SEG ISSUES WORK GROUP REPORT

Work Group Objectives:
The SEG plan calls for the Issues Work Group to:

- Develop answers to key questions, issues and concerns raised by stakeholders where information exists to address each.
- Develop and execute a plan for addressing key questions, issues and concerns raised by stakeholders where information does not currently exist (including indentifying associated costs to develop the information); and
- Providing answers to issues raised in the process of the Outreach & Communications Work Group deploying their plan.

Background Information:
During the June, 2009 inaugural meeting of the SEG, participants identified initial questions, issues or concerns for the Issues Work Group to explore. Participants categorized the subject areas based on whether they believed information did or did not readily exist as follows:

Information exists
How will the following be addressed in the use of biotechnology:

- Alleopathy;
- Invasiveness;
- Genetic diversity;
- For species like the American chestnut, capturing as much native diversity as possible (the intersection of invasiveness and genetic diversity);
- Genetic migration; and
- Physical expressions

Information does not readily exist
How will the following be addressed in the use of biotechnology:

- Consequences;
  - unintended based on what we don't know;
  - unintended based on what we thought we knew; and
  - unintended based on intended performance.
- Ecological dynamics; and
- A restoration plan.

Current Status:
The Work Group has produced the attached draft issue paper on *physical expressions*. This draft paper is intended to generate discussion among the Social/Environmental, Science and Regulatory/Policy Groups to help determine the appropriate format and approach for future issue papers.

Since June, the Issues Work Group has met by conference calls and has exchanged ideas by email, during which the Group identified the following additional thoughts:

- Traditional breeding should be used as the baseline to compare biotechnology.
- Use of Chinese chestnut genes may be an issue that needs to be addressed.
- What is the right research to determine if planting a biotech tree "is appropriate?"
- Experience with existing biotech trees may provide the information necessary to address some of the questions.
- Will never know the answer to all of the questions.
- Principles Work Group should address what is "acceptable level of risk" – what is the tipping point?
- Consider some sort of analysis illustrating at a landscape level what happens if biotechnology is not used.

Issue Work Group Participants:
Anne Law – American Bird Conservancy
Maud Hinchee – ArborGen
Susan McCord – Institute or Forest Biotechnology
Doug Williams – Association of Consulting Foresters
Steve Strauss – Oregon State University
Leila Pinchot – Pinchot Institute
Dana Nelson – USDA-Forest Service
Ray Albright – National Park Service

FHI ISSUE PAPER

Physical Expressions

Issue

How should we scientifically assess physical and biological changes to the environment imparted by transgenic trees.

Definition

"Physical Expressions" is a term that has been coined to capture the effect of an introduced gene on the phenotype (i.e., measurable characteristics) of transgenic trees.

Non-technical synopsis

The goal of this document is to outline how to scientifically assess the ways and extent to which a transgenic tree (modified by genetic engineering methods) has impacts on the physical and biological environment. It includes examples of how assessments for transgenic trees might be done, and experiences from agricultural biotechnology. Assessments should consider expected direct and indirect impacts, as well as to search for unintended effects of transgenic modification. Important expressions to consider include effects on non-target organisms, soil nutrition and toxicity, and the ability of trees to compete and disperse. All assessments should be include field-based comparisons to contemporary forestry practices, such as conventional breeding, planting, and harvesting, so that the relative magnitude of impacts can be determined.

Understanding "Physical Expressions"

Transgenic trees by definition are trees which have had a gene or genes added to the original set of genes that comprises its genetic makeup. The introduced gene contains a specific DNA sequence, which when transcribed into RNA, can produce proteins or affect gene expression of the tree's original gene set. This results in an added feature or trait in the performance of the tree. Let's use two genes involved in the disease resistant American Chestnut project as an example. One gene being introduced is an oxalate oxidase gene obtained from wheat. This gene produces an enzyme that oxidizes oxalic acid and generates hydrogen peroxide, which then effectively inhibits the growth of an invading fungal pathogen. Another gene is the neomycin phosphotransferase gene that comes from *E. coli*. This gene is used to enable the gene insertion process by conferring antibiotic resistance to allow for the selection of cells which have stably incorporated the gene which confers the desired trait.

