**PLANT/PLANT PATHOGEN RESEARCH**

This topic can be divided into two areas which may overlap: research with plant and/or plant pests and research with transgenic plants. The two cornerstones of biosafety are risk assessment and containment. Both of these will be discussed.

**RISK ASSESSMENT**

A risk assessment starts with identifying all the hazards associated with the project and determining the likelihood that it will occur. A hazard is defined as a source of harm or danger. Risk is the likelihood that it will occur. So you are determining what can go wrong, the probability of that occurring and what the effects might be.

In classic biosafety risk assessment the focus was on the prevention of laboratory-acquired infections with human pathogens. Research dealing with nonhuman pathogens/transgenic plants must focus on the effect the biohazard will have on the environment and the resulting economic impact.

**The risk assessment sequence is:**

1. Identify all risk factors or hazards
2. Review guidelines, regulations, publications to determine current standard practice
3. Assess available data, both field and laboratory
4. Consult subject matter experts if necessary
5. Design your mitigation strategy using administrative, work practice and engineering controls.

**QUESTIONS TO ASK**

**For Plant Pathogens/Pests (bacteria, viruses, fungi, arthropods, nematodes, etc.)**

1. What is the nature of the agent?
2. Is the organism pathogenic for plants, animals, humans, and how virulent (aggressive) is it?
3. Is it endogenous or exotic to the United States/North Dakota?
4. What is the route of transmission? Air-borne, water-borne, soil-borne, direct contact etc.
5. What is the host range? A single species of plant or many
6. Are local hosts present?
7. How communicable is it? How easily is it transmitted from plant to plant?
8. What is the infectious dose?
9. How stable is it in the environment?
10. What is the capacity to control or eradicate the organism if it escapes?
11. What is the history of the organism in other new environments?
12. Is there risk information already available for the organism?
13. What are the locations and proximity of suitable hosts and the time of year of the proposed work?
14. What is the potential rate of local and long distance spread?
15. Are there any vectors present (e.g. arthropods, fungi, nematodes)?
16. Are there vectors present in or near the containment facility?
17. How persistent is the organism in the environment and what is its potential for overwintering?
18. What are the environmental requirements for establishment and spread?
19. Will it be cultured? If so, how large of a volume and at what concentration?
20. Will it be restricted to the lab or disseminated in a greenhouse or field plot?
21. What measures will be used to avoid dissemination of the organism (recombinant or wild type) beyond the laboratory or greenhouse?
22. What is the economic and environmental significance of potential pest organisms and their host plants?
23. Are there any biosecurity related risks (e.g. potential for theft and misuse)?

**For a Plant:**

1. What is the nature of the plant?
2. Is it endemic or exotic?
3. Is it a noxious weed?
4. Is it parasitic on other plants?
5. What impact will an accidental release have on agricultural production, forestry or natural environments?
6. Can the plant interbreed with local noxious weeds?

**For Transgenic Plants/Recombinant Organisms:**

A transgenic plant/organism contains a gene or genes which have been artificially inserted instead of through natural acquisition such as pollination in the case of plants or conjugation in the case of bacteria. The inserted gene sequence (known as the transgene) may come from another unrelated plant, or from a completely different species: transgenic Bt corn, for example, which produces its own insecticide, contains a gene from a bacterium. Plants containing transgenes are often called genetically modified or GM crops, although in reality all crops have been genetically modified from their original wild state by domestication, selection and controlled breeding over long periods of time. On the UND Biological Safety webpage we will use the term transgenic to describe a plant which has transgenes inserted.

1. Describe the genes that will be introduced into the plant/microorganism. What is the source and nature of the DNA?
2. Is it a complete genome or a fragment of DNA?
3. What is the vector that will be used to transport the DNA?
4. What is the nature of the expressed protein? Is it a vertebrate toxin or allergen? Is it toxic to other organisms in the local environment?
5. How will the transgenic plants/recombinant microorganism be contained?
6. Will the resulting altered plant be considered a noxious weed?
7. Will the altered plant be able to interbreed with noxious weeds?
8. What impact will an accidental release have on agricultural production, forestry or natural environments?
9. What is the local environment? What is the nature and importance of nearby crops? Are sexually compatible wild or weedy species in the vicinity?
10. What are the experimental procedures for transferring: to and from greenhouse etc.
11. What is the recipient organism and its mode of dissemination?

**Containment**

Plants and plant pests rarely infect or infest healthy humans and therefore pose little direct risk to lab personnel. Some, however, can pose a significant threat to agricultural production, forests or natural ecosystems. As a result it is important that personnel working with plants and plant pests or facilities housing these organisms take steps to prevent the accidental escape of potentially damaging plants or plant pests into the environment. The containment requirements for a particular plant or plant pest are often project-specific and are determined after assessing the risk factors associated with the biology of the plant/plant pest and the impact an escape might have. The risk model in Table 1 demonstrates the general principle of requiring increased levels of containment with increasing risk of escape/establishment and the impacts on the economy, environment, agriculture, forests and trade in the event of an escape.

