installing and maintaining the glazing determines its overall effectiveness. Improperly installed or loose-fitting glazing material can leave gaps through which contained materials or outside contaminants could pass.

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. **FIG. 1** (*see above*) illustrates where silicone sealant is applied within a conduit carrying data cables. The cables are passing through the primary containment barrier and thus require sealing. Firestop products are a good choice for sealing conduits that carry electrical and data cables.



FIGURE 1. Caulking around Service Intrusion*

Screening

When properly sized, installed, and maintained, screens can exclude pests and pollinators from a greenhouse or, conversely, keep experimental organisms in. The integrity of a screening system is determined by several factors, including the nature of the screening material, the size and morphology of the insects being excluded, the screen hole size and shape, and the amount of air pressure that will be applied on either side of the

TABLE 3. Screen Hole Size for Excluding Common Greenhouse Insect Pests[35]

ADULT INSECT	SCREEN HOLE SIZE		
	mesh*	microns	inches
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.

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

* Courtesy Dave Hansen, University of Minnesota, Agricultural Experiment Station

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

screen. The maximum hole size generally capable of restricting common greenhouse pest species is given in **TABLE 3** (*see page 21*). Commercial products that offer fine mesh for excluding small insects have trade names such as Anti-Virus™, Econet™, and No-Thrips™ screening.

Pollen Filtration

Pollen containment can be difficult, requiring specialized materials and equipment. Pollen size, shape, viability, and 'stickiness' affect its ability to become an airborne risk. Filtration media are used to trap spores and pollen. Netting is available with holes as small as 100 microns that can trap larger pollen grains, like maize. However, most pollen is smaller than 100 microns and therefore requires specialized fabric filters. The fabrics, constructed from 'meltblown' or 'spun' fibers, offer a range of pore sizes from less than 1 micron to 100 microns. The widely available high efficiency particulate air filters (HEPA) are 99.97% effective for particles larger than 0.3 microns in diameter. The effectiveness of filtration is dependent upon the correct choice of filter, proper filter installation, and regular maintenance. These criteria are especially critical in the plant growth environment.

Air Pressure

Containment of airborne pollen, spores, and insects is a significant challenge. One containment strategy it is to create negative air pressure within a facility. Maintaining the containment area under negative pressure will keep contaminated air from flowing into adjacent, uncontaminated areas and/or the outside environment and thus reduces the probability of spreading spores, pollen, or insects outside of containment. Negative pressure is created when the amount of air exiting a space exceeds the air intake. Negative air pressure pulls air into a room whenever a door is opened, and therefore it is sometimes referred to as 'inward air flow'. Negative pressure bench-top chambers are often used to 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. 2**, *see below*).

Conversely, it may sometimes be useful to create positive air pressure within a greenhouse to prevent insect pests, pollen, or other contaminants from entering from the outside. An example is research involving a gas exchange plant/insect system in which an unwanted pest or insect could compromise the experiment.



FIGURE 2. Negative Pressure Bench-top Containment Unit

Vestibules

Most insect containment guidelines suggest using double-door vestibules or anterooms, which may be equipped with light traps, airlocks, air wash devices, interlocking doors, and differential pressurization.

Doors should slide or open outward and be self-closing. To deter insects, vestibules must be darker than adjacent rooms and are often painted black to reflect light. Darkness is also required for effective light trap operation. Vestibule lights should turn off automatically when a door is opened. Vestibules are commonly located at greenhouse entries, emergency exits, or other areas housing the organism of interest (**FIG. 3**, *see right*).

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 met. Researchers may fashion cages out of metal, wood, glass, or screen; however, effective commercial models are also available. The Bugdorm® insect cage (**FIG. 4**, *see below*) is available from biological and greenhouse supply companies. The sleeved-style cage depicted here is recommended so that plants and arthropods can be manipulated without breaching containment.

Small seeds such as *Arabidopsis* can easily be carried inadvertently out of a greenhouse or growth chamber. Consequently, specialized growing apparatus such as the Aracon™ system have been developed to both collect and contain *Arabidopsis* seed.

Location

The geographical location of a greenhouse provides an element of physical containment. For example, the study of a tropical plant disease in a location that routinely has severe winter weather may prohibit the survival of the disease-causing organism and/or the plant host outside the facility. Likewise, research involving a crop pest or noxious weed 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 consider what type of agricultural practices and crops might be found in adjacent areas over the lifespan of the greenhouse. Generally, work with GEOs has not required remote or otherwise special siting, since other containment safeguards are usually adequate.



FIGURE 3. Containment Entry Vestibule with Overhead Airwash, Card Reader, and Differential Pressure Monitor*



FIGURE 4. Bugdorm® Insect Cage*

* Courtesy Dave Hansen, University of Minnesota, Agricultural Experiment Station

TABLE 4. Minimum Isolation Distances, Periods of Post-Harvest Land Use Restriction, and Minimum Monitoring Frequency for Confined Research Field Trials*

CROP	MINIMUM ISOLATION DISTANCE	PERIOD OF POST-HARVEST LAND USE RESTRICTION	MONITORING FREQUENCY	
			TRIAL PERIOD	POST-HARVEST PERIOD
Agrostis palustris Huds. (creeping bentgrass)	300 m (without cropping)	3 years	weekly, daily and every 3rd day	every 2 weeks
Beta vulgaris L. (sugar beet)	3 m and harvest before flowering	2 years	weekly	every 2 weeks
Brassica carinata A. Braun (Ethiopian mustard)	200 m from other *Brassica spp.* 50 m from weedy relatives	3 years	weekly	every 2 weeks
Brassica juncea L. (brown mustard)	200 m from other *Brassica spp.* 50 m from weedy relatives	5 years	weekly	every 2 weeks
Brassica napus L. (argentine rape canola)	200 m from other *Brassica spp.* 50 m from weedy relatives	3 years	weekly	every 2 weeks
Brassica rapa L. (polish rape canola)	400 m from other *Brassica rapa* 200 m from other *Brassica spp.* 50 m from weedy relatives	5 years	weekly	every 2 weeks
Capsicum annuum L. (pepper)	20 m	1 year	every 2 weeks	every 2 weeks
Carthamus tinctorius L. (safflower)	400 m	2 years	weekly	every 2 weeks
Cucurbita pepo L. (squash)	650 m	1 year	weekly	every 2 weeks
Glycine max (L.) Merr. (soybean)	10 m	1 year	every 2 weeks	every 2 weeks
Helianthus annuus L. (sunflower)			weekly	every 2 weeks
Hordeum vulgare L. (barley)	10 m	2 years	every 2 weeks	every 2 weeks
Lens culinaris Medik. (lentil)	10 m	1 year	every 2 weeks	every 2 weeks
Linum usitatissimum L. (flax)	10 m	2 years	weekly	weekly
Lolium perenne L. (perennial ryegrass)	300 m (without cropping)	3 years	weekly, daily and every 3rd day	every 2 weeks
Lycopersicon esculentum Mill. (tomato)	20 m	1 year	weekly	every 2 weeks
Medicago sativa L. (alfalfa)	300 m (without cropping)	3 years	weekly, daily and every 3rd day	every 2 weeks
Nicotiana tabacum (tobacco)	400 m	1 year		
Phalaris canariensis L. (canary seed)	10 m	2 years	every 2 weeks	every 2 weeks
Picea spp. (spruce)	removal of seeds and pollen cones	2 years minimum	monthly, twice a week during cone formation	monthly
Pisum sativum L. (pea)	10 m	1 year	every 2 weeks	every 2 weeks
Populus spp. (poplar)	removal of inflorescences	3 years minimum	monthly, twice a week during flowering and budburst	monthly
Sinapis alba L. (white mustard)	400 m from other *S. alba* 50 m from other *Brassica spp.* and weedy relatives	5 years	weekly	every 2 weeks
Solanum tuberosum L. (potato)	one blank row (~ 1 meter)	2 years	weekly	every 2 weeks
Trifolium repens L. (white clover)	300 m (without cropping)	3 years	weekly, daily and every 3rd day	every 2 weeks
Triticum aestivum L. (wheat)	30 m	2 years	every 2 weeks	every 2 weeks
Vitis spp. (grapevine)	bagging of flowers	3 years minimum	monthly, weekly at pollen shed	monthly
Zea mays L. (corn)	200 m	1-year	weekly	every 2 weeks

* Source: Canada Food Inspection Agency, http://www.inspection.gc.ca/english/plaveg/bio/isole.shtml

LAYERING PHYSICAL AND BIOLOGICAL CONTAINMENT

Containment is often enhanced by means of layering—i.e., using more than one type of physical containment method at one time, using a biological confinement method within a containment facility, or combining both physical and biological containment methods. A primary advantage to layering is that by combining methods, the effectiveness of confinement is increased, and hence the requirements may be lowered to next lower biosafety level. For example, 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. Suitable physical containment would be provided by a greenhouse fitted with AntiVirus™ screening or by conducting the experiment in insect-proof cages within a conventional greenhouse. Containment would be further enhanced by removing alternate host plants for the virus both within and outside of the greenhouse and by applying stringent insect control measures in the surrounding area.

Biological containment is defined as the use of biological means to block plant sexual and vegetative reproduction and to prevent the spread and persistence of genetic material in the environment. Many methods of biological containment are available[36], including redundant systems that combine multiple methods of containment. For example, chloroplast engineering restricts the transgene to the chloroplast genome, preventing pollen mediated gene flow. Genetic Use Restriction Technology (GURT) is used to switch off the gene(s) for either a variety (V-GURT) or a trait (T-GURT). V-GURT technology produces plants with sterile seeds; and plants modified with T-GURT produce viable seed but repress expression of the engineered trait until it is 'turned on' by application of a chemical trigger. Virus Induced Gene Silencing (VIGS) is a technique that creates plants with no transgenes in pollen or seed. Plants engineered with this system could be used to express a protein of interest without presenting a risk to the environment from viable propagules.

Physical and biological containment methods are often combined as an added measure of safety. The biological methods that create sterile organisms or nonviable seeds may not always be 100% effective; hence layering with physical containment methods increases the overall efficiency of containment and may permit researchers to pursue this type of experimentation at lower containment levels.

Though not a method of containment, technologies are also available to create transgenic plants lacking antibiotic or herbicide resistant selectable marker genes, further reducing potential risks to the environment. 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 usually have a good understanding of the biological systems involved. Hence, they are at liberty to devise other means of layering containment in their experimental protocols, subject to review by the IBC and/or regulatory agencies.

Containment of Plants

Procedures that can prevent the dissemination of genetic material by pollen or seed include the following examples.

- Use genetic engineering techniques that localize transgenes in non-propagative plant parts, prohibit plant propagules from surviving, or confer plant sterility
- Cover or remove flower and seed heads to prevent pollen and seed dispersal
- Harvest plant material prior to sexual maturity
- 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

[36] National Research Council. 2004. *Biological Confinement of Genetically Engineered Organisms*. National Academy Press, Washington, D.C. 255 p.

Plant breeders commonly bag flowers to prevent cross-pollination with nearby plants. Female flowers are covered to prevent insect pollinators or windblown pollen from landing on a receptive surface. Male flowering structures are bagged to prevent pollen dissemination by insect vectors, wind, or mechanical transfer. Paper and glassine bags are most commonly used to cover flower heads. Flower heads can be removed prior to pollen or seed production when the research protocol does not require seed collection.

To be considered an environmental risk, transgenic pollen must fertilize receptive plants outside the containment facility. To reduce the risk, 'isolation distances' have been determined, which are the minimum distances required between varieties of the same species to prevent cross-fertilization by pollen dispersed by wind or gravity. Isolation distances can be affected by environmental factors, whether pollen is dispersed by wind or insects. **TABLE 4** (*see page 24*) shows the recommended minimum isolation distances, post-harvest land use restriction periods, and minimum monitoring frequencies for the current year of a field trial and for the years of post-harvest land use restriction. Current research on GE maize demonstrates that border rows, which serve as a buffer zone to trap pollen, significantly reduce the isolation distances required to prevent cross-fertilization[37]. Regulations (cited above) for working with PMP/PMIC crops require the addition of border rows to aid in isolation.

Depending on the location of the containment facility, plant material can be confined by carefully choosing the time of year that an experiment is performed. For instance, growing transgenic sunflowers in a greenhouse only during the winter in northern climates ensures that any escaped pollen would not be viable, as no compatible species would be growing in the area at that time of year.

Containment of Microbes

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 or persist if they are dispersed. Biological measures often provide the best containment option. The following methods may help prevent dissemination of microorganisms.

- Avoid creating aerosols when inoculating plants with 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 susceptible plants are not growing nearby
- 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
- Treat runoff water to kill living organisms

Containment of Insects

Insect and mite containment is difficult in a greenhouse facility. Entomologists who raise insects on greenhouse plants continually work 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 escaped 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 insects in greenhouse cages
- Destroy all pollinating insects in cages after pollen transfer

[37] Thomison, Peter. 2004. *Managing "Pollen Drift" to Minimize Contamination of Non-GMO Corn.* Ohio State University Extension Factsheet AGF-153-04.



Section V. Management Practices

CONTAINMENT STRATEGIES ARE EFFECTIVE ONLY WHEN greenhouse personnel understand and adhere to established procedures for handling contained material. Before entering the greenhouse, all staff working around the organisms of interest should be fully informed of 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. Maintaining containment depends on committed staff who not only insist on compliance but are the first to notice unusual conditions and instigate an investigation of the problem.

Access

Routine access to facilities housing confined research material is restricted, regardless of the biosafety level. Such restrictions are intended to minimize the spread of pollen, seed, or other propagative material that could be carried by people moving between rooms or facilities. Public visits are generally discouraged if not prohibited entirely.

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; visitors who have a special interest in the research are escorted. Some facilities require access through a vestibule which may have interlocking doors, light traps, or air wash devices. Exiting through a shower and change room, which can serve as the vestibule, is required for some high containment programs.