Physical expressions can be characterized into three categories: 1) primary effects, 2) secondary effects and 3) pleiotropic effects. The two gene examples above will be used to provide insights into the effects that are part of these 3 different categories.

Wheat oxalate oxidase

<u>Primary effects</u>: catalyzes the oxidation of oxalate (oxalic acid) into carbon dioxide and hydrogen peroxide. Removal of oxalate and production of hydrogen peroxide have a negative effect on the cells, as well as the fungus, in fungal invaded plant cells. This stops the fungal infection process.

<u>Secondary effects</u>: oxidation of glycolic acid and malic acid is also catalyzed by oxalate oxidase into products involved in photosynthesis and amino acid biosynthesis

<u>Pleiotropic effects</u>: none have been reported in the literature, but these would be detectable as the plant grows, and could manifest as changes in non-target phenotypes such as leaf color, plant size, root mass, flowering time, production of secondary products, etc.

<u>E. coli</u> neomycin phosphotransferase

<u>Primary effects</u>: phosphorylation of aminoglycoside antibiotics, including neomycin, kanamycin, gentamicin B, paramomycin, ribostamycin, butirosin, and geneticin (G418), which renders the antibiotic inactive. ribostamycin, butirosin, gentamicin B and geneticin (G418) as substrates and renders the carrier of the trait resistant to these antibiotics.

<u>Secondary effects</u>: No literature was found indicating that substrates other than aminoglycoside antibiotics could be phosphorylated.

<u>Pleiotropic effects</u>: No literature identified any non-target phenotypes, such as stature, form, disease resistance etc) that were associated with the introduction of the nptII gene in transgenic plants.

Risk Identification Associated with Physical Expressions

The following questions are used to identify possible risks.

1. Does the primary effect itself cause harm in addition to the intended positive effect (in the case of our example above – disease resistance in chestnut)?
    a. This is the target effect, and it quantified and characterized in multiple transgenic lines that express this trait
    b. If yes, then does this phenotype have any known risk factors?
        i. Does the expressed protein have any harmful effects to humans, animals, other plants, microbes? This could include toxicity, allergenicity?
        ii. Does this primary effect provide the tree with a characteristic that could have plant pest characteristics (i.e. it is harmful to other plants, does become more invasive?

2. Are there secondary effects?
    a. These are known effects based on the literature, and are experimentally investigated (quantified/characterized) in the transgenic tree.
    b. If yes, are there any harmful or undesirable effects of the secondary effects to other organisms? This may occur by changes to pest resistance, where organisms other than the target pest are affected. It may also occur by changes to soil properties that affect the ability of other plants to germinate or grow (e.g., allelopathy).
    c. Does the secondary effect provide the tree with a characteristic that has plant pest characteristics? A transgenic modification, such as for pest or stress resistance, may affect the ability of a tree to survive and produce propagules, and thus to persist and spread. Such changes may be viewed as desirable, such as for the spread of a pest-threatened species in wild forests or to help promote survival under climatic stress, and actively promoted,. It may also be viewed as undesirable and considered as "invasive" or" weedy," and thus steps taken to mitigate and manage spread.
    d. The source and extent of human modification of the transgenic trees that may spread in the environment may influence how they are studied. Transgenic trees with genes that are viewed as familiar, such as those from a closely related species, or with DNA sequences that are similar to those of closely related species, are likely to more acceptable to some stakeholders and thus require less scrutiny than genes that are novel/exotic to the ecosystem.
    e. Does the secondary effect render the transgenic tree less desirable towards achieving its acceptance by stakeholders, expected performance and other desired desired functional outcome?

