In the United States, three agencies have regulatory oversight at the federal level over genetically engineered plants—USDA-APHIS is one of those three. The EPA becomes involved in the field testing process for pesticidal plants, or, more correctly, plant-incorporated protectants, once field tests reach the 10-acre limit. From a regulatory standpoint, agencies have very specific questions to consider as they make their decisions. What follows is an overview of APHIS regulatory authority and procedures and their application to laboratory work in industry and academia. Also, how the new genetic constructs and approaches to modifying plants fit into that overall regulatory scheme will be discussed.

Much of this workshop will focus on some of the earliest stages of field testing. Figure 1 represents the field tests that have been performed since 1987, and a clear, increasing trend is evident. Plant biotechnology is a very active area of research. In recent years, APHIS typically processed around 1200 - 1400 authorizations for field tests; this past year the number was again about 1400. Since 1987, over 8,700 field tests have been authorized at over 30,000 sites. Additionally, a single authorization from APHIS might cover several field sites. A wide diversity of crop plants (36 species), grasses (10 species), trees (13 species), and ornamentals (9 species) has been field tested since 1987.

A comparison of the types of genes used in 1988 with the types currently used suggests that in 1988 the state of the art of plant bioengineering was fairly straightforward. Currently in 2002, a lot of the plant engineering work is generated by the plant genome projects. In addition, a number of different systems are emerging in which researchers are using animal models and developing plant analogs to answer some very interesting questions about plant biology. These new and diverse systems prompt APHIS to question the assumptions we have previously made about regulating field tests. Are those assumptions still valid when we examine these “newer” types of gene constructs?

APHIS uses two separate mechanisms for authorizing field tests: permits and notifications. They have slightly different requirements, which may present important considerations for this workshop.

APHIS regulations are administered under the Plant Protection Act of 2000, which is a consolidation of several acts including the Plant Pest Act, the Federal Plant Quarantine Act, and the Noxious Weed Act. The regulations have not been changed since the consolidated act was instituted. APHIS regulations were first promulgated in 1987, and then amended in 1993 to outline the system for notification procedure and also to describe the process for granting non-regulated status. In 1997, we amended the regulations again to broaden the eligibility for notifications to virtually all plants, as long as the plants are not noxious weeds in the release area.
Criteria for Field Testing of Plants with Engineered Regulatory, Metabolic, and Signaling Pathways

The permit is the original mechanism APHIS used to regulate field tests. It is a fairly straightforward and paper-intensive procedure. The notification procedure is for plants only; whereas permit authorizations can be used for all regulated articles: plants, microbes, and arthropods.

The time required for review is longer for permits than notification. For field testing, review is a 120-day process under permits and a 30-day process under notification. Importation approvals require 60 days under permits, versus 30 days for notification. Interstate movement approvals take 30 days under permits versus 10 days under notifications. Importation and interstate movements are defined as transport from one contained facility to another. The regulated article must remain in containment during the entire trip so that there is no chance of environmental release. Under both systems, permits and notifications, APHIS interacts with the States so that they have the opportunity to concur with the proposed APHIS authorization.

Under a permit application, there are no restrictions imposed on the types of traits that can be approved for testing, but under the notification procedure certain genes or traits are not eligible. APHIS has had less experience over the years evaluating those traits that are considered ineligible for notification are or those that might pose a higher risk or whose risk is less well characterized.

A notification contains basically two main categories of information: eligibility criteria and performance standards. The eligibility criteria are shown in Table 1. To be eligible, the recipient plant cannot be a noxious weed or weed in the release area. Also, the inserted genetic material has to be stably integrated and its function known.

The phrase “function is known” as it relates to the eligibility criteria for the notification procedure was applied when APHIS was first developing the regulations in 1992. Researchers who were working with disease resistant response genes and who were evaluating these genes in field tests authorized under APHIS permits did not know precisely what the genes did (i.e., what the gene products were). However, they knew that expression of these genes was increased when the plants were inoculated with pathogens. Therefore, as APHIS was writing the eligibility criteria for the new notification procedure, they decided that the level of characterization of gene function for the disease resistant response genes would not meet the criterion for “gene function is known.” These plants can still be field tested, but an applicant must apply for a permit rather than utilize the notification procedure. Conversely, the Bt genes are examples of genes whose function is known and therefore qualify for the notification procedure.

Table 1. Brief summary of the six eligibility criteria for notification.