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 or key card to enter. Signs must be posted at the entryways, indicating that access is restricted for the research program 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 regular personnel, visitors, 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 generally wear their usual street or lab clothing. However, lab coats that remain at the facility are recommended and often required. It is important that no personal materials such as backpacks, coats, or purses be brought into containment facilities without good reason, as they may allow pests to 'hitchhike' out. Special care should also be taken to ensure footwear do not convey organisms from the facility. Eating, drinking, and smoking can create a multitude of problems and should be prohibited. Hands are a primary route of disseminating organisms, so wearing disposable gloves is encouraged upon entry to the facility or when handling live material, and hands should be washed carefully when leaving.

For entry into BL3-P greenhouses, disposable lab gowns, gloves, caps or hair nets, and/or foot coverings are usually required. This apparel must be removed before leaving the facility and decontaminated (usually by autoclaving) before washing or disposal.

BSL-3Ag and 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. Showering upon entering is required only if there is concern that cross-contaminating organisms will be brought into the containment area from the outside. Users are also required to remove all street clothing and put on protective clothing before entering. Likewise, personnel leaving the facility must remove protective clothing before showering and exiting. The *NIH Guidelines* require that clothing is stored in the inner change room and autoclaved before laundering. Disposable apparel can be destroyed by autoclaving or incineration.

Signage

APHIS requires posting signs to indicate that access is restricted to authorized personnel for facilities containing material covered under an APHIS permit, i.e., USDA Regulated Material. The wording on signs depends on the material present, which is clearly explained in the permitting process. 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 sign as long as this is not confidential information. The sign should state the name and telephone number of the responsible individual(s), 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.

Information on signs should not conflict with or compromise security measures. It is prudent, if allowed by regulation, to omit an individual's contact information if to do so may present a security concern. Also, use of the universal biohazard symbol should be reserved for its intended purpose—to protect people from infectious agents. Misuse or overuse of this symbol conveys a danger that may not exist and/or desensitizes people to this important icon. Signage used to identify emergency exits is required as per standard building codes. When under APHIS permit, signs should state **USDA-APHIS Containment Facility—Emergency Exit Only**.

Transgenic material in a greenhouse room must be marked to distinguish it from non-transgenic organisms, such as plants serving as experimental controls or not involved with the experiment. If GEOs under APHIS permit are in a greenhouse with a non-transgenic variety of the same species, APHIS recommends that the two groups (or more) be spatially separated to avoid inadvertent cross pollination. Temporal separation by avoiding overlapping flowering times is also effective.



FIGURE 5. GE Plants Marked with Barcodes*

It is recommended that GEOs have a designated boundary on the bench, using color-coded markers, for instance. In addition, individual pots, bench sections, or entire benches can be marked with stakes or signs to identify the plant and the primary genetic modification; for example, "Soybeans with viral coat protein gene." Barcode labels are commonly used to track research data on individual plants (**FIG. 5**, *see above*). Regulatory information can easily be included in this system. All organisms in the room must be treated in accordance with the highest level of containment required by any experimental material present.

Storage and Handling

Plant parts, cultures, whole plants, and seeds are routinely stored and manipulated in containment facilities. Coolers, freezers, and growth chambers equipped with locks are recommended for storage. Transgenic seed should be stored in a locked cabinet located preferably in a greenhouse room to minimize handling in unconfined spaces, and should be clearly identified and labeled to distinguish it from other stored seeds or materials in the cabinet. Cabinets or storage areas housing material under APHIS permit must be clearly identified with signs. Seed that is stored or handled outside the area of containment, such as in a cabinet or on a potting bench in a headhouse corridor, should be kept in a spill-proof container. Greenhouse personnel should take ordinary precautions to prevent seed germination in unwanted locations. Threshers, seed counters, and related equipment used to process seed should be easy to thoroughly clean. For some operations, dedicated equipment may be required to ensure that mixing between runs or trials does not occur. Regardless, all waste material and unused seed should be decontaminated appropriately for the risk involved.

Transfer of Materials

The *NIH Guidelines* specify requirements for transporting experimental materials to and from

* Courtesy Kent Schnoeker, The Salk Institute

greenhouses for levels BL2-P – BL4-P. For facilities designated BL2-P and higher, transgenic material in the form of seeds or propagules, potted plants, trays of seedlings, etc. must be transferred in closed non-breakable containers. For BL3-P and BL4-P containment, the guidelines require that experimental materials are also enclosed in a secondary sealed container for transport. The exterior surface of the secondary chamber is 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.

Special consideration is given to opening and handling incoming packages. The material is generally moved inside tissue culture equipment, growth chambers, or greenhouses after being opened in a biological safety cabinet or sleeved cage within the containment area. Movement of APHIS permitted material, especially Select Agent registered material, is restricted. Permits specifically ask that all routes of travel are documented.

Termination: Sterilization, Disinfection, and Disposal

To prevent the survival of organisms unintentionally transported outside the greenhouse environment, experimental materials must be rendered biologically inactive (devitalized) before disposal. Termination and subsequent validation 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 GE research materials, taking into consideration the biosafety level of the experiment and the volume of material to be handled. The project PI is considered the responsible party for ensuring proper termination and disposal of all materials. Abandoned or forgotten experimental materials are not an infrequent problem for greenhouse managers. An institutional policy can help to prevent or remediate the problem that occurs when a PI leaves material in the greenhouse due to death, resignation, or simple oversight.

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 by several methods:

- Heat via steam, hot water, incineration, or heating coils
- Chemical treatment
- Freezing
- Composting
- Desiccation

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. Sterilization boxes with electric heating coils that deliver temperatures of 60 – 93 ºC are also common. The standard practice of heating materials to 85 – 100 ºC for 30 minutes will kill almost all plant-associated organisms. To avoid killing beneficial soil organisms, soil is often pasteurized for 30 minutes by adding air to the steam, resulting in a 'cool', 70 ºC steam[38]. APHIS guidelines suggest treating soil and other solid wastes at a minimum of 104 ºC for three hours before disposal when working with fungal, viral, or nematode plant pathogens under permit. Regardless of mission or method, validation is recommended because it is not uncommon to find portions within the media that do not reach the desired temperatures.

Material from smaller experiments can be inactivated by autoclaving all plants, plant parts, containers, and potting media. The recommendation is to autoclave materials at 15 – 30 lbs. pressure and 121 ºC for 15 – 180 minutes, depending on the type and state of the material being sterilized. At higher containment levels, the recommendation is to sterilize *all* materials leaving the greenhouse in an autoclave. A double-door, pass-through system for moving larger items in and out of containment is recommended. For liquids, a batch or pass-through type system that sterilizes effluent before it enters the

38 Horst, R.K.; Lawson, R.H. 1982 Soil sterilization: an economic decision. *Greenhouse Manager*.

sewer is a good choice (**FIG. 6**, *see below*). Liquid effluent normally must be cooled before release.

The standard practice of chemically treating greenhouse soil with methyl bromide, chloropicrin, and similar products is being replaced by steam methods due to toxicity concerns. The chemosterilants ethylene oxide (EO) and vaporized hydrogen peroxide (VHP) are used in high containment facilities but require specialized application equipment. An EO chamber is used when the heat of autoclaves (> 60 °C) would damage equipment that needs to leave containment. VHP is applied using a special generator to sterilize all exposed surfaces. This is an ideal method for decontaminating small laboratories and related work rooms, as there is no toxic residue.



FIGURE 6. Liquid Effluent Sterilizer*

▲▲▲ **Position expensive sterilizers so they can serve multiple zones within a facility.**

Containment laboratories may use common disinfectants such as sodium hypochlorite, phenols, formaldehyde, glutaraldehyde, and alcohol. Chlorine as well as non-chlorine-based greenhouse disinfectant solutions that are safe for applicators and the environment are easily obtained from grower supply houses. The gravel under benches in BL2-P facilities can be decontaminated by, for example, treatment with a 10% sodium hypochlorite (household bleach) or similar solution. Periodic cleaning of all growing area surfaces with standard cleaning solutions or plain soap and water is highly recommended. Cleaning alone can be an effective decontamination method but also serves as preparation for VHP or any other surface sterilization method. A thorough discussion of disinfecting products and methodologies is available in Appendix B of the *BMBL*.

Freezing is a common method for killing adult arthropods but has limited use as a sterilant. For large volumes, composting is an acceptable treatment for experimental plant and soil materials that pose no recognized harm to the environment. Plants without seeds can be devitalized through desiccation simply by withholding water, or they can be chopped or minced into 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. Furthermore, incineration may conflict with local ordinances. 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.

Regardless of the method, decontamination must be appropriate for the organisms of interest. It is foolhardy to believe that simply applying a spray, vapor, or a wipe-down is adequate. Time and

* Courtesy Dave Hansen, University of Minnesota, Agricultural Experiment Station

temperature criteria for the targeted organisms, autoclave test strips, and equipment maintenance and testing are but some of the tools needed for validating termination methods. Materials can be disposed with confidence once decontamination is validated.

Pest Control

The NIH, APHIS, and other guideline sources require a pest control program when working with contained organisms in a greenhouse setting. Rodents and birds can transport seed outside the facility. Insects and other organisms can transfer pollen and pathogens to receptive plants located either within or outside the containment area. Viral, fungal, and bacterial organisms are not uncommon in the greenhouse setting and can cause disease when the environmental conditions favor their development on suitable host plants.

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 or spray application of pesticides can be used to control certain insect pests such as whiteflies. Biological pest control measures may involve the introduction of predators, parasites, and parasitoids. Routine cleaning with hot water and detergent applied with a power washer is a very effective method for reducing pest populations. This technique is best implemented between experimental runs. Also, 'baking out' greenhouse rooms by raising the room temperature to 40 – 45 °C and holding for two to three days is a common practice to reduce pest loads. Care should be taken to not raise temperatures to the point of damaging equipment.

Greenhouse researchers commonly use 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 unwanted pests 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. A stringent pest control program, using physical, chemical, or biological control measures, alone or in combination, should be implemented and monitored for effectiveness.

Protocols should be instituted to avoid the transmission of microbial pathogens both within the greenhouse and to the outside environment. For example, Tobacco Mosaic Virus (TMV) can be spread easily by handling susceptible plants. An example of a practical protocol to avoid TMV contamination is in **Appendix II**.

Training and Reference Manuals

Personnel instruction is a critical component of good management practices. A reference manual should be prepared containing directives covering all safety and permit considerations pertaining to the research. The staff is required to read, comprehend, and agree to adhere to the instructions provided in the manual before entering the greenhouse. Personnel training is best accomplished through interactive sessions that include the PI, greenhouse manager, and other safety-management staff.

For BL2-P and higher facilities, emergency and contingency plans, as well as documents pertaining to routine operations, are required 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 in compliance with containment procedures. Conversely, relevant portions of the manual may be included in the project documents submitted for IBC approval.

Monitoring Containment

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 sentinel 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. Corridor light traps operated at night are useful to indicate the presence of insects that have escaped greenhouse rooms.

In addition to biological systems, many of the equipment systems in a high containment facility require periodic testing to monitor efficacy. For instance, in addition to monitoring for leaks in the greenhouse envelope, it is recommended that HEPA filtration, biosafety cabinets, and sterilization systems be checked annually.

Procedures for Loss of Containment

The integrity of the containment facility 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 a response. At BL2-P or higher, such events would 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 create a potential for loss of containment. Greenhouse systems that monitor automated environmental controls should have integrated local and remote alarms. Instances of human error, such as a door left open or the ordinary disposal of unlabeled transgenic materials, is actually a more common cause of containment loss than facility malfunctions or structural damage. Designated people should be promptly alerted when problems arise so they can make timely decisions about dispatching appropriate response personnel.

For BL-2P and higher facilities, both APHIS and the *NIH Guidelines* require contingency plans for handling emergency situations, including 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. APHIS continues to evaluate this process as lessons are learned. Permit applicants are advised to work closely with regulators to ensure that an unintended release is managed quickly and efficiently.

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, the NIH Office of Biotechnology Activities, and/or other designated authorities. APHIS regulated material that escapes or is stolen must be reported verbally and in writing within 24 hours of the incident. Telephone calls should be made to **1-301-734-5690**. A written description can be sent by email to: **BRSCompliance@aphis.usda.gov** or by courier to:

[Name of Regulatory Specialist]
USDA-APHIS-BRS
Compliance and Inspection Branch
4700 River Road, Unit 91
Riverdale, MD 20737

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. The use of barcode labels is a practical way to track material. 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 because 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. Notification and permit applications provide clear and detailed

instructions for record keeping when working with APHIS regulated material, Select Agents, and PMPs. Select Agent research requires thorough documentation of all plans for biosafety, security, and incident response, as well as transfer, training, equipment, inventory, and personnel access records. A central repository of all records in or near the facility assists both staff and inspectors.

Inspections

Greenhouses should be inspected periodically to ensure that containment measures appropriate for transgenic and other organisms are rigorously applied. 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.

Inspection checklists help ensure that a greenhouse facility meets the necessary physical, biological, and managerial requirements for a given Biosafety Level. The checklists facilitate IBC approval, provide an outline for internal monitoring, and serve as documentation of compliance. A sample of an APHIS "Biotechnology Facility Inspection Worksheet" is found in **Appendix III**. The questions in this worksheet are only examples of questions the USDA inspector may ask during a biotech facility inspection. The inspection officer is not likely to ask all of these questions, and additional questions may be added, depending on the specific situation. The officer records his answers to these questions based on 1) discussions with the researcher, 2) examination of documents, and 3) observations made during the inspection.

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 at minimum ask:

- Who are the responsible parties and how can they be contacted?
- What is the nature of the experiment 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 used to achieve containment?
- Are SOPs available and are they 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 cause of a containment breach?
- How are materials disposed at the end of the experiment?

Re-inspections by greenhouse managers should be conducted periodically. 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, because staff adherence to non-standard procedures may tend to relax over time.

A facility inspection is required to obtain an APHIS-PPQ 526 Permit (*Application for permit to move live plant pests, biological control agents, or noxious weeds*). However, the USDA in general does not certify or otherwise designate a greenhouse's suitability for research materials *unless* the researcher is applying for or operating under a permit from APHIS. Detailed inspections of facilities containing Select Agent organisms are conducted by APHIS or CDC staff.