3. Are there pleiotropic effects?
    a. These are effects that were not initially predicted, but are discovered when the transgenic tree is observed as it grows
    b. If yes, are there any harmful effects to other organisms?
    c. Do the observed pleiotropic effects provide the tree with plant pest characteristics?
    d. Do the observed pleiotripic effects render the tree less desirable towards achieving its acceptance by stakeholders, expected performance and other desired functional outcomes?

Assessment of Magnitude of Risk

Identified risks are characterized by their potential impact. For example, if a transgenic plant exhibits the phenotype of having red leaves instead of green leaves, then it may be deemed as having little harm to other organisms (unless if effects behaviors of beneficial insects or other organisms). This would be considered a low potential risk characteristic. If the transgenic plant has a secondary effect in which a toxic compound is produced that negative impact on the growth and health of other organisms, then this would be considered a high risk characteristic

Comparative Assessment of Risk

To inform scientists and society about the importance of ecological impact, it is desirable to have information to allow meaningful comparisons about the extent of impact. For example, if a transgenic variety affects a soil property such as organic matter, or the abundance of insect herbivores, it would be useful to know how conventional varieties of trees, or common harvesting practices, also affect those same properties. Very small effects, even if scientifically reliable, may not be of ecological or social significance. The comparators should be appropriate for the systems considered. For example, for hybrid poplar plantations the comparisons should be a range of other hybrid varieties that are cultivated or in applied breeding programs, grown under similar conditions. For American Chestnut, comparators could include native ecosystems without viable chestnuts other than stump sprouts to forests with surviving chestnut trees. It might also include Chinese hybrid and/or backcross resistant chestnuts. Field trials are essential for determining impacts under realistic ecological conditions.

Case Example: Assessment of Potential for Insect-Resistant Bt-Corn to Cause Harm When Released Into the Environment

A characteristic that has a high risk profile may not cause actual harm. Harm is caused when a characteristic with a risk potential has the opportunity, via presence or timing, to actuate it's negative impact on another organism. An example of a transgenic plant, with a potentially harmful characteristic but low risk is Bt corn. The primary effect in Bt corn is kill or cause the cessation of feeding by caterpillar pests. These pests are highly damaging and required the extensive use of pesticides in order to generate adequate corn yields. The same Bt protein in the corn plant can also kill Monarch butterfly caterpillars. This is characterized as a potentially harmful secondary effect. The question was posed as to whether Bt pollen could be shed onto milkweed plants, upon which the butterfly larvae obligately feed, which are growing in corn fields. However, it was determined that the potential for actual harm is low because farmers who grow Bt corn manage their crop so that weeds, such as milkweed, are not present in significant amounts in their corn fields. The Monarch butterflies had sufficient milkweed available outside of corn fields and their numbers were not affected.

Mitigation and/or Management of Identified Risks

Once a characteristic is identified as having potential to cause harm, then actions can be taken to reduce any negative impacts.

1. Developers of transgenic trees should do thorough literature reviews to identify in advance the potential for primary or secondary effects to cause harmful effects.

Developers would follow Responsible Use Guidelines, and not release these plants into the environment.

2. Regulatory approval process will make an assessment as to whether the transgenic plant is safe to release into the environment. Their decision can be: no release, release with management/monitoring requirements, release with minimal or no management or monitoring requirements.

3. Mitigate risk.

  a. Modify genes (or use another gene source) to reduce potential for harmful primary, secondary or pleiotypic effects.

  b. Modify gene expression, so that gene is induced only when needed, only in certain cells or tissues.

c. Add other genes which counteract the potential for harmful effects.

4. Manage risk.

a. Remove opportunity for potentially harmful characteristic to have an impact. This could involve limiting or controlling presence, distribution, frequency, density, sales etc of transgenic tree relative to organisms that can be potentially impacted.

i. management requirements associated with regulatory approval/compliance

ii. management requirements established by the tree provider

ii. third party organization establishes management policy and ensures compliance of members with its management policy (eg certification schemes)