1. Recipient is not a noxious weed, or a weed in the release area
2. Stable chromosomal integration of the genetic material
3. Function is known: does not result in plant disease
4. Genetic material does NOT:
   • cause the production of infectious entities,
   • result in toxic effects on associated nontarget organisms, or
   • encode substances intended for pharmaceutical use
5. Plant-derived virus sequences must be:
   • regulatory sequences of known function,
   • sense or antisense genes from prevalent & endemic virus that infects the recipient plant and
   • not functional noncapsid cell-to-cell movement genes
6. No animal or human pathogen sequences that are:
   Viral coding sequences of a likely causal agent of disease

Although the same level of biological confinement occurs under both systems, the mechanisms differ in the types of information required to be submitted to the Agency and States. When applying under a permit, the applicant must provide a detailed list of the protocols used to achieve biological containment. Under notification, built in to the regulations is the stipulation that the applicant must meet performance standards. The type of design protocols used may vary from experiment to experiment, and the applicant is not required to submit a written protocol at the time the request is made for a notification. Rather, the applicants periodically must submit a set of design protocols that they may use at one of their
test sites. APHIS reviews the design protocols for adequacy to ensure that the applicant has a high likelihood of meeting the performance standards if the protocols are followed. Under both systems, field sites are periodically inspected by APHIS personnel and relevant State Department of Agriculture officials.

An additional eligibility criterion stipulates that the genetic material cannot cause the production of infectious entities that could produce toxic effects on associated non-target organisms. Under this criterion, the Bt genes would qualify for the notification procedure. Other excluded classes include those substances intended for pharmaceutical use. Another eligibility criterion pertains to plant virus sequences and is intended to reduce the chance of generating new viral components during field testing. The final criterion addresses animal and human pathogen sequences that are likely to cause disease.

Performance standards comprise the second part of the notification procedure. Basically, performance standards require that plants in a field test are grown so that nothing is left behind in the environment when the test is completed. This stipulation has been used in field testing in the U.S. and elsewhere, whether the test is conducted under a notification procedure or under permits or some other procedure.

To achieve confinement of transgenic plants, scientists must consider the potential for outcrossing and pollen and seed dispersal by either biological or physical mechanisms. Some methods for achieving confinement at the test site include: termination of the test prior to flowering; male sterility; inhibiting or removing flowers; bagging; spatial separation (using isolation distances such as those proposed by the Association of Official Seed Certifying Agencies (AOSCA)) from sexually compatible species; and temporal separation of flowering cycles. Additional mitigation measures may be taken such as planting windbreaks or border rows. A key to establishing confinement measures in the United States and elsewhere is to take the knowledge gained from traditional plant breeding for minimizing the persistence of material in the environment and apply it to genetically engineered plants. Beginning in 1987, in the course of preparing an environmental assessment for each field release permit, APHIS discussed how to apply the knowledge underlying the AOSCA seed purity standards used to maintain seed stock purity to design appropriate confinement measures for field testing. This approach has been recognized within the scientific community as a good place to start and is used around the world in designing confinement measures for field testing plants.

Regulatory compliance mechanisms include inspections and reporting requirements. Records, facilities, and sites are inspected during planting and harvesting of the plant material and following the field test. In addition, a field test report has to be submitted to the agency following the test. If anything unplanned or unusual occurs during the field test, the responsible party must notify APHIS. APHIS has the authority, under the Plant Protection Act, to levy substantial fines for non-compliance, if necessary. Fortunately, compliance is very high, so the agency rarely has to impose fines.

The following is a summary of the context in which regulatory decisions are made by APHIS when considering the field testing of plants engineered with these newer, “complex” genes (an imprecise term chosen to simplify the discussion).

First, do these genes meet the eligibility criteria?
That question has two components: (1) is the function known and (2) is it unlikely to affect non-target organisms? These two key questions determine whether the field test will be handled under the notification or permitting system.

Second, what are the confinement protocols?
Performance standards stipulate that the genetically modified material has to be confined during the test and that essentially nothing is left behind when the test is over. Confinement is the issue, whether the field test is handled under a notification or a permit. Likewise, the likelihood for the impact on non-target organisms is an issue for both mechanisms. To restate this second point about the confinement, the determination depends on the assumptions we have made. When making a relatively small genetic change, the assumption has been that, for the most part, the plant is going to behave in the environment like the unmodified parent plant. It will likely flower
about the same time and have the same interaction with other organisms. Issues affecting confinement such as reproductive biology and changes in the vegetative biology are important considerations. Another consideration is the likelihood that the modified plant will impact non-target organisms.

One of the questions APHIS needs to answer when considering some of these unusual constructs, or “complex genes” as they are referred to, is “Should our assumptions be the same or different from the assumptions that have been widely used for the less complex genes?” We want to consider how we reach our conclusion, i.e., what scientific information is available to inform our assumptions.