USDA-PPQ uses a 22 page checklist questionnaire, titled "Inventory of Containment Facility for All Plant Pathogens" (Revised 11/01)[39], that provides an excellent inventory of the containment features and procedures for facilities that desire to import and contain any plant pathogens. The PPQ staff uses the completed inventory to determine if containment of a particular organism is possible in the facility before they issue a permit to the researcher. The inventory, which is to be completed only by a PPQ officer, includes an examination of the construction, equipment, and operational standards.

APHIS inspectors not only observe containment features but ensure that good laboratory practices are followed. The inspection process is detailed during the permitting process. After inspection, a letter is issued indicating the facility's adequacy for containing the organisms of interest. The permit process is then continued or finalized. APHIS may choose to conduct unannounced re-visits to facilities housing organisms under federal permit. Unannounced inspections occur during normal business hours and are a Standard Permit Condition.

Security

Vandalism is a continual concern for greenhouse managers. Individuals and organizations opposed to recombinant DNA research have targeted greenhouse and field trial research projects, often 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 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. In response to these threats, the USDA has authorized using an armed on-site security force to patrol the premises of BSL-3 and higher Federal facilities.

The use of Select Agents (SA) triggers a rigorous set of security requirements because these organisms are potential tools of bioterrorists. Anyone who handles SA is required to undergo a personal security risk assessment. Those without clearance are denied access to SA materials by physical barriers—locks or keycards on doors and storage containers—or by personnel. Maintenance, repair, and cleaning staff without approved security risk assessments are escorted by appropriate personnel while in areas housing SA but are not allowed direct access to the organisms. Physical facility security is high and access to SA materials is protected by at least three locking compartments. Compliance requirements for handling SA are reportedly challenging and time consuming.

Standard Operating Procedures

A required management tool when working with organisms under APHIS permit is the development and use of standard operating procedures (SOPs)[40]. The containment officer or similar responsible authority develops, updates, and implements the SOPs, which describe, for example, how to conduct the following activities:

- Use, maintain, and disinfect the facility and its equipment
- Respond to emergencies
- Maintain security
- Manage visitors
- Handle a containment breach
- Replace glazing in glasshouse

39 http://www.hawaii.edu/ehso/bio/plant_pathogen_checklist.pdf

40 http://www.aphis.usda.gov/plant_health/permits/downloads/containment_sop_outline.pdf

SOPs should be considered 'living' documents that may be modified as new permits are received, research practices change, equipment and personnel are added, and technological innovations arise. Annual reviews of SOPs are encouraged if not required. An SOP outline created by APHIS-PPQ is contained in **Appendix IV**.



Section VI. Designing and Building for Containment

If you have a choice between a complex solution or a simple solution with the same end result, choose the simple system. Someone in the future will thank you.

▲▲▲ **JON CRANE,** AIA

THIS SECTION ENCOMPASSES THE DESIGN AND CONSTRUCTION of containment greenhouses, including support facilities. Most hardware elements described in Appendix P of the *NIH Guidelines*, plus those listed as 'Suggestions' for meeting APHIS-PPQ Containment Facility Guidelines[41], are covered. Suggestions for various containment options are provided so that the reader can appropriately match features, i.e., reconcile cost with need.

Today's research greenhouses often meet BL1-P containment standards or can be retrofitted at minimal cost. Naturally, as containment requirements increase, so do the costs of building and operating facilities. Therefore, a new greenhouse intended for containment purposes should be designed and built with sufficient quality to maintain functionality over its lifespan. Employing a qualified and experienced design team is crucial for achieving high containment; also, as a general rule, high quality design and construction also provide the best long term value.

Building a Design and Construction Team

A team of experts is required to create a greenhouse containment facility. The researchers and staff who use and maintain the facility have the greatest knowledge of the biological aspects of the research. They work with designers—architects and perhaps engineers—to prepare plans and documents for construction firms, who are hired independently of the design team. Commissioning agencies are brought in to ensure that all systems work properly and that the construction complies with design standards. IBC members and regulators from APHIS and state agriculture departments should be notified and updated regularly as well as invited to join the design team. APHIS employs Containment Facility Evaluation Specialists who can discuss your intentions, review your design, and

41 http://www.aphis.usda.gov/plant_health/permits/organism/containment_facility_inspections.shtml

arrange for inspections, even though they do not attend regular meetings. The process of creating a containment greenhouse is greatly facilitated when good working relationships are maintained within this team of experts, especially when facing difficult decisions or encountering mistakes.



Perhaps the most important lessons learned were by visiting other facilities. In touring similar containment facilities elsewhere, we were able to learn from their successes and failures to determine the best choices for our facility. …Some ideas we found useful and others were abandoned after having seen how successful or unsuccessful they were in other facilities.

▲▲▲ **ROSEMARIE DE CLERCK-FLOATE, PATRICK PLUE, TIM LEE**

A TOUR OF THE LETHBRIDGE RESEARCH CENTRE INSECT-MICROBIAL CONTAINMENT FACILITY

Much valuable information can be gained from those who have experience building a high containment facility. Therefore designers of new facilities benefit by consulting with experienced managers and other end users of research greenhouse facilities early in the design process. In addition, the Association of Education and Research Greenhouse Curators[42] provides an electronic mail forum, web site, and annual meetings from which detailed information can be gathered. See **Appendix V Resources** for organizations that may be of assistance.

Construction Overview

The construction process consists of four basic phases: programming, design, construction, and commissioning. The programming and design phases are generally partitioned into several steps: pre-planning, planning, pre-design, schematic design, design development, and construction documents preparation. Each step in the process provides an increasing level of detail. The construction process uses either a design-bid-build or design-build approach. The latter approach allows construction to proceed as design details are finalized, which can be advantageous and time-saving for complex construction projects; but disadvantageous and costly if changes have to be made after construction has begun.

Regardless of stated contractual obligations, it is critical that the owner, designers, and builders meet regularly throughout all phases of the construction process. Likewise, all relevant 'owners', including facility managers, containment officers, greenhouse managers, and researchers, must convene frequently during the planning and programming phases. Usually ideas are gathered and budget costs are assigned in several iterations before plans are finalized. The owner group, architects, engineers, and regulators are all involved in the design phase, and often numerous versions of the design are formulated in the process.

Construction documents are prepared by the design team either in conjunction with the builder or as a separate process. Bids and pricing can then be finalized. Once builders are chosen and the project enters the construction phase, progress is tracked via weekly or bi-weekly construction meetings attended by the owner representative(s), architect, general contractor, sub-contractors, and commissioning agents. Although less end user input is required in this phase, a strong owner presence is needed and regular walk-through inspections are recommended. Changes in the design made after construction has begun are costly, as the design and construction already initiated may have to be removed, re-designed, priced, and reconstructed. This illustrates the importance of thorough and accurate planning prior to construction.

Finally, commissioning agents work with contractors to test and document that all systems operate correctly and according to specifications. This is the most critical phase; it may be carried out in-house or by system suppliers, but is often performed via contract with a third party to impart objectivity. The initial training of maintenance staff and other users may be included in the commissioning contract. Once commissioning is complete, equipment warranties may be started. APHIS inspectors prefer to make an initial visit once all systems are online. End users can begin operating the facility at this point.

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

Location

The major advantage greenhouses have over other types of growth facilities is the ability to capture natural light. However, this aspect may sometimes be in conflict with other considerations. For example, APHIS guidelines stipulate that greenhouses must be located in areas that minimize risk to the local environment, agriculture, and humans. Certainly high level containment benefits from a separate, dedicated facility located in a region where escaped organisms would have little chance of survival or otherwise affect the local agriculture or the natural environment. Consequently the choice of a building site may require finding a balance between the need to minimize potential risks versus other practicalities such as natural lighting, convenience, access, or affordability. Other location considerations range from climatic conditions to the effects of street traffic and the ease of access by personnel. On-grade space for constructing windbreaks may need to be considered. For convenience and other reasons, building a rooftop greenhouse is an option some may consider; however, APHIS discourages rooftop greenhouses for some types of containment because of increased wind exposure.

Layout

A containment greenhouse is seldom an entity unto itself. Supporting workspaces—headhouse preparatory space, laboratories, growth chambers, incubators, tissue culture facilities, inoculation chambers, and maintenance areas—are either located

Users—stay involved from the onset and make no assumptions that information is being distributed if it is not done formally. Ask for what you need first. There are cost/budget implications to everything!
▲▲▲ **DANN ADAIR,** UNIV. MINNESOTA, ST. PAUL, MN

Establish a commissioning budget early. ▲▲▲ **SANDY KELLEY,** UC DAVIS, CA



FIGURE 7. Typical Containment Facility Floor Plan*

* Courtesy RSP Architects, Minneapolis, MN.

directly adjacent to or within a reasonable distance from the greenhouse. Traffic patterns, process flow, and security measures should be analyzed to determine a layout that will optimize efficiency for all intended programs. The configuration should provide variable containment and growing conditions, control of access, and ease of movement. **FIG. 7** (*see page 39*) depicts an efficient and practical floor plan of a facility that could accommodate various levels of containment.

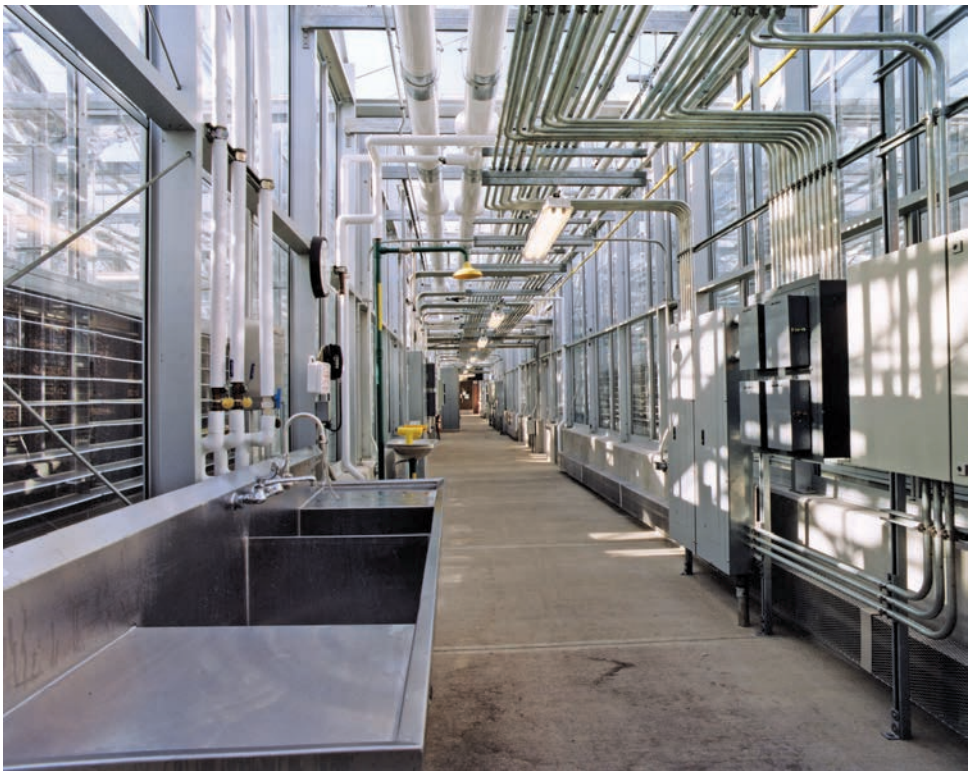


FIGURE 8. Common Corridor for Access, Preparation, and Mechanical Equipment*

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. For example, in a large room containing exempt, BL1-P, and BL2-P material, all research must conform to BL2-P containment standards. However, in many existing research greenhouses, interior space is divided into relatively large rooms with a common central corridor. This arrangement forces personnel to pass through each room or workspace to get to the succeeding one, making it difficult, if not impossible, to restrict access to an individual room and adhere to the *Guidelines*. A better layout is an array of small rooms or cubicles opening off one or more common walkways (**FIG. 8**, *see above*). This compartmentalized arrangement of small rooms will facilitate the coexistence of different containment levels and individualized environmental conditions within the larger structure.

Security Equipment

The same security systems commonly used in modern buildings can be used to prevent entry to the greenhouse of unauthorized persons, be they vandals, bioterrorists, or the simply curious. In addition to the measures described under Entry Doors and Locks (*see page 44*), the initial facility design should include security measures such as fencing, bollards, security cameras, and sensors. APHIS suggests that facilities be encircled with at least a 15 foot-wide, plant-free buffer zone, generally consisting of gravel or pavement, with a six foot or higher fence erected around the perimeter. Parking bollards are positioned to prevent vehicles from striking the facility. Closed circuit television cameras or webcams can be configured to detect and record activity near the greenhouse. Wired or wireless motion or sound detectors are also commonly used.

* Courtesy Rob Eddy, Purdue University

Glass-break sensors, for example, that are finely attenuated to sounds will signal only in response to the sound of breaking glass, thus reducing or eliminating false alarms. Window films that protect glass from breakage may be useful for certain applications. The loss of UV light that occurs with these films should be considered before applying to greenhouse glazing, however.

Select Agent (SA) rules stipulate that these agents or toxins must be secured behind at least three levels of locked barriers. SA protocols require periodic testing of all security equipment as well as keeping extensive logging records. Card readers are suggested not only for entry doors but also on freezers and other storage equipment.

While providing for maximum security, such as fencing, card key access, motion sensor HID, and/or cameras, do not engage in "overkill". An infrared beam motion detection system was installed in addition to the four other security systems. However, the six figure system remains unusable as it is too sensitive – not taking into account the almost daily fog that is endemic to the area.

▲▲▲ **DAVID HANTZ,** USDA, ALBANY, CA



Structure

Greenhouse structures are engineered to support cladding and other component loads as well as to withstand environmental stresses. High level containment facilities require a reinforced, rigid frame for both security reasons and to accommodate the weight of required double-paned, break-resistant, sealed glass. Regardless of construction method or purpose, building codes must be met or exceeded to ensure a quality, long-lasting structure. Climate conditions significantly vary across locales, affecting wind and snow loads placed on the structure. Hence, it is advisable that locally licensed engineers review and approve the structural design to certify that all relevant codes are met.