The U.S. government and other entities have published sets of guidelines for containment of plants and plant pests. The most common guidelines cover the following:

1. noxious weeds and parasitic plants
2. nonindigenous, phytophagous arthropods and their parasitoids and predators
3. plant pathogenic nematodes
4. bacterial plant pathogens
5. fungal plant pathogens
6. viral plant pathogens and their vectors

United States Department of Agriculture Animal and Plant Health Inspection Service

Containment Facility Guidelines for Noxious Weeds and Parasitic Plants
<http://www.aphis.usda.gov/plant_health/permits/downloads/noxiousweeds_containment_guidelines.pdf>

Containment Facility Guidelines for Arthropods
<http://www.aphis.usda.gov/plant_health/permits/downloads/arthropod_biocontrol_containment_guidelines.pdf>

Containment Facility Guidelines for Nematodes
<http://www.aphis.usda.gov/plant_health/permits/downloads/nematodes_containment_guidelines.pdf>

Containment Facility Guidelines for Non-indigenous Snails
<http://www.aphis.usda.gov/plant_health/permits/downloads/snails_containment_guidelines.pdf>

Containment Facility Guidelines for Bacterial Plant Pathogens
<http://www.aphis.usda.gov/plant_health/permits/downloads/bacteria_containment_guidelines.pdf>

Containment Facility Guidelines for Viral Plant Pathogens
<http://www.aphis.usda.gov/plant_health/permits/downloads/plant_viral_pathogens_containment_guidelines.pdf>

Containment Facility Guidelines for Fungal Plant Pathogens
<http://www.aphis.usda.gov/plant_health/permits/downloads/plant_fungal_pathogens_containment_guidelines.pdf>

**DESCRIPTIONS OF THE PLANT BIOSAFETY LEVELS**

**BL1-P:** This designation provides for a low level of containment with 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. An example would be an experiment with transgenic potato plants containing cloned genes for insect resistance obtained from primitive potato cultivars.

This biosafety level also applies to DNA-modified microorganisms that cannot spread rapidly and are not known to have any negative effects on either natural or managed ecosystems. This would include experiments using a transgenic *Rhizobium* strain containing *Agrobacterium* genes known to affect root colonization.

**BL2-P:** This level is assigned to experiments with transgenic plants and associated organisms, which if released from 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 may be capable of interbreeding with weeds or related species growing in the vicinity. An example would be greenhouse tests with transgenic sunflower containing wheat genes able to confer resistance to the fungus *Sclerotinia*. This would be classified as BL2-P because sunflower is capable of hybridizing with wild relatives and becoming established as a volunteer weed.

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

**University of North Dakota does not have the facilities for BL3-P and BL4-P work.** These levels deal with exotic agents that can have a detrimental impact on the local environment. For more specific information, consult one of the above references.

**Table 1 Suggested Criteria for Assigning Biosafety Levels**

|  |  |  |  |
| --- | --- | --- | --- |
| **CRITERIA** | **TRANSGENIC PLANTS** | **TRANSGENIC MICROBESExotic Non-exotic** | **INSECTS/ANIMALS/ASSOCIATED MICROBESWILD TYPE OR TRANSGENIC** |
| 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 BL-1P+ |  |  |  |
| Contains complete genome of non-EIA\* | BL2-P or BL-1P+ |  |  |  |
| Contains genome of EIA | BL3-P orBL2-P+ |  |  |  |
| Treated with an EIA | BL3-P orBL2-P+ |  |  |  |
| Detriment to environment |  | BL3-P orBL4-P\*\* | BL2-P or BL-1P+ | BL3-P or BL-2P+ |
| Involves EIA with detriment to environment | BL3-P or BL-2P+ |  |  |  |
| May reconstitute genome of infectious agent *in planta* | BL3-P or BL-2P+ |  |  |  |
| Contains vertebrate toxin | BL3-P | BL3-P | BL3-P |  |

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

**Table 2 Standard Practices for BL1-P and BL2-P Containment**

|  |  |
| --- | --- |
| **BIOSAFETY LEVEL 1-P** | **BIOSAFETY LEVEL 2-P** |
| Discretionary access | Access limited to individuals directly involved with experiments |
| Personnel must read and follow instructions | Personnel must read and follow instructions |
| Procedures followed are appropriate for organisms | Greenhouse manual to advise of consequences: give contingency plans |
| Record kept of experiments in facility | Record kept of experiments and movement in/out of facility |
|   | Containment required for movement in/out of greenhouse |
| Biologically inactivate experimental organisms at end of experiment | Biologically inactivate experimental organisms at end of experiment; decontaminate gravel periodically |
| Pest control program | Pest control program |
| Appropriate caging and precautions for escape of motile organisms | Appropriate caging and precautions for escape of motile organisms |
|   | Sign for restricted experiment in progress with plant names, responsible person and special requirements |

**PERMITS**

Permits are required under specific USDA Animal and Plant Health Inspection Service Regulatory Authorities to import designated plants, plant products and soil into the U.S., transport designated plants and plant products through the U.S., import plant pests and biological control organisms into the U.S., and move plant pests and biological control organisms between states. View this page for more information about permitting. The FDA and EPA may also be involved in the regulation of transgenic plants. See Table 3.

<http://www.aphis.usda.gov/plant_health/permits/index.shtml>

**Table 3 Interagency Permitting**

|  |  |  |
| --- | --- | --- |
| **NEW TRAIT/ORGANISM** | **REGULATED REVIEWCONDUCTED BY:** | **REVIEWED FOR:** |
| Viral resistance in food crop | USDAEPAFDA | Safe to growSafe for the environmentSafe to eat |
| Herbicide tolerance in food crop | USDAEPAFDA | Safe to growNew use of companion herbicideSafe to eat |
| Herbicide tolerance in ornamental crop | USDAEPA | Safe to growNew use of companion herbicide |
| Modified oil content in food crop | USDAFDA | Safe to growSafe to eat |
| Modified flower color in ornamental crop | USDA | Safe to grow |
| Modified pollutant degrading soil bacteria | EPA | Safe for the environment |

**References**

1. NIH guidelines for research involving recombinant or synthetic nucleic acid molecules.
2. A Practical Guide to Containment: Greenhouse research with Transgenic Plants and Microbes, Information Systems for Biotechnology, Virginia Tech.
3. Saint Louis University Plant/Pathogen Research.