FIGURE 9. Aluminum Framing*

The structural system consists of a primary roof, a secondary structure, columns, foundations, and cladding. Modern greenhouse structures are framed with aluminum (**FIG. 9**, *see above*) or galvanized steel; though many older facilities are framed in wood or metal pipe. Prefabricated frames can be assembled from aluminum or galvanized steel trusses to speed construction. Rigid frames are built to accommodate secondary structural components—purlins, glazing bars and caps, and other materials that accept the cladding. Alternatively, curtain wall construction permits integration of the structure and glazing systems. The best design is one that reduces or eliminates hiding places for pests and organisms, offers good security, is long-lasting, is relatively easy to clean, and can withstand repeated disinfecting.

Although many construction styles are available, common designs for research facilities include: even-span with a standard peak; Venlo; attached even-span; lean-to; and gutter-connected ridge and furrow.

* Courtesy Rob Tanzer, Rough Brothers, Ohio

Make sure that all greenhouse equipment and areas have access for maintenance. At our facility, the side walls are 12', which makes the ridge vents, motors, screens, and rack and pinions over 20'. No allowance was made to access them with scaffold supports and maintenance from a 28' ladder is disallowed for safety reasons.

▲▲▲ **ROSEMARIE DE CLERCK-FLOATE, PATRICK PLUE, TIM LEE**

A *TOUR OF THE LETHBRIDGE RESEARCH CENTRE INSECT-MICROBIAL CONTAINMENT FACILITY*

Greenhouse construction also includes the headhouse and hallways, which, if constructed immediately contiguous to the greenhouse, are considered part of the containment area. Knee walls made of masonry concrete or block are often recommended to provide an extra measure of security at the greenhouse base. All masonry materials must have an epoxy coating or be otherwise sealed for BL3-P and higher facilities.

Environmental control and containment is enhanced through proper installation and fitting of all materials. Standards and guidelines on structural materials, as well as other greenhouse materials, can be found in the *Book of Standards* authored by The National Greenhouse Manufacturers Association (NGMA)[43].

Glazing

Greenhouses are clad with a variety of materials, ranging from clear glass to opaque insulating panels. Different glazing materials have widely varying degrees of light transmission, longevity, flammability, selective measures of strength, and infiltration by air and water. Standards of performance have been developed by agencies such as the Association of Standards and Test Methods (ASTM) or the American Architectural Manufacturers Association (AAMA). Performance test criteria and results are listed in the NGMA *Book of Standards*.

Standard greenhouse glazing material will satisfy the requirements for BL1-P, BL2-P, and some permitted material. Clear glass glazing is the most enduring and provides the greatest amount of natural light. Tempered, laminated, chemically strengthened, and/or multi-layer (double or triple) glass is preferred for high containment greenhouses and also serves to meet codes for occupancy; therefore it is not uncommon to find these types of glass in modern research greenhouses. Glass can be manufactured in lengths that extend from eaves to ridge, though lengths over six feet may be impractical. As glass size and weight increase, so do the size of framing members and the difficulty of installation.

Glass panels are traditionally overlapped. Though overlapping does not provide as tight a seal as gasketed glass, it is perfectly adequate for many applications. Properly installed glazing provides low air infiltration and generally affords a high degree of containment. Bedding putty for traditional lapped glass greenhouses wears out long before the glass, a condition that may precipitate glass cracking and

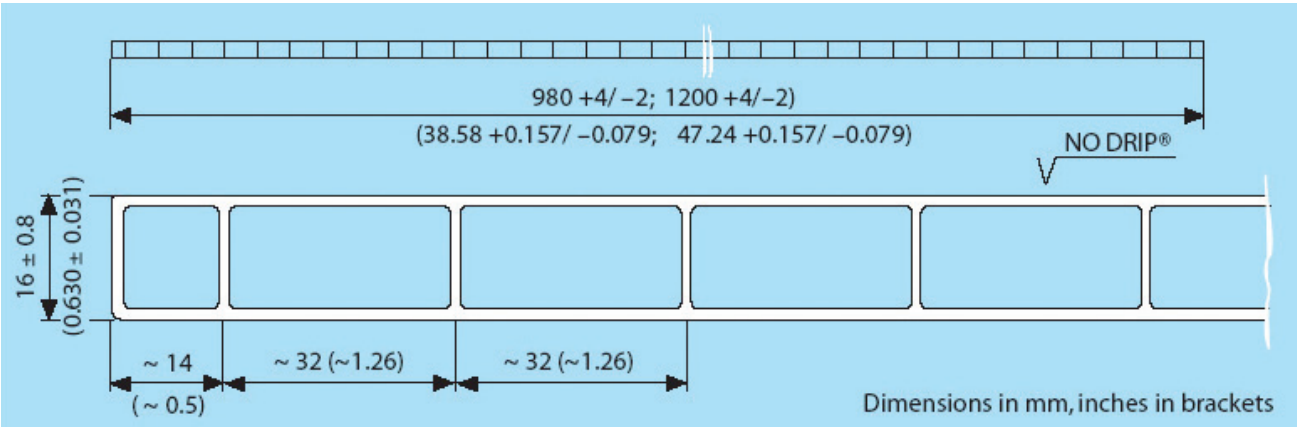


FIGURE 10. Cross Section of Double Walled Glazing Panel*

[43] *Book of Standards*. 2006. National Greenhouse Manufacturers Association (NGMA). www.ngma.com

* http://www.deglasamericas.com/greenhouse/products/products.html

breakage. If an older 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 provides tighter containment, better environmental control, and an energy cost savings.

Sheets of rigid thermoplastic such as Lexan™ polycarbonate or Exolite™ acrylic glazing are available as single, double, and triple layered material. Double and triple layered sheets are built with structured channels that significantly increase insulation value as well as security (**FIG. 10**, *see page 42*). Polycarbonate costs less and is more fire resistant than acrylic; acrylic glazing, however, lasts longer and permits better light transmission. These materials shift significantly within their framing with temperature fluctuations; therefore, inspections should be made seasonally for gaps. Cracks may develop over time as well. New formulations offer high impact resistant thermoplastics, which are ideal in areas that experience hail storms. APHIS allows the use of rigid thermoplastics for containment greenhouses as long as security requirements are maintained.

Various types of film plastic glazing are commonly available, e.g., polyester, polyethylene, polyvinyl chloride, and so on. Double-layer plastics rely on a fan to inflate the space between sheets. Plastic films 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. Newer films have a longer life, improved light transmission, and may resist hail damage better than rigid materials. Though these materials are common in commercial greenhouses, they offer minimal security and therefore their use would be limited to the lowest level of containment, BL1-P at best. Hence, film plastic glazing is not the preferred choice for most situations and is specifically listed as unacceptable in APHIS guidelines.

Standards for higher containment require that windows are closed, sealed, and resistant to breakage. This requirement can be met by using double-paned sealed glass, laminated glass, or, in some situations, rigid, double-walled plastic panels. All glazing systems must be long-lived and able to withstand temperature extremes, flexing of glazing and structure, UV radiation, and disinfectants. Gaskets and sealants can be dry (e.g., TPE, EPDM), wet (e.g., silicon), or a combination. Silicon products are commonly used to seal under glazing bars and gaps. A specific type of curtain wall construction, Structural Silicon Glazing, seals glass to framing by building up layers of silicon.

It may be possible to retrofit an existing structure, including glazing, to meet higher containment standards, but it is not necessarily practical. The structure and glazing may be thoroughly sealed with silicon or similar material if installed with care. A complete new glazing system is required to reglaze single pane glass greenhouses with rigid sheet material or double-pane glass.

It is important to install glass breakage sensors, which utilize sound, motion, or pressure, in high containment facilities. Sensors can elicit a quick response, thus minimizing or avoiding loss of containment. If cracking or breakage occurs, consult the SOPs, which should stipulate procedures for making temporary as well as permanent repairs.



Glazing selection is a balance between light transmissivity and security of the quarantine boundary. It is our judgment that a commercial, 'off the shelf' greenhouse envelope approach is inadequate for containment purposes. At the same time it is important that the builder of the greenhouse be schooled in the unique objectives of his task of providing a secure envelope. We have selected insulated glass units composed of tempered glass outer panes and laminated glass inner panes. The glazing system and its attachment to the greenhouse frame with structural silicone, and connection to the quarantine headhouse are critical to the continuity of the quarantine boundary and should be reviewed carefully during design.

▲▲▲ **ROSEMARIE DE CLERCK-FLOATE, PATRICK PLUE, TIM LEE,** *LESSONS LEARNED DURING THE DESIGN OF AN ARTHROPOD AND PATHOGEN QUARANTINE FACILITY*

Floors and Drains

Requirements for greenhouse floors vary according to the designated biosafety level (**TABLE 6**, *see page 53*). 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 suggested for lower level containment. Regardless of the requirement, solid concrete flooring adequately sloped toward drains is preferred for all research greenhouses. Retrofitting a greenhouse with concrete floors and walkways can substantially improve containment and sanitation practices.

Brush finish floors or other rough finish floors will be slightly harder to clean but will limit the slipping hazard.

▲▲▲ **JAMES D. KRAMER,** *University Greenhouse Design Consideration*



We have custom built drain baskets with 100 mesh screen to prevent small-seeded, regulated material from leaving the greenhouse. You need to have enough screen surface area exposed to be able to clear (with brush, hands, or fingers) debris to allow for drainage. Dedicated, trained individuals must maintain the screened drains.

▲▲▲ **KRISTEN CURLEE,** DOW AGROSCIENCES

APHIS guidelines recommend installing impermeable floors that can withstand repeated applications of disinfectants and suggest placing filters or screens in the drains when working with small arthropods or plant pathogens (**FIG. 11**, *see right*). Properly sealed or coated (e.g., a slip resistant polymer floor system) concrete flooring is the most practical way to meet these and other high containment guidelines. BL3-P and BL4-P facilities must have non-porous floors that can be disinfected, as well as a system to collect all runoff. Runoff is drained to a decontamination tank or treatment facility before released to a standard sewer or other disposal system (*see 'Termination', page 30*). At BL4-P, the *NIH Guidelines* state that sewer vents must be HEPA filtered and certified annually.

Entry Doors and Locks

Greenhouse doors should be given particular attention because containment and security breaches occur most often at points of entry. A self-closing, locking, steel door is always recommended, though not always required. 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. Extended-height kick plates can protect doors from structural damage caused by rolling carts. High containment facilities must have a *double* set of self-closing, locking, gasketed doors at entryways (*see Vestibules page 45*).

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 (**FIG. 12**, *see right*) or a short plastic brush attached to an aluminum holder that can be fastened to any relatively flat surface. Although sweeps cannot restrict all small insects that are intent on penetrating a space, they can meet lower



FIGURE 11. Filter Sock on Floor Drain*

* Courtesy Darren Rose, University of Sheffield

containment standards by excluding rodents, birds, and larger flying insects. For higher containment, doors should be sealed to the door frame using magnetic seals and solid or air-filled rubber or Neoprene gaskets. BSL-3Ag facilities may use air-lock doors with inflatable perimeter seals and electromagnetic locking systems.



FIGURE 12. Neoprene Door Sweep*

Shoe baths and floor sticky mats should be placed at doorways and vestibules to trap pollen and seeds that could be carried on footwear. They are especially useful when working with *Arabidopsis* in a greenhouse, growth room, or laboratory. Sticky mats and shoe baths can be positioned in the walkway so they are used only when personnel exit the room, which prolongs the life of mats and reduces the need to frequently change shoe bath solution. For higher containment, shoe covers are often recommended.

Containment facilities at all levels limit public access. Traditional cylinder door locks provide good security as long as strict key control is maintained. Newer electronic and electromagnetic systems utilizing key cards provide highly restricted access and a log of all entries and exits. Using an electronic system, fewer keys are issued, key loss is minimized, and codes can be changed quickly and easily. The distribution of greenhouse keys or key codes should be carefully controlled and monitored. Individual rooms dedicated to containment can be re-keyed to ensure access is limited to authorized personnel only. It is also advisable to limit the total number of keys issued, and especially to strictly limit the number of master or sub-master keys available.

Building codes prescribe the presence and placement of emergency exits, regardless of containment needs, so that personal safety is never compromised. For safety, emergency exit doors should have panic bars on the interior and have no exterior hardware. Local officials must be consulted before amending or creating entrances and exits.



FIGURE 13. Vestibule Retrofit**

Vestibules

Entrance and/or exit vestibules are recommended, if not required, by APHIS for plant pathogen and arthropod work. A connected walkway, headhouse, or prep room may serve as a vestibule in some

* Courtesy Dave Hansen, University of Minnesota, Agricultural Experiment Station

** Courtesy David Hantz, USDA-ARS-PGEC

situations, thus providing another layer of isolation between greenhouse material and the environment. A double-door entry system, with a dark vestibule sandwiched between the doors, aids in effective insect containment. Black light traps should be placed within vestibules to catch flying insects. APHIS further recommends a span of at least six feet between vestibule doors. The door should be interlocked so that only one door can be opened at a time. Shower rooms or other controlled spaces may act as vestibules in high containment facilities. Air curtains over doors that fan individuals exiting a contained area can help blow organisms and propagules back into containment (**FIG. 3**, *see page 23*). There may be occasions in which retrofitting entrances with a small vestibule will be sufficient to allow some containment work to proceed (**FIG. 13**, *see page 45*).

Screening

Screens used in either new or retrofit construction must be chosen and installed with care. Many types of screen size and composition are available. Screen mesh size should be gauged to the size and shape of the organisms of interest. A comparison of commercial screening materials[44] indicates that in some instances screens with a larger hole size may have exclusion 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 the hole shape. Further, thread diameter and mesh composition also influence exclusion properties. Relatively rigid stainless steel mesh may offer better exclusion than softer mesh with a similar hole size. Insects may chew through softer screens such as those made of polyester. APHIS suggests using 80-mesh, metallic screen for arthropod containment, and much larger, 3.25-mesh when working with noxious weeds or parasitic plants.

Screen size can greatly affect airflow, cooling efficiency, CO_2 retention, humidity level, and light transmission. For example, a piece of 64-mesh screen with a thread thickness of 0.008 in. has only 23.8% open space. Therefore, it is critical to size the screen in accordance to the ventilation system, regardless of the type of cooling systems installed—passive, fan only, fan and pad, or mechanical (air-conditioned). Dust accumulation on screens can also affect their efficiency; therefore, as the screen opening size decreases, the need to clean screens by washing or vacuuming increases. Consider the ease of replacing and cleaning screens before purchasing; in general, fine mesh screen requires more maintenance.

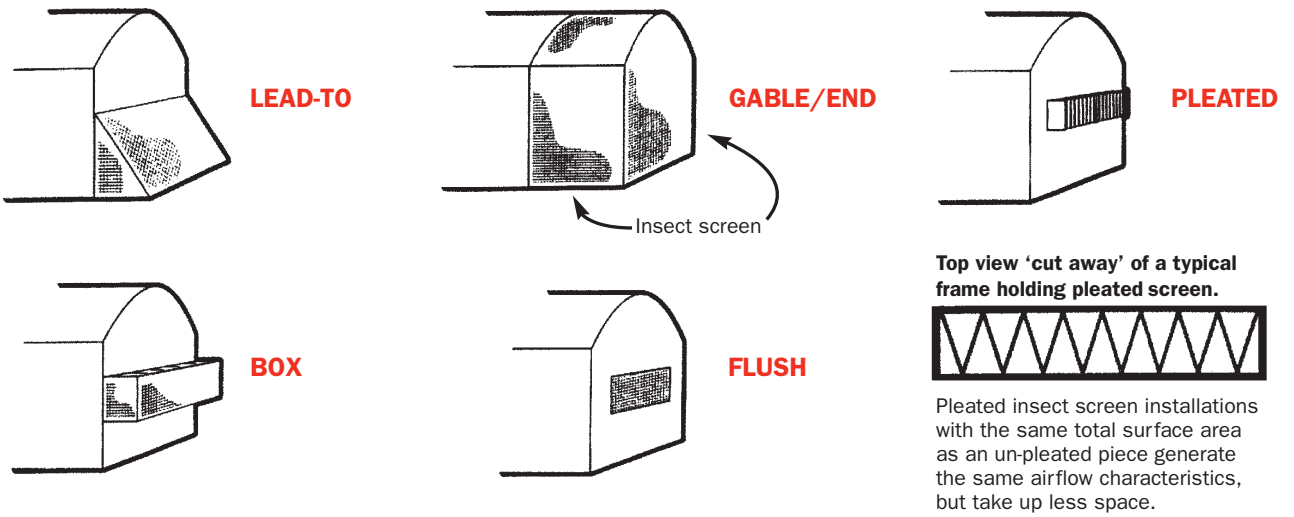


FIGURE 14. Screen Box Styles for Increasing Screen Surface Area*

[44] 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.

* From *Book of Standards*, National Greenhouse Manufacturers Association. Reprinted with permission. http://www.ngma.com/

For containment purposes, screened side vents are recommended for BL1-P and required for BL2-P. One should be especially careful, however, to consider the effect on airflow when installing screens on ventilation intake vents or fan housings. If evaporative cooling pads made of aspen fiber or corrugated cellulose are used on intake side vents or cooling units, screening is still useful because insects can find their way through these materials.

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 address airflow restriction is to build a "screen box" outside the cooling pad frame (**FIG. 14**, *see page 46*) to provide adequate surface area for airflow though the cooling pads. Another creative approach is to place such screen frames inside the greenhouse structure, which would also facilitate maintenance and increase longevity. The best method for determining if a screen retrofit or addition will negatively affect airflow is to take static pressure measurements. Fan suppliers are a good resource to assist in calculating if the pressure loss caused by adding screening will negatively affect airflow, fan performance, and subsequent cooling.

Ventilation, Cooling, and Heating

Precise temperature setpoints are challenging to maintain within a greenhouse due to the constantly fluctuating solar load and other ambient conditions. Cooling a greenhouse is usually difficult, so choosing the appropriate system is essential. Greenhouses are cooled by natural ventilation, shade systems, exhaust fans, evaporative methods, and mechanical air conditioning. Different systems for summer and winter cooling may be employed, depending on locale and cooling methods. The most common and energy efficient method is simply to employ natural ventilation using motorized and/or manual hinged vents located at the roof ridge and/or sidewall. Shade systems also effectively provide an energy efficient form of passive cooling by reducing solar load. Louvered exhaust fans accelerate the exchange of warm air with outside ambient air and are often combined with evaporative cooling pads. Motorized louvers should be sequenced to open and close with fan startup and shutdown. Although evaporative cooling pads are frequently installed in sidewall vent openings, they are also available as stand-alone fan and pad cooler units. The effectiveness of cooling pads depends on proper sizing, installation, and maintenance.

A range of measures are required to preserve containment when installing cooling systems. Insect screening is recommended for BL1-P and required for BL2-P for all vent openings and motorized or gravity-driven exhaust fan louvers. Generally, the vent operator arms or racks that pass through screen are fitted with brushes or flexible barriers to prevent rodents and other large pests from entering the greenhouse. Maintenance on cooling systems is required to sustain containment and includes ensuring that gaps around cooling pads are minimized or eliminated, fan louvers seal tightly when closed, and screens are clean.

High pressure fog is an evaporative cooling system that can be used when the structure and climate permit. Fog droplets, ideally 20 microns or less in size, evaporate before landing, so free water is not deposited on plant leaves. A fog cooling system must use a clean water source such as water purified by reverse osmosis. A well-designed fog system offers a more uniform temperature throughout the greenhouse than fan and pad systems. This type of system is also better for containment because it does not require cooling pads in vent openings.

When reducing or eliminating outside vents is required, mechanical cooling is preferred over evaporative cooling systems, even though construction and operation costs are higher than other methods. A closed greenhouse heats rapidly regardless of location, even in minimal sunshine. Therefore mechanical cooling, i.e., air conditioning, is the only cooling option for a closed containment greenhouse. Mechanical cooling works by passing air over coils containing refrigerant, chilled water, or other chilled solution. When properly designed, this approach offers the most precise temperature



FIGURE 15. Bag-In, Bag-Out HEPA Filter System Attached to Air Handler*

setpoints and uniform conditions. Because mechanical cooling tends to dry the air, humidification is recommended.

At BL3-P or higher, greenhouse exhaust air must be filtered and the room held under negative pressure. Intake air is also routinely filtered to prevent introduction of organisms from the environment into the enclosed space. Filter systems can be designed to trap pollen, spores, and other small particles. High efficiency particulate air (HEPA) filters can remove very small particles while allowing gases to transfer across the filter media. For fungal pathogen work performed under APHIS permit, tandem 99.97% efficiency HEPA filters that can trap particles .03 microns and larger are suggested. Roughing pre-filters (ASHRAE standard 52) are sometimes used to protect the HEPA filters from premature clogging. Protocols for monitoring and changing the filters regularly are necessary.

A commercial air handling unit (AHU) placed in an adjacent mechanical space passes conditioned and filtered air throughout the greenhouse. Fresh air can also be incorporated into an AHU air stream, which assists in maintaining the correct balance of air gasses and preventing the depletion of CO_2. The incorporation of five to ten percent fresh air into the total volume of recirculated air should suffice to replenish required gases, including CO_2. Additional air handling components, such as heating and cooling coils and humidifiers, may be added to further condition air within the greenhouse.

In a laboratory setting, containment is related to worker safety. Ventilation in these settings is often accomplished with 'single pass air', i.e., the use of

* Courtesy Dave Hansen, University of Minnesota, Agricultural Experiment Station

100% outside air. Single pass air is required for BSL-3Ag facilities, although greenhouses seldom contain material hazardous to human health. Continually conditioning air that passes through a greenhouse comes with high energy and maintenance costs. Furthermore, the facility must be very carefully designed and built to avoid increasing the risk of a containment breach compared to a non-single pass approach. An alternative is to recirculate conditioned air within the growth space, while supplying and exhausting HEPA filtered air (**FIG. 15**, *see page 48*). This approach, if acceptable to regulators and researchers, allows for directional, pressurized airflow, reduces energy costs for conditioning the air, and may actually lower the risk of containment breach.

Base cooling and heating will be provided by the central air handling unit and supplemented by individual fan coil units for each greenhouse compartment. This differs from some facilities we visited where each greenhouse compartment has its own air handler. It was felt that the space temperature and air flow rates of the compartments of our planned greenhouse can be adjusted to meet individual research requirements and to address energy conservation. While moisture from the plants and soil within insect cages will influence humidity at the micro level, we also have the ability to add humidity to each compartment independently. This feature also will provide the flexibility to deal with the possibility of inadvertent dehumidification of the space arising from the cooling mode of the fan coil.



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Typical greenhouse heating systems include hot water radiation, steam radiation, infrared electric, solar, and forced air. The types of heating equipment can be quite variable as well; including in-floor heating, finned-tube radiators, unit heaters, refrigeration coils, and bench heating. Air can be distributed through overhead tube assemblies or horizontal air flow fans. All of these systems are adequate for every containment level, if care is taken not to create spaces that are difficult to clean and disinfect. Design firms and specialty suppliers or manufacturers are the best sources of the specialized knowledge required for designing and installing heating, cooling, and ventilation systems.

Pressurization and Infiltration

High containment facilities with multiple labs and other workspaces must have the capability to maintain differential air pressures between rooms. Pressure differences should be configured to direct airflow sequentially from the least hazardous or clean areas (held at positive or the least negative pressure) to the most contaminated areas (held at the most negative pressure) where the organisms of interest are generally handled. A difference of 0.05 wg between adjacent functional spaces is sufficient to maintain this differential air pressure gradient. The design is implemented by conducting air balancing exercises after all equipment is installed. Magnehelic® or digital pressure gauges on transducers are installed so occupants can easily determine if a room is at the proper pressurization (**FIG. 16**, *see page 50*). When pressures reach unacceptable levels, alarms should alert the appropriate individuals to make corrections. If cross contamination from ambient air or adjacent spaces must be strictly avoided, then growing areas need to be held in positive pressure. In this case, the pressure gradient principles described above would be reversed.

Research greenhouses, like any structure, cannot completely eliminate air infiltration (**TABLE 5**, *see page 50*). However, to preserve containment the infiltration rate should be controlled to allow an exchange rate of no more than one complete internal volume of air per hour, according to ASABE (American Society of Agricultural and Biological Engineers) standards. Tight greenhouses have air exchange rates of well under one per hour, whereas older ones may experience three or greater. A smoke test can be used to detect sources of air infiltration.

For high containment facilities, air tightness tests must conform to standards set for curtain wall and other building systems. For example, ARS stipulates[45]

[45] http://www.afm.ars.usda.gov/ppweb/242-01m.htm.

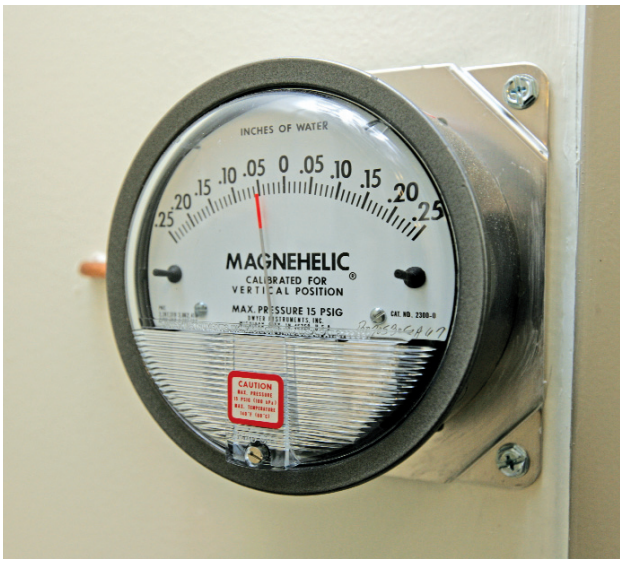


FIGURE 16. Magnehelic Gauge for Visual Confirmation of Pressurization

that BSL-3Ag greenhouses "will undergo the following tests, or the latest subsequent standards: (a) an air infiltration test conducted according to ASTM E 283-91; (b) a static pressure water resistance test conducted according to ASTM E 331-93; and (c) a dynamic pressure water resistance test conducted according to AAMA 501.1-94". The standard guidance provided by NIH for testing BL4-P greenhouses stipulates "… an air leak rate (decay rate) not to exceed 7 percent per minute (logarithm of pressure against time) over a 20-minute period at 2 inches of water gauge pressure. Nominally, this is 0.05 inches of water gauge pressure loss in 1 minute at 2 inches water gauge pressure"[46]. However, 2 inches of water gauge pressure is so extreme that many facilities cannot withstand the test without compromising internal structural integrity. At best, a test at that pressure can only be cautiously conducted in a robust and very well sealed greenhouse.

CO_2 decay tests are also routinely performed to test infiltration rates. By tracking the decay of a measured amount of CO_2 over time, one can derive the infiltration rate. This test must be conducted on a windless day and with all fans and other air handling equipment turned off. The test works best if conducted for several hours during the night when ventilation is not required.

Benching

Many different types of benching can be used in research facilities. Benches made of aluminum, galvanized steel, and certain plastics provide the longest wear and are easiest to clean. Wood is a poor choice because it may conceal pests. Benchtop materials that let water drain to the floor are most common because they permit drainage under plant containers and enhanced air circulation. Expanded metal benchtops of galvanized steel or aluminum are preferred, as these materials are resistant to water and most chemicals. In addition, these benches are readily available, meet higher containment

TABLE 5. Estimated Infiltration Rates for Greenhouses by Type and Construction*

TYPE AND CONSTRUCTION	s–1	h–1
NEW CONSTRUCTION:		
double plastic film	$2.1x10^{-4}$ – $4.1x10^{-4}$	0.75 – 1.5
glass or fiberglass	$1.4x10^{-4}$ – $2.8x10^{-4}$	0.50 – 1.0
OLD CONSTRUCTION:		
glass, good maintenance	2.8x10-4 – $5.6x10^{-4}$	1.0 – 2.0
glass, poor maintenance	5.6x10-4 – $11.1x10^{-4}$	2.0 – 4.0

1) Internal air volume exchanges per unit time (s–1 or h–1). High winds or direct exposure to wind will increase infiltration rates; conversely, low winds or protection from wind will reduce infiltration rates.

[46] http://www.afm.ars.usda.gov/ppweb/PDF/242-01M.pdf, page 254.

*From ANSI/ASAE EP406. 4 Jan, 2003

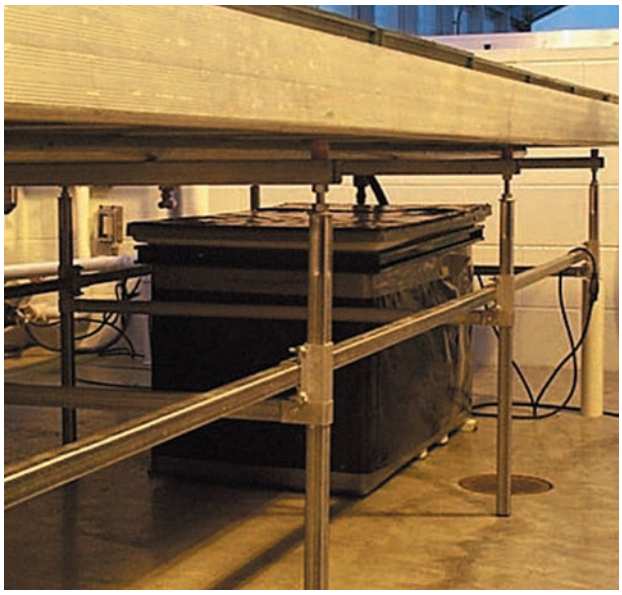


FIGURE 17. Ebb and Flow Bench with Collection Tank

standards, and can be thoroughly cleaned, which benefits a pest control program, regardless of the research protocol. Bench material that can withstand repeated applications of disinfecting products is always a good choice and a requirement for high containment.

A bench that collects water for recirculation, called an ebb and flow bench (**FIG. 17**, *see above*), may be modified to drain runoff into a holding tank for treatment with chemicals or heat before it is released to the sewer or ground. This approach may be practical when runoff water needs to be collected only occasionally. For high containment, collection and treatment of all liquid effluent is required as described under 'Floors and Drains' (*see page 44*).

The use of rolling benchtops can significantly increase usable growing space, which is especially prudent when building expensive, high containment greenhouses. Alternately, collapsible or removable benches offer flexibility, as long as adequate space is available to store unused benches.

Lighting

Supplemental lights are commonly added to research greenhouses to aid plant growth and for task lighting. Lighting may impact containment due to the attraction of arthropods to light and may also be a problem if the luminaire design allows arthropods to persist inside the greenhouse. Even though environmental conditions and a lack of food will cause most species to die, the presence of insects should not be overlooked.

High pressure sodium (HPS) and metal halide (MH) high intensity discharge lamps are the most common types of supplemental plant growth lights. HPS lamps normally use an open bulb arrangement, whereas MH lamps require a jacketed bulb or lens, due to the risk of rupture. Both of these lamps radiate a large amount of heat, which minimizes the possibility of arthropod survival. Task or other fluorescent lighting gives off significantly less heat, which may allow arthropod survival and potential reproduction.

Control and Electrical Systems

The need for precise control and supplemental lighting in research greenhouses necessitates that high quality, large capacity electrical systems are installed. High containment facilities must have an even greater capacity because they require equipment redundancy and a backup method of electricity generation. High containment facilities also require that all electrical receptacles, outlets, and conduit are

Automated shading, lighting, and irrigation systems for each of the four greenhouse compartments will provide flexibility in growth conditions. Benching systems have been chosen to facilitate operations rather than to maximize the bench to floor area ratio. For all compartments, we will use stationary (i.e., versus rolling) benching which can be reconfigured dependent on the experiments that are being conducted.



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sealed to prevent arthropod or other organism escape.

Greenhouse control systems technology has become highly advanced, reliable, and cost effective. It is strongly recommended that any control system used in a greenhouse be designed and manufactured exclusively for greenhouses and not to use typical building control systems, which cannot readily meet the exacting specifications for a research greenhouse. Several vendors offer control systems that incorporate the latest digital technology and allow precise environmental control, logging, sensing, alarm, remote access, and related functions. Non-digital controls—analog, pneumatic, or mechanical—are suitable for research at BL1-P and BL2-P. Separate, stand-alone systems are needed for recording and logging environmental data, if desired. Older control systems may be updated, which is an economical means of achieving better environmental control and enhanced containment.



FIGURE 18. Caulking around Service Intrusions*

It is useful in any research greenhouse, and critical in high containment greenhouses, to have the capability to remotely respond to alarms. Inexpensive systems with an auto-dialer that continues to call numbers until a response is received can be installed in any greenhouse. Ideally, the responder can then log on a computer control system to correct the alarm condition without physically entering the facility, although it is prudent to physically monitor an alarm situation at the first opportunity. Email, telephone, and text message notification is also available with some computer-based systems. Several vendors include a weather station that continually feeds data to the control system so that it can anticipate ambient conditions by trending historical data. This function can result in very accurate environmental control in the greenhouse.

In high containment, sensors can monitor differential air pressures and security functions, as well as environmental control. Sensor data may feed to either the greenhouse or building control system. The important point is that all critical systems are monitored and have a mechanism for alarm response.

Piping

Heating, watering, and fertilizing systems are typically piped into and throughout the greenhouse. For containment purposes, piped systems should be installed with a minimal number of intrusions. Good greenhouse design also routes piping so it does not shade plants. All new and existing intrusions should be tightly sealed with a durable material (**FIG. 18**, *see above*). Further measures are required for high containment. Conduit and piping that penetrate the containment barrier must be sealed on both the outside and the inside of the barrier. This ensures that differential pressures can be maintained and organisms cannot penetrate.

Automatic watering and fertilizing systems are advantageous because they reduce the amount of traffic into the greenhouse, thus decreasing the opportunity to spread pollen, seed, and other propagative materials. The relative ease and affordable cost of installing these systems makes them an attractive option, though they cannot replace frequent monitoring by staff.

* Courtesy Dave Hansen, University of Minnesota, Agricultural Experiment Station

Summary of Greenhouse Features

TABLE 6 provides a comparison of the important features of a containment greenhouse facility. The features are recommended or required, as noted, for the prescribed biosafety levels or criteria of containment, according to guidelines issued by the NIH, USDA-ARS, and USDA-APHIS agencies. This table is compiled for the convenience purposes; for details relating to specific containment requirements, the appropriate agencies should be consulted. (The page number refers to the relevant discussion of the feature in the Guide text.)

TABLE 6. Important Features of a Greenhouse Containment Facility.

Feature	Criteria	NIH				ARS	APHIS					Pg #
		BL1-P	BL2-P	BL3-P	BL4-P	BSL-3Ag	Arthropods	Bacteria	Fungi	Virus	Weed	
ACCESS	Training prior to access **required**	●	●	●	●	●	●	●	●	●	●	27
	Entry pre-authorization **required**			●	●	●	●	●	●	●	●	
	Hand washing station at exit			●	●	●					●	
	Shower on exit, depending on experiment			●			●					
	Shower required upon exit				●	●		●	●	●		
	Entry logbook required, users				●	●						
	Entry logbook, visitors						●	●	●	●	●	
	Visitors prohibited				●	●						
	Materials enter and leave through a double door autoclave, airlock, or fumigation chamber			●	●	●			●			
APPAREL	Street clothing acceptable	●	●									28
	Lab coat, coverall, or additional covers upon entry which remain in facility or disposed						●	●		●	●	
	Complete clothing change **required**			●	●	●			●			
	Lab clothing autoclaved prior to laundering or disposal			●	●	●			●	●	●	
SIGNAGE	Indicate restricted experiments in progress and include relevant contact information		●	●	●	●	●	●	●	●	●	28
	Post signs at emergency exits stating “USDA-APHIS Containment Facility–Emergency Exit Only”						●	●	●	●	●	

Feature	Criteria	NIH				ARS	APHIS					Pg #
		BL1-P	BL2-P	BL3-P	BL4-P	BSL-3Ag	Arthropods	Bacteria	Fungi	Virus	Weed	
TERMINATION	Clean and disinfect facility			●	●	●	●	●	●	●	●	30
	Experimental material rendered biologically inactive before disposal	●	●	●								
	Autoclave recommended for treating material before removal from facility			●	●	●	●	●	●	●	●	
	Double door pass-through autoclave **recommended**						●	●	●	●	●	
	Double door pass-through autoclave **required**				●	●						
	Liquid waste sterilized before entering sewer system				●	●	●	●	●	●	●	
PEST CONTROL	Pest control program **required**	●	●	●	●	●	●	●	●	●	●	32
RECORDS	Movement of live materials in and out of greenhouse facility		●	●	●	●	●	●	●	●	●	33
	Record all personnel entry and exit			●	●	●						
	Documentation and reporting of inadvertent release of microorganisms/experimental material to appropriate authority		●	●	●	●	●	●	●	●	●	
INSPECTION	Inspection conducted by regulators before permitted work begins followed by scheduled or unannounced reinspections					●	●	●	●	●	●	34
SECURITY	Facility surrounded by a security fence or equivalent measure (CCTV, bollards, motion sensors)			●	●	●	●	●	●	●	●	35
	A personal security risk assessment required to access experimental materials						●					
STRUCTURE	Foundation of concrete, concrete block, brick, or similar material			●	●	●	●	●	●	●	●	41
	Rigid, reinforced greenhouse framing (aluminum and/or steel typical)			●	●	●	●	●	●	●	●	
	Resistant to disinfecting chemicals			●	●	●	●	●	●	●	●	

Feature	Criteria	NIH				ARS	APHIS					Pg #
		BL1-P	BL2-P	BL3-P	BL4-P	BSL-3Ag	Arthropods	Bacteria	Fungi	Virus	Weed	
GLAZING	Rigid thermoplastic acceptable	●	●	●			●	●	●	●	●	42
	Sealed and break resistant glazing material			●	●	●	●	●	●	●	●	
	Glass break sensors recommended			●	●	●	●	●	●	●	●	
FLOORS AND DRAINS	Concrete floor **recommended**. Porous material under benching acceptable	●	●									44
	Impervious to organisms and tolerant of disinfection			●	●	●	●	●	●	●	●	
	Ability to collect and decontaminate liquid waste			●	●	●						
	HEPA filtration of sewer vents				●	●			●			
DOORS	Rigid, e.g., steel, self-closing doors with sweeps			●	●	●	●	●	●	●	●	44
	Double set of locking, self–closing doors			●	●	●						
	Emergency doors without exterior hardware, with audible alarms upon opening			●	●	●	●	●	●	●	●	
	Key card or equivalent entry system tracking recommended			●	●	●	●	●	●	●	●	
VESTIBULES	Interlocking, sealing doors installed in facility entryways					●	●	●	●	●	●	45
	Consider air curtains and/or light traps for insects						●			●		
SCREENING	Standard, 30 mesh or higher insect screen on openings to the exterior	●	●				●	●		●	●	46
VENTILATION	Allow evaporative pad and free air cooling on vents	●	●									47
	Air conditioning system designed for containment			●	●	●	●	●	●	●	●	
	Install filters/screens on air handling system						●	●		●		
	HEPA filtration of exhaust air: Bag-in, bag-out system that is recertified annually or upon filter change. Filters must be sterilized before disposal.			●	●	●			●			
	System designed to accommodate fumigation						●	●	●	●		

Feature	Criteria	NIH				ARS	APHIS					Pg #
		BL1-P	BL2-P	BL3-P	BL4-P	BSL-3Ag	Arthropods	Bacteria	Fungi	Virus	Weed	
PRESSURIZATION	Directed airflow to maintain negative pressure			●	●	●			●			49
	Install differential pressure transducers to read conditions and trigger alarms			●	●	●			●			
INFILTRATION	Perform annual testing for greenhouse tightness				●	●			●			49
BENCHING/WORK SURFACES	Tolerates frequent disinfecting; easy to clean and inspect			●	●	●	●	●	●	●	●	50
CONTROL AND ELECTRICAL SYSTEMS	Computer control with remote access capability recommended or required			●	●	●	●	●	●	●	●	51
	Can maintain containment under loss of power			●	●	●	●	●	●	●	●	

NOTE. *These are general approaches to containment at various regulated levels. Individual situations may require more or less stringent strategies.*

Screenhouses

Screenhouses are only acceptable for research involving GEOs if they meet the requirements for BL1-P or BL2-P level greenhouses, including floors, and contain organisms that would have a 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. Screenhouses are designed and constructed using many of the same standards listed for greenhouses. Common upgrades to existing screenhouses include the addition of concrete floors, well-fitting lockable doors, individual compartments, sealed joints and utility intrusions, and special screening. APHIS permits may be granted for experiments conducted in an approved screenhouse requiring BL1-P or BL2-P level containment; however, experiments conducted at BL3-P are not allowed. APHIS almost always requires that only metallic screen is used, due to the reduced integrity and security of non-metallic screening.

Growth Chambers and Rooms

Reach in growth chambers and walk-in growth rooms deliver some containment advantages over many greenhouses. According to the *NIH Guidelines*, growth chambers may be used for containment at BL1-P and BL2-P levels under certain conditions, and even for level BL3-P if more stringent conditions are implemented within the building. APHIS routinely permits the use of a growth chambers, provided the facility meets containment standards and passes physical inspections.

Some containment advantages of growth chambers include:

- Precise environmental control in any season
- Flexibility—can be placed in multiple locations within a building
- Greater resistance to earthquakes, weather, vandalism
- Enhanced security
- Equipment maintenance is performed outside the containment area
- Exhaust air is HEPA filtered
- Foot traffic is reduced
- Interior surfaces are smooth and easy to clean

Growth chambers or rooms must be located within a containment laboratory, greenhouse headhouse, or dedicated facility—an arrangement referred to as the 'room within a room' concept. The larger 'room' enhances containment by providing filtered air and runoff decontamination, for example. Growth rooms are also manufactured with a vestibule that provides an additional layer of isolation between the growth area and the larger facility.

As with greenhouses, growth chambers or rooms used for BL3-P and other high containment situations require special features such as HEPA filtration of exhaust air and directional airflow. Though standard growth chambers and rooms provide basic containment features, they are not entirely suitable for high containment. Though it is possible to direct airflow into a growth chamber to minimize escape of organisms, inward airflow may conflict with the standard airflow design. Flexible barriers around door openings can minimize but not entirely prevent the egress of contained material. Also, a growth chamber is seldom built 'tight' enough to restrict all airborne organisms or propagules to a prescribed area within the chamber; therefore material may accumulate in unwanted parts of the unit.

The most practical way to have a 'tight' chamber suitable for high containment is to initially stipulate that the chamber meet specific containment standards. It may be possible, in some instances, to retrofit or modify a growth chamber, depending on the chamber style. HEPA filtration units can be installed on growth chamber or growth room exhaust ports (**FIG. 19**, *see page 58*). Materials within a growth chamber or room may sit on solid trays so that runoff water and debris can be collected and stored for disinfecting or autoclaving. Collecting runoff at the drain may be more problematic, unless the drain is connected to an autoclave, kill tank, or similar disinfection equipment.

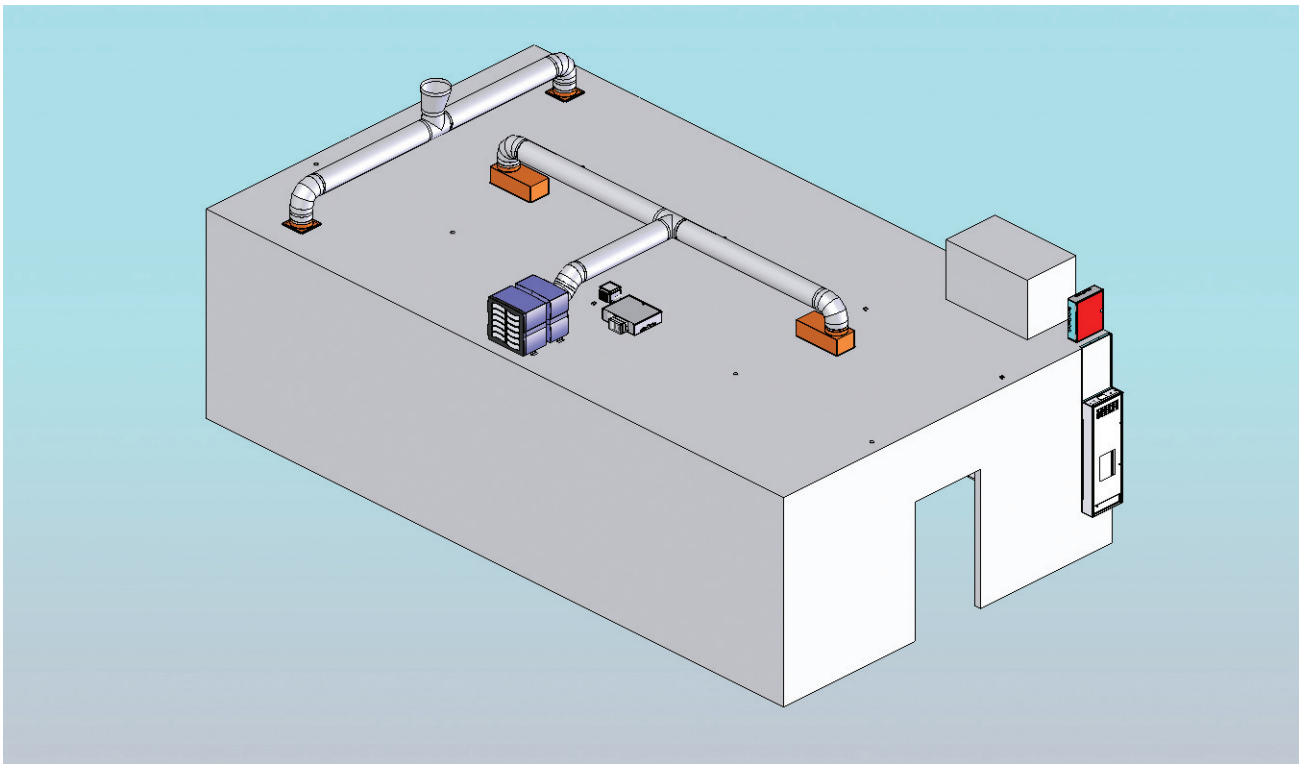


FIGURE 19. **Growth Room with HEPA Filtration**

Retrofitting Greenhouses

It is probably cheaper to retrofit a conventional greenhouse to meet BL1-P and even BL2-P containment standards than to build a new facility. Requirements for meeting BL3-P standards are more extensive and may involve basic structural changes; therefore, retrofitting is not likely to be feasible or cost-effective for high containment situations. Similarly, if a greenhouse is structurally unsound or suspect, rebuilding may in many cases be a better option than retrofitting. BSL-3Ag and BL4-P standards require a dedicated, highly engineered, and isolated facility, which excludes the possibility of retrofitting existing greenhouses.

Existing greenhouse facilities should be closely 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, and/or experienced consultant before proceeding with any major renovation.

Appendix I.

Sample Biosafety Review

CONSIDERATIONS FOR HANDLING BIOLOGICAL MATERIALS AND ORGANISMS CONTAINING RECOMBINANT DNA MOLECULES

NOTE. *Some areas are dimmed or sections deleted that are not relevant to current review.*

Complete and return to Biological Safety Officer. If a question does not apply, or was addressed in a different section, please indicate with an "n/a" or other written designation.

The Principal Investigator (P.I.) is responsible for staff instruction and training in safety practices and techniques related to this project.

Date: _____ **IBC Review Required?** ☐ Yes ☐ No

1. **Project Title:** _____

2. **Group:** _____

3. **Investigator** (name, highest degree, title) _____

Other personnel associated with this project: _____

4. Locations where materials related to the project will be used. _____

Project Protocol: In a paragraph(s) describe the general intent/objectives of the project(s). Proprietary information need not be provided.

Project Classification: Choose from the categories below those that best classify this project. Check all that apply. For each category checked, please complete the appropriate section in this document.

☐ Recombinant DNA (rDNA) molecules (Section A)
☐ Plants - including rDNA hosts (Section B)
☐ Plant Pathogens and Symbiotes (Section C)
☐ Field Tests (Section D)
☐ >10 liter - Large Scale Cell Culturing (Section E)
☐ Animal Systems (non-primate) (Section F)
☐ Human-derived Materials/Primary Human Cell Isolates (Section G)
☐ Animal Pathogens (Section H)
☐ Vertebrate Toxins and Toxin Expression (Section I)

7. **Related Projects:** If material is to be submitted for analysis, separation, purification, etc. to laboratories not covered by this Biosafety Review, forward a copy of this review in order to communicate associated hazards and precautions.

8. List any Laboratory Related Illnesses during the Preceding Year.

9. List general specific safety containment and safety procedures for listed protocol.

10. **Waste Disposal:** Describe procedures for disposing of solid and liquid biological waste.

11. **Emergency procedures:** Describe emergency procedures for accidental spills and personnel exposures. Include name and phone number of contact person(s).

CONTACTS:

12. **Medical Surveillance (baseline blood archiving, immunizations, health risk assessments) required?**

13. Hazard communication

14. **Proposed physical containment level for this project?** (BL1, BL2, BL3, BL4.)
Use NIH guidelines Appendix G and P to determine this level.

NOTE. *Any change in the program that would change the magnitude of the risk (e.g., quantity of material handled, change in biological material, or change in facilities) may require a review of this approval.*

Section A. Recombinant DNA (rDNA) Molecules

Characterize rDNA systems including the following:

1. **Prokaryotic rDNA vector systems:**
   a. Bacterial
      1) Type:
      2) Host:
      3) Source of vector DNA:
      4) Synthetic or naturally occurring:
      5) Conjugative or non-conjugative:

2. **Viral rDNA system description:**
   a) **Description**:
      1) **Host:**
      2) **Pertinent information:**

3. **Plastid rDNA systems:**

4. **Yeast rDNA systems:**
   a. **Yeast**
      1) **Host:**
      2) **Vector Name:**
      3) **episomal or integrated?**

5. **Nature of inserted DNA(s) in above systems.**

6. **Scale**: Indicate quantity, frequency and manner in which organisms or cell cultures will be used.

7. **Are there any research activities ongoing which are exempt from the *NIH Guidelines*? If exempt, IBC notification of activities is required.**

Section B. Plants— including rDNA hosts

NOTE. *For all experiments involving rDNA plants, Section A must also be completed.*

Please characterize plant(s) for the following:

a. Species
b. Presence of rDNA transposible elements
c. Mode of reproduction: asexual, open pollination, self-pollination, apomixes
d. Potential for release of pollen in the work area
e. Is the plant a common cause of pollinosis, contact dermatitis, or other effects?
f. Genetically modified traits being evaluated
g. Environmental invasiveness/weediness of plant

2. **List locations of propagation of recombinant plants:**

3. **Indicate plant biosafety containment level.** If necessary, reference NIH Guidelines Appendix P–Physical and Biological Containment for Recombinant DNA Research Involving Plants. (pg. 5)

☐ BL1-P
☐ BL2-P
☐ BL3-P
☐ BL4-P

4. List specific safety practices that address contamination including:
   a. Control of undesired species and motile macro-organisms:
   b. Laboratory, growth chamber and greenhouse design:

Section C. Plant Pathogens and Symbiotes

Characterization of plant pathogens and symbiotes:

1. Insect nematode fungal bacterial viral

2. **Pertinent information:**

3. **Was/were the agent(s) acquired under USDA-APHIS permit?** If so, please list associated permit numbers

2. **Containment level for handling plant pathogens:**

☐ BL 1
☐ BL 2
☐ BL 3
☐ BL 4

3. **List specific safety rules for handling the pathogen:**

Principal Investigator Agreement:

I agree to comply with the NIH and associated regulatory agencies in requirements pertaining to use, shipment and transfer of recombinant DNA materials. I am familiar with and agree to abide by the provisions of the current NIH Guidelines pertaining to the proposed project. To the best of my knowledge the above information is correct as stated.

Name (Please print)**:**

Signature

Institutional Biosafety Committee Approval:

I certify that the IBC has reviewed the proposed project for recombinant DNA research within "Lab Name" and has found it to be in compliance with the NIH Guidelines.

IBC Chair Date

SPACE BELOW RESERVED FOR BIOLOGICAL SAFETY OFFICER AND REVIEWERS

Reference Number:

Courtesy of Dean Rochester, Donald Danforth Plant Science Center

Appendix II.

Sample Guideline on TMZ Quarantine

INFECTED PLANTS TO BE DISPOSED BY: _____

1. Plant owners should not enter other plant growing areas after working with infected plants in this zone. Please plan tasks accordingly.
2. Wear latex gloves and lab coats when touching plants and change gloves in between plants. See Rob to order disposable sleeves if you prefer not to wear lab coats. Alternatively, dip hands in milk (whole or skim).
3. Spray plants with whole or skim milk 24 hours prior to handling. Smokers should dip hands in milk prior to handling plants.
4. Avoid any contact with sleeves, tools, hose nozzles or any item that might spread TMV to other plants.
5. Plant owners should space/prune plants to minimize contact between plants and to keep plants from being brushed against during normal greenhouse tasks. New plants should be separated physically from established plants.
6. Greenshield and 3% trisodium phosphate (TSP) are the most effective agent for disinfecting tools and surfaces. 70% ethanol is not effective.
7. Neither Greenshield or TSP are safe to wash hands with. Milk (skim or whole) will inactivate TMV spores and is safe to wash hands with.
8. Plants to be discarded will be autoclaved by greenhouse staff, even if not transgenic.
9. Plant owners are responsible for managing spread of TMV using similar measures in other plant growth areas they use.
10. Plant owners will need to take meristem cultures, perhaps combined with heat treatment to propagate infected plants. Other vegetative propagation means will spread the disease. Seeds need to be acid- or bleach-treated to be rid of TMV.

Courtesy of Rob Eddy, Purdue University

Appendix III.

BIOTECHNOLOGY FACILITY INSPECTION WORKSHEET*

All questions will be given Yes, No, N/A, options for answers. Answers of No or N/A will require explanation in the Summary of Findings (SOF) section.

General Considerations

1. Do SOPs establish proper techniques for handling recombinant DNA/transgenic material?
2. Was a copy of SOPs given to the inspecting official?
3. Does the facility have an institutional biosafety committee?
   (Indicate name and phone number of the chair of the biosafety committee in the SOF)
4. Is the biosafety committee chair aware that this research involves USDA regulated transgenic material?
5. Is the scientist who is conducting the research listed on the permit application?
   (Please list the names of researchers, including project leader, in the SOF.)
6. Does the scientist conducting the research have a copy of the permit application and SOPs? (Please verify that they have contact information for BRS.)
7. Do SOPs, or other documents, include detailed instructions for reporting and correcting unintended environmental release?
8. Are other personnel working on this project trained in accord with written SOPs?
9. Does the responsible researcher have records that other personnel working on the project have received proper training?
10. Will movement of the regulated material occur within the facility? (Yes or N/A)
    (Please list areas, by room number, greenhouse number/letter, or growth chamber serial number, which will be used for this research in SOF)
11. Has the responsible researcher provided the inspecting official with a copy of floor plans indicating which areas will be used for this research? *(If the answer is No, please draw a floor plan or obtain one upon follow-up)*

Facility Security and Prevention of Commingling

12. Is the general area secure from public access?
13. Can individual laboratories be locked?
14. Are there signs on the walls or doors of individual rooms for research stating that USDA Regulated Material or Genetically Engineered Organisms are present?
15. Are there signs posted stating, “Authorized Personnel Only”?
16. Are there lockable cabinets or storage areas for storing all regulated materials? (seeds, tissue cultures, microbial material, etc.)
17. Is each storage cabinet/area identified as containing USDA Regulated Material or Genetically Engineered Organisms? (If the answer is **No** to 14, 15 or 17, indicate in SOF when sign(s) will be posted.)
18. Will regulated material be clearly marked by color coding and/or labeling?
19. Will markings or labeling be clear and durable? (Ask to see an example)
20. Do SOPs clearly specify methods for clean-up/disposal of spilled seed/regulated material?
21. Do SOPs cover the cleaning or disposal of equipment, including personal protective equipment, such that regulated materials are not inadvertently released into the environment? (seeds, pollen, microbes, etc.)
22. Do SOPs clearly specify methods to be used for devitalization and disposal of regulated material after work with material is completed? *(Please specify these methods in the SOF)*
23. Will non-transgenic sexually compatible species be absent from research areas during the entire length of the trial?
24. If the answer to above question is **No**, will marking or labeling be sufficient to segregate regulated material from non-transgenic material?

*NOTE. *This worksheet provides a non-exhaustive list of sample questions one may be asked during an APHIS-BRS inspection.*

25. If the answer to question 23 is **No**, will transgenic plants/organisms be prevented from reaching sexual maturity during the entire length of the trial?
26. Are records (log or inventory) maintained regarding receipt, propagation and destruction of regulated material?pread the disease. Seeds need to be acid- or bleach-treated to be rid of TMV.

Laboratory

27. Does this trial/research involve use of laboratories? (If the answer is No, skip to the section on Growth Chambers)
28. Will seeds, tissue cultures, plant material, etc. be grown or germinated in the laboratory? (Yes or N/A)
29. Is the area free of any cracks or irregular surfaces that could trap seeds?
30. Is the area free of obvious places that seed may be lost or lodged?
31. Will regulated material work be conducted in a biosafety cabinet or hood? (Yes or N/A)
32. Is the entire laboratory free of any water drains?
33. If the answer to above question is **No**, do the drain(s) flow into a special waste trap?
34. Are water drains screened with an appropriate screen size for the material being used in this research?
35. Are methods for disposal/devitalization of collected seeds/material clearly specified in SOPs? (Please indicate these methods in the SOF)

Growth Chamber

36. Does this trial/research involve use of Growth Chamber(s)?
    (If No, skip to the section on Greenhouses)
37. Can the growth chamber(s) be locked?
38. Is the growth chamber dedicated for use with transgenic material?
39. Will this transgenic research be the only work being done in the growth chamber?
40. Will plants/microbes be prevented from reaching sexual maturity in the growth chamber?
41. If the answer to above question is **No**, does the venting/HVAC system likely prevent flow of pollen/spores into the environment outside of the facility?
42. Is the growth chamber free from any water drains?
43. If the answer to above question is **No**, do the drains flow into a special waste trap?
44. Are water drains screened with an appropriate screen size for the material being used in this research?
45. Are methods of disposal/devitalization of collected seeds/material clearly specified in SOPs? (Please indicate these methods in the SOF)

Greenhouse

46. Does this trial/research involve use of Greenhouse(s)?
    (If the answer is No, skip to the SOF section)
47. Is the greenhouse accessible to authorized personnel only?
48. Is the greenhouse manager aware that this research involves USDA regulated transgenic material?
49. Is the greenhouse manger aware of the Permit conditions? (SOPs, Standard Permit Conditions, Supplemental Permit Conditions)
50. Do greenhouse doors and all alternate exits have locks?
51. Does the greenhouse have a double door entry system or a head-house to help prevent escape of regulated material into the surrounding environment?
52. Will plants be prevented from reaching sexual maturity in the greenhouse?
53. If the answer to above question is **No**, will flower bagging or removal be used to prevent pollen flow?
54. If the answer to above question is **No**, is the vent and exhaust fan/HVAC system likely to prevent flow of pollen/seeds/spores to the surrounding environment?

55. Will non-transgenic sexually compatible species be absent from the greenhouse during the entire length of the trial?
56. If the answer to the above question is **No**, will marking or labeling be sufficient to segregate regulated material from non-transgenic material?
57. Will soil used in this research be re-used again? (Please list method of soil treatment/devitalization or disposal in the SOF)
58. Do roof and/or side vents open manually? (Yes or N/A)
59. Do roof and/or side vents open automatically? (Yes or N/A)
60. If the answer to above question is **Yes**, do greenhouse controls have an over-ride to prevent vents from opening automatically?
61. Are roof and/or side vents screened with an appropriate screen size to prevent movement of most insects?
62. Are greenhouse exhaust fans enclosed in screen structures? (Yes or N/A)
63. Do exhaust fans have louvers that are working properly? (close automatically when fans turn off, well lubricated, intact)
64. Is all greenhouse screening generally intact without noticeable holes or gaps?
65. Is the integrity of the greenhouse walls, floors and doors adequate to exclude rodent/varmint vectors?
66. Does the greenhouse have "sticky" board or tape type insect traps?
67. Does the greenhouse have black light traps?
68. Does the greenhouse have other kinds of traps to prevent insect or rodent pollen/seed vectors? *(Please list other types of traps in the SOF)*

Inspecting official—please list any other concerns about the capability of this facility to safely contain the regulated organisms in the Summary of Findings (SOF) section. *(Please include photos of labs, growth chambers, greenhouses, and storage areas.)*

Appendix IV.

APHIS-PPQ OUTLINE FOR STANDARD OPERATING PROCEDURES

I. Introduction

1. Background information on the facility
2. Major objectives and activities (arthropods, plant pathogens, noxious weeds, biological control agents, etc.)
3. Location of the facility

II. Physical Containment Standards

Describe the physical characteristics of the facility in detail using the guidelines.

1. Description of site (e.g., distance from commercial crop production areas, airports, international borders, highways, etc.)
2. Fence
3. Buffer area
4. Demarcation of the facility
5. Schematic floor plan of the facility on 8" X 12" paper
6. Mechanical floor plan (if available) on 8" X 12" paper (reduced to 50%)
7. Blue prints on 8" X 12" paper (reduced to 50%)
8. Description of the facility with safeguards in each compartment of the facility (see guidelines) depicted by photographs (e.g., entrance and exit doors, vestibules, shower facilities, corridors, various laboratories, soil preparation room, growth chamber rooms, greenhouses, sterilization room, emergency exit, etc.
    a. Walls, ceilings, and floors
    b. Windows
    c. Exterior doors
    d. Heating, ventilation, and air conditioning - detailed written description (e.g., screens, filters, HEPA filters, negative pressure, biocontainment testing, etc.)
    e. Benches, cabinets, etc. in laboratories
    f. Electrical system
    g. Plumbing system
    h. Communication system
    i. Vacuum cleaning system
    j. Vacuum aspiration system

III. Equipment Standards

Describe the equipment present in the facility—follow guidelines.

1. Benches, tables and other furniture
2. Solid waste sterilization–type of autoclave, time, temperature, pressure, quality control, etc.
3. Liquid waste sterilization–description of effluent treatment systems
4. Sterilization of non-autoclavable articles (e.g., camera)
5. Cages and containers
6. Biosafety cabinet

IV. Operational Standards

Describe the general operating procedures–follow guidelines.

1. Containment director–designation of a containment director (name, address, and telephone number of the containment director)
2. Responsibilities of containment director
3. Signs
4. Accessing the facility
    a. Before entering the facility (e.g., personal apparel, hand washing, etc.)
    b. Entering the facility
    c. Exiting the facility
5. Sanitation
    a. Sanitizing miscellaneous articles and equipment
    b. Sanitization of personal belongings and use items
    c. Removal of articles from containment
    d. Maintenance and repairs in the containment
    e. Probable local emergencies and contingency plans to manage them. Detailed description is needed.
6. Cleaning and disinfesting the facility
7. Opening and handling packages from foreign sources
8. Start, grow, and store cultures
9. PPQ regulatory requirements

V. Description of special procedures for handling plant pests (arthropods, plant pathogens, noxious weeds, biocontrol agents, etc. and infected/infested plants)

Describe in detail specific procedures used for handling plant pests under permit.

Appendix V.

Resources

Regulatory Contacts

USDA-APHIS
4700 River Road, Unit 147
Riverdale, MD 20737
Web: http://www.aphis.usda.gov/

USDA-APHIS-PPQ
For general inquiry and permits:
Phone: (301) 734-0841
Fax: (301) 734.5392
Email: Pest.Permits@aphis.usda.gov

USDA-APHIS-BRS
For general inquiries or to contact a biotechnologist:
Phone: (301) 734-7324
Email: biotechquery@aphis.usda.gov
For accidental release of regulated article or compliance issue:
Phone: (301) 734-5690
Fax: (301) 734-8669
Email: BRSCompliance@aphis.usda.gov.

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

National Greenhouse Manufacturers Association (NGMA)
4305 North Sixth Street, Suite A
Harrisburg, PA 17110
Phone: 800-792-NGMA, 717-238-4530
Fax: 717-238-9985
Email: ngma@ngma.com
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.

USDA NCERA-101 / NCR-101
Committee on Controlled Environment Technology and Use http://ncr101.montana.edu/

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)
http://www.aergc.org/

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. Interested individuals from other institutions such as botanical gardens or private companies are welcome as members also. 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.

Glossary

Air circulation The process of moving or mixing air within a greenhouse to control temperature, humidity, and carbon dioxide distribution.

APHIS regulated article Term used by USDA-APHIS for an organism that has been genetically engineered (via recombinant DNA techniques) from a donor organism, recipient organism, vector, or vector agent that is a plant pest or contains plant pest components.

Autoclave A pressurized vessel using saturated steam under pressure to sterilize or decontaminate materials and equipment.

Baking out The process of raising the room temperature to 40–45˚C for two to three days to kill pest loads in the greenhouse.

Biological containment The use of biological means to block plant sexual and vegetative reproduction and to prevent the spread and persistence of genetic material in the environment.

Biosafety levels A combination of administrative controls, work practices and procedures, equipment, and facilities required to achieve a designated level of containment.

BSL-3Ag A special facility designed, constructed and operated at a unique containment for research involving certain biological agents in large animal species. BSL-3Ag facilities are specifically designed to protect the environment by including almost all of the features ordinarily used for BSL-4 facilities as enhancements. All BSL-3Ag containment spaces must be designed, constructed and certified as primary containment barriers.

Containment facility A structure for the storage and/or propagation of a biological control agent that is designed to prevent the escape of the enclosed plant pest organisms.

Current Good Manufacturing Practice (cGMP) A set of standards for the food and drink industry aimed at ensuring that products are consistently manufactured to a quality appropriate to their intended use, first published in 1987.

Decontamination A process whereby viable microorganisms are removed from solutions, surfaces, or materials by filtration, heating, radiation, or chemicals to an acceptable level.

Evaporative cooling The addition of moisture to air to reduce its temperature.

Exotic and Invasive Species Any species, including its seeds, eggs, spores, or other biological material capable of propagating that species, that is not native to that ecosystem; and whose introduction does or is likely to cause economic or environmental harm or harm to human health.

Glazing The transparent or translucent covering of a greenhouse, usually glass, plastic film, or rigid plastic panels.

Glutaraldehyde A colorless liquid with a pungent odor used to disinfect equipment.

Good Laboratory Practice (GLP) A set of principles that provides a framework within which laboratory studies are planned, performed, monitored, recorded, reported and archived.

Greenhouses Controlled environment structures having a transparent or translucent covering and used for growing plants.

Growth chambers and growth rooms Self-contained controlled environments created specifically for plant research.

High Efficiency Particulate Air (HEPA) filter A disposable extended/pleated medium, dry-type filer composed of a mat of randomly arranged fibers and designed to remove at least 99.97% of all 0.3 micron spherical particles in aerosol.

Inches of water gauge A unit of pressure equal to the weight of a column of liquid water 1 inch high at 20˚C. The measurement is used to measure the air pressure in some high security containment facilities.

Insect vector Any insect capable of transmitting a pathogen from one host to another.

Institutional Biosafety Committee (IBC) Established under the NIH Guidelines for Research Involving Recombinant DNA Molecules, these committees, comprised of at least five people, provide local review and oversight of nearly all forms of research utilizing recombinant DNA.

Isolation distance The minimum distance required between varieties of the same species to prevent cross-fertilization by pollen dispersed by wind or gravity.

Light traps Insect traps that use some type of light as an attractant.

Magnahelic gauge An instrument used to measure differential pressure.

Nonregulated status Term used by APHIS for an organism that does not present a plant pest risk.

Permit An authorization to move into or through the United State a plant pest, regulated article, product or means of conveyance.

Plant pest Any living stage of any insects, mites, nematodes, slugs, snails, protozoa, or other invertebrate animals, bacteria, fungi or other parasitic plants or reproductive parts thereof, viruses, or any organisms similar to or allied with any of the foregoing, or any infectious substances which can directly or indirectly injure or cause disease or damage in any plants or parts thereof, or any processed, manufactured, or other products of plants.

Plant-made Industrial Compounds (PMIC) Compounds for industrial use produced in live plants.

Plant-made Pharmaceuticals (PMP) A category of therapeutic agents (pharmaceutical proteins) produced in live plants.

Protective clothing Boots, shoe covers, overalls, and hats worn in some high security containment facility .

Recombinant DNA Genetically engineered DNA prepared by transplanting or splicing genes from one species into the cells of a host organism of a different species. Such DNA becomes part of the host's genetic makeup and is replicated.

Ridge and furrow A type of greenhouse construction where modular units are connected at the gutters to cover large ground areas.

Screen Any woven, matted, flat sheets of material that may be used to block entry or exit of arthropods, mollusks, nematodes, or other plant pests.

Screenhouses Structures that are screened for insect or plant containment (or exclusion) but that offer little environmental control.

Select Agents/Toxins Agents that Department of Health and Human Services considers to have the potential to pose a severe threat to human health. High Consequence Livestock Pathogens and Toxins are agents that the USDA considers to have the potential to pose a severe threat to animal or plant health, or to animal or plant products. The plant pathogens listed by USDA have been deemed a threat to plant health or products. Agents that post a severe threat to animal health, animal products and also public health are referred to as "Overlap Agents." These agents appear on both the HHS and USDA list of agents and toxins.

Standard Operating Procedure (SOP) Codified best laboratory practices for handling biological control agents in quarantine or containment.

Ventilation rate The volume of air exchanged per unit of time per unit floor area.

Vestibule A hall or room between two rooms or between the outside and the interior of a building.

VHP Vaporized hydrogen peroxide used as a chemosterilant.

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